

PhD. Thesis

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Doubly Photopolymerised Holographic Sensors

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Abstract

With expanding and aging populations globally, pressure on healthcare systems is expected to increase in coming decades. The requirement for next generation point-of-care biosensors to monitor population health is an emerging necessity for medical providers to maintain patient treatment quality. Point-of-care devices can permit patients to obtain medical data without medical professional involvement, potentially increasing measurement frequency and reducing the requirement for doctors or patients to attend appointments. Holographic sensors are uniquely placed to fulfil this requirement through low-cost fabrication, and colourimetric, reversible, and rapid readouts. The scope of this thesis is to expand the seldomly reported, doubly UV photopolymerised holographic sensor platform, including chemical functionalisation and provide evidence of viability in point-of-care applications. Double photopolymerised holographic sensors utilise a single coherent UV laser pulse (5 ns, 355 nm) to photopolymerise highly crosslinked nano-fringes, fabricating a Bragg mirror structures within a responsive hydrogel material from two distinct polymers. Holographic sensor parameters have been computationally modelled through COMSOL Multiphysics[®] to determine the dynamic relationship between recording, response, and fabrication parameters. Viable colourimetric sensors for real-time determination of pH and glucose concentration have been fabricated and verified in biological samples (blood serum and urine, respectively) with Bragg wavelength shifts of 90-170 nm over physiological ranges (pH = 7-9, glucose = 0.0-9.4 mmol L⁻¹). Each sensor utilizes a different hydrogel base network (pH: (hydroxyethyl)methacrylate, glucose: acrylamide) indicating the platforms potential for future applications. This thesis presents a nanoparticle free holographic sensing platform which has been functionalised through co-monomer incorporation for application in point-of-care testing, with optimisation of parameters applicable to future systems for controlling sensitivity, response time, replay wavelength, and diffraction efficiency.

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Declaration of Originality

This statement is to certify that all research within this thesis was completed by myself and is the outcome of independent work. All collaborative works have been appropriately referenced.

Samuel Davies

London, United Kingdom

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Publications

The following thesis chapters have been adapted from previously published literature:

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Introduction

In medical treatment the identification of biomarker concentrations is vital to monitoring patient homeostasis. Previously, the analysis of biofluids has required conventional lab analysis, which is both costly and time consuming. The development of new point-of-care (POC) technologies permits the low-cost monitoring of biomarkers at the bedside or outside of medical settings. The devices deliver accurate, rapid results, reduce medical staff workload, and improve patient outcomes by identifying disease warning signs earlier than previously possible.¹ The strength of POC devices was shown in the COVID-19 pandemic, where simple tests permitted the public to determine their infection status rapidly to minimise transmission. The global application of POC devices demonstrated a paradigm shift in medical care ethos to preventing illness occurring in contrast to treating patients once illness has developed.² POC devices can improve the data collection frequency, delivering healthcare providers a comprehensive overview of patient health, which combined with big data analysis could help identify warning signs of illnesses in the future.

The sensing technology applied in POC devices is varied, however, the main platforms used are electrochemical cells and optical platforms. All platforms will be important to the development of POC devices, each possessing characteristics applicable to different situations. Electrochemical sensors present highly accurate analysis; however, they can be prone to signal drift and require a transducer to permit realisation of the data collected. Optical sensors present unique attributes that are desired in the design of biosensors. At present, optical devices are a focus of research due to their label free response allowing the design of simple devices which can be used by patients outside of medical settings. This would potentially remove the requirement for costly analytical equipment, hospital appointments, or involvement of medical professionals in common illness monitoring.

Optical sensors are varied in their functioning mechanism, with the two main branches being fluorescent probes and photonic crystals. Fluorescent probes offer colourimetric confirmation of data, however due to the chemistry of the platform there are often specialised excitation requirements and a vulnerability to photobleaching that can hinder wider application. Holographic sensors (HS) are a branch of photonic crystal sensors with growing interest for the simple analytical platform they offer. Previously demonstrated HSs use a range of methods to produce a photonic structure, including silver nanoparticle (NP) photo deposition. The technique requires an extensive 12-step fabrication process which can hinder the ability to scale up the technology. Alongside this, the incorporation of silver NPs can impede sensors potential application to *in vivo* monitoring due to suspected links to toxicity. The doubly photopolymerised (DP) fabrication studied throughout this research eliminates these previous limitations. The seldomly reported technique uses a simple UV photopolymerisation process to produce nanoscale variations in refractive index across a responsive hydrogel matrix. The 4-step fabrication removes a major hurdle for the scale up to commercialisation of the sensing device.

The objective of the research demonstrated herein is to expand the understanding of the UV DP HS fabrication technique through computational modelling of sensor recording and response. This thesis also aims to demonstrate the chemical functionalisation of DP HS to analytes such as pH, and glucose through the incorporation of functional *co*-monomers to the hydrogel material. Throughout the development of these devices studies of parameters that affect sensor performance such as drying time and polymer composition are explored whilst optimising sensor response.

Preface

Chapter 1 contains an introduction to HSs, reviewing and discussing the recently published literature in the field, examining the fabrication techniques, and applications to the biotechnology field.

Chapter 2 demonstrates the methods and materials utilised throughout this work. Materials purchased and employed throughout the research are listed with technical information and purchasing details. Experimental methods include descriptions of all aspects of computational model design, sensor fabrication and readout analysis.

Chapter 3 details the computational study of parameter optimisation and the dynamic swelling characteristics of the two distinct polymer layers within the hydrogel sensors is examined.

Chapter 4 demonstrates the development of a chemically functionalised doubly UV photopolymerised HS for pH. Through the incorporation of functional pH sensitive *co*-monomers a wound monitoring optical device is established, with sensors validated in biological solutions.

Chapter 5 highlights the development of a doubly UV photopolymerised HS for urinary glucose determination. Further computational modelling is demonstrated to examine the effect of drying time on sensor properties. Hydrogel compositions are optimised, and sensor properties are exhibited with device response validated in human urine.

Chapter 6 draws final conclusions demonstrating the developments, limitations and further works required within the field.

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List of Abbreviations

Abbreviation	Definition
1D	One dimensional
2D	Two dimensional
3-AAPB	3-acrylamido phenylboronic acid
3D	Three dimensional
AM	Acrylamide
AU	Artificial urine
BAP	1,4-bis(acryloyl)piperazine
CAGR	Compound annual growth rate
CCA	Crystal colloidal array
CLI	Clindamycin hydrochloride
cm	Centimetre
COC	Cyclic olefin copolymer
CSF	Cerebrospinal fluid
CW	Continuous wave
<i>d</i>	Grating constant
DC	Dibenzo-18-crown-6
DI	Deionised water
DMAEA	Dimethyl aminoethyl acrylate
DMPA	2,2-Dimethoxy-2-phenylacetophenone
DMSO	Dimethyl sulfoxide
DP	Doubly polymerised
DSC	Differential scanning calorimetry

EGDMA	Ethylene glycol dimethacrylate
EMI	Electromagnetic interference
EWFD	Electromagnetic waves frequency domain
FDTD	Finite-difference time-domain
FEM	Finite element methods
h	Hour
hCE1	Human carboxylesterase-1
HEMA	2-(Hydroxyethyl)methacrylate
HMPP	2-hydroxy-2-methylpropiophenone
HPLC	High-performance liquid chromatography
HQ	Hydroquinone
HS	Holographic sensor
IF	Imprinting factor
IL	Interference layer
IPA	Isopropyl alcohol
L	Litre
L₁	Length of the multilayer Bragg structure
LFT	Lateral flow test
LIL	Laser interference lithography
M	Molar concentration
MAA	Methacrylic acid
MBA	Methylenebisacrylamide
MICA	Molecularly imprinted colloidal array
min	Minutes
MIP	Molecularly imprinted

mJ	Milli Joule
mm	Millimetre
mmol	Millimole
MRI	Magnetic resonance imaging
mW	Milliwatt
n_0	Effective medium refractive index
NC	Nanocrystal
NHS	National health service
NICA	Non-molecularly imprinted colloidal array
nm	Nanometre
NP	Nanoparticle
PBA	Phenylboronic acid
PBS	Phosphate buffered saline
PDMS	Poly(dimethylsiloxane)
PEGDA	Polyethylene Glycol Diacrylate
pK_a	Acidic association constant
PMMA	Poly-methacrylic acid
POC	Point-of-care
PVC	Polyvinyl chloride
PWE	Plane wave expansion
R	Diffraction efficiency
RH	Relative humidity
RI	Refractive index
RM	Responsive matrix
RSC	Royal society of Chemistry

SEM	Scanning electron microscope
SLM	Single longitudinal mode
TBC	Tetraethyl 4-tert-butylcalix[4]arene
TEM	Transmission electron microscope
TFMPA	2-(trifluoromethyl acrylic acid)
TRIS	Tris(hydroxymethyl)aminomethane
USD	United States dollar
USAF	United States Airforce
UV	Ultraviolet
V	Volt
v/v	volume per volume
WO	World intellectual property organisation
Δn	Refractive index modulation
ϵ	Strain
θ	Diffraction angle
λ	Wavelength
μL	Microliter
μm	Micrometer
μs	Microsecond
$\varnothing$	Diameter

Chapter 1. Holographic Sensors in Biotechnology

Note: The following section has been previously published as a manuscript in the journal. **Advanced Functional Materials,**

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As populations expand worldwide, medical care will need to diversify its data collection techniques to be able to provide adequate healthcare to global populations, this could be achieved through POC analysis by wearable analytical devices. HSs are reusable optical biosensors with the capability to continuously monitor variations, generating the prospect of *in vivo* monitoring of patient homeostasis. Holographic optical sensors have emerged as an opportunity for low cost and real-time POC analysis of biomarkers to be realised. This thesis aims to summarise the fundamentals and fabrications of HSs; a key focus will be directed to examining the biotechnology applications in a variety of analytical settings. Techniques covered include surface relief gratings, inverse opals, metal NP and NP free HSs. This article provides an overview of holographic biosensing in applications such as pH, alcohol, ion, glucose, and drug detection, alongside antibiotic monitoring. Details of developments in fabrication and sensitising techniques will be examined and how they have improved the applicability of HSs to POC analytics. Although HSs have made significant progress in recent years, the current challenges, and requirements for advanced holographic technology to fulfil their future potential applications in biomedical devices will be discussed.

1.1. Introduction

HSs belong to the category of optical sensors which use light as a method for detailing variation in analytes or conditions. The optical sensing platform is seen to be of increasing in significance in recent years with applications spanning industrial, environmental, food monitoring systems,

and biosensing to mention a few.³⁻⁹ Currently, a rise in value of the optical sensor market can be observed, with further increase being projected. The POC market is expected to increase 7.3% annually reaching \$23.36 billion by 2027.¹⁰ Some of the factors on this growth can be attributed to the rise in wireless sensing technologies, need for enhanced security and safety solutions, and better alternatives for conventional sensing technologies.¹¹ The benefits of HSs which give them a market advantage include high accuracy and reliability, immunity to electromagnetic interference (EMI), and a projected high return on investment in the long run.¹² Moreover, holographic devices are usually low cost, easily transportable, and offer less complex readouts than conventional sensing methods.¹³ They also require less energy input, and therefore are more environmentally friendly.¹¹ However, lack of awareness, high initial investment and insufficient technological standards are aspects that can be seen as limitations in market growth of optical sensors and should be further addressed.¹¹

1.1.1. History of Holographic Sensors

Optical diffractive structures are present in numerous common objects and animals observed in the world around us. Animal and insect species often display structural colouration on their bodies giving them iridescent colour (Figure 1a).¹⁴⁻¹⁶ An example of this phenomenon is the wings of *Morpho* butterflies (Figure 1b).¹⁶ Electron microscopy of the wings of these insects reveals the periodic structure responsible for the colouration (Figure 1b-ii).¹⁷ The surface of the wings is covered in a highly ordered 3D array of nanostructures which reflect a narrow band interaction with light in the blue region of the spectrum.¹⁷

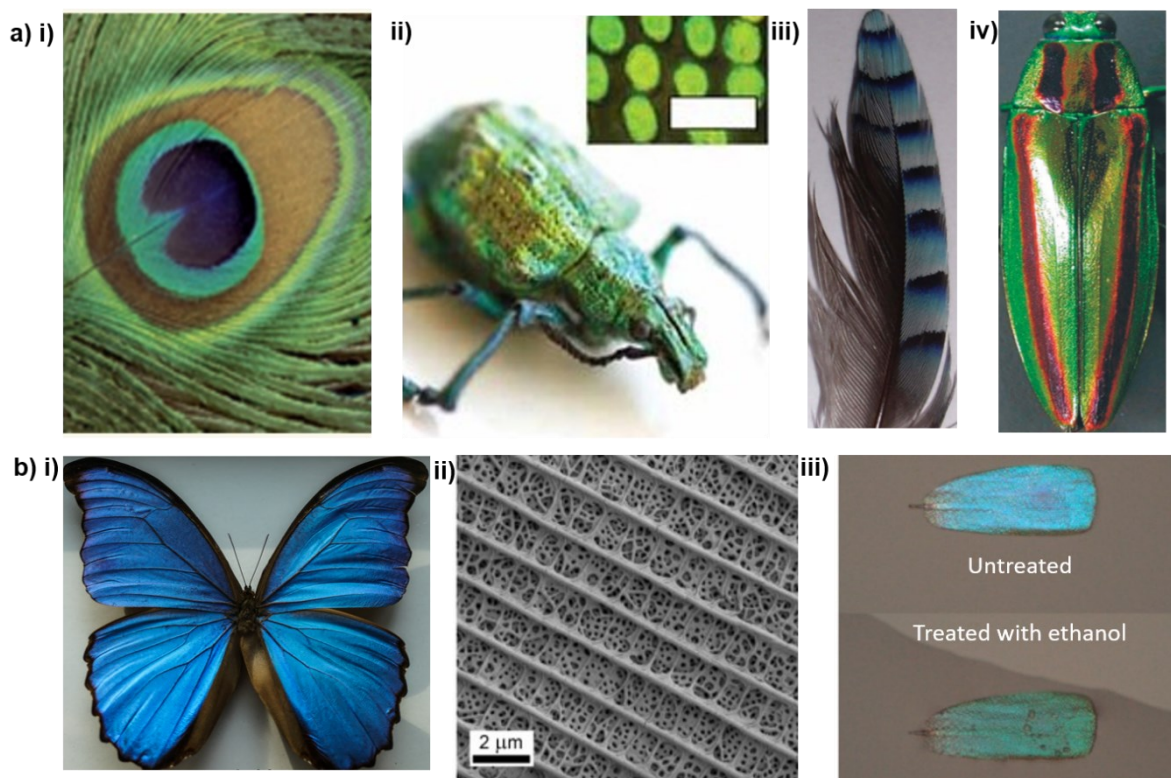


Figure 1. Demonstration of the natural occurrences of optical structures and the responsive nature of the grating. a) i) Structural colouration observed in the iridescent colour of peacock feathers, reproduced with permission from Springer Nature;¹⁵ ii) Photograph of weevil *L. Augustus*, inset demonstrates the green coloured photonic scales, reproduced with permission from Wiley Publishing;¹⁴ iii) Periodically patterned Jacy feather, Open access from Nature Scientific Reports;¹⁸ iv) Image of the structural colouration of the Japanese jewel beetle, *Chrysochroa fulgidissima*, reproduced with permission from The Royal Society,¹⁹ b) i) Photograph of *Morpho* butterfly, reproduced with permission from Springer Nature; ii) Transmission electron microscopy (TEM) image of wing scales of a *Morpho* butterfly, iii) *Morpho* butterfly wing as observed in the wild (top), *Morpho* butterfly wing treated with ethanol (bottom), Reproduced from MDPI, Open Access.¹⁷

Holographic recordings have been established since the mid-20th century. Dennis Gabor a British-Hungarian scientist often referred to as the ‘Father of Holography’, initially was able to generate holographic gratings using the interference pattern between a reflected wave front and reference wave emitted from a relatively uncoherent light source.²⁰ It was for this work

that Gabor alone was awarded a Nobel Prize in 1971. Although Gabor initially published his findings in 1947, the work was unable to be utilised widely due to the technology at the time being unable to produce monochromatic coherent light. The explosion of holographic research was later in 1960, with the invention of the laser²¹ which allowed Emmett Leith to record the first 3D hologram.²² Holographic recording can be considered similar to normal photography where light is directed onto a photosensitive medium which records the incident light which, post development, shows an image of the scene. Normal photography records a single front of light whereas HSs utilise a complex interference pattern between two coherent waves. One wave front is reflected from the object, which contains the image wave, the other light is a reference beam.²³ Both components are directed onto the photosensitive recording material and the interference pattern between the two waves gives rise to the holographic recording containing a 3D image of the object. Up until this point holographic gratings served data storage and artistic purposes, in modern science holographic recordings have found application in developing sensors. By utilising smart materials as a substrate for simple holographic structures, wavelength selective Bragg mirrors with tuneable responses to chemical and physical stimuli are achievable.²⁴ The variation in wavelength can be calibrated against the volumetric responses to physical or chemical stimuli within the hydrogel film. The development of both smart materials such as functionalised hydrogels and HSs have worked in tandem. The functionalisation of films to correspond to change in biomarker concentrations allows for simple biosensors to be developed *via* chemical functionalisation.

1.1.2. Requirement for Holographic Sensors in Biomedical Applications

Recently, a lot of focus has been placed on the practical aspects of sensing, for instance, introducing simple readout methods to improve affordability and accessibility to healthcare worldwide.^{25, 26} HSs can deliver label free sensing to remove a number of complex processing steps often required by conventional chemical analysis, therefore reducing the level of training

and intricate equipment required to obtain reliable data.²⁷ Smartphone applications can be designed to convert sensor response into a qualitative result.²⁸ This in tandem with the opportunity to fabricate sensors using biocompatible fibres. Biocompatibility can allow for the long-term exposure of biological systems to sensing platforms, offering truly continuous monitoring. These characteristics offer the opportunity for HSs to be truly disruptive in the POC biosensor industry.

There has been rapid development in the use of functionalised nanostructured surfaces to allow for quantitative and accurate measurement of biological analytes with the development of sophisticated substrates such as hydrogel based ionic skins.²⁹ HSs for analysing hydration,¹⁷ monitoring respiration,¹⁸ urinalysis,³⁰ tear fluid analysis,^{31, 32} and alcohol intoxication³³ within the body have been highly investigated in recent years due to the growing drive to personalised medical care.³⁴ The development of these devices offers the possibility for healthcare providers to analyse the realtime concentrations of biomarkers using simple inexpensive analytical equipment. Due to the inherent holographic sensing mechanism utilising the reversible binding of analytes continuous monitoring is achievable, giving a whole new dimension to the field of medical treatment. Offering real-time data of a patient's condition, permits the personalisation of medical treatment. An example would be in the absorption, distribution, elimination, and metabolism of drugs, the rates of these processes vary in every individual. If a dose regime has a narrow therapeutic index, this could be due to high interindividual drug variability or due to toxicity risks, dosing accuracy is vital.^{35, 36} Conventional chemical analysis methods for example high-performance liquid chromatography and liquid chromatography-mass spectroscopy would not be viable due to cost and training requirements. HSs are classed as an optical sensor, utilising light as a medium to indicate variation of analytes. The deployment of metabolite sensitive HSs to patients could report variation in metabolites directly to patients or medical professionals remotely permitting personalisation of medical treatment. This capacity

for a patient or doctor to monitor the body more frequently than in conventional appointments allows treatment to be administered when required rather than utilising a general time frame, ensuring the maintenance of the therapeutic effect.^{37,38} In the previous two years we have seen how powerful POC analytics have been in the COVID-19 pandemic.³⁹ Rapid lateral flow devices have allowed global populations to be better informed of their infection status without time consuming polymerase chain reaction tests, this benefit could be applied to several illnesses through the application of holographic devices.

HSs exhibit characteristics specifically beneficial to biosensing functions when compared to their electrochemical counterparts. The lack of requirement for electrical currents or large metallic components of HSs, permits remote monitoring in numerous applications within demanding environments i.e. high magnetic fields.⁴⁰⁻⁴² In modern medicine, Magnetic Resonance Imaging (MRI) scans have become a powerful diagnostic tool dependent on the use of high magnetic fields.⁴³ The strong magnetic field by which MRI scan images are generated can lead to several complications when metallic components of electrochemical cells are introduced. Metals interacting with the magnetic field generate distortions known as ‘artefacts’ within the images obtained, giving unreliable readouts which can lead to misdiagnosis. In tandem with this interference, metals within a magnetic field will be physically pulled towards the magnet source, alongside electromagnetic heating to temperatures capable of causing permanent damage to the patient.⁴⁴ HSs are able to remove this interference and risk due to the absence of metallic wires and the use of only light as the sensing medium. Allowing HSs to be applied in tandem with MRI scanning which may in future this will allow for new avenues of diagnosis to be explored, for example analysis of biological processes within the brain whilst simultaneously recording MRI scans. This development creates a possibility of obtaining previously inaccessible data about degenerative diseases furthering our understanding.⁴⁵

1.1.3. Optical properties and Sensitisation

The fabrication methods explored in this thesis contain periodic nanostructures with varying refractive index (RI) capable of interacting with light to generate an optical response. Change in RI alters light passing through layered structures giving rise to structural colouration as a Bragg mirror.⁴⁶ RI is defined by Eqn. 1, where n is the RI, c is the speed of light and v is the phase velocity of light.

$$n = \frac{c}{v} \quad (1)$$

The colour observed is dependent on the fringe spacing between changes in RI of these scales, defined by Bragg's law (Eqn 2), where d corresponds to the grating period, n is an integer, λ is the reflected wavelength and θ is the angle of diffraction from the normal. This interaction occurs when light interacts with a periodic structure, causing a specific wavelength of light to be scattered by the matrix. Alteration of this d spacing tunes the wavelength of light reflected (Figure 2a). This correlates with the responses seen from the other optical structures whereby their surroundings can alter the response observed.

$$n\lambda = 2d\sin(\theta) \quad (2)$$

The recording of HSs is dependent upon the use of constructive interference between incident and reflected light to generate points of high and low intensity (Figure 2b).⁴⁷ A range of laser wavelengths can be used to generate the holographic grating. The interference between the two propagating waves generates nodes and anti-nodes where the fringe structures are recorded.⁴⁷ This dependency of initial fringe spacing on the recording laser has a direct impact on the replay wavelength observed. Holography has origins in the chemistry utilised in photography, but significant differences clearly separate the two processes in their application and future scope. Photographic images are the recording of a wave front reflecting from an object, whereas HSs depend upon the recording of the interference pattern of the beam reflected from the object and a reference beam. The interference between these two counter-propagating

beams gives rise to points of high and low intensity which creates a perspective in the image recorded. By controlling the way in which these two beams interfere holographic gratings can be recorded in two different conformations, either reflection or transmission. The main difference between these two recording methods is the direction from which the two incident beams interact. If the two beams come from opposing sides, then the hologram recorded will be in reflection, whereas if the two beams come from the same side the hologram will be in transmission. The resultant interference patterns between these two arrangements give rise to the gratings illustrated in Figure 2c. These two conformations differ in how they interact with light, reflection gratings will selectively reflect a single wavelength dependent upon the periodic d spacing of the fringes, whereas transmission gratings will create a diffraction pattern spreading the light that passes through them dependent upon the d spacing.⁴⁷

Sensitisation to specific biomarkers is achieved by utilising responsive hydrogel materials which initiate a volumetric change upon the introduction of external stimulus.⁴⁸ These polymer networks are modified with sensitising moieties, receptive to specific analytes, which stimulate swelling of a material dependent on the concentration of an analyte in the test solution. The volumetric response of the network leads to alteration of the layer spacing and therefore a variation in the optical properties observed. This thesis will aim to look at the various methods of formulating nanostructures within smart hydrogel networks and how different formulation methodologies can be utilised to generate a variety of sensing platforms.

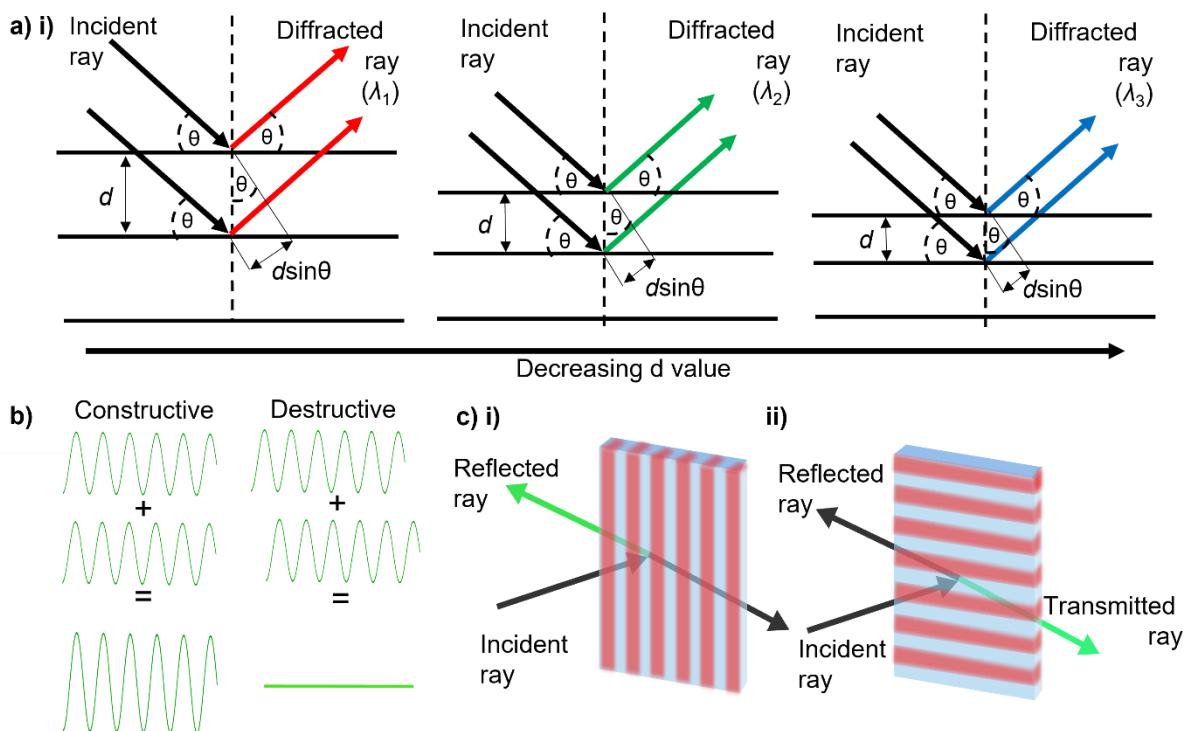


Figure 2. Overview of some of the fundamentals of holographic interactions with light. a) Dynamic relationship of reducing layer spacing and the tuning of the diffracted wavelength (λ); b) Constructive and destructive interference between wavelengths; c) Diffraction gratings recorded in i) reflection and ii) transmission and how the grating orientation affects the interaction with light.

The holographic sensing mechanism is intrinsically built into the structure, not requiring an external electrical input to stimulate detection offering a route to continuous monitoring of analytes increasing the level of collectable data. Holographic colourimetric sensing permits users to observe variation of specific analytes via the reflected wavelength variation without the requirement for expensive analytical equipment. Alongside this, outside the biosensing field, these properties can allow for a rapid identification of contaminants or leaks in industrial settings without human stimulus, which may both save lives and highlight the presence of an issue prior to a major incident.^{49, 50} With electrochemical cells, it is a necessity to recalibrate the device regularly due to signal drift.⁵¹ Recalibration although tedious, is less of an issue for non-medical devices or monitoring of samples, but if a device is

to be used *in-vivo* recalibration becomes a significantly more difficult task. Optical structures rarely require a re-calibration once formulated as they are less prone to signal drift.⁵¹ Reduction in the requirement for recalibration decreases overall impact on patients day to day activities. Electrochemical cells are currently considered to be capable of a higher level of accuracy in the of monitoring analytes than optical structures, this thesis aims to explore the current developments of optical sensors.

1.2. Fabrication of Holographic Sensors

The photonic structures required for the generation of HSs can be produced in several ways. Due to the optical properties being dependent upon the spacing of fringes, nanoscale fabrications are often required. This can range from casting an optical template to give a final product with optical properties or *via* the use of interferometric light interactions to stimulate regularly spaced changes within a substrate on a nanoscale.⁵² Within this thesis several of the most prominent and established fabrication methods will be examined.

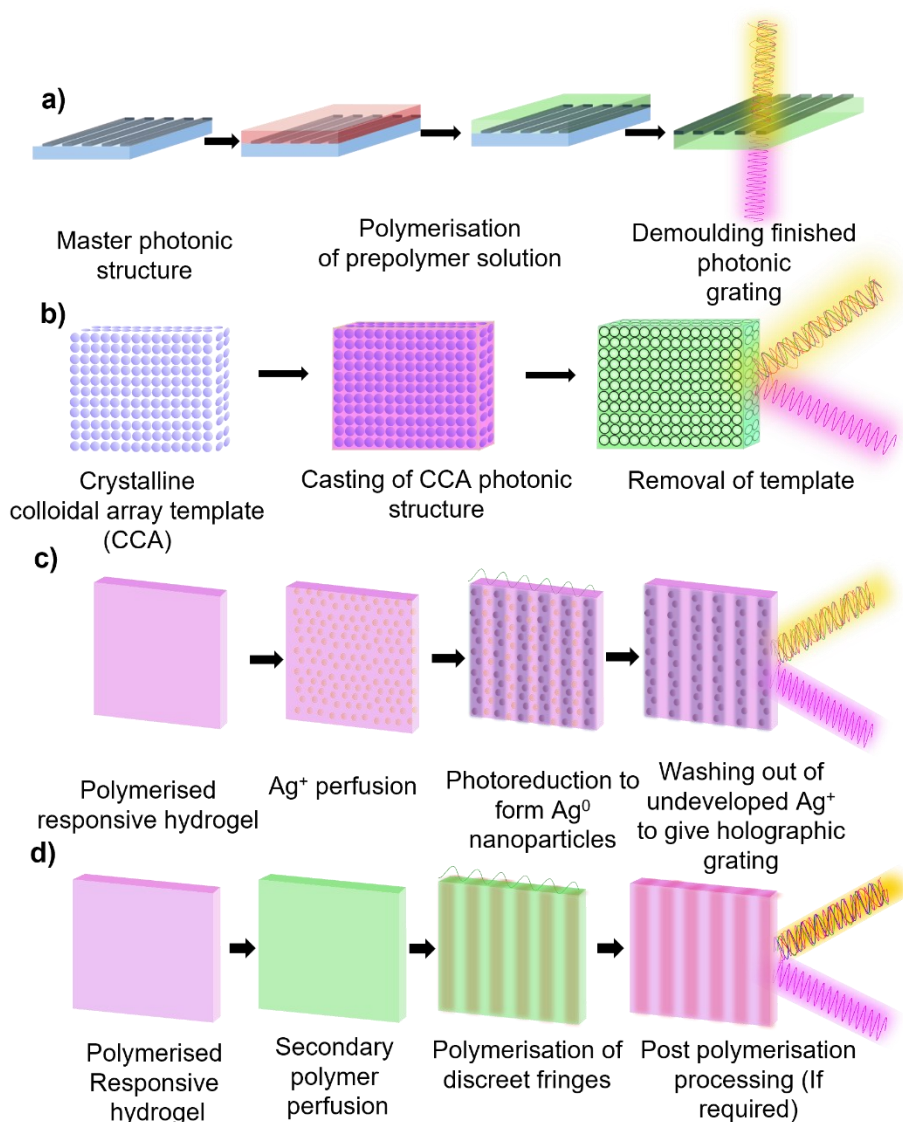


Figure 3. Fabrication techniques of holographic gratings. a) Photonic stamp casting of responsive hydrogels generating holographic surface gratings; b) Inverse opal fabrication *via* casting of a crystalline colloidal array; c) *In-situ* photo reduction of metal salts to form metal NP fringes; d) Photopolymerization of secondary polymer fringes.

1.2.1. Surface Relief Gratings

Holographic casting is a fabrication method that involves the copying of an optically active ‘master’ template with a responsive hydrogel material. The resultant copy obtained from this process produces a device with chemical sensing properties alongside the optical

characteristics of the template (Figure 3a). The technique is advantageous as the production of optical devices can be relatively straightforward and low cost.⁵³ This process produces a grating structure on the exterior of the material which can interact with light as a simple wave guide. This fabrication method can be utilised with a wide range of substrates such as responsive monomers or responsive liquid crystals.⁵⁴ The simplicity permits optical structures to be fabricated on large scale and offers opportunities for attachment to novel probes such as optical fibre for medical devices.⁵⁵ Sensors of this type have been demonstrated with sensitivity to pH, glucose, alcohols, ionic strength, ions, temperature, and humidity.⁵⁵⁻⁵⁸

1.2.2. Inverse Opals

Inverse opals differ from surface relief gratings due to the polymeric structure being a negative cast of a colloidal crystalline array (CCA) (Figure 3b).⁵⁹ The CCA is responsible for imparting the photonic structure of the sensor, which can be fabricated through simple colloidal self-assembly. This simple master optical structure fabrication removes the requirement for expensive initial investment for a coherent laser source to generate the fringe structures therefore reducing overall costs of production. After polymerisation, the CCA template can be dissolved generating an optical structure completely formulated from polymer. This is beneficial as the sensor does not possess any NPs which permits simpler approval for use in biological systems.⁶⁰ The sensing properties are defined by the polymer used therefore the limitations on sensing are defined by the ability of hydrogels to respond to analytes. Inverse opals have been utilised to detect changes of pH,⁶¹ humidity,⁶² and macromolecules.⁶³ Although the main focus in this thesis will be on the optical properties of inverse opal sensors, the unique morphology of the structures can be applied to controlled release and sorption of compounds in chemical applications due to their porous nature.⁶⁴ Overall optical structure casting is a simple and powerful technique for producing optical sensors without the requirement for coherent laser light.

1.2.3. Metal Nanoparticles

Photolithographic production of metal NPs is one of the most common methods used to fabricate holographic gratings. The process is comparable to development of silver nitrate film in black and white photography. To generate metal NP holograms, photochemical reactions are utilised to produce ultrafine layers of NPs within a chemically responsive material (Figure 3c). Metal halide (i.e. AuBr/AgBr) solutions are diffused into a chemically responsive material which is exposed to coherent laser light.²⁴ Interference between incident and reflected laser light can generate points of high and low intensity to reduce metals such as silver or gold. After photoreduction of the metal ions and development, stable NPs are formed. Silver NPs create a high change in RI producing a Bragg mirror structure within the polymer material. This production process is advantageous in several ways as the technique is reliable and gives rapid responses to chemical analyte changes. NP HSs have previously been applied in the detection of numerous stimuli, i.e. glucose, enzymes, pH, ions, ionic strength, and cocaine.⁶⁵⁻⁷¹ Metal NP formulation methods were the genesis of photolithographic HSs and are a cornerstone of the field. In future with the development of novel NP/small molecule technology,^{72, 73} NP holography could be functionalised with complex chemical properties.

1.2.4. Nanoparticle-free Holographic sensors

Techniques previously described for the fabrication of holographic structures depends upon materials of inorganic materials or physically printed fringes to generate changes within a material to vary the RI. Another photolithographic method is the use of a secondary polymeric material to generate holographic structures.⁷⁴ To fabricate NP free sensors a monomer solution with a high crosslinking concentration is soaked into the sensitive hydrogel matrix. Once saturated the hydrogel is exposed to laser light, where constructive and destructive interference between incident and reflected beams polymerise fringes at points of high intensity producing a grating structure. Variation of RI is achieved by the increased crosslinking density to produce

a wavelength selective Bragg mirror within a responsive hydrogel (Figure 3d). At present NP free holograms have been applied in sensing alcohol, glucose, ions, temperature, and humidity.⁷⁴⁻⁷⁷ The removal of NPs from holographic gratings removes some of the barriers that have previously hindered the NP based systems due to the potential health risks of NP leaching.⁷⁸ Benefits are also observed in the reduction in the complexity of processing of holograms, by removing the development and fixation steps required in metal NP HSs the production of holographic gratings on a mass scale is more achievable. These chemical processes also require the use toxic chemical reagents which can be carried over into the finished product, by removing these steps entirely the potential risks are mitigated. The improved scalability can also be attributed to the reduction in cost of raw materials, NP free holograms only require monomer compounds to create holographic gratings and do not require the use of silver and gold, two of the most precious elements on the planet. Overall NP free HSs are not as well established as NP-based systems but with time these systems could allow for greater expansion.

1.3. Applications of Holographic Sensors

HSs have been expanding at a rapid rate improving the selectivity and sensitivity to compete with that of conventional biosensing techniques. This has been achieved with techniques such as the molecular imprinting of hydrogel materials and increasing the selectivity of chemical binding sites through specific chemical interactions. This section aims to demonstrate the recent developments in holographic optical sensors, highlighting the improvements in selectivity, sensitivity, and simplicity of readout.

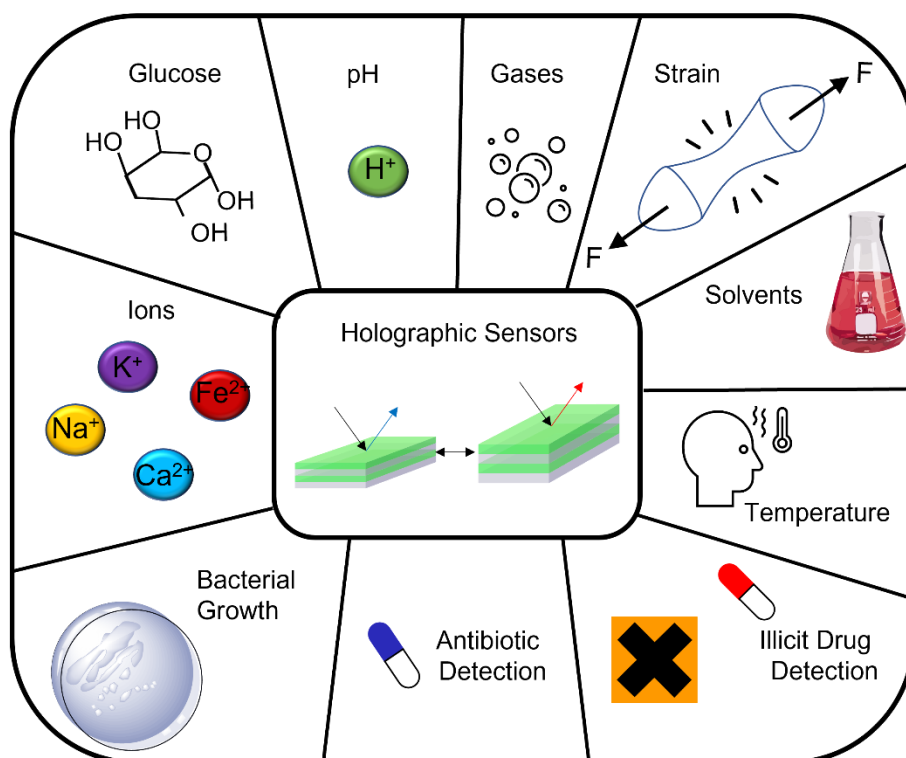


Figure 4. Summary of the analytes to be covered throughout this thesis.

1.3.1. pH Monitoring

Monitoring of pH is universal across many physiological processes, pH indicates respiratory issues,⁷⁹ wound healing,⁸⁰ and diagnosis of gastro oesophageal reflux disease.⁸¹ A pH sensor was fabricated in the form of 1D slanted photonic crystal flakes.⁸² The fabrication process involved preparing the monomer solution consisting of 2-hydroxyethyl methacrylate (HEMA) (91.5 mol %), ethylene glycol dimethacrylate (EGDMA) (2.5 mol %), and methacrylic acid (MAA) (6 mol %) which was then polymerized using free radical UV polymerization on a PMMA surface (chosen as a temporary substrate). AgNO_3 salt was used as a source of Ag^+ for embedding Ag^0 NPs in the p(HEMA-co-MAA) matrix which was exposed to a 5 ns Nd:YAG pulsed laser ($\lambda = 532 \text{ nm}$, 350 mJ) (Figure 5a). The sample was immersed in the exposure bath at an inclination of 5° from the surface plane of the mirror (Figure 5b). The film was detached from the PMMA substrate via immersion in a water/ethanol solution (50%v/v), where films would detach to be subsequently shaped into round photonic crystal flakes.

The pH response of the sensor is based on ionization of the carboxylic acid groups in the hydrogel matrix as the pH increases. As the MAA was ionized, the Donnan osmotic pressure increased, resulting in water uptake into the p(HEMA-*co*-MAA) matrix, therefore increasing Ag⁰ NP lattice spacing, shifting the Bragg peak to longer wavelengths. The grating was illuminated with a supercontinuum white light laser and the backscattered light was documented. When the pH was increased from 4.0 to 8.0, the Bragg peak of the photonic crystal flakes shifted from 510 to 620 nm, which was accompanied by colour change (Figure 5c-d). This shift corresponded to a pH sensitivity capable of identifying variation of 0.1 pH units. The Bragg peak shift by free-standing flakes was approximately half of the Bragg shift by the pH sensors attached to the PMMA substrate. This could be attributed to the flakes having a greater degree of freedom to expand than comparable sensors attached to a substrate, but further investigation would be required to confirm this hypothesis. The sensor offers several advantages, such as label free detection, qualitative measurements, and immunity to photobleaching. The volumetric change caused by change in pH was found to be reversible, giving the sensor the potential for reusability. Moreover, the pHEMA matrix can be further functionalized to tune the pH sensing range.

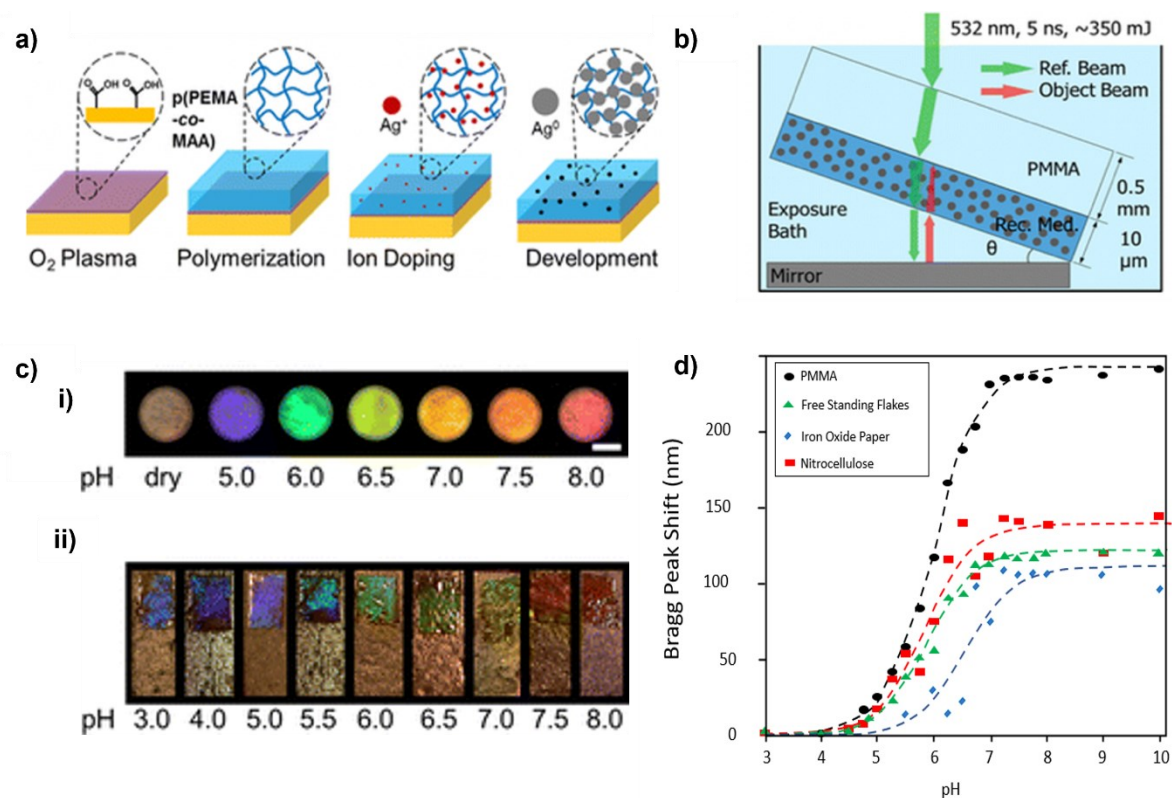


Figure 5. Overview of MAA modified film pH responses.⁸² a) Schematic of sensor preparation; b) Schematic of laser writing of 1D photonic crystal matrix; c) Photographs of i) free-standing flakes and ii) paper backed flakes in response to pH range 5.0-8.0; d) Bragg peak shifts as a function of pH in pH sensors of different forms. Reproduced with permission from American Chemical Society.

1.3.2. Ion Sensor

Surface relief gratings have long been hoped to offer a cheap and reliable alternative to ion concentration determination in biological samples. Naydenova *et al.* demonstrate a methodology whereby holographic surface relief structures are formed and coated in a ionophore containing polyvinyl chloride (PVC) matrices.^{83, 84} The methodology described illustrates that the grating is initially formed utilising a Nd:YAG laser which is separated and directed onto the photopolymer surface. To sensitise the grating structure a secondary layer is spin coated onto the surface containing the binding ligand dibenzo-18-crown-6 (DC) or tetraethyl 4-tert-butylcalix[4]arene (TBC), PVC and plasticizers to give rise to a sensitised

coating. These two sensitising compounds are ionophores expected to show selective binding for K^+ and Na^+ respectively. Volumetric changes in this outer coating tunes the periodicity of the grating, altering the gratings diffraction intensity.

DC having a significantly larger ring structure has a higher binding affinity for K^+ ions due to the larger ionic radii, this conclusion is confirmed by the results obtained within the article. Where the alteration in diffraction efficiency for DC decreases from 1 to 0.6 for K^+ whereas for Na^+ the drop is significantly reduced to 1 to 0.9. The reverse was observed for TBC, the Na^+ response observed the diffraction decrease from 1 to 0.52, in comparison results obtained for K^+ of 1 to 0.8. For application in a medical device the technology shows real promise and could be a true asset to the monitoring of ionic concentrations within the body. Although the methodology is promising and there is further work required to bring the sensitivity of the device to utilisable levels, it is a novel demonstration that there is capability of optical sensors to begin to rival the conventional devices in use today.

Yetisen *et al.* report a NP free methodology for solvent determination and selectively sensing Cu^{2+} and Fe^{2+} utilising a pulsed laser reflection set up.⁷⁵ Deficiencies of Cu^{2+} and Fe^{2+} in blood serum can be an indication of chronic illnesses, such as Wilsons disease and anaemia.^{85, 86} The research demonstrates the way in which chemical stimulus can alter the fringe spacings between areas of high and low RI within hydrogel networks leading to a change in the reflection spectrum observed (Figure 6a). This development removes several matters that have hindered the utilisation of silver halide NPs in previous years. Inconsistent diffusion of Ag^+ ions into the hydrogel film prior to exposure decreases the sensitivity of grating significantly, due to disparity in diameter and deposition of NP's.⁷⁵ This is also paired with the problem that often Ag^+ ions can interfere with a variety of sensitising agents prior and post fabrication. Yetisen *et al.* describe a methodology which removes the requirement for Ag NP by utilising a functionalised crosslinking photosensitive monomer (Figure 6b).⁷⁵ By

undertaking a condensation reaction between pyrrole and 4-formylbenzoic acid, a porphyrin structure was synthesized. To incorporate into the polymer films modification of the sensitizing agent with 3-(4-hydroxyphenoxy)propyl acrylate *via* an esterification reaction inserts a double bond unit capable of undergoing a free radical polymerisation reaction. The sensor presented sensitivity to solvents, effectively identifying concentrations of methanol, ethanol, DMSO and isopropanol, with the effective colourimetric determination of ethanol concentration demonstrated in Figure 6c. The porphyrin ring synthesized has free amine lone pairs in an orientation capable of binding ions selectively. The ring structure when incorporated into holographic gratings showed selectivity for Cu^{2+} and Fe^{2+} , with quantitative colorimetric readouts (Figure 6d). By integrating this functional porphyrin ring into a pHEMA matrix and utilising a green pulsed laser (350 mJ, 532 nm, 6 ns), a Bragg reflection grating was formulated with a replay wavelength within the visible region. Binding of ions adjusts the fringe spacings within the film generating a corresponding change in the reflected wavelength. This research has been able to demonstrate the selective binding of ions but also a NP free path to a new generation of HSs which can remove several barriers such as cost and limited functionality creating opportunities for their widespread application.

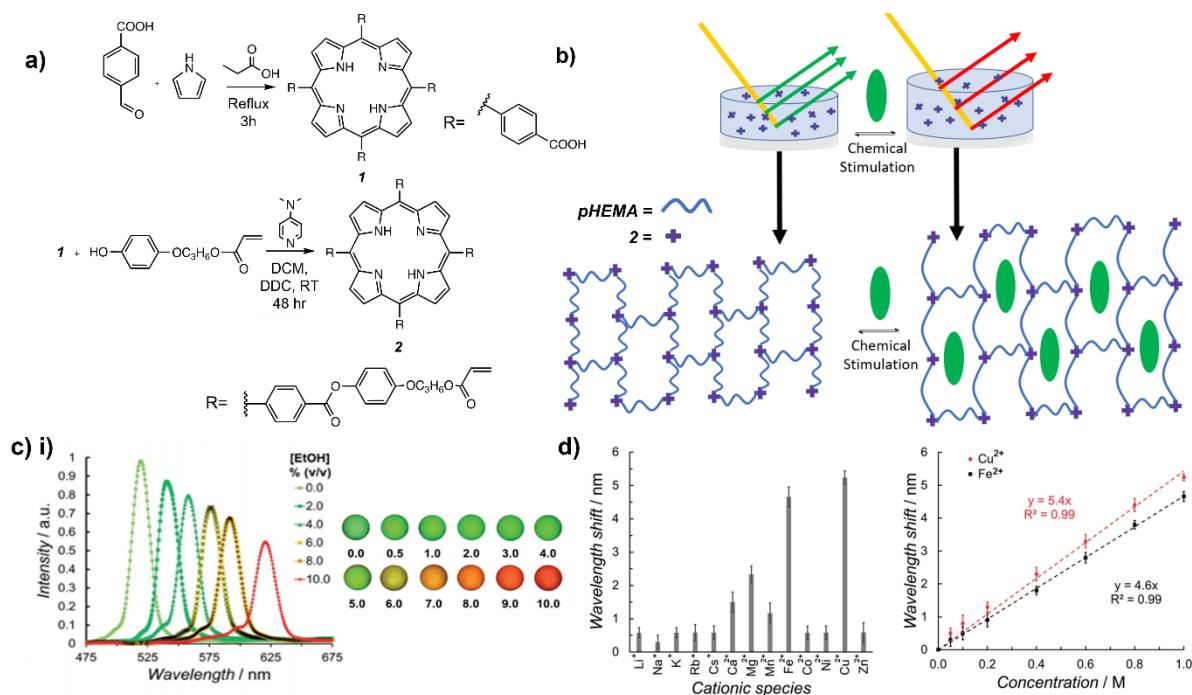


Figure 6. Selective NP free holographic Cu²⁺ and Fe²⁺ sensor.⁷⁵ a) Schematic to demonstrate the response and reversible expansion of the hydrogel films upon chemical stimulation; b) Synthetic pathway in the fabrication of porphyrin ring structure capable of binding metal ions; c) Graph and colourimetric images showing the wavelength shifts observed for the holographic metal ion sensor to ethanol; d) Graphs illustrating the selective binding of Fe²⁺ and Cu²⁺; Reproduced via open access from MDPI.

1.3.3. Temperature and Humidity Sensing

With the further expansion of the exploration of photopolymers in the preparation of holographic gratings, there have been interesting developments in the hybridisation of printed photopolymer structures with a variety of sensing components. The formation of composite materials allows for greater functionalisation of biosensors with alternative functionalities.⁸⁷ By employing zeolites within the hydrogel network, alternative functionalisation's can be achieved. Naydenova and Mintova *et al.* have demonstrated the utilisation of the hydrophilic and hydrophobic properties of zeolite nanocrystals (NCs) in the inkjet printing of alcohol sensitive holographic gratings (Figure 7a).⁸⁸ In the article there is description of the formulation

methodology for both transmission and reflection holograms, the method differs but the components utilised do not vary. The presence of zeolite NCs is to create a change in RI between differing layers of the holographic grating, this leads to requirement for particles of homogenous particle diameter. Disparity in diameter produce irregularities with diffusion rates, optical properties and creates a larger surface area which reduces the reliability of measurements. The inkjet printing of zeolite NCs onto photo polymer films is beneficial in the production of reflection holograms, but this formulation method does not lead to significant benefits in the production of transmission holograms as current technology does not allow for sufficient resolution or accuracy to produce gratings capable of interacting with light effectively. To generate transmission holograms zeolite NCs are dispersed within the base polymer film, then on to this a holographic grating is recorded creating areas of differing RI.

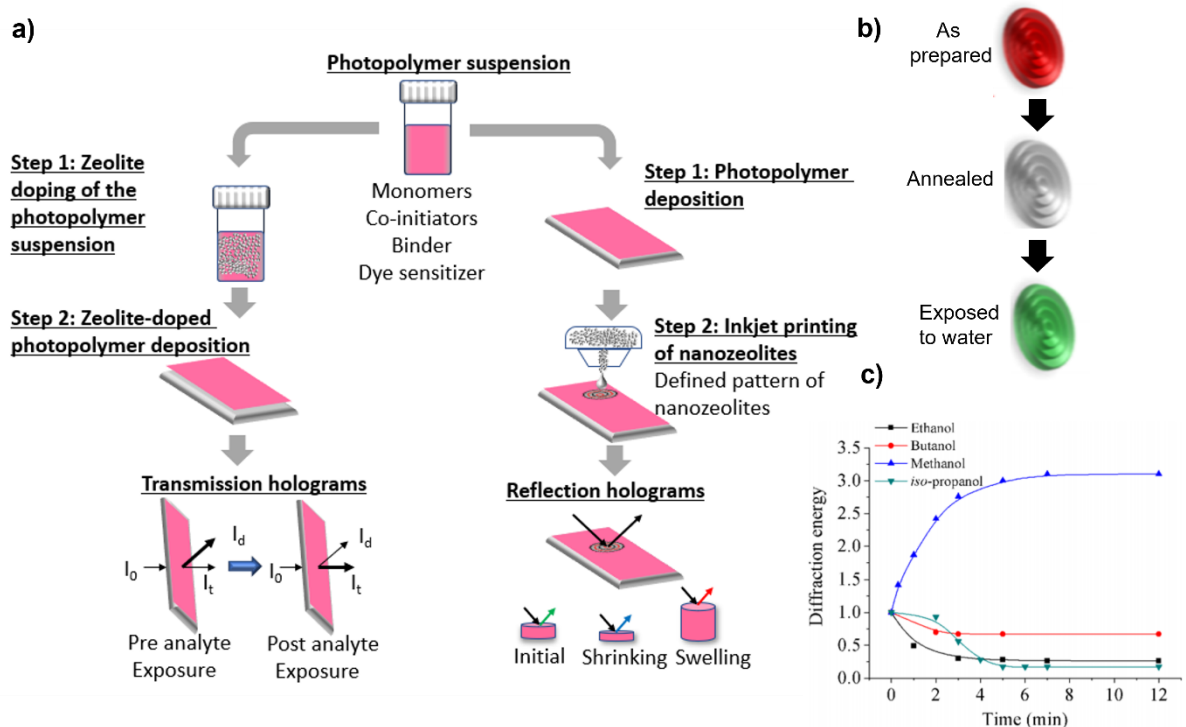


Figure 7. Overview of inkjet printed zeolite NP functionalised holographic gratings for humidity determination;⁸⁸ a) Fabrication of zeolite NP based holograms; b) Visual response of holographic films pre and post exposure to water; c) a graph to display the response of sensors

when exposed to various simple short chained alcohols, Reproduced with permission from American Chemical Society.

Two different crystalline structural conformations of zeolite NC, MFI and EMT, are highlighted within this work. The main difference in these two arrangements is the channel formation, MFI conformation bares channels running straight through the material and an EMT conformation where the flow of analyte is obstructed. These properties make the two structurally different materials ideal for different purposes, MFI for reversible measurements and EMT for non-reversible. The capability of these printed zeolite nanocrystalline holograms in the irreversible detection of analytes such as H₂O is highlighted. By depositing zeolite NCs in regularly patterned concentric circles with the highly hydrophilic EMT structured zeolites. The sensors initially display a red colour but after an annealing process lose visible colour due to the removal of water present in the formulation process. Removal of water diminishes the fringe spacings to a shorter wavelength outside of the visible spectrum. Upon exposure to a humid environment the zeolite NCs take up water leading to an expansion in the fringe spacings and therefore the observed colour (Figure 7b). The highly hydrophilic nature of the EMT structure leads to the capturing of water that could not be removed even when exposed to low humidity sequentially. The irreversibility of the sensor response allows users to identify the occurrence of changes in humidity between monitoring checks. The sensors developed were designed to test for the presence of alcohols, the testing was carried out within a gas chamber where the respective alcohols were in the gas phase. A specificity for methanol was shown over ethanol, isopropanol, and butanol, where the diffraction efficiency decreased by 0.2, 0.2, and 0.75 respectively except for methanol which increased by a factor of 3.2 (Figure 7c). This is hypothesized within the paper to be due to two factors, the ability to be taken up by the zeolite crystals and the nature of the swelling. The expected response of the NCs is for a

decrease in the diffraction efficiency as is observed for the longer alkyl chained alcohols. The response observed is caused by the increased uptake of the methanol due to its shorter alkyl chain giving a significantly increased shift in comparison to the other alcohols and due to the high polarity of methanol giving an opposing response in the hydrophilic NCs.

A HS developed for detecting changes in temperature and relative humidity is described by Mikulchik *et al.*⁷⁶ This investigation explores various photopolymers for reversible sensing of relative humidity and temperature, as well as the possibility of developing humidity and temperature stable sensors. The response of the sensor can be tuned by altering its polymer composition. Three different photopolymer layers were investigated, using varying concentrations of PVC, AM, and MBA for polymers A and B, and PVC, diacetone acrylamide, and MBA for photopolymer C, with sensors recorded in transmission and reflection mode. Photopolymer layers were prepared by deposition of photosensitive solutions on levelled glass slides. Volume phase transmission and reflection gratings were recorded using a Nd:YVO₄ laser (532 nm) (Figure 8a-c). Afterwards, the gratings were exposed to UV-light to polymerize all residual monomers using a UV Exposure unit. The humidity response was investigated by measuring the diffraction efficiency of slanted transmission gratings and the spectral response of reflection gratings as relative humidity was varied. A controlled environment chamber with humidity and temperature control system was utilized to obtain different humidity levels at constant temperature. Different temperatures were maintained by a temperature-controlled plate. Differential scanning calorimetry (DSC) was used to analyse the amount of moisture desorbed from the photopolymer layers under heating. For the transmission gratings, the change in diffraction efficiency was measured by recording Bragg selectivity curves in RH range 20-90% and normalized to diffraction efficiency at RH = 20% (η_{20}). Figure 13d-i shows that the changes in diffraction efficiency of photopolymer C were very significant i.e. increase of the diffraction efficiency from 6 to 84% was observed as the relative humidity

changes from 20 to 90%. Minimal variation was shown by photopolymers A and B. Reversibility of the humidity induced changes was confirmed for RH up to 70% for photopolymer A, up to 90% for photopolymer B and up to 60% for photopolymer C.

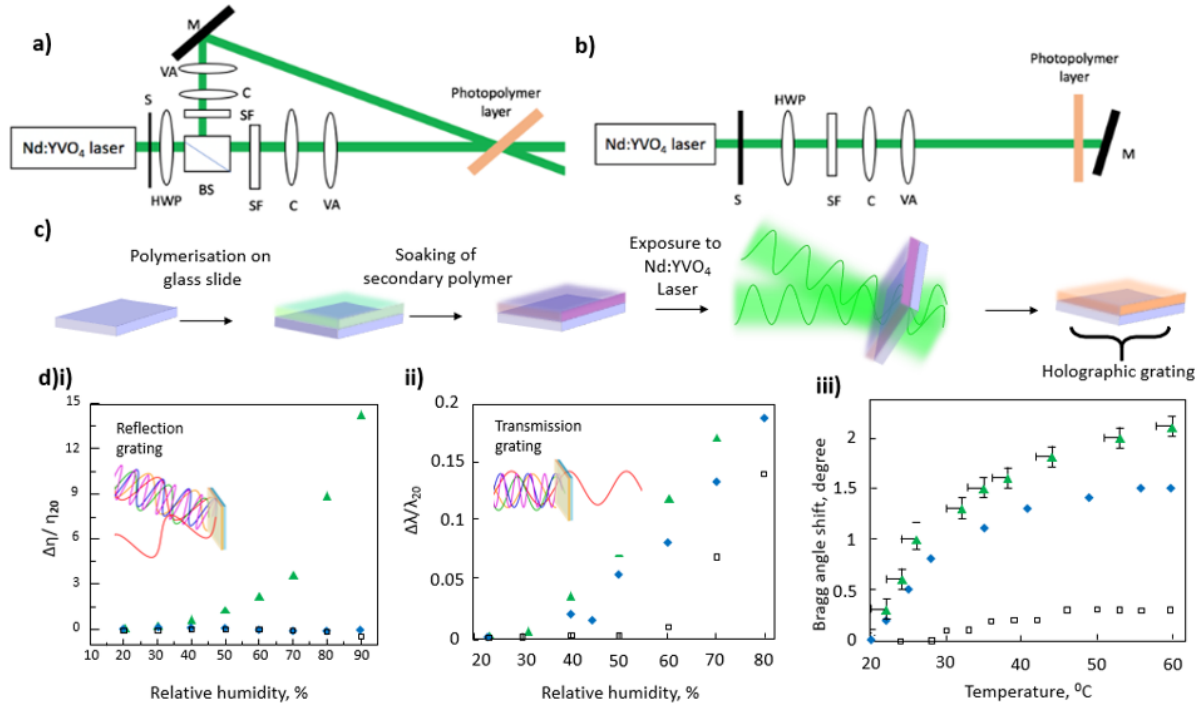


Figure 8. Experimental set-up for the recording of a) slanted transmission grating and b) slanted reflection grating; S – electronic shutter; HWP – half-wave plate; BS – beam splitter; SF – spatial filter; C – collimator; VA – variable aperture; M – mirror; c) Schematic of the holographic grating fabrication; d) i) Relative changes in the on-Bragg diffraction efficiency of slanted transmission gratings recorded in photopolymers versus relative humidity; ii) Spectral response of reflection gratings recorded in photopolymers versus relative humidity; iii) Temperature dependence of the Bragg angle shift of slanted transmission gratings recorded in photopolymers. Photopolymers: photopolymer A (♦), photopolymer B (□) and photopolymer C (▲). Reproduced with permission from Mikulchyk *et al.* (2017). Copyright, Elsevier B.V.

Measuring the response of reflection gratings to change in relative humidity was conducted by monitoring the position of the peak intensity in the spectral response of reflection gratings in range 20–80% RH and normalising to the wavelength of the diffracted light at RH = 20% (λ_{20}). In Figure 8d-ii, photopolymer A demonstrates a non-linear response. Photopolymer B is relatively unresponsive to variations in RH below 60% but has an almost linear response at RH > 60%. Photopolymer C displays significant sensitivity, especially within the range of 40–70% RH. The shift in the position of the maximum intensity in the spectral response of each photopolymer layer was determined to be reversible to within 4.8 ± 2 nm. The humidity response of the photopolymers is attributed to their permeability to water vapor. Photopolymer C, with the most hygroscopic compounds, shows the greatest change in the optical properties of the holographic gratings. The different responses of photopolymer layers can be explained by their ability to release water due to increase in temperature, largest in photopolymer C, which experiences the most shrinkage. Since photopolymer C is also responsive to humidity, temperature response of sealed layers was also investigated to eliminate effects due to humidity and only account for the thermal effects. The temperature response of slanted transmission gratings was investigated in 20–60 °C range. In photopolymer C, exposure to temperature in this range causes shrinkage of the layers and Bragg angle shift up to 2° (up to 1.5° for A and 0.3° for B).

1.3.4. Strain Sensor

The accurate determination of strain is complex due to the requirement for complex laser-based systems to monitor minor deformations in a structure such as buildings, aircrafts, and bridges which can lead to catastrophic failures.⁸⁹⁻⁹¹ Strain sensors are of great importance with a number of both hydrogel and holographic strain sensors have been demonstrated in recent years using a range of fabrication techniques.⁹²⁻⁹⁴ AlQattan *et al.* developed a holographic strain sensor through use of holographic interference patterning to record curved nanostructures on

adhesive tapes, which upon deformation vary their diffraction properties.⁹⁵ Common black ink (Staedtler Lumocolor) deposited onto a glass substrate was utilised as the recording medium. Holographic gratings were produced using a Nd:YAG laser system creating either Fresnel lens or a curved grating nanostructure by controlling the point of reflection from the concave mirror, with grating spacing tuned through the variation of recording angle (Figure 9a). The gratings recorded could interact with broadband light to generate a rainbow diffraction pattern, by varying the angle of illumination the degree of diffraction increased, allowing researchers to identify a variation in the angle relative to the normal (Figure 9b). In addition to these rotational experiments, by adhering the gratings to a tape substrate (4 cm) the effect of strain could be studied. The tape bearing the holographic grating was stretched over 300 increments (5 $\mu\epsilon$ per stretch), giving a total expansion of 0.6 cm. Increasing the distance stretched lead to an increase in the level of diffraction. This correlated with stretching elongating the fringe spacing within the sensor, this variation in diffraction efficiency could be correlated with the level of strain. The sensors developed within this work showed little vulnerability to interference from water or pH solution due to the hydrophobicity of the ink utilised. Low wettability is vital when monitoring stress in real world situations due to rain or external influence. Alongside the lack of interference these sensors are easily fabricated from simple materials, a readily available ink and a simple substrate of glass or tape, presenting the potential for low cost and easily accessible devices. The fabrication method could be easily modified to generate images in the gratings, such as lettering illustrated in Figure 9c which was responsive under strain.

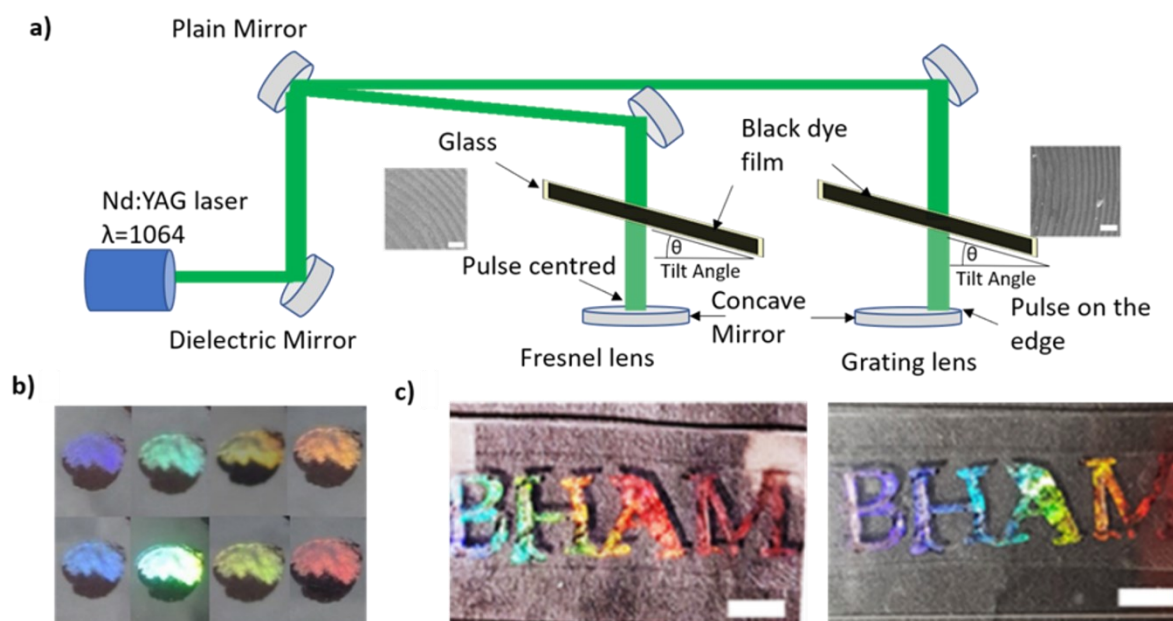


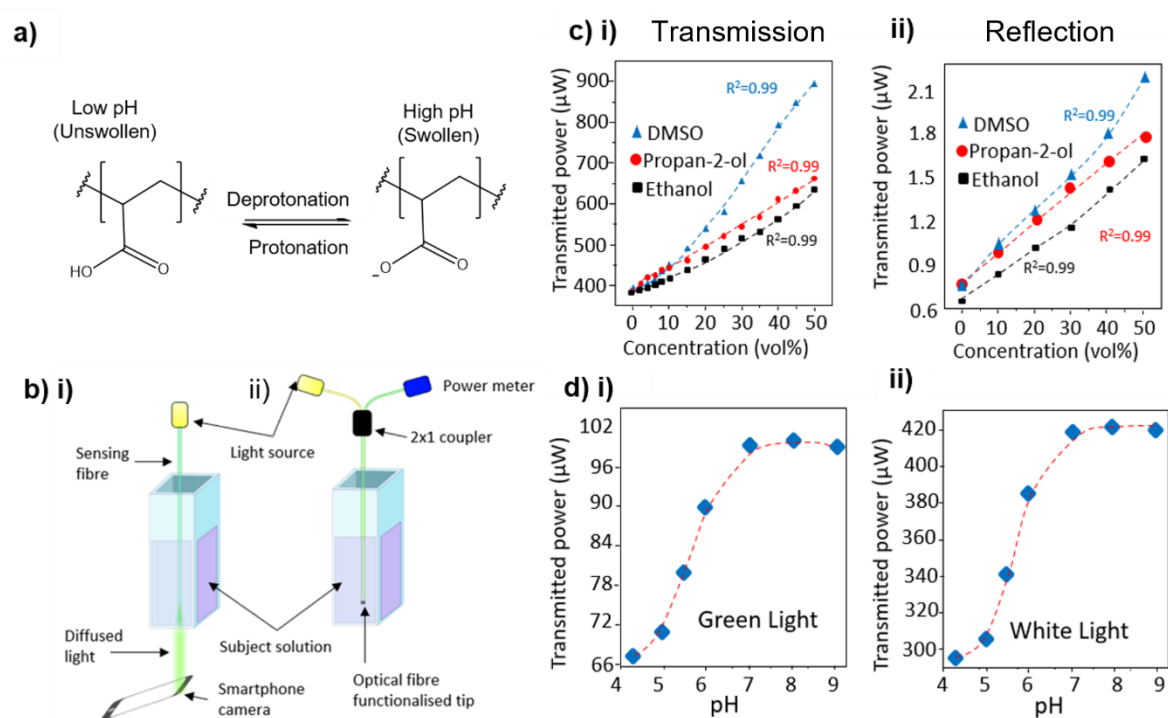
Figure 9. Black dye diffraction gratings for the identification of strain; a) Fabrication set up for the production of either Fresnel or grating lenses dependent on the point of reflection from a concave mirror inset SEM images of Fresnel lens and Grating lens respectively, scale bar 100 μm ; b) Colourimetric response of sensors upon rotation of the diffraction grating; c) Nanograting of BHAM logo to demonstrate the images recordable and the variation under strain. Reproduced with permission from American Chemical Society.

1.3.5. Solvent Sensor

Elsherif *et al.* developed holographic fibre optic sensors responsive to alcohols at 0-50 vol% and pH.⁹⁶ The hydrogel sensors were prepared from monomer solutions consisting HEMA and EGDMA. Acrylic acid was added as a *co*-monomer for hydrogel functionalization in the pH sensor (Figure 10a). For the optical fibre fabrication, ethylene glycol diacrylate was mixed with 2-hydroxy-2-methylpropiophenone (HMPP) in DI water. The prepared solution (200 μL) was injected into a polyvinyl chloride tube and polymerized under UV light generating a flexible optical cable. Affixing of the holographic grating was achieved *via* silanization of the tip and submerging in either alcohol or pH-sensitive monomer solutions on asymmetric micro lens

arrays and the tube was UV cured to produce a negative of the master photonic structure. The sensors were evaluated in both of transmission and reflection modes (Figure 10b), either using a smartphone or an optical power meter. Output signals measured via spectrophotometer validated smartphone readout as a viable, simple, and accessible analysis technique.

Figure 10. The pH and alcohol responsive films fabricated by surface relief casting;⁹⁶ a) Equilibrium reaction to initiate the swelling and shrinking of the hydrogel film; b) Setup for



testing in i) transmission mode and ii) reflection mode; c) Interrogation of the fibre optic probe for alcohol sensing in the transmission and reflection configurations: i) The maximum optical transmitted power versus alcohol concentrations; ii) The reflected power versus alcohol concentrations; d) Interrogation of the fibre optic probe for pH sensing in the transmission and reflection configurations: i) The maximum transmitted power versus pH; ii) The reflected power versus pH. Reproduced with permission from Elsherif, *et al.* (2019). Copyright, Royal Society of Chemistry.

The alcohol-responsive sensor was tested for response to ethanol, propan-2-ol, and

dimethyl sulfoxide (DMSO). In both modes, the highest sensitivity was with respect to DMSO. The sensor responded to all three analytes in the range 0-50 vol% with the limit of detection of 2% and was only linear for propan-2-ol. The trends observed in both of transmission and reflection modes were similar, although the sensitivities in reflection mode were significantly smaller than in transmission mode (Figure 10c). The pH-responsive sensor showed good sensitivity in pH range 5.0-7.0 but a diminished response at pH higher than 7.0 (Figure 10d). Sensitivity was decreased in reflection mode, which could be attributed to the readout setup and the angle of illumination of the sensor. The approach presented offers a real-time sensing method with simplistic readouts and fabrication methods. The sensor can be fabricated using biocompatible fibres, which improves suitability for *in vivo* applications and the optical sensing mechanism is immune to electromagnetic interference occurring in common diagnostic techniques such as MRI scans. Relatively minimal analyte volumes (1 mL for sensor attached on the glass slide) are required for analysis. The sensors are highly reproducible and have a rapid equilibration time ($60 \text{ s} \pm 10 \text{ s}$ for pH sensor and approximately 60 s for alcohol sensor, depending on alcohol type and concentration). Sensors could be modified to show sensitivity at higher pH and enhance the sensitivity of biocompatible fibre systems.

By employing a NP free fabrication method Jiang *et al.* formulated a holographic alcohol sensor capable of determining the concentrations of methanol, ethanol, 1-propanol and isopropanol *via* UV photopolymerization of a secondary highly crosslinked polymer.⁷⁷ By utilising in situ photopolymerization the requirement addition of dyes such as Safranin O is removed.⁷⁴ The base hydrogel matrix consists of a loosely crosslinked hydrogel matrix of HEMA (93.9 mol%), glycerol dimethacrylate (4.2 mol%), and HMPP (1.9 mol%). The low level of crosslinking present within this film allows the hydrogel to change its volumetric swelling dependent on the external stimuli (Figure 11a). Holographic gratings were fabricated through the recording of interference pattern between a single flash of incident and reflected

coherent 355 nm Nd:YAG laser light was directed onto samples in a simple reflection grating set up to initiate free radical polymerisation of a secondary polymer in ultrafine fringes (Figure 11b-c). RI of the two distinct polymer layers was achieved by increasing the level of crosslinking in the secondary polymer HEMA (13 mol%), EGDMA (84 mol%), HMPP (3 mol%). The benefit of this ‘double polymer’ system is the lack of requirement for chemical developments post exposure. In comparison to AgNP systems where development and fixation are required the sensors developed within this work only require a simple washing in an ethanol water bath (1:1, v/v) step to prepare samples for use. This streamlines the synthesis process, increasing applicability to mass production and widespread use. Sensors demonstrate a clear colour shift for the detection of alcohols up to 60%, with a higher sensitivity to isopropanol when compared to other short chain alcohols (Figure 11d). Although this higher level of shifting was observed, the response time went in contrary to this, with shorter alcohol chains showing the faster rate of equilibration, isopropanol (15 min) and methanol (7 min), attributed to a faster rate of diffusion within the film. The sensors clear colour changes are distinguishable with the naked eye but offer the opportunity for quantitative analysis through a spectrophotometer. Overall, the sensors developed in this work show a strong response to the variation of alcohol concentration through a simple double polymer fabrication.

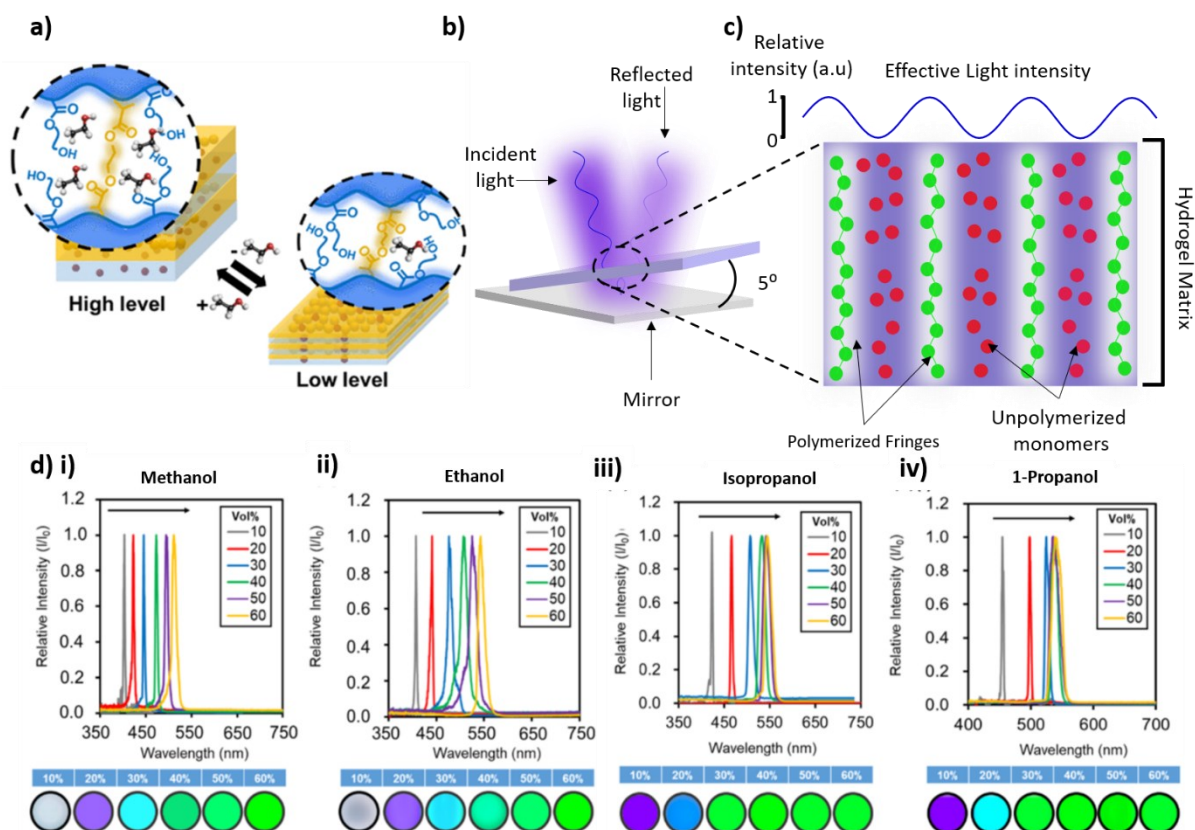


Figure 11. Short chain alcohol sensor fabricated *via* double photopolymerization of hydrogels;⁷⁷ a) Influence of increasing alcohol concentration of polymer layer swelling; b) Incident and reflected coherent laser light with the hydrogel sample raised to 5° , c) Effective laser light intensity passing through the film from constructive and destructive interference and its polymerisation effect on the hydrogel film; c) Bragg peak shift and respective colour variation observed for short chain alcohols i) methanol, ii) ethanol, ii) isopropanol, and iv) 1-propanol. Reprinted with permission from American Chemical Society.

1.3.6. Gas Monitoring

The monitoring for the presence of potentially harmful gases has applicability to a wide range of industries such as workplaces⁹⁷ with even minor exposures showing detrimental effects on health.⁹⁸ Rho *et al.* produced a responsive liquid crystalline holographic meta surface using photonic structure casting to create an optical sensor capable of determining the presence of

volatile organic compounds giving a clear image response to the viewer.⁹⁹ A meta surface hologram fabricated from hydrogenated amorphous silicon was utilised to generate alternative images dependent on the polarisation of incident light. The one step fabrication process utilising spin coating of polyethylene terephthalate on to a range of substrates to cast a photonic mould produced optically active films post polymerisation. To alter the polarisation of incident light a responsive liquid crystal material 4-cyano-4'-pentylbiphenyl is utilised. Light passing through the nematic liquid crystal phase varied the passage of either left or right circularly polarised light, illuminating either 'alarm' or 'safe' holographic images respectively. Upon introduction of volatile compounds, the ordered structure is disrupted which reduces the retardation for right circularly polarised light to pass through the device. Sensors identified the presence of many common organic volatiles such as chloroform, acetone, and toluene with response time of 1.3, 1.6 and 5.2 seconds, respectively. Whilst also maintaining high reversibility between the 'alarm' and 'safe' messaging, allowing for sensors to be left in situ and report real time variation in air contamination. The devices developed in this research are low cost with simple manufacturing processes, rapid response times, simple image readout, and high reversibility without the requirement for electrical or mechanical components.

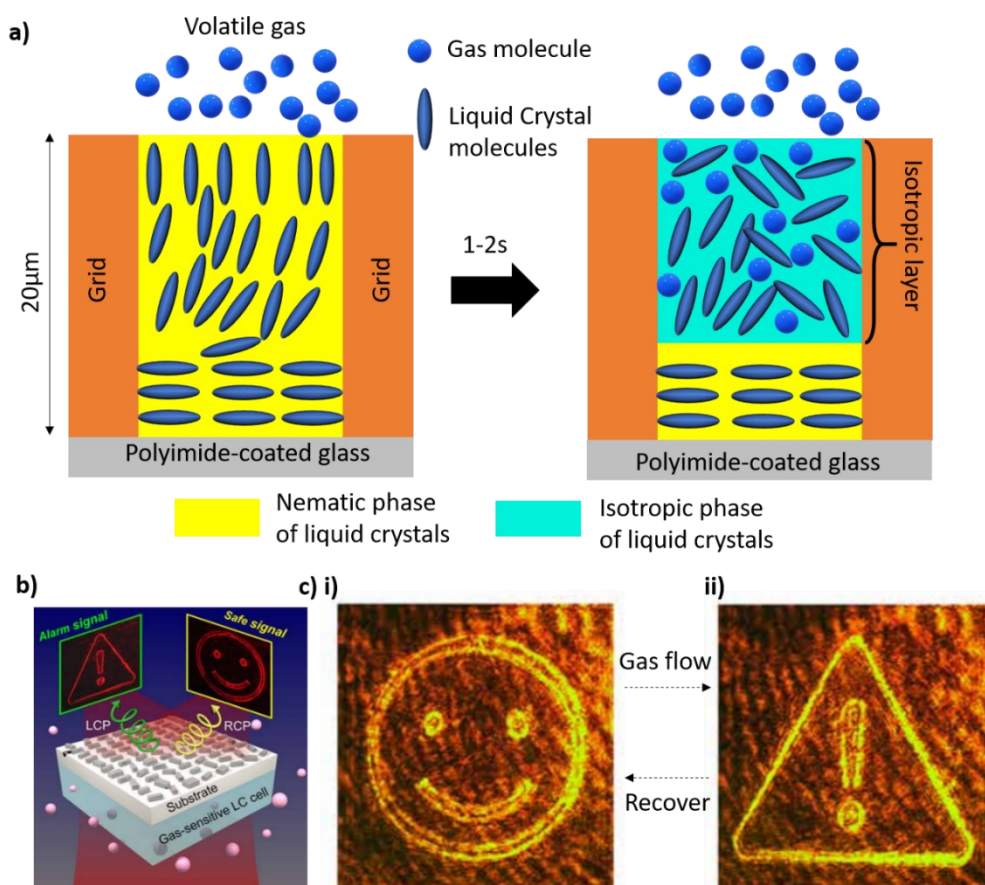


Figure 12. Optical volatile organic gas sensor based on liquid crystal meta surface holograms, a) functional properties of the phase change within the gas sensitive LC cell upon introduction of volatile gas compounds; b) The fundamentals of how light interacts with the sensor illustrating either the ‘alarm’ or ‘safe’ signage; c) Experimental result of introduction of gas molecules to sensor surface when illuminated with 600 nm light. Reproduced from open access copywrite American Association for the Advancement of Science.

1.3.7. Glucose monitoring

With the pandemic rise in diabetes, glucose has become one of the most monitored metabolites worldwide.¹⁰⁰ Although treatments are improving in dealing with a range of diabetic illnesses, identification of diabetic glycaemic index variation is vital.¹⁰¹ Current technologies such as, finger prick testing, although accurate have several points for improvement.¹⁰² A core

drawback with these devices is the invasive nature, requiring the breaking of skin and bruising leading to patient discomfort and over time fatigue with the monitoring process. Non-invasive techniques have become a major point of interest for sensing to try and combat patient monitoring fatigue.¹⁰³ Butt *et al.* developed imprinted hydrogel sensors, capable of rapid continuous and quantitative blood glucose concentrations determination utilising phenylboronic acid (PBA) functionalised hydrogel films. The 2.5D optical imprinted structure casting method utilised is illustrated in Figure 13a.⁵⁵ These devices counter the infrequency in measurements, and give colorimetric responses analysed quantitatively with a mobile phone camera. This reduces the requirement for sophisticated and expensive equipment to obtain reliable results. The hydrogel matrices employed reversibly change their volume upon the binding of glucose to the PBA modified hydrogel which has been imprinted with an optically active honeycomb 2.5D structure by casting a polymer on a PDMS stamp. The fabrication process is simple and low cost, which is readily scalable to a mass production level, due to only requiring minimal processing. The honeycomb structure imprinted on the hydrogel matrix can interact with light to give 1st and 2nd order refractions, the angle of this refraction is indicative of the glucose concentration present in the analyte (Figure 13b). This work was able to demonstrate reliable results within a 15-min analysis window.

Binding of glucose to PBA groups embedded within the matrix leads to an overall expansion of the imprinted structure on the hydrogel surface and therefore the response observed when interacting with light (Figure 13c). Boronic acids are efficient in the reversible binding of cis diol groups which are present on several common sugars such as glucose, fructose, galactose, and mannose. The mechanism of binding is dependent on several factors such as pH and pK_a of the boronic acid group. At pH > 10 the boronic acid group is anionic and in the tetrahedral conformation where there is a higher efficacy for diol binding.¹⁰⁴ James *et al.* identified that by positioning a tertiary amine within the boronic acid monomer it is

possible to bring down the pK_a of the group therefore allowing for binding at physiological pH values.¹⁰⁵

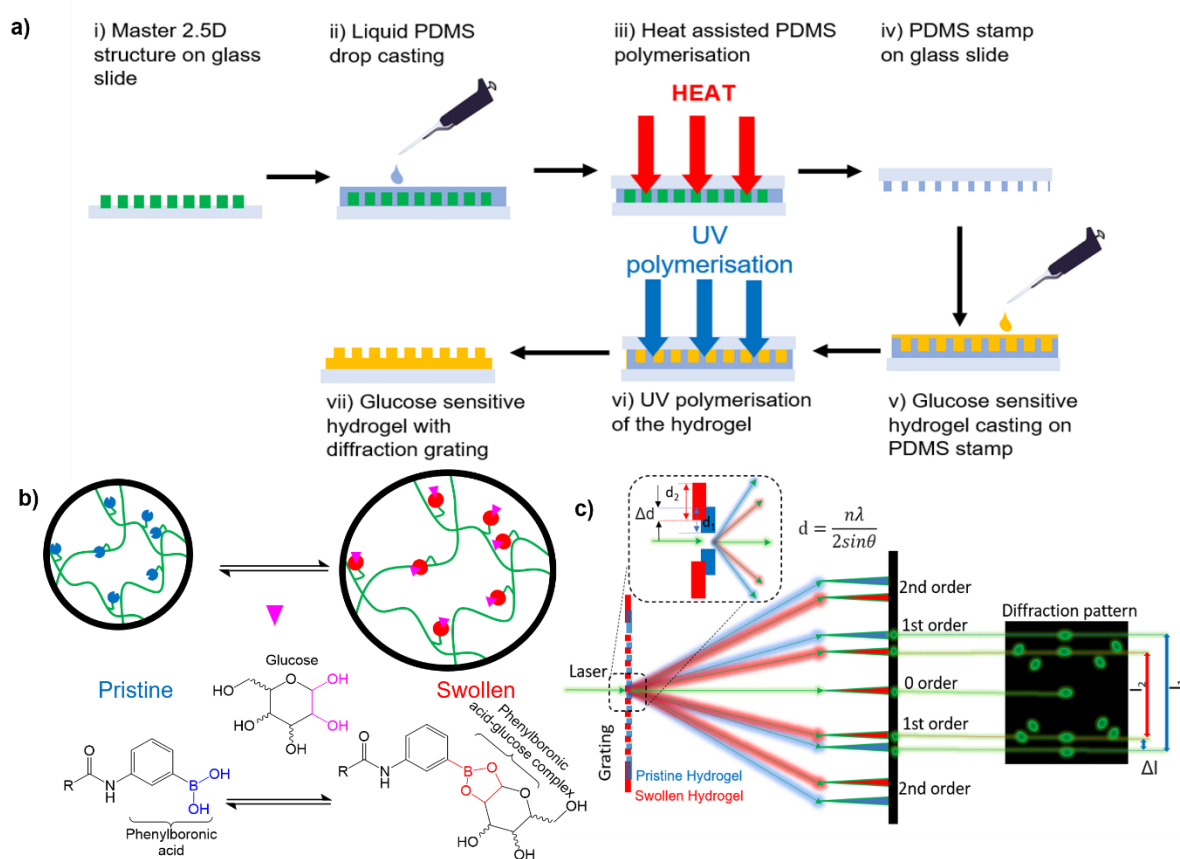


Figure 13. Boronic acid-sensitive photonic sensors by molecularly imprinted casting;¹⁰⁶ a) Fabrication process of 2.5D molecularly imprinted polymers via casting of PDMS stamp; b) Equilibrium reaction profile of the boronic acid binding mechanism; c) The principle of the diffraction mechanism observed with 2.5D imprinted sensors when swollen (wet) or pristine (dry), Published by The Royal Society of Chemistry (RSC) on behalf of the European Society for Photobiology, the European Photochemistry Association, and RSC.

A glucose sensor was fabricated by Jiang *et al.*, using silver halide chemistry and laser interference lithography (LIL).¹⁰⁷ The hydrogel matrix was sensitized to glucose utilizing a 3-acrylamido phenylboronic acid (3-AAPB). A monomer solution containing AM, PEGDA, 3-AAPB, and HMPP in DI water was pipetted on a silanized glass slide. The hydrogel film was

formed by free-radical polymerization under UV exposure for 3 min. Ag^+ ions were diffused into the synthesized hydrogel film which was then exposed to a solution containing LiBr and photosensitive acridine orange dye (later removed from the matrix), which converted Ag^+ ions to photosensitive AgBr NCs (Figure 14a). The reflection grating was recorded using a continuous wave (CW) laser diode ($\lambda = 532 \text{ nm}$, 5 mW) (Figure 14b). The fabrication process took less than 10 min. The hydrogel was shaped into round flakes of diameter 2 mm. The measurements of hydrogel response to glucose solutions were performed using a spectrophotometer under broadband light. Glucose–boronic acid complexation resulted in increased Donnan osmotic pressure of the system and hydrogel swelling. The response of the sensor to glucose was tested in the applicable physiological range 0–20 mmol L^{-1} . As the glucose concentration increased by 5 and 20 mmol L^{-1} , the hydrogel flake diameter expanded by 1.4% and 7.7%, respectively, within 40 min (Figure 14c). The Bragg peak shifted by 5 nm and 12 nm, respectively, within 1 h (Figure 14d). Without functionalizing the hydrogel with 3-AAPB molecules, the flakes showed no significant expansion. The average sensitivity was 0.2 mmol L^{-1} . The response of the hydrogel was found to be reversible. Selectivity studies showed that the hydrogel responded by expansion, due to contact with fructose and lactate solutions as well (Figure 14e). The sensor offers cost-effective sensing method for glucose within the applicable clinical range. The fabrication is quick and has the potential to be implemented on mass scale. The hydrogel matrix could be functionalized for detection of other analytes. There are some improvements to be made, such as increasing the diffraction efficiency above 9% and improving the selectivity for glucose.

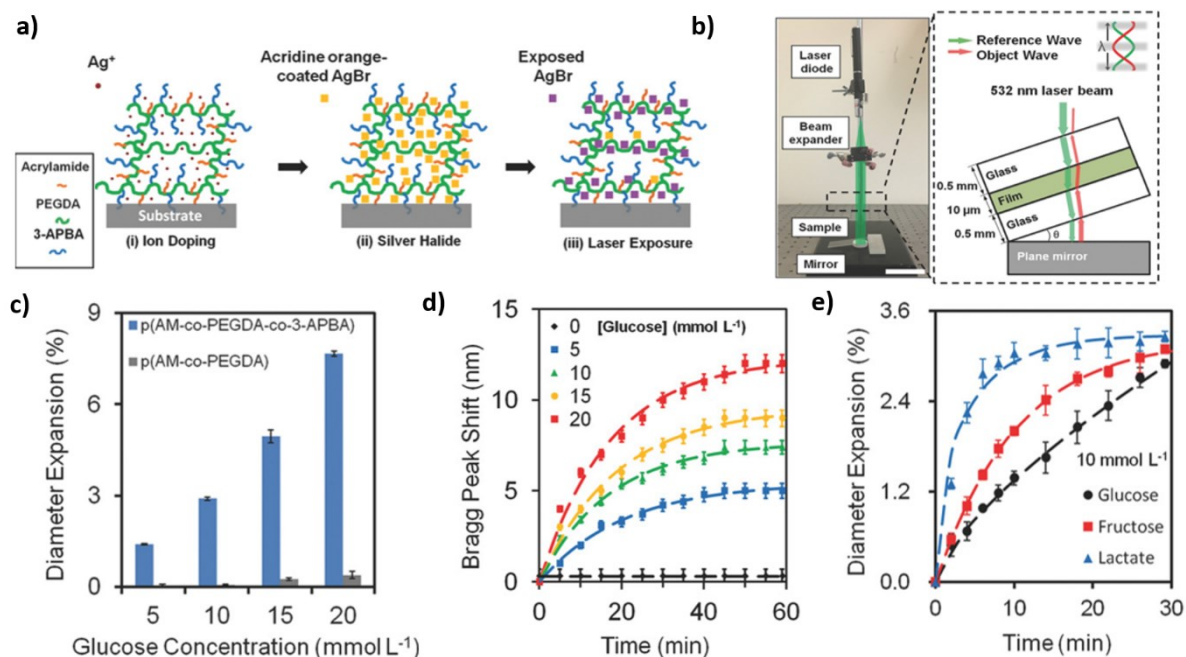


Figure 14. Boronic acid glucose sensor fabricated through silver NP photolithography.¹⁰⁷ a) Sensor fabrication; b) Recording of the setup for recording of reflection grating (scale bar = 5.0 cm), reproduced with permission from Elsevier; c) Diameter expansion of the 3-AAPB functionalized and non-functionalized hydrogel versus glucose concentration; d) Bragg peak shift of the 3-AAPB functionalized hydrogel in time at different glucose concentrations; e) Response of the sensor in time to glucose, fructose and lactate. Reproduced with permission from John Wiley& Sons, Inc.

Lowe *et al.* developed a novel methodology for the formulation of holographic gratings *via* a double polymerisation mechanism.⁷⁴ The approach utilises an initial polymer layer which is lightly crosslinked (P1), into this layer a secondary monomer solution is soaked in, which contains a significantly higher crosslinker concentration. The secondary polymer (P2) is polymerised *via* exposure to reflected laser light where constructive and destructive interference between incident and reflected waves of light generate a holographic grating.⁷⁴ The holographic grating is generated due to the difference in RI between the two polymer layers within the film. The functionalisation group utilised within this work is the boronic acid bearing

ligand 3-AAPB. It is described that the hologram responses were complicated due to the two polymer components displaying initially an expansion followed by a contraction response to the presence of glucose, variation in results observed would be time dependent. The initial expansion effect was attributed to the formation of a negative charge on the boronic acid which in turn led to the rapid penetration of H₂O and cations into the film to balance the anionic charge generated in the 1:1 binding reaction. The contracting response is hypothesized to be due to the sluggish formation of a 1:2 binding complex with glucose until an equilibrium was achieved. To overcome these response variations, Lowe *et al.* added an equimolar equivalent of glucose to the P1 film formulation which was then subsequently removed prior to P2 soaking, this effort to imprint the polymer with glucose was to ensure the spatial positioning of boronic acid groups would more easily facilitate the formation of 1:2 complexes upon binding. The results obtained from this work followed this hypothesis where the equilibration point was achieved more rapidly but the wavelength shift was significantly decreased. Although this methodology showed promise it is thought that at higher concentrations there will be a preference for the 1:1 conformation due to the excess of glucose present within the analyte. The response time for these films varied with formulation but at approximately 20 min an equilibration point was reached. These sensors have been capable of showing a novel double polymer mechanism of forming holographic films is viable, but more work is required to bring their specificity and the equilibration of the response time down to more actionable values.

1.3.8. Hormone Sensing

Mayes *et al.*, demonstrated a novel methodology for detecting testosterone whereby molecularly imprinted films were generated by directly structuring metal free holographic gratings within polymer substrates during synthesis.¹⁰⁸ By recording holograms within a molecularly imprinted (MIP) film selectivity was achieved, this partnered with the visible colour response of a holographic grating offers the opportunity for the determination of the

presence of analytes. The paper published utilises complex precursor solutions consisting of imprinting factors (IF), functional *co*-monomers, crosslinkers and photoinitiators to formulate the porous MIP structures, into the polymer based optical gratings are recorded utilising a Nd:YAG laser ($\lambda = 532$ nm). The sensor described within this work demonstrated a selectivity towards testosterone when compared to a competing analyte (5 α -androstane). When comparing these two analytes the chirality and backbone structure is comparable, but due to the absence of the alcohol and ketone group on the 5 α -androstane the affinity experienced is significantly weakened when compared to testosterone, allowing for higher selectivity.

1.3.9. Cocaine Detection

Illicit drugs are a significant problem in society as their production and sale provides financing to criminal groups.¹⁰⁹ At present law enforcement officers utilise a simple colourimetric dye to identify the presence of cocaine on surfaces, which show low selectivity and can incorrectly identify substances.¹¹⁰ Conventional nuclear magnetic resonance techniques are impractical due to device size, safety hazards, and complexity demonstrating the requirement for selective analytical techniques which can be used in field locations. Due to HSs low cost and high sensitivity chemical sensing platform the sensors have found application in detection of illicit drugs. Oliveira *et al.* developed a sensor for cocaine detection based on Ugi synthesis of a ligand to mimic cocaine-protein interactions and Bragg diffraction grating comprising gold NPs.⁷¹ Cocaine (Figure 15a-i) was found to interact with human carboxylesterase-1 (hCE1) and catalytic antibody GNL7A1. Screening of the combinational library for compounds for Ugi synthesis aimed at producing a ligand most likely to mimic the cocaine-protein interactions. The formation of Ugi ligand was assessed indirectly by fluorescence microscopy. Eventually, ligand A19C28I5 (Figure 15a-ii) was chosen for this HS due to its high binding affinity for cocaine (Figure 15b). To fabricate the sensor, polymer film was synthesized on glass slides from a monomer precursor of HEMA (80 mol%), EGDMA (5 mol%), and

methacrolein (15 mol%). Monomer solutions were diluted with 1-propanol (1:1 (v/v)), and free radical initiator (DMPA, 1% (w/v)) was added. The films were polymerised for 40 min with a UV lamp ($\lambda = 254$ nm). Ugi ligands were synthesized on the polymer films according to a procedure previously described. Gold holograms were fabricated by exposure to a single 6 ns pulse of a 532 nm laser (Figure 15c). A smartphone application was also tested for use in cocaine detection by the colour response of the sensor.

The response of the sensor to cocaine was tested in the applicable range of 10-50 mmol L⁻¹, with maximum swelling of $\Delta\lambda = 30.5$ nm at a concentration of 50 mmol L⁻¹. The limit of detection was found to be 7.1 mmol L⁻¹. The response time of the sensor was determined as 15 min and the response was found to be reversible. Smartphone readout was also tested and demonstrated to be successful in detecting cocaine in street seized samples. Specificity studies, comparing response of the sensor to cocaine to that of naloxone and ketamine (Figure 15d), showed that the sensor response to naloxone is diminished compared to that of cocaine. The sensor had a specific interaction with cocaine at concentrations above 36 mmol L⁻¹ and showed little to no response to ketamine. The sensor offers advantages of appropriate sensitivity, ability to detect real drug samples, short response time, low cost, and reusability. The smartphone-based readout suggested in the paper offers a simple readout method. Utilisation of smartphone cameras as analytical devices removes barriers to accessing quantitative analysis. By utilising applications or add on components, it can be possible to achieve analytical chemistry lab quality results using simple paper based devices.²⁸

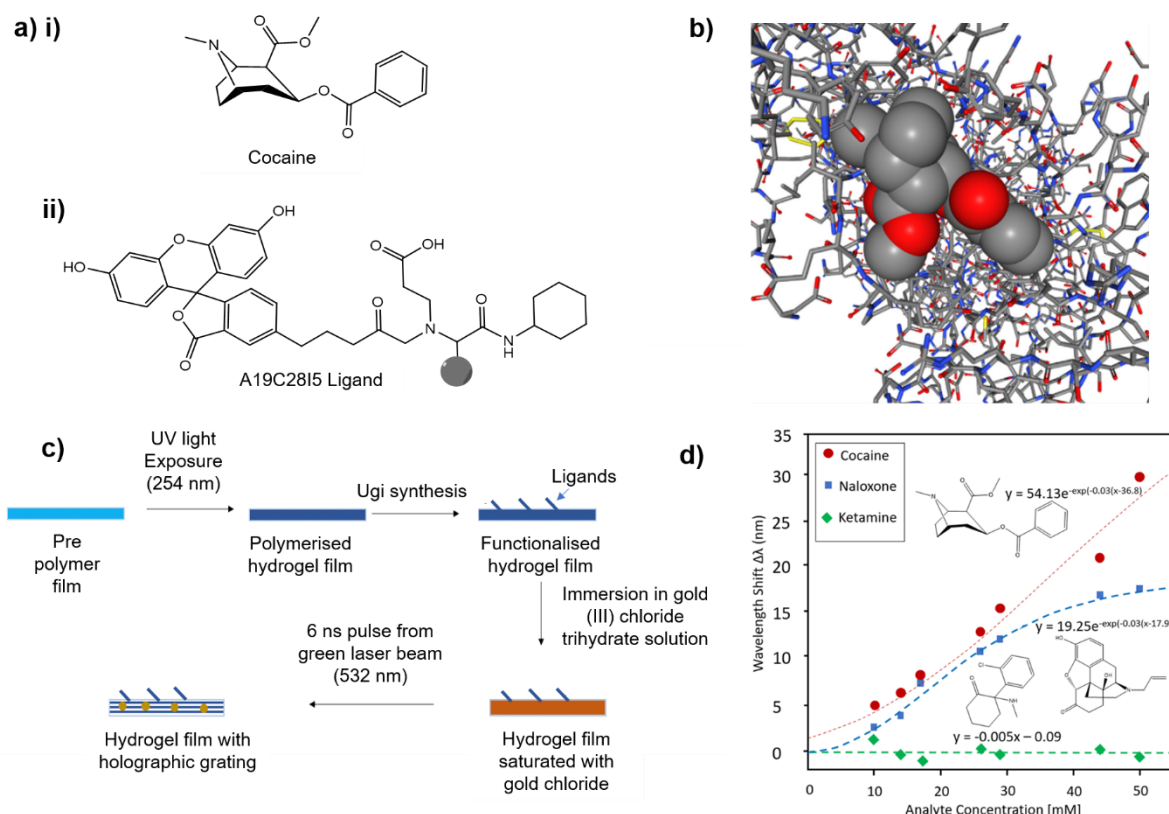


Figure 15. Ugi ligand cocaine sensor fabricated utilising gold NP HSs;⁷¹ a) i) Structure of cocaine ii) Structure of the A19C28I5 ligand synthesized on the holographic matrix; b) X-ray crystallography of the A19C28I5 ligand interacting with cocaine; c) Schematic of sensor fabrication; d) Response of the sensor to cocaine, naloxone and ketamine concentrations within 10-50 mmol L⁻¹. Reproduced with permission from Oliveira *et al.* (2018). Copyright, Elsevier B.V.

1.3.10. Antibiotic Detection

Molecular imprinted polymers have been a topic of great interest in recent years with the technology offering new avenues in a variety of optical structures to analyse complex analytes. To achieve this binding an IF with similar chemical properties is cast within a polymer mould capable of binding the target analyte. After polymerization, the IF is removed generating a pocket with higher affinity for the analyte due to the special configuration of the binding site (Figure 16a). The powerful technique can be widely applied in a wide array of uses such as

chiral compound separations and sensing.^{111, 112} MIP technology has resulted in issues with capability when scaled to industrial levels but this has not hindered the science being investigated widely with over 4000 patents filed at the WO mentioning MIPs (Apr 2020). This technology has been implemented in a wide array of optical applications to create nanostructures within a responsive medium with higher binding affinities for complex analytes.

Antibiotics revolutionised medical treatment of previously fatal illnesses. Although lifesaving, these compounds often lead to pollution of natural resources such as water supplies, sediment, and long-term deposition within the human body. Long term exposure to these compounds can have serious medical implications.¹¹³ This is overshadowed by the risk of development of antimicrobial resistance, by exposing bacteria to low concentrations of antibiotics it is possible for bacterial colonies to amplify resistant strains.¹¹⁴ This is an imminent risk to modern medicine, if resistance is obtained to core antibiotic scaffolds treatment of common infections could be obsolete. Qiu *et al.* describe a methodology whereby optical crystal structures can be utilised to indicate the presence of antimicrobials.¹¹⁵ By utilising a optical crystalline colloidal array (CCA) which contained molecularly imprinted cavities sensitive to clindamycin hydrochloride (CLI), a common antibiotic for treatment of minor bacterial infections, the paper described a device with a sensitivity range of 0.01 mmol L⁻¹ to 1 mmol L⁻¹ with a response time <10 min.¹¹⁶ Previously to analyse the concentrations of CLI required chemical processing or separation techniques to accurately determine concentrations¹¹⁷ since then a number of powerful techniques such as HPLC,¹¹⁸ and mass spectroscopy¹¹⁹ have been utilised. Although these techniques provide accurate analysis, they require expensive equipment and trained individuals, Qiu *et al* aimed to overcome this with a POC analysis device. Readouts from the sensor are obtained with the use of a monochromatic blue/violet light source which illuminates the 2D optical crystal giving rise to a Debye ring (Figure 16b).¹²⁰ Analyte absorption modifies the spacings within the nanostructured hydrogel,

changing the diameter of the Debye ring, with rapid response times of under 10 min for up to 1 mmol L⁻¹ (Figure 16c). Demonstration of the improved sensitivity was shown by comparing the molecularly imprinted colloidal array (MICA) against a non-molecularly imprinted colloidal array (NICA). It was observed that there was a spacing change of 41 nm for the MICA and 1 nm for the NICA with a CLI concentration change of 1 mmol L⁻¹ (Figure (16d)). Selectivity is shown to be high against a comparable antibiotic, *via* the analysis of lincomycin in parallel with CLI. Although there was a similar response profile for the presence of lincomycin over concentrations ranging 0.01-1.00 mmol L⁻¹ (Figure 15e). The sensor displayed a spacing change of 10 nm for lincomycin and 40 nm for CLI at a maximum concentration of 1.0 mmol L⁻¹, demonstrating selectivity for the desired analyte.

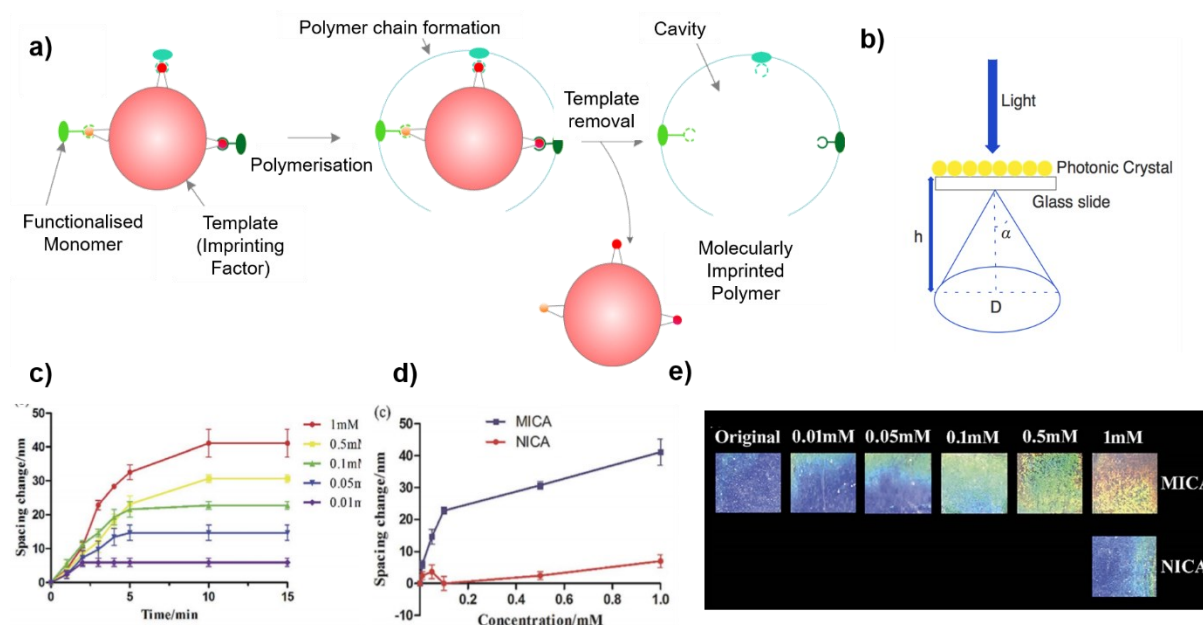


Figure 16. Molecularly imprinted colloidal array antibiotic sensor;¹¹⁵ a) fabrication of MIP hydrogel films; b) Debye diffraction and a comparison of the change in spacing observed with molecularly imprinted colloidal array (MICA) and non-imprinted colloidal array (NICA); c) Response time of the MICA to CLI at varying concentrations; d) Response of CLI to both MICA and NICA; e) observable colour change upon exposure to CLI for both the MICA and NICA, reproduced with permission from Royal Society of Chemistry.

The research establishes a methodology whereby the concentration of an antimicrobial contaminant can be identified rapidly at the point of analysis. The sensors binding of analytes is reversible allowing the device to be reusable, overall increasing the availability of the sensor to a wider population due to reduction in costs. Although a clear environmentalist application is intended the monitoring and removal of these compounds from the environment can reduce the risk of antimicrobial resistance occurring. CLI is chosen as a general antimicrobial within this research but in future research, application of the molecular imprinting fabrication could open an opportunity for optical antimicrobial detection in a range of settings. A further point of research would be the application of these technologies on to urine analysis technologies, by performing these tests on urine monitoring and dosage of antimicrobials to maintain a healthy therapeutic window can be achieved. In tandem this will treat bacterial infections more efficiently, squandering fewer resources and mitigating the risk of emergent antimicrobial resistance.¹²¹

1.3.11. Bacterial Growth Monitoring

A holographic pH sensor was fabricated by Chan *et al.* with the purpose of using change in pH attributed to bacterial metabolism of glucose into lactic acid for monitoring the growth of *Lactobacillus casei* Shirota.¹²² Gel precursor solution consisting of a monomer: HEMA, cross-linker: EGDMA, solvent: isopropyl alcohol (IPA), pH-sensitive moiety: 2-(trifluoromethyl acrylic acid) (TFMPA), photoinitiator: 2,2-dimethoxy-2-phenylacetophenone (DMPA) and polymerization inhibitor: hydroquinone (HQ) was prepared, introduced between the sample substrate and the COC (cyclic olefin copolymer) cover, and polymerized under UV light through a chrome-glass photomask (Figure 17a). The diffraction gratings were generated via the *in-situ* formation of Ag⁰ NPs, by diffusing silver perchlorate and lithium bromide into the gel matrix and exposure to a 532 nm continuous wave SLM laser. The HS was then enclosed

in a microfluidic chip by bonding the substrate with a poly(dimethylsiloxane) (PDMS) cover chip with microchannels.

The sensor was tested by spectrometer in reflection mode,¹²³ the colour of reflected light being determined by the alteration of Ag⁰ NP fringe spacing at the given pH (Figure 17b). The gel matrix swells or shrinks depending on the position of equilibrium between protonated and deprotonated form of the pH sensitive ligand. Here, as pH increases and leads to a net charge in gel matrix, water and counterions get absorbed in the gel matrix, causing expansion of the gel and increase in the Ag⁰ NP interfringe distance, inducing a red shift of the diffracted light. The final fabricated HSs elevation and appearance is illustrated in Figure 17c. Conversely, contraction of the matrix by decreasing pH result in a blue shift of the diffracted light (Figure 17d). Over a pH range from 3.0 to 6.0, the peak wavelength of the HS shifted from 450 nm to 540 nm. The sensor has accuracy comparable to a standard pH meter and offers a non-invasive method for monitoring of microorganisms in a nanobioreactor, with minimal volume of analytes needed for analysis and simple readout with a camera.

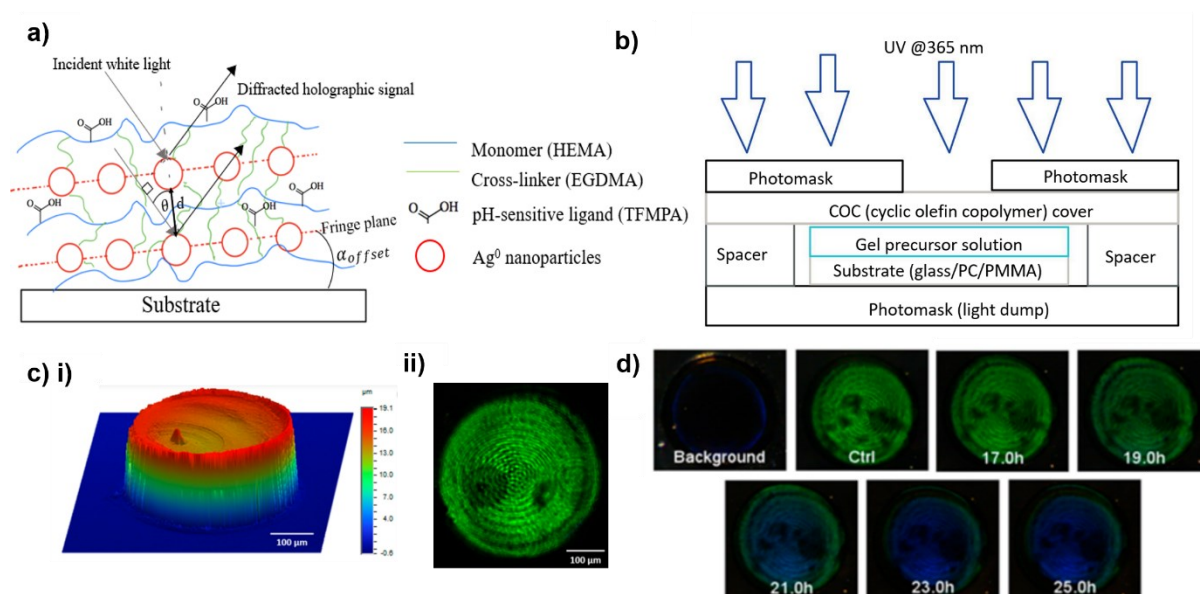


Figure 17. Monitoring of bacterial colony growth sensor fabricated through silver NP photolithography;¹²² a) Schematic of the setup for hydrogel fabrication; b) Representation of

the sensor function in reflection mode; c) i) Visual representation of 3D elevation of HS after fabrication, ii) Photograph of HS taken by CMAS camera whilst immersed in pH 6.0 McIlvaine buffer; d) Colorimetric response observed upon exposure of holographic gratings to *Lactobacillus casei* Shirota, reproduced with permission from American Chemical Society.

1.4. Conclusion

HSs in biotechnology cross an intersection of physics, chemistry, and biomedical engineering, as we see research within these respective fields progress the fabrication methods will evolve as they already have improving sensitivity, reliability, and accessibility. Over the past decades, HSs have been advancing into new fields. Significant progress has been achieved due to the improvement of hydrogel selectivity through advances such as molecular imprinting and improved ligand efficacy, but there is still considerable improvement required to reach a commercialisation stage.^{124, 125} The trend in development of sensors is to improve biocompatibility and remove toxic compounds, such as metal NPs. It has been clearly demonstrated the variety of analytes that can be identified using HSs, from biomarkers including ions, glucose, and temperature to illicit drugs. Quantitative analysis of HSs *via* smartphone cameras can be utilised to facilitate quantitative analysis in the field.¹²⁶ It is clear from current economic trends and the COVID-19 pandemic that personalised medicine and rapid POC devices are going to be a corner stone in future medical treatments. In the past three years we have observed the correlation between the ability for a country to rapidly test its population for COVID-19 and its mortality rate.¹²⁷ HSs can deliver simple colorimetric analysis of biomarkers reliably and could be a great asset in the future detection of illness without the requirement for medical professionals. Fabrication techniques for holographic devices have been summarised, showing simpler low cost, rapid production, and relatively minimal steps required for synthesis, allowing for easier scalability.¹²⁸ As presented throughout this thesis imprinted structures and polymer based holographic devices are removing the requirement for

NP. NP presence in films has hindered HSs use in biomedical applications but the novel techniques described herein abates many health concerns.¹²⁹ Response time is a factor that will need to be addressed for the devices to be able to truly function as a biological sensor, at present sensors are dictated by the rate of diffusion throughout the hydrogel to initiate sensing, showing response times too long to be viable, when compared to fluorescent or electrochemical biosensors.^{130, 131} For holographic devices to achieve their full potential in the application to analysing biological analytes collaborative works between medical professionals, chemists, and engineers to identify binding targets, design ligands with high specificity to fabricate sensors with ideal characteristics will be required.

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Chapter 2. Materials and Methods

2.1. Computational Modelling

Computational Simulations: Model design and study execution were conducted on COMSOL Multiphysics® 5.6 Wave Optics module based on a Lenovo ThinkPad E14, Intel Core i7 processor, 64-bit, running Windows 10. Below are highlighted the wave equations extracted from COMSOL Multiphysics®, due to the complex equations utilised within the model further information can be obtained from the COMSOL Multiphysics® Wave Optics module handbook.

2.1.1. Holographic Recording Modelling

Modelling of holographic recording mechanism was adapted from COMSOL Multiphysics® ‘Single Bit Hologram’ tutorial, with parameters and properties optimised to allow for application to doubly photopolymerised holographic biosensor recording set up. The geometry consisted of a union between a square to represent the external medium (height = 30 μm , thickness = 30 μm), and a rectangle (height = 20 μm , thickness = 10 μm . RI of the external medium and the internal hydrogel structure were set to 1.00 and 1.47 respectively. Ports were positioned at opposing ends of the model, to adequately represent the holographic recording set up utilized for reflection holography. A wavelength of 355 nm was selected to coincide with the recording wavelength utilised in UV photopolymerised holographic sensor fabrication. Induced modulated RI post recording was collected *via* a 2D cut line through the hydrogel with an inferred exposure threshold of 0.4.

2.1.2. Holographic Readout Modelling

HS readout models were designed with a fixed size exterior medium positioned at either end of a grating structure. The readout model was designed with positioning of the two exterior

mediums (height = 4000 μm , thickness = 4000 μm , RI = 1.33) at points relative to the grating thickness to minimise the risk of overlap. Periodic input and output ports were positioned on opposing vertical faces of the model, with a Floquet periodic condition applied to the lateral boundaries. The grating array was designed with individual parameters and position relative to the opposing layer thickness (height = 3000 μm , thickness = 50-300 μm). Parameters such as RM/IL spacing, refractive indices, angle of incidence, were all set prior to sensor design to arbitrary values to permit optimisation of the model. Blank materials were utilised for the interior medium with RI set relative to the input parameters highlighted throughout this thesis. An extra fine physics controlled quadratic mesh was selected to ensure the correct resolution and reliability of studies. Electromagnetic wave frequency domain physics package was utilised for computation of optical response. Parametric sweeps facilitated the study of individual parameters with wavelength range set to 350-700 nm to correspond with the visible range. Angle of incidence was altered at the incident port, with angles varied *via* parametric sweep (0° - 25°). Results of the electric field distribution and reflectance were exported from COMSOL Multiphysics[®] or to Excel to allow for simple processing and analysis.

2.1.3. Recording Thickness and Readout Modelling

Computational model design and parameter setting were adapted from the COMSOL Multiphysics[®] “single bit hologram” tutorial, with hydrogel thickness varied over values of 6, 9, 12, 15, and 18 μm . Points of constructive interference with high intensity were assigned as points of polymerisation with the total number counted to determine the effective layer number recorded. Holographic response modelling was varied through the application of parameters directly related to the glucose sensor with RI values of RM=1.4, IL=1.6 applied. Internal fringe spacings were set relative to the layer number simulated, with 5, 10, 15, 20, and 25 layers set to initial RM spacings of 310, 300, 290, 280, and 270 nm expanding through 6 iterations of 5%, 4%, 3%, 2%, and 1% respectively.

2.2. Experimental Sensor Procedure

Equipment

Microscope slides and Memmert™ Universal Oven UN with Natural Convection were purchased from Fisher Scientific, UVP crosslinker was purchased from Analytik Jena. Nd:YAG frequency tripled Quantel Q-smart (5 ns, 355 nm) solid-state laser purchased from Lumibird, France. A pulsed Nd:YVO4 FOBA 0020.UV laser marking system (355 nm) was purchased from FOBA. A plano-concave lens (−75.0 mm, Ø1" UV fused silica plano-concave lens, uncoated), optical posts (Ø12.7 mm, L = 20 mm), pedestal post holder (L = 20 mm, Ø12.7 mm), power and energy meter interface (PM100USB), UV extended Si photodiode, motorized precision rotation stage (Ø1"), collimated diode-pumped solid-state laser module (532 nm, 4.5 mW), laser diode module mounting kit (Ø11 mm), and iris (mounted standard iris, Ø12 mm max aperture, TR3 post) were purchased from Thorlabs, United States. A 25 mm dielectric 355 nm Nd:YAG laser line mirror was purchased from Edmund Optics, UK. Fisherbrand classic vortex mixer was purchased from Fisher Scientific, Bishop Meadow, UK. pH Meter. Mettler Toledo FiveEasy Plus™ pH benchtop meter was purchased from Mettler-Toledo Ltd. Holographic responses were analysed using UV-VIS bifurcated fibre optical cables, a 20 W tungsten halogen broadband light source, Flame-S-VIS-NIR-ES spectrophotometer and OceanView software (1.6.7) purchased from Ocean Insight. Abbe 5 Refractometer purchased from Bellingham + Stanley. Leica DM2700 P Upright Microscope was purchased from Leica Microsystems, Wetzlar, Germany.

2.2.1. pH Sensor

Materials

All chemicals were analytical grade. Hydroxy ethyl methyl methacrylate (HEMA), 2-(dimethylamino)ethyl acrylate (DMAEA), 2-hydroxy-2-methylpropiophenone (HMPP),

ethylene glycol dimethacrylate (EGDMA), 3-(trimethoxysilyl)-propylmethacrylate, isopropanol, methanol, ethanol, potassium chloride, tris(hydroxymethyl)amino methane (TRIS), adult bovine serum, hydrochloric acid (1M) and sodium hydroxide were purchased from Sigma Aldrich.

Synthesis of Responsive Matrix

Microscope slides were submerged in a solution of 3-(trimethoxysilyl)propylmethacrylate in acetone at a ratio of 1:50 (v/v). Slides were left to silanize overnight in a fume hood. Once silanization is completed slides are wiped clean and stored in darkened conditions until required. Monomer solutions containing HEMA (>61 mol%), EDGMA (2-6 mol%), HMPP (1 mol%), and DMAEA (5-25 mol%) were prepared in brown glass bottles to volumes of the mol% specified by the experiment. The monomer solution was diluted at a ratio of 1:1 with isopropanol. Throughout the experiments, the concentration of HMPP as a photoinitiator was maintained at 1 mol%. Once all components were prepared, solutions were mixed *via* vortex. Onto the polyester side of a flat polyester aluminised film, a 50 μ L of the monomer solution is pipetted and a silanized slide is carefully placed onto the droplet ensuring not to trap air pockets. It was then exposed about 26 cm from a bank of 4 UV (A) strip lights for 30 min. Once fully polymerized slides were removed and washed (>30 min) in a stirred methanol/water solution (1:1, v/v). To ensure the reproducibility of results, excess hydrogel on the edges was removed with a scalpel.

Laser Exposure

Under low orange light conditions, a slide with the responsive matrix (RM) was placed into a hot methanol/water solution ($\sim 60^{\circ}\text{C}$, 1:1, v/v) for 3 min. The slide was then removed and gently wiped with a tissue and without delay placed with hydrogel side up onto a clean glass sheet. Then under low orange safe lighting, 150 μ L of the secondary monomer solution (interference layer) was immediately pipetted onto it and spread evenly over the surface. It was then left to

soak into the RM for ~10 min. Then, the slide was given a quick single wipe with a tissue to remove any excess monomer from the surface. It was then dried under a cool airflow (4 min). The slide was then placed hydrogel side down into the laser set up at an angle of 5° from the normal of the surface plane of the mirror (Figure 2c). Slides were exposed to a single flash from a frequency tripled Nd:YAG laser (5 ns, 355 nm, 40 µs delay) with an irradiance area of ~1.5 cm. By moving the slide along in measured steps each time, the exposure was repeated over the entire length of the slide.

Still, in safelight conditions, the slide was washed in ethanol:water bath (1:1, v/v) (>3 h) to remove any remaining insufficiently polymerized material. Bright holograms were visible after short washings (<10 min), but then using slides after a short wash time often led to unreliable results, a minimum of 3 h is recommended but overnight is preferred.

Hologram Interrogation

Holograms were marked and cut out using a glass cutter (approx. 8 mm wide) and placed into a cuvette, hydrogel side facing the solution. Buffer solutions were prepared to range pH 7-9 according to their required parameters with tris(hydroxymethyl)aminomethane (TRIS). Ionic strength was corrected with KCl, and pH corrected to corresponding values with small volumes of HCl (1 mol L⁻¹) or NaOH (1 mol L⁻¹). The pH of buffer solutions was confirmed by a pH meter and regularly tested to ensure there had been no variation in pH over time. It was important for the meniscus of the solution in the cuvette to always be below the top of the 1-inch-long grating, otherwise, the glass side of grating was inclined to not stay in contact with the side of the cuvette.

A broadband light source was used to illuminate the HS *via* an optical fibre cable with a collimating lens. An Oceanview Flame spectrophotometer was utilised to analyse the spectral response from the grating and a smartphone camera to take colour images, *via* a bifurcated fibre optic cable. Realignment of the receiving fibre was required between samples as the angle

of diffraction would vary slightly, this process could be difficult to achieve, however by rotating or changing the height of the fibre a clear reproducible signal could be observed. Throughout measurements, the room temperature was maintained as constant (20 °C) and stirring was achieved *via* magnetic stirring by a micro flea. Changing of pH solutions was done by rinsing the grating in the cuvette with the buffer solution (3 times) and adding fresh to ensure the solution being measured was representative of the value being tested. To monitor the angular dependency of the grating reflection, the cuvette was maintained at a constant angle and elevation of 0° relative to the broadband light source. The receiving fibre was affixed to the rotational stage and rotated through a range of angles (5-50°). The collection of data and control of the mechanism was achieved *via* LabView software which supplied raw data that was processed utilising Origin 2018.

Minimum analyte volumes were determined *via* horizontal spectrophotometer analysis (Figure 28), where concurrent buffer solutions (TRIS, 50 mmol L⁻¹, 100 µL) were pipetted onto a section of the holographic grating. To overcome the natural buffering capacity of bovine serum pH was adjusted over a physiological pH range (pH 7-8) utilising high molarity TRIS buffers (1 mol L⁻¹). Before holographic analysis pH was monitored of each stock solution to ensure reliability of the result. Continuous measurements were achieved through the concurrent submerging of HSs in a range of prepared bovine serum samples (pH 7-8). Concurrent pH changes were achieved by rinsing the grating within the cuvette with the following analyte (3 times) and finally replaced with a fresh analyte to ensure a reliable pH within the cuvette.

2.2.2. Glucose Sensor

Materials

All chemicals utilised were analytical grade. Acrylamide (AM), 3-acrylamidophenylboronic acid (3-AAPB), methylenebisacrylamide (MBA), 2-hydroxy-2-methylpropiophenone

(HMPP), 3-(trimethoxysilyl)-propylmethacrylate, phosphate buffered saline (PBS), D-(+)-Glucose, 1,4-bis(acryloyl)piperazine (BAP), dimethyl sulfoxide (DMSO), hydrochloric acid (12 mol L^{-1}), sodium hydroxide, fructose, citric acid, creatinine, sodium chloride, lactic acid, sucrose, sodium sulfate, potassium chloride, calcium chloride, ammonium chloride, potassium oxalate dihydrate, magnesium sulfate heptahydrate, sodium hydrogen phosphate mono basic, disodium hydrogen phosphate, urea, and uric acid were all purchased from Sigma Aldrich. Human urine was supplied by a healthy 27-year-old fasting male and utilised immediately after collection.

Holographic Sensor Fabrication

Microscope slides were functionalised for polymerisation through immersing in a solution of (trimethoxysilyl)propylmethacrylate in acetone (1:50, v/v) and left exposed overnight in a dark room, with slides then washed with methanol and placed into an opaque storage container. RM solutions were prepared corresponding to the requirements within the sensor tested. Monomer solutions comprised of polymeric backbone (AM, 68-87 mol%), crosslinking agent (MBA 20% w/v, 1 mol%), functional *co*-monomer (3-AAPB, 10-30 mol%) and UV sensitiser (HMPP, 1 mol%), samples were dissolved in a DMSO:H₂O solution (1:1, v/v) at 0.5 g/mL. Interference layer (IL) monomer solutions contained a polymeric backbone (AM, 61 mol%) a crosslinking monomer (BAP, 29 mol%), and UV sensitiser (HMPP, 10 mol%), dissolved in a DMSO:H₂O solution (1:1, v/v) at 0.17 g mL^{-1} . Thin hydrogel films were prepared *via* the pipetting of RM monomer solution (25-100 μL) on to mirror backed plastic sheeting. Silanized glass slides were slowly placed on top of the solution, ensuring the contact with the solution is void of air pockets, and exposed to UV light (30 min). Once polymerised, hydrogels were washed in warm water ($\sim 40^\circ\text{C}$, 30 min), with the excess water removed with a soft tissue post washing. Optical

functionalisation was achieved in safe lighting *via* pipetting IL monomer solution (100 μL) onto a glass surface and placing the washed RM hydrogel side down to soak (5 min), ensuring no air pockets form between the monomer solution and the hydrogel. Slides were removed from the glass surface and wiped once with a soft tissue and placed under a hot air flow (10 min). Slides were kept under safe lighting and exposed to a single UV pulse from an Nd:YAG laser (frequency tripled, 355 nm, 5 ns, 30 μs delay) at a 5° angle with an irradiance area of ~ 1.5 cm. Whilst maintaining safe lighting, hydrogels were washed with DI water for 2 hours, with longer wash time often improved sensor reliability and reproducibility however sensors were able to be observed rapidly (< 3 min).

Holographic Sensor response monitoring

HSs were cut out in strips (width = 9 mm, length = 22 mm) and placed hydrogel side inward to a cuvette, filling with PBS solution (1.5 cm^3). Due to the requirement for temperature control, a water bath spectrophotometry set-up was established. A cuvette holder was manufactured with hooks to permit the suspension of a cuvette on the internal edge of a glass water bath atop a hotplate fitted with a thermocouple (Figure 31a). The cuvette was placed with the glass side of the sensor facing outwards and allowed to equilibrate with microflea stirring on a low speed (1 hr). Sensors were illuminated with a broadband light source *via* a fibre optic cable fitted with a collimating lens. The sensor response was monitored *via* a bifurcated receiving fibre optic cable, with a spectrophotometer and phone camera facilitating quantitative and qualitative analysis, respectively. To adequately monitor sensor response the receiving fibre was required to be angled to observe the angular reflection. This property varied between gratings due to their position within the cuvette, however by moving/rotating the fibre slightly with respect to the grating reflection a peak of maximum intensity can be obtained. Glucose concentration was altered through the removal of 150 μL of PBS from the cuvette and the

addition of 150 μL of a glucose solution (20.0 mmol L^{-1}). This process was repeated giving rise to concentrations of 2.0, 3.8, 5.4, 6.9, 8. 2 and 9.4 mmol L^{-1} , respectively.

Chapter 3. Computational Modelling of Doubly-Photopolymerised Holographic Sensors

*Note: The following section has been previously published as a manuscript in the journal, **Advanced Theory & Simulations***

*Reference: Davies, S.; Hu, Y.; Guo, D.; Jiang, N.; Montelongo, Y.; Naydenova, I.; Yetisen, A. K., **Advanced Theory and Simulations** **2022**, 5 (8), 2200082.*

Note: Design of the readout model was completed in collaboration with D. Guo as part of his MSc studies, where I supervised the project and assisted in the model and experiment design. D. Guo's contribution was focused on geometry design of the 1D readout model and assisting with the data collection for this component. Post completion of his MSc project S. Davies continued to optimise the model and further expanded the studies and remit of the project.

In the following chapter computational modelling is utilised to simulate the recording and swelling characteristics of doubly photopolymerized (DP) holographic sensors. DP holographic gratings are achieved through *in-situ* photopolymerization of a highly crosslinked polymer to produce nanostructured RI modulation. The unique swelling characteristics of DP holographic sensors necessitates the development of system-specific computational modelling. Hydrogel parameters, including film thickness, RI change, layer number, and external medium RI are examined for their effect on reflection spectra. Optimised computational models are utilised to study the effect of differential swelling rates of individual layer spacings on sensor response, indicating an idealised reduction in swelling of 50% for the highly crosslinked region. A 2D photonic crystal geometry with additional periodicity is developed, to inform further sensor design opportunities. Optimised parameters for both 1D and 2D photonic structures will assist the further development of DP holographic sensors.

3.1. Introduction

Throughout the COVID-19 pandemic, the importance of rapid medical diagnosis has been paramount, with prompt testing and action for positive cases directly mitigating the health risk to the general population.¹ POC diagnostics are key in these situations, through decentralised, fast, sensitive, and cost-effective attributes, which have the potential to revolutionise the approach to medical data collection. The pandemic catalysed healthcare providers to realise the potential therapeutic improvements offered by POC devices.² POC devices have previously been developed utilising electrochemical and fluorescent sensors due to their rapid response time and simple fabrication techniques; however issues such as signal drift, complex readouts, and lack of selectivity have hindered widespread use.³ Holographic sensors are ideally placed to facilitate the progression towards POC devices, due to their real-time, label-free readout, low cost, and reversibility.⁴ Recent holographic technologies show a primary focus to biomedical applications,⁵ such as sensing pH,⁶ heavy metals,⁷ glucose,⁸ ions,⁹ and lactate.¹⁰ Holographic recordings can be constructed with additional information such as text to convey further information to users offering both qualitative visual analysis and quantitative measurements through the combination with smartphone applications.¹¹

Holographic sensor fabrication techniques and readouts vary significantly depending on the applications and analytes. Common examples are surface relief gratings¹² and phase transmission¹³ or reflection holograms.¹⁴ Holographic sensors can be fabricated through the physical casting of surface-based gratings,¹⁵ inkjet printing,¹⁶ and photolithography.¹⁷ Phase holograms obtain optical functionality through RI variation, *via in-situ* NP reduction¹⁸ or polymerisation of highly crosslinked nano fringes.¹⁹ However, the technology has been hindered due to a range of issues, mainly focused on the use of potentially non-biocompatible silver halide (AgBr) NCs, complex production techniques, and high cost of materials.^{20, 21} DP hydrogel holograms utilise photolithographic *in-situ* polymerisation to generate nanostructured

crosslinking density variations in alternating layers with different RI.^{22, 23} The brightness and colour of the hologram can be varied by volumetric change in layer spacing *via* interaction with analytes.²⁴ With exact determination of the nanostructure layer and swelling degree difficult to be achieved experimentally, computational modelling offers a solution to gain better understanding of sensor response. Computational modelling has the capability to guide the experimental process through simple and rapid optimisation, expediting the prototyping process without the requirement for expenditure of financial and chemical resources.

Computational modelling is a fundamental tool in scientific research, engineering, and product development.²⁵ Several modelling computational techniques have been demonstrated in the modelling of holographic sensors such as finite-difference time-domain (FDTD),²⁶ plane wave expansion methods (PWE),²⁷ and finite element analysis (FEA).²⁸ Computational software such as MATLAB (FDTD) and COMSOL (FEA) have been established as viable platforms for the study of holographic sensor fabrication and readout^{29, 30} with both modelling techniques yield comparable results. However, FEA produces results more rapidly and shows a greater ability to suitably investigate to complex geometries as more accurate meshing can be applied. Due to the relative novelty of the DP system, few modelling studies have yet been undertaken on the complex swelling characteristics. Jiang *et al.* utilised FDTD theory in MATLAB to study the recording and readout of DP sensors.²⁶ Parameters including RI and spacing of the two polymeric layers through a sinusoidal recording of the nano fringes were simulated. FDTD produces the ability to study structures over a range of time steps, and FEA can be established within a MATLAB interface. Difficulty with MATLAB is encountered when varying individual layer thickness as the hydrogels mechanical properties are considered consistent. COMSOL Multiphysics® offers a modelling interface whereby a geometry can be designed with individual parameter variation that allows for users to conduct FEA studies of individual sensor characteristics. Although a sinusoidal grating structure is unable to be

simulated simply through COMSOL, the flexibility and simplicity of the software generates the opportunity to deepen comprehension of the swelling characteristics of sensors.

Utilising COMSOL Multiphysics[®] wave optics module, the recording, readout, and optimisation of a DP holographic sensor are demonstrated. Interference patterns between two counter-propagating 355 nm laser beams indicate regions of polymerisation and simulate effective RIs throughout the sensors. Upon this modelling, an initial internal geometry of the DP structures can be deduced. Computational models have been developed within COMSOL Multiphysics[®] to examine the idealised swelling characteristics of generic hydrogels, where simulated results adhere to the expected optical response determined by Bragg's law. Taking forward optimised swelling characteristics and to improve the potential applications of DP sensors, a second periodicity is proposed to fabricate a 2D grating, potentially minimising the physical restrictions induced by higher crosslinking density. The computational modelling established herein has applicability to DP holographic sensors, providing approximations for future works in studying the swelling characteristics and key parameters in the optical response of developed DP holographic sensors.

3.2. Results & Discussion

3.2.1. Grating recording simulation

Holographic sensors can be produced in several different orientations to generate various optical responses, through alteration of recording angle or the utilisation of multiple laser interference, complex patterns can be fabricated.³¹ Constructive and destructive interferences between coherent laser light generate regions of high and low light intensity respectively, which can be used in a photosensitised hydrogel film to generate holographic gratings.³² Orientation of the two coherent laser beams has a significant impact on the characteristics of the optical properties. If recording beams propagate from the same side through the material, the hologram recorded is in transmission.³³ When illuminated, light diffraction can be observed

on the opposing side to the probing beam. If the beams enter the material from opposing sides, the hologram will be recorded in reflection mode and light diffraction is observed reflected on the same side as the incident beam. COMSOL Multiphysics® has been employed as a computational platform to study the interference patterns and recording process involved in producing holographic sensors. Here, modelling focus is placed on reflection gratings, the geometry utilised to simulate the propagation of reference and object beams is illustrated in Figure 18a. Two ports (marked in red) on opposing sides of the hydrogel film illustrate where the reference and object beams originate. RI of 1.47 was set across the hydrogel film, with a thickness of 10 μm . The propagating wavelength was set to 355 nm to correspond with the UV laser light utilized in the fabrication of DP HS. The RI of external medium (light blue) was set to 1.00 to correlate with recording in an air environment, with scattering boundary conditions bordering the model. As specific polymer materials are not being investigated, an assumption of beam attenuation is made of <30% when passing through the hydrogel giving an optical density of approximately 0.5. This is considered negligible due to visibility at boundaries being ~ 0.98 , however with hydrogels where optical density is greater visibility may diminish and consideration of beam intensity decay would be required.

Figure 18b demonstrates an interference pattern between the two counter-propagating waves. The region of high energy initiates the polymerisation of a highly crosslinked IL within the base hydrogel RM. Crosslinking density disparity between these two layers generates a nanostructured RI variation and photonic band gap capable of tuning reflection wavelength. An expanded view of the electric field interference pattern is shown in Figure 18c, where alternating high and low intensity regions are present throughout the hydrogel film. Regions of varied laser intensity are observable throughout the hydrogel film; potentially linked to counter-propagating wavelengths not being in phase. The photo-polymerisation in the IL is dependent upon free radical generation by the photolysis of initiator compounds such as HMPP

or DMPA.³⁴ Figure 18c demonstrates the laser energy profile across the film throughout exposure, effectively highlighting the expected gradient of polymerisation. The intensity of laser light is directly correlated with the extent of polymerisation, and therefore the effective change in RI throughout the film. The electric field intensity of the interference between the two counter-propagating beams is displayed in Figure 18d. A sinusoidal pattern is observable, demonstrating the production of nodes and antinodes with an electric field intensity of 3.5 V/m. Efficiency of photoinitiator photolysis can be affected by parameters such as concentration, oxygen inhibition, laser power and exposure time.³⁵ The electric field intensity gradient between maxima/minima suggests that the degree of polymerisation can be increased with lower initiator photolysis activation energy. In development of experimental prepolymer solutions, results indicate that the lowering of photolysis threshold of the initiator compounds could tune the thickness of initial nanostructured layers of the gratings. Figure 18e reports the effective RI change across the film post-polymerisation. A modulation factor of 0.1 was applied relative to a RI value of 1.4, ensuring results showed relevance to the DP system. Both of RM and IL showed thicknesses ranging from 0.048-0.072 μm throughout the film. Experimentally holograms are recorded in an anhydrous state; therefore upon submerging in an aqueous medium, it is expected that both layers will expand due to the high hydrophilicity of the biocompatible polymers utilised. Herein, by observing position of individual polymerisation points, although expansion will occur throughout the measuring process, the results demonstrate the individual polymer layers origin.

In experimental HS recording, photosensitive hydrogels are positioned at an angle of 5° for laser exposure. The slight angle allows for the separation of diffracted light from the specular reflection of the probing broadband light. To observe the effect of recording angle variation, the object beam was modelled with an orientation angle of 5° respective to the hydrogel surface, and the corresponding interference pattern was recorded (Figure 18f). The

generated electric field demonstrates interference planes at an angle of -5° relative to the hydrogel surface, with a sinusoidal intensity pattern. The simulation indicates produced holograms will show the inverse angle of the recording beam. Although this is not vital to produce simple mirror holograms, complex patterned recording templates should be inverted to make ensure that the image is not reversed when illuminated. Simulation of the interference pattern of counter-propagating beams at both 0° and 5° has effectively determined the position and thickness of distinct layers within the hydrogel, improving understanding of the fabrication technique.

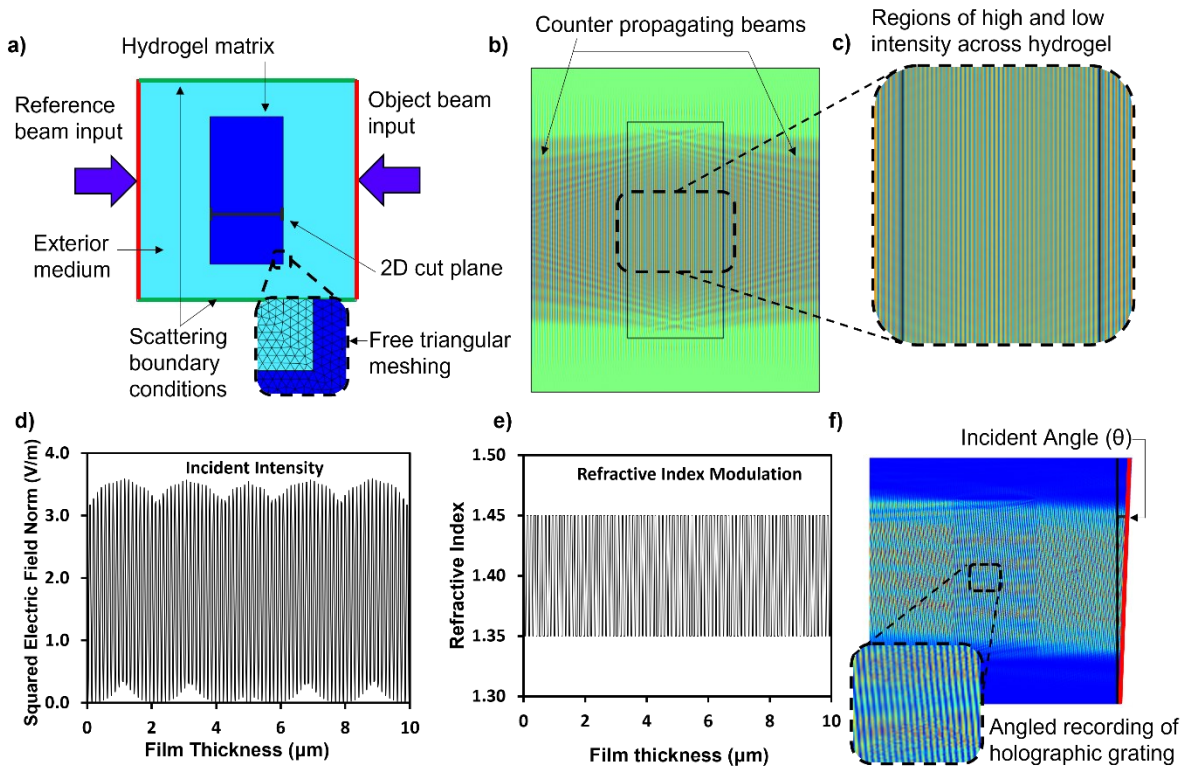


Figure 18. Schematic depicting the key points of the recording simulation. a) Overview of the geometric structure of the model, inset displays the free triangular meshing utilised; b) Electric field intensity plot of counter-propagating 355 nm reference and object beams; c) Expanded view of the interference pattern; d) Graph to show the effective spatial intensity of the propagating waves across the hydrogel film; e) Effective RI recorded within the hydrogel film; f) Interference pattern observed as the object beam offset to an angle of 5° .

3.2.2. 1D Grating Readout Modelling

Improved understanding of the response in the materials is vital to the development of the DP HS. The alternating RI of the nanostructured layers within HS generate a selective mirror for wavelengths that conform to the Bragg condition. The smart hydrogels volumetric response to external stimuli introduces variations in the layer spacing, and therefore tunes the wavelength which can be correlated to a quantitative sensing platform. COMSOL Multiphysics® Wave Optics module was chosen to study the sensing performance of holographic sensors. Modelling was achieved through the following fundamental steps (Figure 19a). Prior to geometry design, initial parameters including RI of hydrogel and external medium, layer spacing, and layer number were defined at arbitrary values. Geometry design was guided by theory and holographic structures, as it is expected that the recording pattern is analogous.²⁸ A 3D projection of the light path through the modelling grating is demonstrated in Figure 19bi. External medium size was set to a constant value independent of the grating thickness and spacing, to permit sufficient visualisation of the reflected or propagating wave. Electromagnetic Waves Frequency Domain (EWFD) physics settings were applied with a wavelength domain study selected. EWFD is applicable to solving for calculation of time-harmonic electromagnetic field and wavelength domain studies that permit the examination of sensor response over a range of wavelengths. Therefore, appropriate for studying the relationship of film expansion and reflection wavelength.

Application of physics to the model boundaries required the selection of a periodic input and output, Floquet periodic boundary conditions were applied to the lateral exterior boundaries (Figure 19bii). These conditions indicated that the external boundary did not interact with the propagating waves, comparable to experimental conditions. Two internal arrays were input to represent the alternating layers of high and low RI for RM and IL, respectively (Figure 19bii). Due to the lack of curved or triangular components, a physics

controlled quadratic meshing was selected; however, both quadratic and free-triagonal meshing yielded similar results. To study the interaction between the grating and the propagating wave, the electromagnetic field distribution was examined to observe the efficiency of the reflection (Figure 19c-d). Figure 19c illustrates the expected response of the grating when no major interaction occurs, small harmonic interactions are observable, and the electric field across the model is relatively consistent. When fringe spacings of the grating and the propagating wave satisfy the Bragg condition, a reflection interaction is observed (Figure 19d), where minimal propagation occurs past the grating. Therefore, model geometry was established with physics defined and the determination of the holographic sensor reflection interaction was identified.

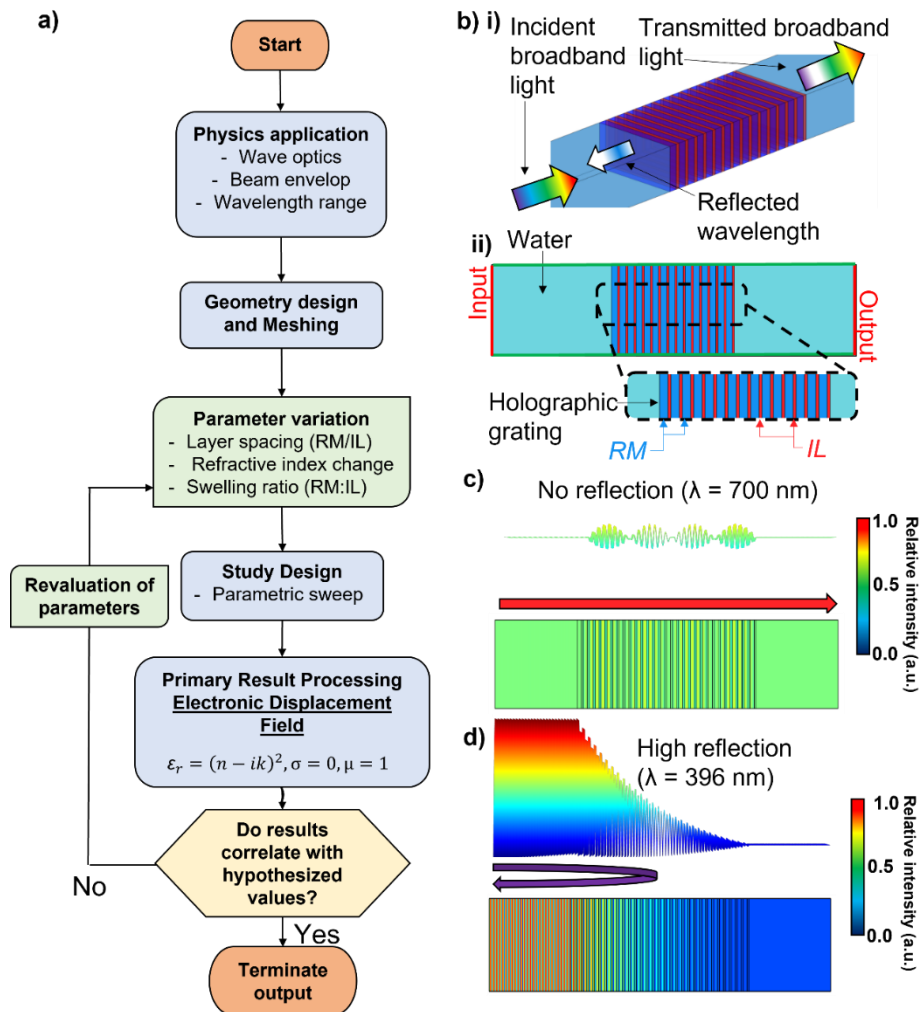


Figure 19. Setting up of simulations and overview of geometry. a) Flow chart to illustrate the process of model design, parameter setting, and output optimisation to obtain viable results; b) i) 3D projection

of the holographic grating geometry and the light path throughout the structure, ii) schematic depicting the overview of the modelling geometry defining specific regions such as RM and IL; Sensor output for c) low reflection and d) high reflection. The parameter design steps, and the readout mechanisms were the product of a collaboration with D. Guo.

3.2.3. Optical Sensor Readout Optimization

The angle of incidence was varied to observe the angular dependency of the fabricated holographic sensors. Angled recording of holographic gratings separates tuned reflections from the specular reflection reducing interference. Bragg's equation demonstrates sensor response is dependent on the angle of incidence. The focus of these simulations is the identification of potential disparity between simulated planar gratings and the angled gratings expected to be produced experimentally. Angles were varied over a range of 0-25° (Figure 20a). Minimal variation in diffraction efficiency or wavelength occurs for illumination angles of 0-5° (Figure 20b). Upon increasing incident angle from 10-25°, replay wavelength decreased while diffraction efficiency increased. This phenomenon is conducive as at shorter wavelengths the differences in phase were more significant as RI modulation remained constant. The minimal differentiation of the peak replay wavelength for 0-5° validates that modelled results are viable. In experimental processes, if a reflection angle of more than 5° is necessitated, replay wavelength and efficiency of reflection can vary.

A computational study of key parameters in DP holograms was conducted to simulate their influences on the spectral readout. A range of parameters can influence diffraction efficiency, for example, RI modulation and layer number are identified as key in the simulation process. RI modulation is directly linked to the diffraction efficiency of incident light. The RI of polymeric materials generally range from 1.30-1.70. As the hydrogels in RM are comprised predominantly of water due to low crosslinking density and high hydrophilicity, RI for the RM was set to 1.335. The RI of IL is expected to be higher due to the increased crosslinking density

(1.4~1.65). RI variation upon swelling is expected in response to stimuli. The exact RI of IL remains an unknown parameter as nano fringes are not discernible through experimental optical analysis. Furthermore, the exact degree of mixing/polymerisation is unable to be identified; therefore, the degree of polymerisation and mixing of the two polymeric materials cannot be confidently calculated. To determine the effect of RI variation in IL, a range from 1.35-1.65 was examined, whilst RM RI was fixed at 1.335 (Figure 20c). As RI of the IL layer increases, the diffraction efficiency of reflection peaks and the harmonic peaks increased. This phenomenon is due to the high RI modulation between two layers generating a more efficient holographic structure. When observing the diffraction efficiency change of the main reflection peak (~400 nm) and the harmonic peak (~550 nm), two different rates of increase occur (Figure 20d). This can be attributed to the harmonic interactions being more dependent on the RI of IL. As harmonic peaks are rarely observed in holographic devices, it is assumed that the effective RI of the IL layer must be below where the harmonic peak is present. The RI of IL of 1.47 gives the result with the highest diffraction efficiency feasible whilst diminishing the harmonic peak and was therefore taken forward. As it is expected due to the high level of crosslinking density present in the IL layer that swelling will be minimal, the value determined was considered constant throughout the following simulations.

Although RI has a significant effect on the ability of holographic sensors to interact with propagating light waves, the thickness of holographic sensors also determines diffraction efficiency. Experimentally, photosensitised hydrogels are dried to a minimal thickness pre-exposure with nodes and antinodes between counter-propagating laser light producing points of polymerisation. Controlling overall hydrogel film thickness regulates the number of recorded layers in the holographic sensor. Thin hydrogel films are ideal for sensors as thicker films hinder the rate of diffusion through polymeric networks reducing response time. The number of layers relates to the individual repeating unit, one pair of RM and IL constitute a

layer. A range of 5-30 layers increasing in increment of 5 was set (Figure 20e). Diffraction efficiency increases linearly until 15 layers with negligible additional interaction in the remaining section which can be described by the “weak grating approximation” (Eqn 3).³⁶

$$R = \tanh^2\left(\frac{\pi\Delta n L_1}{\lambda}\right) \quad \text{Eqn. 3}$$

Where, R is diffraction efficiency, Δn is the RI modulation, L_1 is the length of the multilayer Bragg structure and λ is the wavelength, from this a Δn value of 0.003 can be calculated. The result demonstrates a point at which film thickness can be optimised for the highest diffraction efficiency whilst minimising the negative characteristics of thicker films (Figure 20f). However, it must also be considered that the values are for an optimised RI modulation, with lower RI modulation it is expected that a higher layer number would be required to obtain comparable results. Throughout the following 1D model experiments, 15 layers were carried forward to reduce computation complexity without hindering output. Here, parameters with high diffraction efficiency have been examined and optimised to inform and guide the sensor design process.

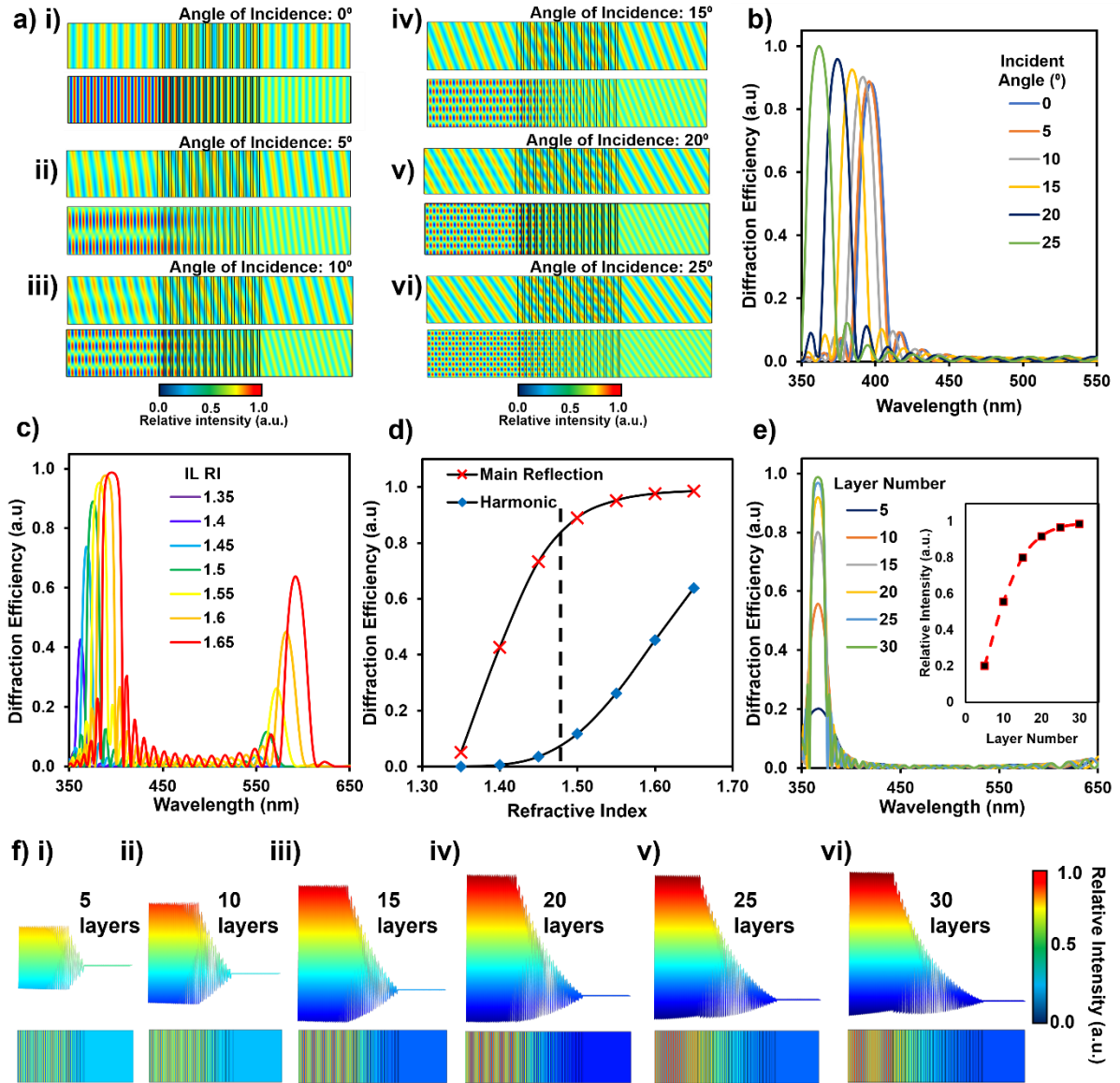


Figure 20. Optimization of model analysis parameters; a) Surface plots showing the wave propagation with varying angle of incidence for i) 0° , ii) 5° , iii) 10° , iv) 15° , v) 20° , and vi) 25° at minimum (upper) and maximum (lower) interaction respectively; b) Simulated reflection spectra demonstrating the effect of incident angle variation; c) Spectral response of IL RI variation over the rational experimental range, d) Comparison of the diffraction efficiency between main reflection peaks and the harmonics; e) Simulated spectral graph depicting the diffraction efficiency variation of reflection spectra for different layer numbers; f) Surface plot with height to demonstrate the wave propagation through the grating for layer numbers 5-30 respectively, inset compares the diffraction efficiency for increasing layer number. The data collected here is the product of a collaboration with D. Guo where we both would decide on design parameters and implement them together.

3.2.4. 1D Hologram Swelling Optimisation

The reflected wavelength is determined by grating periodicity, which can be tuned through the expansion or shrinking of the hydrogel film. Swelling behaviours of the DP sensors have not yet been established in experimental measurements, as the photonic structure is difficult to examine through conventional analytical techniques such as optical microscopes and electron microscopy. Maintaining RI, layer number and incident angle parameters established in previous optimisations, layer spacing was varied to examine the response. Regardless of the variation in crosslinking density across the hydrogel film, both RM and IL are varied at the same rate. Therefore, an initial spacing range of 200-300 nm with an increment of 20 nm was set (Figure 21a). Figure 21b demonstrates the trend in peak variation compared to the expected values dictated by Bragg's law, alongside the corresponding colorimetric response. Disparity in the simulated wavelengths when compared to the expected values is observed; however, the rate of wavelength change is comparable to layer spacing. It is plausible experimentally that the value for IL may be below that of RM due to a lack of polymerisation efficiency, for example if the activation energy of photoinitiators is greater than $\frac{1}{2}$ the total exposure energy. Therefore, IL was examined over 80 to 105 nm whilst maintaining an RM spacing of 200 nm to obtain an ideal Bragg replay wavelength (Figure 21c). IL shifting displays a linear trend, with an optimum reflection wavelength for IL at 90 nm delivering a response closest to that of the expected wavelength at 400 nm (Figure 21d). The expansion of IL with a high crosslinking density is minimal when introduced to an aqueous environment. Therefore, IL was maintained at the idealised value (90 nm) and RM was varied through a 200-300 nm range (Figure 21e). It was observed that the rate of change in Bragg replay wavelength reduced agreement with theory, indicating that both RM and IL must swell simultaneously to maintain the idealised response (Figure 21f). As the IL is polymerised within the RM, it is expected that an expansive

force would be induced due to stimuli-responsive *co*-monomers. Initial RM and IL values of 200 nm and 90 nm respectively show the strongest correlation with theory; however, a requirement for both layers to swell simultaneously at different rates has been identified.

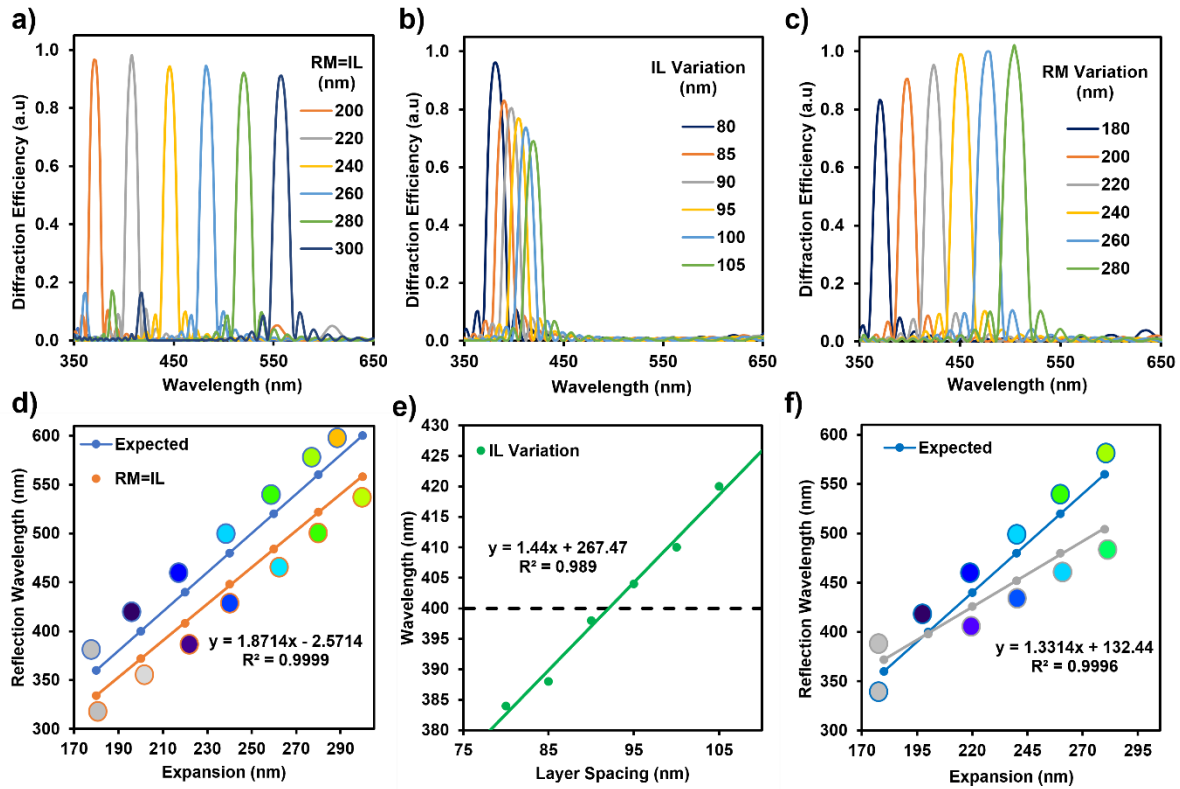


Figure 21. Simulation for the variation of both RM and IL swelling. a) Reflection spectra where both RM and IL are varied at equal rate; b) Simulated reflection spectra whereby RM is fixed at 200 nm and IL is varied over a range of 80-105 nm; c) Simulated reflection spectra from swelling of RM with fixed IL spacing; Comparison of maximum diffraction wavelength change plots where RM and IL parameters are varied at d) RM and IL at an equal rate, e) RM set at 200 nm and IL varied from 80-105 nm, f) RM (200-280nm) with IL set to 90 nm, with comparison to the expected wavelength change. Inset images demonstrate the corresponding wavelength colour for obtained simulated values. The data collected here is the product of a collaboration between myself and D. Guo.

To examine the optimal layer spacing variations, RM and IL were increased at different rates to reflect expected results more closely. Relative IL expansion values of 25, 50, and 75%

were set to optimise film response (Figure 22a–c). The RM layer was set to 200, 220, 240, 260, 280, and 300 nm, IL was set to an initial value of 90 nm. The ratioed swelling of the film can facilitate more accurate estimations of the degree to which the IL expands.

Figure 22a highlights the simulated Bragg reflection spectra at a relative expansion of 25%. With RM varied from 200 nm to 300 nm, IL spacing was set to 90, 95, 100, 105, 110, and 115 nm respectively. Over the IL spacing range of 90-100 nm, diffraction efficiency increased around 10% and a maximum is attained at IL spacing higher than 100 nm. Harmonic peaks occur between 350-400 nm, hypothesized to be linked to additional resonances within the structure. The correlation between peak wavelength and layer spacing remains below the predicted values. 50% swelling shows a significantly improved sensing performance (Figure 22b). Throughout the range of expansions, diffraction efficiency of approximately 100% is maintained without harmonics. Figure 22c demonstrates the simulated spectral response at a relative expansion of 75%. Diffraction efficiency diminishes comparing the 84% diffraction efficiency at RM = 300 nm of 75% swelling to 25% and 50% where diffraction efficiency is near 100%. IL expansions of 135, 150, and 165 nm harmonic peaks reoccur over the 350-450 nm range invalidating the expansion ratio. Figure 22d illustrates the reflection peak shift for each swelling ratio when compared to the expected Bragg's law response, with 50% showing the highest agreement. Figure 22e demonstrates the electric field surface plot of the maximum expansions of RM at 25%, 50%, and 75% with an expected replay wavelength of 600 nm. It can be observed that the maximum expansion at 50% shows strong interactions with high diffraction efficiency whereas 25% and 75% display weak interactions. Limitation of IL

expansion at 50% for RM is consistent with the predicted wavelength spacing relationships. Therefore, will be carried forward throughout the following modelling studies.

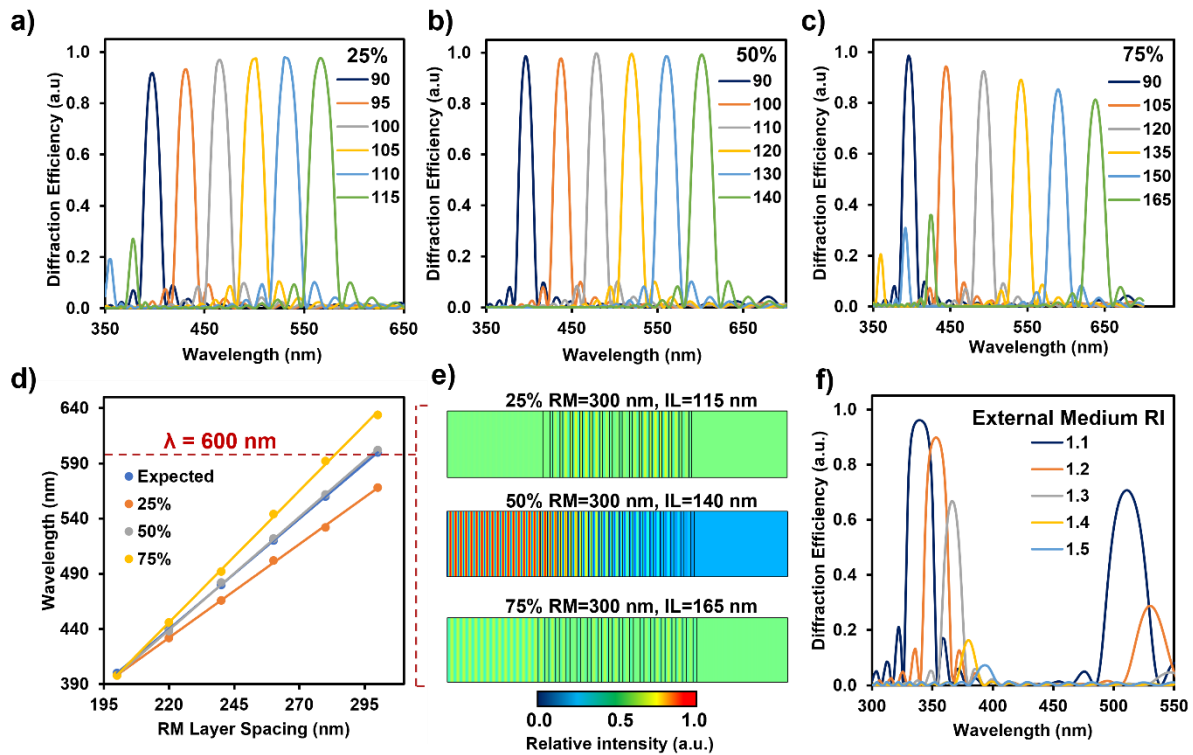


Figure 22. Optimization of peak replay wavelength through correlated simultaneous swelling; a–c) Simulated spectral response of gratings where IL thickness is varied at a) 25% ($y = 1.6943x + 59.42$), b) 50% ($y = 2.0457x - 10.762$) and c) 75% ($y = 2.3857x - 78.762$) of the rate of IL; d) Simulated peak wavelength change compared to the expected wavelength response; e) Surface plot of the spectral responses of the three expansions at the expected wavelength of 600 nm; f) Simulated reflection spectra of the holographic sensor in a variety of external medium RI. The data collected here is the product of a collaboration between myself and D. Guo.

DP holographic sensors have shown the sensitivity to alcohols via the use of pHEMA hydrogel systems.²⁶ Sensing properties of hydrogels were not affected by minor external RI variation in the presence of alcohols. Future sensors may be capable of sensing analytes with significantly higher RI's. The effect of external RI was studied with values varied from 1.1 to 1.5 to cover a range of feasible potential analytes. Figure 22f highlights that as external RI is

increased, the diffraction efficiency of the sensor decreased significantly. As external medium RI increased, the RI modulation between the internal layers is diminished, and therefore decreased diffraction efficiency of the gratings. Since the developed holographic sensors have been optimised for an aqueous environment, external RI was decreased inducing a rise in diffraction efficiency due to the increase in effective RI modulation relative to the external medium. Experimental works should consider the RI of analytes in the sensor design for the application along with functionality. If there is a requirement to utilise sensors in excessively high or low RI environments, the materials utilised should be adjusted accordingly.

3.2.5. 2D Optical Structure Design and Readout

To enhance the sensing performance of holographic hydrogels through the reduction of highly crosslinked regions, a computational 2D photonic structure was proposed. The increased crosslinking density within DP holograms can affect hologram sensitivity due to a decreased ability to swell, a hypothesis to utilise a 2D photonic structure with periodicity in both of x and y directions was developed. A schematic of the proposed 2D photonic structure is highlighted in Figure 23a. The removal of optical grating sections is expected to impede the response of the holographic sensors alongside the introduction of new optically active structures. Due to periodicity being present on the x-axis (RM_2/IL_2 , Figure 23b), a transmission grating interaction can be a potential source of interference. The 2D holographic grating was set to a thickness of 10 layers with a vertical layer number of 7.

Fabrication of RM_2/IL_2 periodicity can be considered analogous to experimental recording processes using photomasks. To facilitate experimental measurement, hydrogel sensing films are chemically attached to a glass substrate, this could however produce a high level of restriction in the direction normal to the surface due to few degrees of freedom. Therefore, the expansions of both RM_2 and IL_2 are maintained comparable. To investigate the effect of layer spacing on the diffraction efficiency of the gratings, RM_2 was set to values of

50–100 nm (Figure 23c), whilst maintaining the optimised parameters for spacing and RI for RM_1 (200 nm, 1.335) and IL_1 (90 nm, 1.47). As RM_2 increased from 50–100 nm, a general decrease in the diffraction efficiency from 32% to 26% occurs. Diminishing diffraction efficiency can be linked-to y-axis periodicity that impedes the interaction. As the designed model aims to examine reflections normal to the input port, additional transmission diffraction orders occur in the y-axis may not be visible in this model. The decrease in diffraction efficiency does not affect the wavelength of the reflection, indicating that the wavelength shift of the grating is independent. Individually examining the effect of IL_2 highlighted its opposing effect on diffraction efficiency over a parameter range of 50–100 nm, where diffraction efficiency increased from 8% to 18% (Figure 23d). This is intuitive as the proportion of the hydrogel structure containing low RI layers remained constant, whilst the high RI regions are expanding. Minimal diffraction efficiencies of the 2D photonic structure can be overcome experimentally by utilising a brighter or more intense broadband light source to improve the intensity of observable reflections.

For further understanding of how the additional freedom of movement modifies readout, layer spacing was varied at the previously optimised rate of 2:1 $RM_1:IL_1$ with initial values at 200 nm and 90 nm respectively, whilst RM_2 parameter values were set to 50–100 nm (Figure 23e). Diminishing of diffraction efficiency associated with the individual expansion of RM_2 was present within the spectra displayed, highlighting that the trend is consistent with previously demonstrated wavelength variation. Figure 23f displays the effect of IL_2 variation set to values of 50–100 nm whilst $RM_1:IL_1$ are increased. A continuation of the trend in increasing diffraction efficiency could be observed whilst maintaining consistency in wavelength shift, further confirming the conclusion that diffraction efficiency is determined by the RI modulation throughout the grating whereas wavelength is established through the x-axis expansion. Finally, all periodicities are varied simultaneously to simulate a real-world

expansion of the 2D holographic grating (Figure 23g). Diffraction efficiency is maintained consistently across the spectra with minimal variation. The equal variation of both RM_2 and IL_2 obtained a readout of continuous diffraction efficiency of reflection, indicating a competing effect. It is hypothesized that the ratio of $RM_2:IL_2$ determines the diffraction efficiency. Figure 23h shows the electric field density plot of the respective expansions at the wavelength of highest interaction, where a clear reflection interaction occurs. Simulations of the 2D holographic grating have informed the experimental process of several parameters which need to be maintained to achieve a viable sensor. Replay wavelength is unaffected by additional periodicity in the y-axis; however, the diffraction efficiency is reduced. The IL reduction may allow for easier expansion of the holographic sensor and facilitate higher sensitivity to analytes.

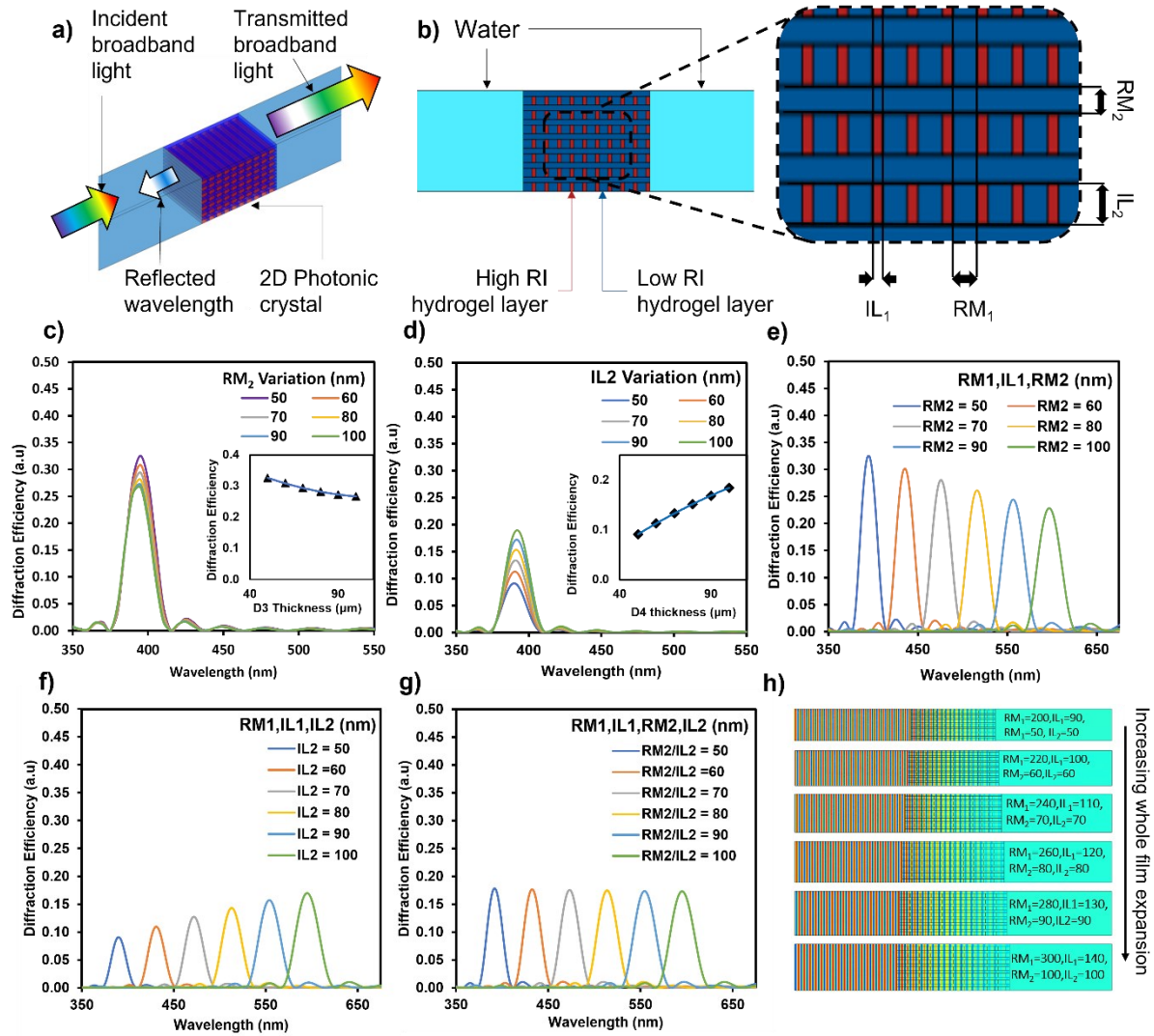


Figure 23. 2D photonic crystal modelling, a) 3D projection of proposed 2D structure demonstrating modelled light path. b) Schematic of 2D photonic structure model displaying the additional RM_1 - IL_2 spacing parameters. The magnified diagram shows the modelling of structure and meshing. c) Expansion of RM_2 whilst maintaining all other variables. d) Variations of IL_2 whilst maintaining all other variables. e) Variations of RM_1 and IL_1 at a ratio of 2:1 for volume change. f) Variation of RM_1 , IL_1 , and IL_2 whilst maintaining RM_2 . g) Variation of all parameters in a ratioed fashion, h) Electric field spectra of finalised swelling behaviours.

3.3. Conclusion

DP holography shows promise to assist in the development of POC devices, due to their rapid, label-free, and low-cost fabrication.³⁷ Sensors have been simulated to inform the development

of biocompatible POC sensing devices. Computational models were designed utilising the COMSOL Multiphysics® Wave Optics module to study the fundamentals of recording, readout, and optimisation within DP holographic sensors. Recording simulations indicate the points of polymerisation throughout the hydrogel structure, facilitating informed assumptions about internal polymeric network structure. Film thickness and RI variation between interior layers and external medium were found to be significant parameters in correlation with the diffraction efficiency.³⁸ Sensors with 15 layers with an IL RI of 1.47 demonstrated results that correlated with responses predicted by Braggs law. Studies of the ratioed swelling behaviours within 1D photonic structures demonstrated readouts correlating with theory, assisting in the expansion of understanding of the response mechanics. Optimised swelling characteristics were identified as an initial spacing of 200 nm and 90 nm for RM and IL respectively, following a relative expansion of 2:1 (RM:IL). To advance sensitivity of the holographic sensors, a 2D grating was proposed and optimized to minimise swelling restriction. Future developments of this computational modelling could focus on the use of a sinusoidal RI change within the grating to reflect the experimental structure. The generic model designed herein can be utilised to inform the development of DP devices once stimuli responsive hydrogels have been selected, the incorporation of chemical characteristics such as swelling rate, rate of diffusion or activation energy of photoinitiator compounds could improve the model's application to sensor design. Overall, the comprehension of the DP systems characteristics and internal mechanics have been expanded, with the experimental process informed of the key parameters in sensor design characteristic optimisation. As experimental projects are advanced modelling developed here can be utilised to highlight the potential routes to improving sensor characteristics.

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Chapter 4. Doubly Photopolymerised Holographic pH Sensors

Note: The following section has been previously published as a manuscript in the journal,

Biosensors & Bioelectronics

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Continuous monitoring of physiological conditions and biomarkers *via* optical HSs is an area of growing interest to facilitate the expansion of personalised medicine. Here, a facile laser-induced dual polymerization method is developed to fabricate holographic hydrogel sensors for the continuous and reversible colorimetric determination of pH variations over a physiological range in blood serum (pH = 7-9). Laser lithography for holographic hydrogel sensor fabrication is achieved *via* a single 355 nm laser pulse to initiate polymerization of ultrafine hydrogel fringes. Eliminating the requirement for complex processing of toxic components and streamlining the synthetic procedure provides a simpler route to mass production. Optimised pH-responsive hydrogels contain amine bearing functional *co*-monomers demonstrating reversible Bragg wavelength shifts of 172 nm across the entire visible wavelength range with pH variation from 7.0 to 9.0 upon illumination with broadband light. Photolithographic recording of information shows the ability to convey detailed information to users for qualitative identification of pH. HS reversibility over 20 cycles showed minimal variation in replay wavelength supporting reliable and consistent readout, with optimised sensors showing rapid response times of <5 min. The developed sensors demonstrate the application to continuous monitoring in biological fluids, withstanding interference from electrolytes, saccharides, and proteins colourimetrically identifying bovine serum pH over a physiological range. The HSs benefit POC pH analysis of biological analytes which could be

applied to the identification of blood gas disorders and wound regeneration monitoring through colorimetric readouts.

4.1. Introduction

Analysis of pH is important to a wide range of fields linked to modern life, including agriculture,¹ environmental monitoring,² pharmaceutical manufacture³ and biomedical applications.⁴ With the growing movement towards personalised medicine, the demand for POC diagnostic devices is of paramount importance.⁵⁻⁷ Medical professionals have expressed the consistent desire for POC devices to assist in the diagnosis, management, and treatment of acute or chronic illnesses.⁸ Factors such as cost or lack of specificity and reliability hinder the adoption of optical technologies,⁹ advances in biosensor technology has had to ensure devices that are accessible *via* the development of low-cost, accurate, and user-friendly diagnostic systems.^{10, 11} In this regard, optical sensors demonstrate their advantages of superior stability, selectivity, and simplicity of readout. HSs function in the visible wavelength region to be interpreted qualitatively by the naked eye without the requirement for specialised equipment.^{12,}¹³ This simplistic readout has been previously utilised to achieve both qualitative and quantitative measurements to specific analytes, such as organic solvent,¹⁴ alcohols,¹⁵ ions,¹⁶ glucose,¹⁷ and ionic strength.¹⁸ HSs can display colorimetric response through chemically stimulated volumetric changes to a hydrogel with nanostructured variation in RI.¹⁹ Quantitative measurements *via* a spectrophotometer analysis through widely available technology such as smartphone cameras permits expansion of POC devices.²⁰ At present, POC monitoring utilises electrical, colorimetric, and fluorescent sensors. Electrical sensors are often susceptible to signal drift over time and are incompatible with several modern medical techniques such as magnetic resonance imaging.²¹ Conventional electrochemical sensors are bound by the requirement for an electrical power source to initiate sensing; however HSs are power-free and label-free devices, potentially expanding applications.²² Colorimetric and fluorescent sensors

are the most widely utilised optical POC devices such as lateral flow assays.^{23, 24} Despite their fast and sensitive response, fluorescent sensors are often susceptible to photobleaching, signal interference from concentration variation, and environmental change.^{25, 26} Unlike fluorescent sensors, HSs allow for reversible and continuous monitoring of homeostasis without the risk of photobleaching. Therefore, the development of holographic optical biosensors will assist in the evolution of medical care.

HSs rely upon RI variation which can be achieved through a range of techniques such as photolithographic recording of nanocomposites,²⁷ and inorganic NPs.²⁸ With the most prominent technique in literature being *in-situ* photo-reduction of metal ions such as silver to form NP fringe structures (i.e. AgNP).²⁹ Metal NPs deliver the change in RI required for HSs, but improvements are required for mass production and application to biological sensing. The complex chemistry used in the reduction and fixing of metal NPs mandates a high number of processing steps, which has been a major hindrance to mass production and widespread application. The removal of the requirement for NP components can be achieved *via* the use of secondary hydrogel polymerisation to reduce the requirement for inorganic species. Utilising the dual photo-polymerised systems, the number of steps is reduced significantly improving the opportunity to move towards widespread use. Moreover, there is a hesitation within regulatory bodies about the *in vivo* application of metal NPs, linked to a lack of information of their metabolism within the body and possible linkage to carcinogenic behaviour, impeding their approval in medical devices. The absence of metal NPs in the dual photo-polymerized holographic structures decreases the possible risks in biomedical monitoring.^{29, 30}

pH is one of the most important physiological conditions monitored in healthcare. Nearly all biological processes are affected by pH variations such as enzyme conformation,³¹ ion transport,³² and cell membrane tension.³³ Wound pH can be correlated with angiogenesis, protease activity and bacterial infection.^{34, 35} Healthy skin pH varies from 4.0-6.0, upon injury

pH rises to a more neutral pH (7.4), due to exposure to the biological material beneath.³⁶ Variation can differ depending upon wound severity with chronic injuries previously recorded at neutral to alkaline (7.2-8.9) values.³⁷ Healing occurs more readily in an acidic environment. However, alkaline or extremely acidic pH values can indicate a bacterial infection.³⁸ Monitoring pH in wound tissue may provide an analytical platform, ultimately enabling determination of response to treatment. Alternatively, pH sensing can be applied to arterial blood pH analysis in the diagnosis of blood gas disorders such as acidosis or alkalosis. With a healthy blood pH between 7.35-7.45, variations outside of this range can be correlated to imbalances in dissolved gases, possible indication of uncontrolled diabetes, haemorrhage, and pulmonary failure. Conventional blood sampling often incurs patient discomfort, reducing adherence to the testing regime and ultimately inducing a lack of patient devotion to medical regimes that reduces data reliability.³⁹ Biocompatible polymers, such as HEMA, can be used in the fabrication of optical hydrogels sensors and can be located *in-situ* for continuous measurement. Therefore, facilitating earlier diagnosis, and treatment of disease due to ease of use and reversible readout. Consequently, there is a currently unmet requirement for biocompatible continuous and reversible optical pH monitoring to improve the management of many ailments.

Here, a single-flash ultraviolet dual photo-polymerized method is developed to produce HSs for reversible and continuous analysis of physiological pH. The synthesis utilised a single pulse from a Nd:YAG laser system ($\lambda_m = 355$ nm) to initiate photo-polymerization of highly cross-linked fringes within a loosely cross-linked pH responsive hydrogel matrix to produce HSs. Demonstrating rapid monitoring of pH over a physiological range with simple optical readouts capable of being determined both qualitatively through visual inspection and quantitatively through a spectrophotometer analysis. The research presented details the chemically functionalised DP system with the effect of *co*-monomers concentration,

crosslinking density, and thickness variation. Properties of the holographic hydrogel pH sensors such as angular dependency, diffraction efficiency, and reliability of wavelength are also described. The developed sensors show high selectivity to pH independent from common biological contaminants, minimal replay wavelength variation over 20 cycles, and sensitivity of $0.0068 \text{ pH nm}^{-1}$ with optimised sensors showing response times of <5 mins.

4.2. Results and Discussion

4.2.1. Fabrication of Holographic Hydrogel pH Sensor

The DP pH HSs are composed of two alternating hydrogel layers of differing RI. A low RI pH sensitive RM which consists of a low crosslinking density hydrogel, into this a second-high RI IL containing high crosslinking density is polymerized by laser photo-lithography. These structures tune the reflection of a narrow band of wavelengths which can be correlated with chemically stimulated expansion of the hydrogel. Concentrations of monomers are varied throughout to further examine their effect on the sensor response, the pH RM was prepared by a free radical polymerisation of a hydrophilic monomer (HEMA, $>61 \text{ mol}\%$), and a functional *co*-monomer (dimethyl aminoethyl acrylate, DMAEA, $5\text{-}25 \text{ mol}\%$) and crosslinker (EGDMA, $2\text{-}6 \text{ mol}\%$) initiated by a photoinitiator (HMPP, $1 \text{ mol}\%$) (Figure 24a-i). The IL is composed of a highly crosslinked polymer network utilising HEMA ($13 \text{ mol}\%$), EGDMA ($84 \text{ mol}\%$) and HMPP ($3 \text{ mol}\%$) (Figure 24a-ii). The low crosslinking density in the pH RM of the hydrogel allows for large volumetric change depending upon the protonation and deprotonation of the *co*-monomer. The smart hydrogel employed in the RM contains functional *co*-monomer, DMAEA, which bears a tertiary amine capable of being protonated and deprotonated at different pH values.⁴⁰ The level of protonation is dependent on the acidic association constant (pK_a) of the amines lone pair of electrons and their ability to donate electron density to protons within solution.⁴¹ DMAEA has been identified as an ideal candidate for hydrogel

functionalisation, due to the slightly basic pK_a , providing a sensitivity range encompassing the normal physiological ranges of biological fluids, i.e. blood (7.35-7.45) and cerebrospinal fluid (CSF) (7.33-7.52). Although DMAEA was selected due to the pK_a , a variety of *co*-monomers such as methacrylic acid or 2-acrylamido-2-methylpropane sulfonic acid could be applicable to tune the sensitivity range of the sensor to alternate pH ranges. The responsive hydrogel utilises a relatively low crosslinker concentration (2-6 mol%), whereas the IL contains 84 mol% of crosslinker. Disparity in crosslinking density generates the change in RI, when swollen required to produce a Bragg nanostructure.⁴² The IL differs by the concentration of crosslinking, with the pH-responsive layer and IL containing <6 mol% and 84 mol% of EGDMA respectively, restricting swelling and increasing RI producing the holographic nanostructure (Figure 24b-c). The visual difference between the two hydrogels can be observed in Figure 24c, where the highly crosslinked hydrogel shows an opaque white colour, while the RM with low crosslinking shows transparency. Effective RIs of the two distinct hydrogels were determined to be 1.34 and 1.65 for the pH RM and IL, respectively.

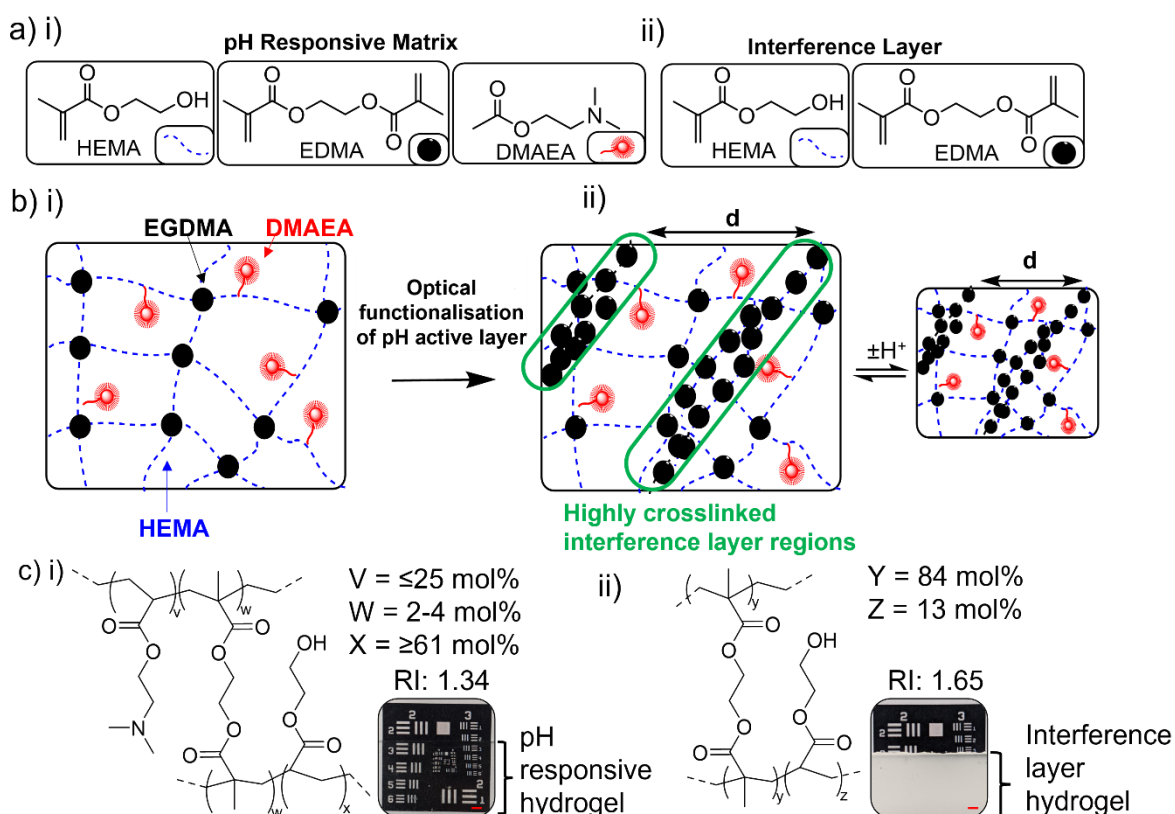


Figure 24. Chemical structures and fundamentals of the holographic hydrogel pH sensor. a) Chemical structures of i) pH RM monomers and ii) IL monomers inset images demonstrate the two hydrogels post-polymerisation, insets show photographs of the polymerised monomer solutions; b) Schematic to display hologram construction i) with and ii) without ILs recorded, where d illustrates the spacing between the IL fringes; c) The respective polymeric networks of the i) RM and ii) IL alongside photographs of the two hydrogels separately polymerised on transparent glass slides covering the bottom half of the USAF resolution target (scale bar = 2 mm).

A schematic overview of the fabrication process and mechanism of optical sensing is illustrated in Figure 25a. Through the optimisation of hydrogel composition, the DP fabrication method is utilized to generate a response over the whole visible spectrum. Monomer solutions throughout this process were freshly prepared for each experiment and aerated prior to each experiment, as it is hypothesized dissolved oxygen in monomer solutions inhibits radical migration in the recording process which can affect grating consistency.⁴³ There are 4 main

steps to the fabrication process: RM polymerization, incorporation of IL monomers, recording of the HS followed by the development of the grating (Figure 25a). Firstly, the responsive hydrogel is polymerized onto a glass slide which has been silanized with 3-tri(methoxy silyl)propyl methacrylate (Figure 25a-i). This was done so that a methacrylate group can be covalently bonded to the glass surface *via* Si-O bonds, then photo-polymerized with the pH-responsive hydrogel, this is imperative for the effective attachment of the hydrogel to the glass slide. Polymerized hydrogels were washed in a hot methanol bath (50 vol%, 60°C) and the secondary monomer solution for the IL was subsequently applied (Figure 25a-ii). Samples were left to soak for 10 min before drying under a cool air flow. As the recording wavelength is in the UV region, shrinkage of the hydrogel post recording led to a replay wavelength in the deep UV, outside the visible region. Therefore, by recording the hydrogel in a dry state, submerging into an aqueous analyte solution post exposure leads to hydrogel swelling, bringing the replay wavelength into the visible region.

The holographic structures fabricated depend upon the use of a coherent frequency tripled Nd:YAG laser source (5 ns, 355 nm, 40 μ s delay) to initiate polymerization of alternating multilayers at regular spacings within a pH-responsive hydrogel. A single flash was utilised to generate polymerization, by utilising a single flash, the risk of multiple recording points is minimised due to lack of any nano-movements. HSs were recorded in reflection mode where the interference pattern between the incident beam and the reflected object beam form nodes and anti-nodes, areas of high and low intensity UV light, fringe spacings of gratings were determined by the wavelength of incident light (Figure 25b).⁴⁴ Anti-nodes, areas of constructive interference, decompose HMPP to initiate free radical photo-polymerization of the IL within the pH-responsive hydrogel. In contrast nodes, areas of destructive interference, lack photo-polymerization (Figure 25a iii-iv) with unpolymerized monomers removed *via* subsequent washing steps in a fresh MeOH:H₂O bath (1:1, v/v). Protonation of DMAEA,

produced a bound ionic charge within the hydrogel network, changing the hydration level of the hydrogel due to changes in osmotic and electrostatic forces from the movement of counter ions across the layers of hydrogel (Figure 25a v-vii).⁴⁵ These changes in solvation alter the swelling/shrinkage of the hydrogel; and therefore the spacing of the ILs, tuning the replay wavelength. Figure 25b illustrates the exposure set up utilised to induce the counter propagation of incident and reflected beams to create the interference pattern required for reflection holography (Figure 25b-ii). Figure 25c highlights the readout set up, where the tuned reflected wavelength is separated from the specular reflection due to the 5° recording angle. Sensors show a high degree of reproducibility in the fabrication process, within this research sensors have been refabricated several times whilst maintaining comparable replay wavelength ranges.

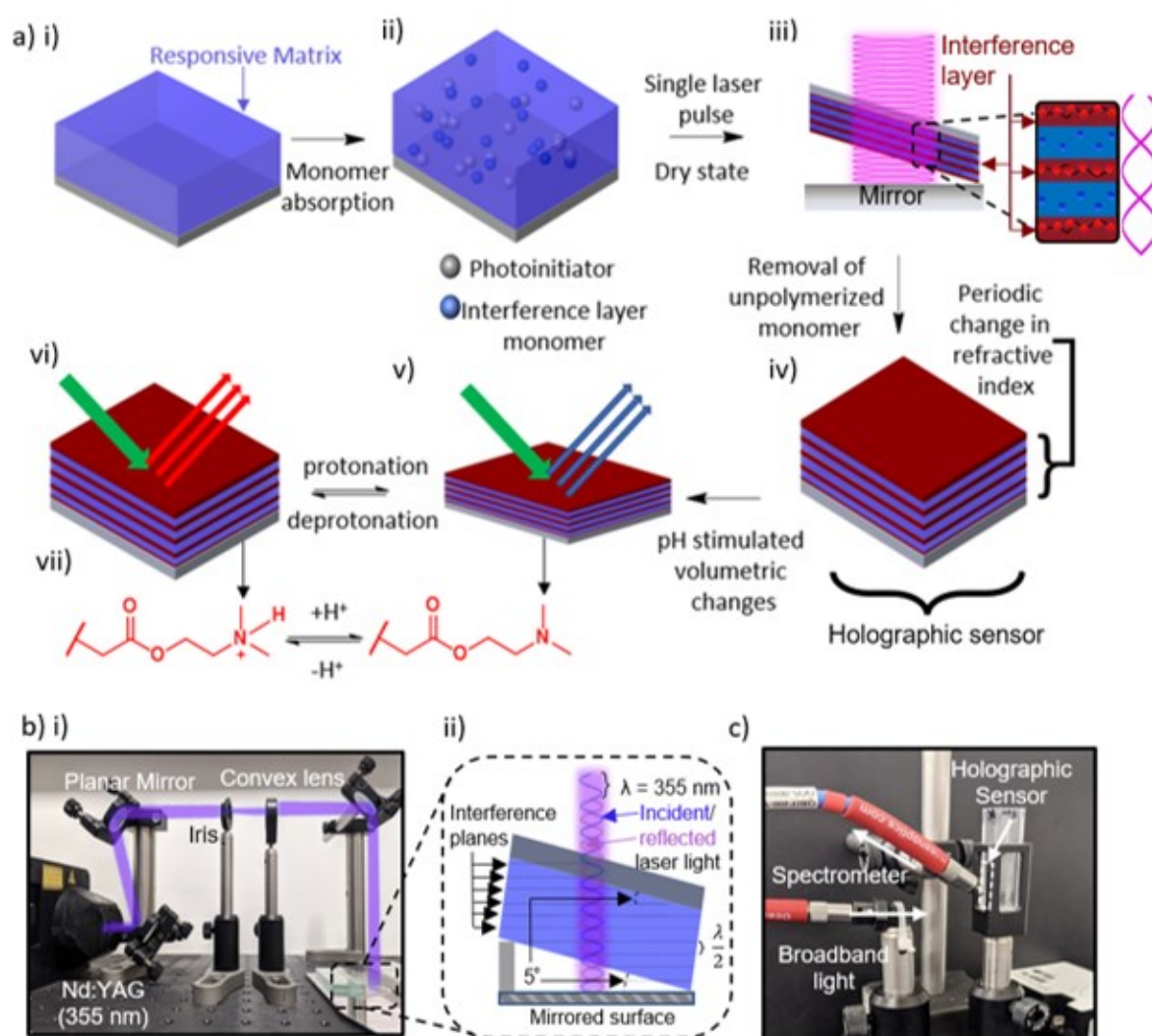


Figure 25. Fabrication and interrogation processes of dual photo-polymerized holographic pH sensors. i) Hydrogels containing functional co-monomer DMAEA, ii) diffusion of monomers into functionalised hydrogel matrix, iii) exposure of photosensitised hydrogel at a 5° angle to a single UV pulse laser (355 nm), iv) removal of unpolymerized monomers, v) blue shifting of replay wavelength at high pH, vi) red shifting of replay wavelength at low pH, vii) equilibrium process that incurs the swelling/shrinking behaviour of the hydrogel pH sensor. b) Photograph and schematic to demonstrate the fabrication process of the pH sensitive optically active hydrogel. i) Photograph to illustrate the optical set up utilised to fabricate the holographic hydrogels, ii) detailed illustration of the interference pattern from the incident laser light to generate the alternating points of polymerisation within the hydrogel sensor. c) Grating interrogation set up including a broadband light source and a spectrophotometer.

Figure 26 highlights the replay wavelengths of 3 separately prepared HSs at pH 7, where an average replay wavelength of 631.0 nm with an error margin of 3.2 nm. The HS developed throughout this work are highly reliable in fabrication, however the utmost care is required to ensure that conditions are kept the same throughout the process. In experiments conducted throughout variations in minor conditions such as the humidity or delay between drying and exposure drastically effected the sensors produced. It was found that conducting the drying of the sample prior to exposure was best done immediately prior to exposure, this was hypothesized to be linked to the environmental humidity altering the initial thickness of the hydrogel between steps.

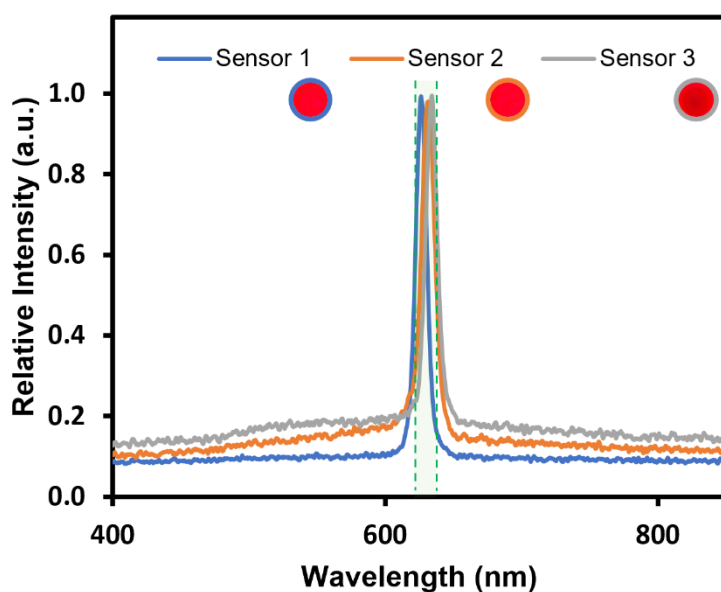


Figure 26. HS 1, 2 and 3 were prepared separately utilising a 25 mol% DMAEA concentration with replay wavelength tested at pH 7, insets highlight cropped smartphone images of the holographic response.

4.2.2. Characterisation of Holographic Hydrogel pH Sensors

To investigate the effect of the thickness on the diffraction efficiency of the HSs and the rate of wavelength variation, the volume of monomer solution for the pH RM was varied. The thicknesses of the hydrogel sensor with 25 μm (T1), 28 μm (T2), 30 μm (T3), 35 μm (T4), and 38 μm (T5) were prepared with RM monomer volumes at 25 μL , 37.5 μL , 50 μL , 62.5 μL , and 75 μL respectively (Figure 27c i-v). The hydrogel sensors were dehydrated under vacuum for 24 hrs, to ensure minimal residual hydration and consistent swelling across all samples. To determine the optimum thickness of the hydrogels, two characteristics were monitored, namely relative diffraction intensity of the hologram and rate of peak equilibration in TRIS buffer at pH 7 (50 mmol L⁻¹). The relative intensity of the reflection rises until a plateau for thicknesses above 30 μm , showing relative intensities of 0.32 (T1), 0.55 (T2), 0.93 (T3), 0.92 (T4) and 0.95 (T5) (Figure 27a). The slight red shift of replay wavelength observed in T4 sits within the error margin expected for these sensors. It is hypothesized to be linked to the decreased the number of layers recorded within the RM in thinner hydrogels, resulting in reduced diffraction efficiency. Equilibration time was monitored of all hydrogel sensors to determine the optimized thickness. This was achieved by timing the interval for Bragg replay wavelength to stabilise when shifting from pH 7-9. Replay wavelengths were monitored at intervals (30 s) until peak stabilisation was achieved. Determined rates for each thickness were 2.5 min (T1), 3.5 min (T2), 5 min (T3), 5.5 min (T4), and 7 min (T5) (Figure 27b-c). It can be concluded that the thickness of the hydrogel sensor is directly related to the rate of movement of the peak. T3 was selected to be ideal, as the intensity of the peak was on the same order of brightness as that of thicker hydrogels with an acceptable response time. Thus, a volume of 50 μL was identified as the optimized monomer volume for the polymerization of the responsive hydrogel matrix. Diffraction efficiency was determined *via* illumination with a 532 nm laser, with the respective diffractions monitored by using a power meter (Figure 27d). By utilising a rotational stage for the receiving spectrophotometer, the angular dependency of the reflection was established

(Figure 27e). As previously described in the fabrication, the hologram is recorded with an elevation of 5° to ensure reflected light is easily distinguishable from specular reflection. Slanted HSs therefore demonstrate an angular variation directly linked the volumetric swelling of the hydrogel (Figure 27f). The dependency on swelling is directly related to Bragg's law, with diffraction angle varying minimally between the swollen and unswollen state. The angle of highest intensity decreases upon shrinking of the hydrogel (swollen = 25° , unswollen = 15° (Figure 27g). The variation of angle can lead to a requirement for the repositioning of the spectrophotometer receiving cable which should be considered when monitoring hologram readout. The reflection is clearly visible to the user for qualitative measurements however to achieve the brightest possible reflection a collimating lens was used to focus the white light on the sensor. This in turn creates a very specific angle at which the receiving fibre must be positioned to monitor response quantitatively. For an experienced user this is no issue, however it may provide difficulty for novice users. This issue could be rectified by using alternative recording methods such as demonstrated by Naydenova *et al.* to produce speckled holographic gratings.⁴⁶

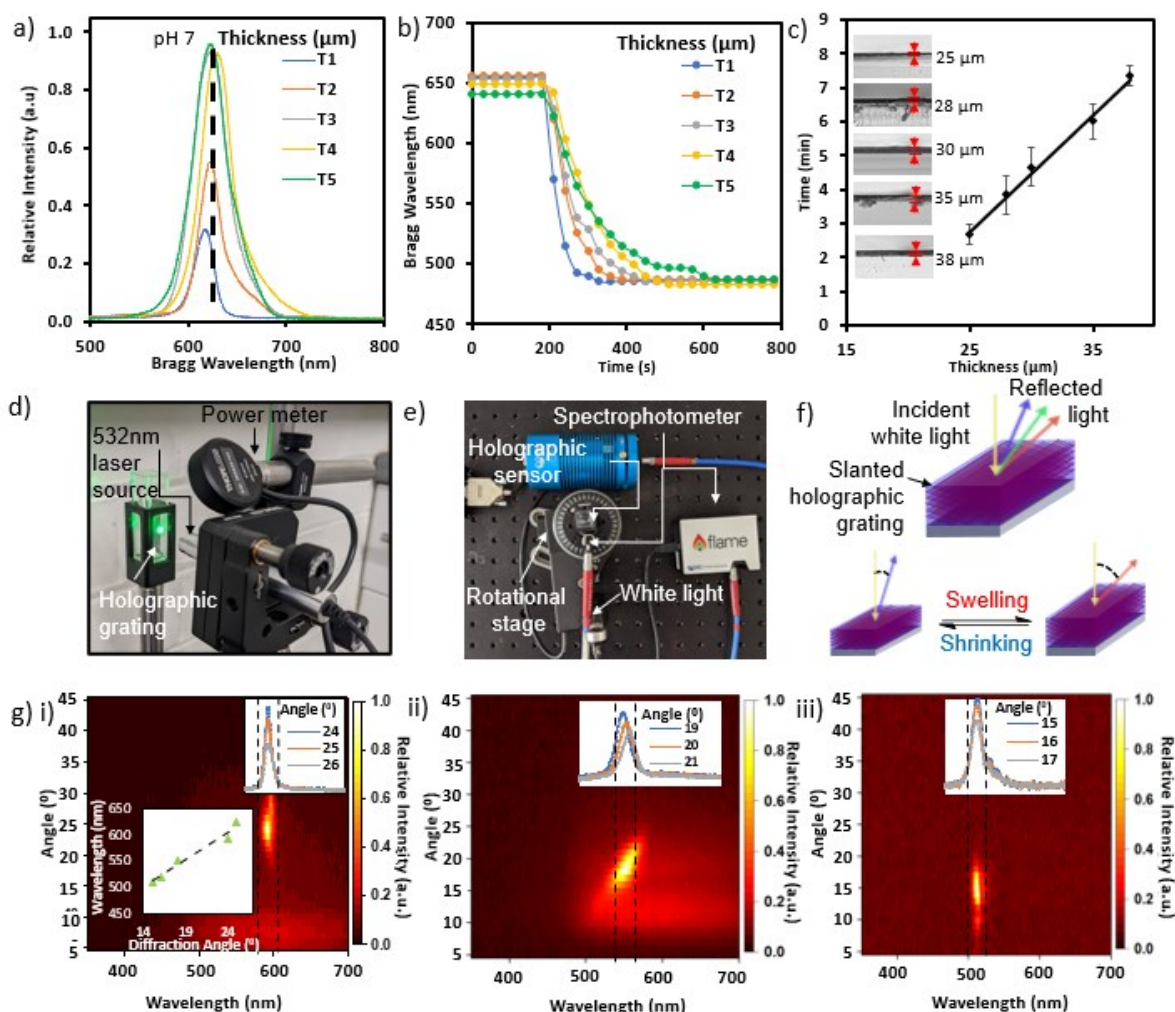


Figure 27. Thickness and angular replay dependency of the HS; a) Bragg replay wavelength as a function of thickness in tris-buffer solution (pH 7, 50 mmol L⁻¹, KCl). b) Rate of movement for the sensors with differing thickness. c) A graph to demonstrate the relationship between hydrogel thickness and response time, microscope images of holographic hydrogels with thicknesses 25, 28, 30, 35, and 38 μm , where error bars represent the calculated standard error of 3 independent sets of results. d) Diffraction efficiency measurement set up with 532 nm laser and power meter. e) Optical set up utilised to record the angle of highest intensity reflection. f) Relationship of diffraction angle to reflection wavelength recorded from HSs. g) Angular measurements from rotational stage for HSs with replay wavelengths of i) 592 nm, ii) 550 nm, and iii) 520 nm, inset highlights the diffraction at angles of greatest intensity.

Minimum sample volume was determined to be 100 μL , through the utilisation of a horizontal optical set up (Figure 28a). Droplets of buffer solutions at pH 7 and 9 were alternately placed onto an illuminated section of holographic grating and the optical response observed (Figure 28b). Sensors showed no clear error in sensitivity and maintained a total shift of 179.5 nm over three cycles. At values lower than 100 μL effective coverage of the HS was not reliably achievable.

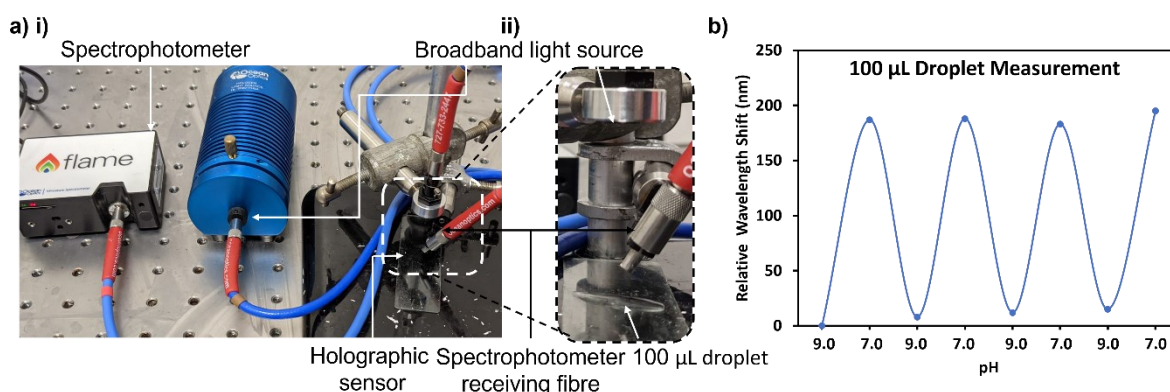


Figure 28. Minimum volume analysis utilising horizontal set up. Tris buffer solutions (100 μL , pH 7 and 9) are pipetted concurrently on to HS.

4.2.3. IL Formulation Development

Key to the production of holographic sensors is the development of the IL formulation, as this defines the novelty of the system in comparison to the AgNP systems previously described. Design of these formulations was dominated by two factors, achieving a crosslinking density variation and the solubility of the monomers contained within. Initial IL formulations were comprised of AM (42 mol%), MBA (5 mol%), ethylene glycol (22 mol%), glycerol 1,3-diglycerolate diacrylate (GDD, 30 mol%), and HMPP (1 mol%) diluted to 50% w/v water/methanol (1:1). The rationale behind this formulation was the utilisation of AM and GDD to generate a chemical variation between the two distinct polymer layers. The AM/GDD IL formulation dominated initial research in the development of DP HS, however whilst optimising the RM formulation to achieve greater shifting it was found that peak broadening

occurred producing a reflection with multiple peaks of maximum intensity and an unreliable signal (Figure 29). This was hypothesized to be linked to the difference in hydrophilicity/hydrophobicity between the IL and RM layers producing alternative rates of swelling across the substrate however this is difficult to determine experimentally. Through further trial and error utilising different ratios of crosslinker and dilution no improvement was achieved, often obtaining no visible holographic recording or an extremely broad peak. Therefore the decision was made to revert the IL crosslinker to EGDMA comparable to the RM, relying purely on crosslinking density to produce the required RI variation for holographic sensors. Screening of formulations was done to determine the required amount of crosslinking to produce a viable grating. It was found gratings in the EDGMA system were producible with IL crosslinking concentrations down to 20 mol% however the reliability of the peak observed was diminished. As the shift increased from the recording wavelength, shoulder peaks would begin to reappear producing a less reliable shift and reproducibility was severely hampered. Increasing IL crosslinking density to 84 mol%, produced highly reliable reflection signals void of produce shoulder peaks when shifting and good reproducibility between samples.

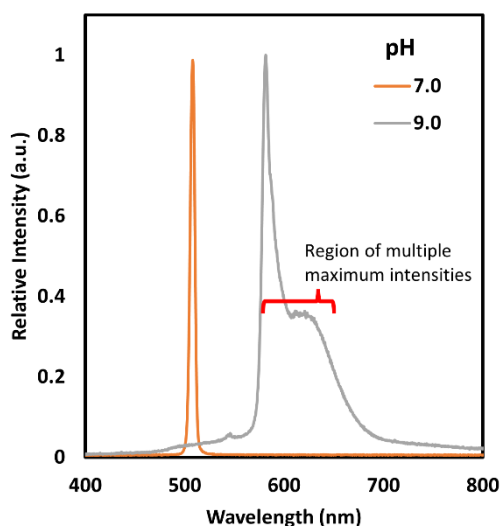


Figure 29. Demonstration of peak broadening occurring with prior formulations, as the pH was increased shoulder peaks occur with multiple points of maximum intensity.

4.2.3. DMAEA Concentration Variation

Sensitisation of developed hydrogels to pH was achieved *via* the incorporation of *co*-monomer DMAEA. Figure 30a demonstrates the change in Bragg peak wavelength with increasing DMAEA concentration over a physiological pH range (7-9). The concentration of the functional *co*-monomer impacted the swelling generated *via* changes in the electrostatic and osmotic forces with variation of bound ionic charge in the hydrogels (Figure 30a-c). Increased DMAEA concentration expanded shifting range, comparing 5 mol% vs 25 mol%, with Bragg peak shift ranges of 45.8 nm and 172.7 nm, respectively. An effective pK_a of the gratings was calculated to be 7.89, in contrast to the unpolymerized pK_a of DMAEA of 8.3, a variation of -0.41 pK_a units.⁴⁷ Various factors determine the pK_a of the polyelectrolyte, such as the hydrophobicity of the environment and the level of hydrogen bonding within the structure.⁴⁸

⁴⁹ Dual photo-polymerized holograms when compared to the AgNP systems previously demonstrated offer a slightly lower range of shifting, due to increase crosslinking density.⁴⁰ High crosslinking density within ILs effectively increases crosslinking density throughout the hydrogel, diminishing the ability to obtain large peak shifts with low concentrations of *co*-monomers (2-8 mol%). Therefore, DMAEA concentration was increased to improve the spectral range. Figure 30b-c demonstrates the replay wavelengths and total shift range of the varied DMAEA formulations. Formulations from 5 mol%-15 mol% show a linear relationship where total shift increases with increasing DMAEA concentration. DMAEA concentrations above 15 mol% produce a plateau in total shift, however the wavelength range varied although the drying time prior to exposure was maintained. Initial replay wavelength can be correlated with drying time prior to exposure, however sensors presented here demonstrate variation which can be correlated with DMAEA concentration. The maximum replay wavelength ranges of 15 mol% DMAEA is from 565 nm (pH 7) to 413 nm (pH 9), whereas 25 mol% of DMAEA shows a range from 648 nm (pH 7) to 475 nm (pH 9). Increasing the baseline shift improves

contrast between the colours observed, i.e. 15 mol% colour range is blue to green, whereas 25 mol% shifts from blue to red. Colorimetric changes are discernible through the naked eye, allowing simple identification of 0.25 pH changes. Images highlighted within the figure are cropped smartphone images through the fibre optic cable, images of the whole illuminated hologram are demonstrated in Figure 30i. The homogeneity of colour with increasing wavelength can be seen to be consistent with minimal variation over the illuminated area. Quantitative analysis allows for more accurate determination of pH. A linear trend was observed from pH 7.25 to 8.50, with a wavelength shift of 159 nm. Translating to a shift of 106 nm per pH unit, offering an ability to distinguish fluctuations of 0.009 pH units per nanometre. The optimized concentration of DMAEA was identified to 25 mol%, with a reflection peak range of 172.7 nm.

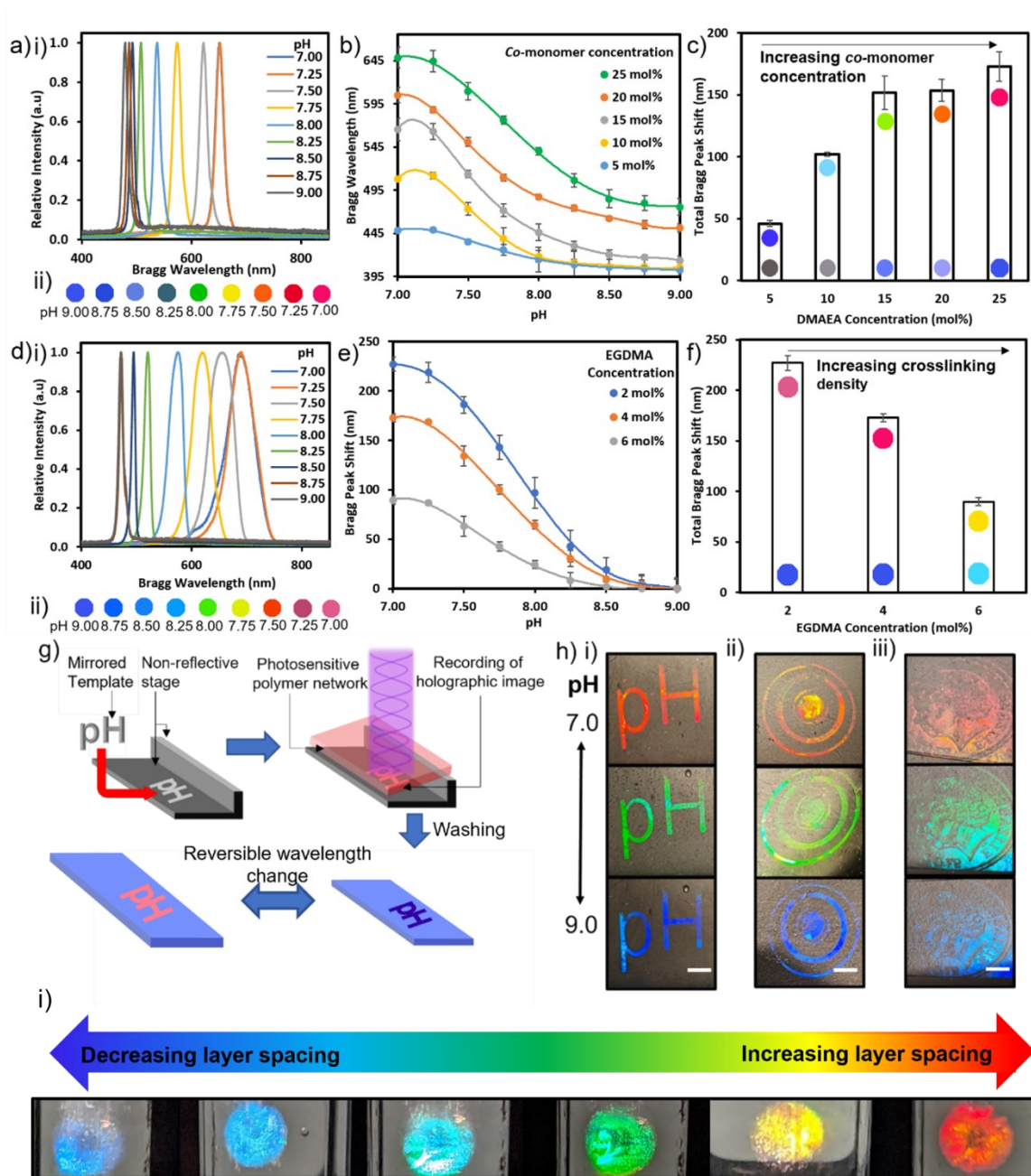


Figure 30. Effect of the monomer concentration on the hydrogel sensing. a) i) Normalised Bragg reflection wavelength from gratings containing 25 mol% of DMAEA over a pH range of 7-9, ii) cropped smartphone images of colorimetric sensor response. b) Bragg wavelength change observed for hydrogel sensors containing functional DMAEA concentrations of 5-25 mol%, where error bars represent the calculated standard error of 3 independent sets of results. c) Total change in Bragg peak that occurs with the change of pH from 7-9 along with the colour changes observed, where error bars represent the calculated standard error of 3 independent sets of results. d) Normalised Bragg reflection

peaks of a grating containing EGDMA (2 mol%) from pH 7-9, ii) cropped smartphone captured images of colorimetric sensor response. e) Plot of the relative Bragg reflection peak change for crosslinking monomer concentrations (2-6 mol%), where error bars represent the calculated standard error of 3 independent sets of results. f) the total shift observed for each grating and its corresponding cropped smartphone image of the colour change, where error bars represent the calculated standard error of 3 independent sets of results. g) Schematic illustrating the recording process of artistic holograms. h) Observable colour change of HS when changing from pH 7-9 recorded with i) 'pH' character, ii) concentric circle pattern, and iii) a coin to illustrate sensing properties (scale bar = 4 mm), images for each sensor were collected with a smartphone positioned at 20° relative to the hologram surface. i) Images to demonstrate the colour change of HSs over the whole illuminated section.

4.2.4. Cross-linker Density Dependency

As HSs swell, the fringe spacings expand, tuning the wavelength reflected.⁵⁰ Crosslinker density is directly related to the equilibrium swelling of the system, with higher crosslinking reducing the swelling capability of the sensor, and therefore diminishing sensitivity.⁵¹ Optimisation of hydrogel sensors was achieved with alteration of pre-polymer solutions where crosslinking densities were set to 2 mol%, 4 mol%, and 6 mol%. To ensure comparability, the second hydrogel formulation was kept consistent. Functional *co*-monomer concentration and dilution were kept constant at 25 mol% and 1:1 (v/v), respectively. Variation in Bragg peak shift is illustrated in Figure 27d-f. Comparing the shifting range of 2 mol% at 227 nm (Figure 30d) and 6% at 89 nm, the relationship is clearly established between crosslinker density and shifting range. Decreased crosslinker density of sensors allows for a greater expansion ranging from the high UV to the low IR (Figure 30e). At 6 mol% of EGDMA, considerable shifting of the Bragg wavelength is still observed, but response is muted in comparison. 2 mol% of crosslinker density displayed the largest Bragg peak shift, with a linear relationship from 691 nm at pH 7.25 to 471 nm at pH 8.75, translating to a sensitivity of 146 nm/pH unit (0.0068 pH

nm⁻¹) (Figure 30f). However, consistency of the wavelength observed was diminished, reducing readout reliability. As EGDMA concentration is reduced, the peak equilibration range increases. 4 mol% of EGDMA was selected as the ideal crosslinking density, offering adequate shifting range from the red region at pH 7 (648 nm) to the blue region at pH 9 (475 nm) whilst maintaining reliability. Herein, hydrogels were demonstrated to show optimum responses with whole spectrum shifting range and fast response time, when the RM was fabricated with a 25 mol% of DMAEA, 4 mol% of EDGMA crosslinker and a hydrogel thickness of 30 μ m.

Optimised hydrogel sensors were applied to holographic recordings of more complex images utilising mirrored templates structures to photopattern HS and convey further information to the user. This is achieved *via* the use of a mirrored stencil on a non-reflective background to record shapes or letters (Figure 30g). The recording of the additional information for the user can improve the clarity of results to viewers and minimises the risk of confusion when analysing *via* the spectrophotometer. Figure 30h highlights the response of artistic holograms when analyte pH is varied from 7-9. A homogenous colour change across the lettering was observed, indicating the variation of conditions within the analyte, with the lettering recorded highlighting the stimuli the sensor is responsive to pH. The recording of text opens the opportunity for POC applications, an example may be in the form of a plaster-based device that can identify the change in physiological conditions and indicate the stimuli without the requirement for any additional equipment. This vital information can better inform care providers of some of the initial warning signs to assign treatment faster and reduce the severity of the ailment.

4.2.5. Application of holographic hydrogel pH sensor

HS reliability and selectivity in the presence of common biological contaminants was determined *via* the variation of pH from 7.4-9.0 in a variety of analyte solutions (Figure 31a). Independence of the salt utilised was confirmed by maintaining ionic strength with NaCl in

replacement of KCl. A negligible variation of 10 nm in total shift was observed. To test the possible interference of physiological concentrations of carbohydrates and proteins, glucose (100 mmol L⁻¹) and albumin (3.94 g/L) were added to TRIS buffers, at pH 7.4 and 9.0. The results clearly show a similar relationship for both samples to the blank KCl buffer with minimal effect on the replay wavelength. The holographic sensing platform developed clearly exhibits selectivity to solution pH without interference by common biological contaminants, due to the absence of functional groups capable of binding or interacting with contaminant analytes. Sensor resilience against interference is linked to the composition of the polymer, both HEMA and EGDMA are unable to generate a fixed ionic charge within the hydrogel, and therefore unable to initiate the required volumetric change within the sensor. The tertiary amine group of DMAEA reduces the probability of irreversible covalent or substitution reactions occurring with contaminants within the sensor due to the strength of the C-N bond. Although theoretically the lone pair of electrons would be able to interact with electrophilic species, the affinity is expected to be below the threshold to facilitate binding and swelling. The absence of interaction can be observed in the minimal effect of changing the ionic species between K⁺ and Na⁺.

Reliability and reversibility of the holographic pH sensors are imperative in their application to continuous monitoring of metabolites. To demonstrate the high reversibility and applicability of sensors to continuous measurement, HSs were repeatedly submerged in pH 7 and 9 (TRIS, 50 mmol L⁻¹) buffer solutions 20 times to observe variation in replay wavelength (Figure 31b). Sensors exhibited minimal variation in reflection wavelength when swollen at pH 7, error range of ± 16 nm. At pH 9, an error range of ± 7 nm is observed. It is hypothesized that due to the greater level of swelling at pH 7, more variation can arise and therefore incur minor alterations in replay wavelength. Although the change is relatively small, this is a factor that requires further attention in future works. The sensors, however, clearly demonstrate a high

level of reversibility between pH measurements, allowing devices to be utilised for continuous monitoring without recalibration.

The volumetric change of HSs is correlated with the generation of ionic species within the pH sensitive layer leading to the movement of ions across the hydrogels. Ionic strength interference required testing to determine the sensor dependency.^{52, 53} Ionic strength of buffers can be affected by variations in the ionisation of functional groups and the presence of salts within solutions. In previous monomer variation experiments, to ensure pH was the stimulus of swelling behaviour, buffer concentrations remained constant and ionic strength was corrected *via* addition of a mono ionic salt (KCl). To demonstrate that the replay wavelength is independent of ionic strength, a HS was submerged in a range of test solutions where the buffer concentration is maintained at 50 mmol L⁻¹ and ionic strength is increased from 50-300 mmol L⁻¹ (Figure 31c). There was observable variation upon initial changing of solution, hypothesized to be linked to sensor equilibration from increases or decreases in ionic strength. However, it can be assumed that the influence of ionic strength over the physiological range is minimal, with a variation of 14 nm from maxima to minima.

To further demonstrate the opportunities and potential applications, the developed HSs were used to analyse the pH of bovine serum samples (Figure 31d). To adequately stabilise pH of the analytes a high molarity TRIS buffer (1.0 mol L⁻¹) was utilised over a pH range of 7-8, increasing in increments of 0.2 pH units. Calibration curves comparing biological analyte response against response observed in a TRIS buffer (50 mmol L⁻¹) (Figure 31e), insets demonstrate the reflected colour observed from a fibre optic cable, with a linear relationship for pH values from 7.2 to 8 permitting quantitative colourimetric pH identification over this range. A similar trend in results is observed with the replay wavelength plateauing below pH 7.2 and decreasing in a linear trend as pH is increased, validating the applicability of the developed devices to application in biological analytes. Although response is slightly

diminished when compared to the buffer system, it is hypothesized to be linked to the significantly higher ionic strength and high number of contaminants present within serum interfering with the hologram response minimally. To achieve a viable continuous monitoring device, replay wavelength consistency over several cycles is vital. To highlight the reproducibility of results, sensors were cycled from pH 7-8 in bovine serum to mimic a biological system (Figure 31f). The sensors deliver a viable continuous response in serum with minimal hysteresis in the response, and visually distinguishable colour changes between each incremental pH change. To demonstrate the HSs viability as a POC testing device, the HS was tested on an *ex vivo* porcine skin model (Figure 31g). The HS was placed hydrogel facing downwards onto an absorbent antireflective polyester material and TRIS buffer solutions (pH 7-8) were pipetted on to the polyester material, washing three times prior to obtaining a measurement. The antireflective coating was utilised to minimise interference from specular reflection from the skin surface. HSs have been demonstrated viability in a real point of care analysis technique comparable to the use of a plaster or sweat sensing application. The wavelength shifting observed is highlighted in Figure 28h, where no hysteresis is shown between prior experimental results and *ex vivo* monitoring.

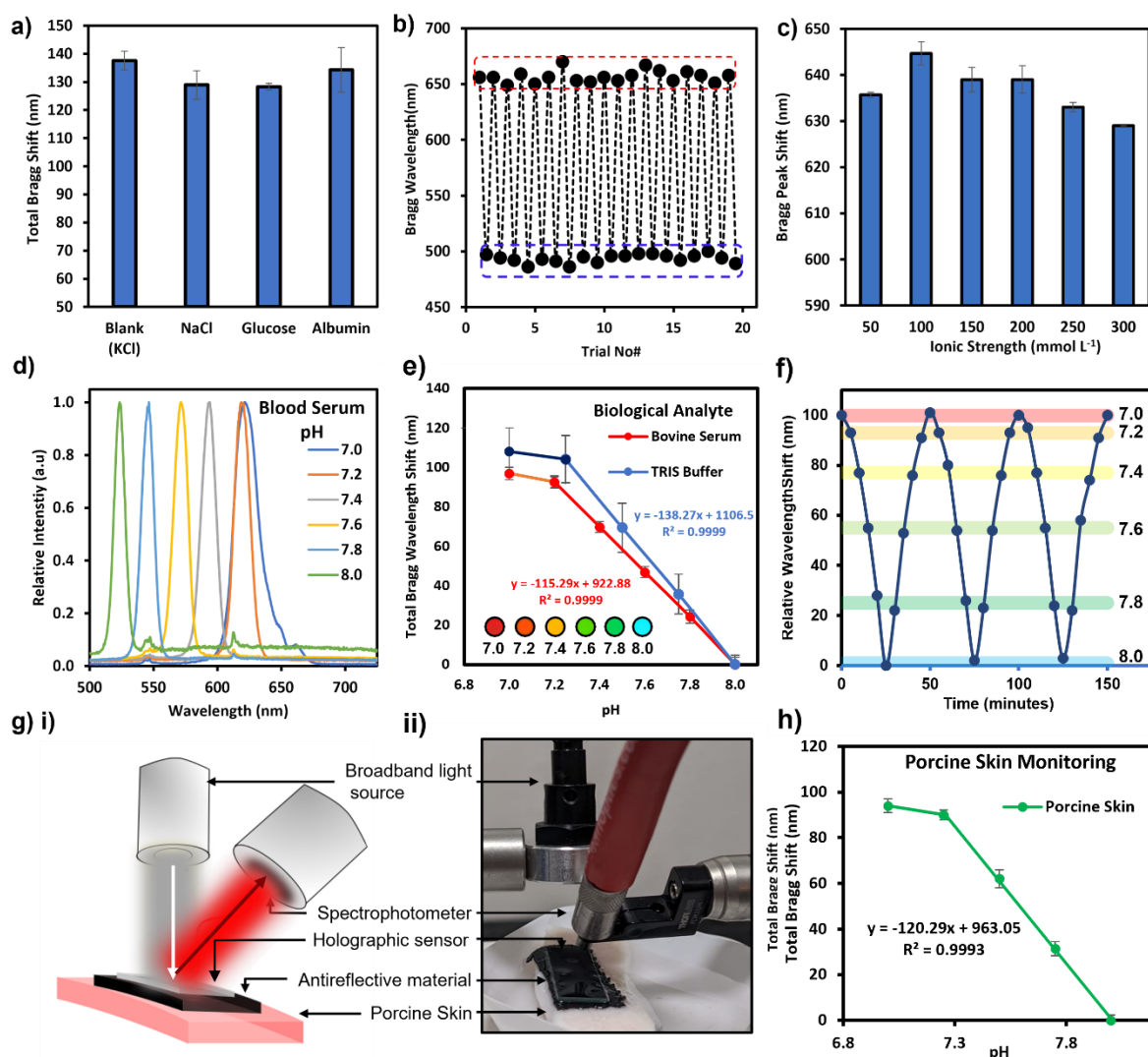


Figure 31. Recording of patterned holographic gratings and measurements to demonstrate sensor characteristics with a composition of DMAEA (25 mol%) and EGDMA (4 mol%). a) Demonstration of the total Bragg peak shift for selectivity experiments varying pH from 7.4-9.0 by changing the ionic salt and contaminating buffers with common biological substances. b) Reversibility measurement cycling to demonstrate reusability of the sensor, where red and blue dashed boxes highlight the region of variation for pH 7.0 and 9.0 respectively. c) HSs were moved between solutions of constant buffer concentration whilst ionic strength was varied from 50 mmol L⁻¹ to 300 mmol L⁻¹. d) Normalised Bragg reflection wavelength collected throughout the blood serum measurements, where error bars represent the standard error of. e) Calibration curves comparing spectral response in bovine serum vs buffer solutions over the range of pH 7-8, insets highlight the colour reflected at specified pH values in bovine serum, where error bars represent the standard error of 3 independent sets of results. f) Relative wavelengths change of HSs continuously monitoring of bovine serum pH from pH 7-8. g) Schematics

highlighting the spectrophotometer setup utilised to analyse HS response in an ex vivo setting. h) Total change in Bragg peak that occurs with the change of pH from 7-8 in ex vivo model plotted against monitoring of pH in TRIS buffer solutions, where error bars represent the calculated standard error of 3 independent sets of results.

4.3. Conclusion

A single flash UV photo-polymerized fabrication technique has been utilised to produce NP-free holographic hydrogel pH sensors. This approach removes a barrier which hindered the application of HSs in implantable devices.³⁰ DP sensors have been functionalised to show high sensitivity and selectivity in biological fluids. Variation of nanoscale fringe crosslinking density within a smart pH responsive hydrogel material facilitates the tuning of reflection wavelength. The HSs are capable of quantitatively monitoring a pH range of 7-9 via a simple spectrophotometer analysis.⁵⁴ Replay wavelength range was extended over the entire visible spectrum by increasing DMAEA concentration to 25 mol%. Qualitative measurements of 0.25 pH units are clearly discerned by the naked eye, reducing the equipment requirement. Ideal hydrogel thickness for response time and relative brightness (30 μm , 5 min) was determined. Sensitivity compares to conventional electrochemical sensors with the maximum sensitivity of 0.0068 pH nm⁻¹. Device angular dependency, selectivity, reliability over an extended usage cycle and independence from biological contaminant have been demonstrated. In future research, response time could be improved by increasing the porosity of the RM to enhance the diffusion across the hydrogel.⁵⁵ Sensor miniaturization and readout techniques through exposure optimisation should be undertaken to facilitate application into a POC device. Sensor pK_a has been tuned for the application to specific biological analytes ranging from 7-9. Variation of the functional *co*-monomer to other weakly acidic/basic monomers (methacrylic acid, 2-acrylamido-2-methyl-1-propanesulfonic acid) could tune hydrogel pK_a.

Functionalisation with *co*-monomers of different reactivities could achieve colorimetric detection of glucose, DNA or Urea and can offer real opportunity for colorimetric biosensing.⁵⁶⁻

⁵⁸ Sensors have shown as viable in biological analytes, such as bovine albumin, effectively identifying pH variation without interference.

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Chapter 5. Doubly Photopolymerised Holographic Glucose Sensors

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Diabetes has been well established as a widespread, incurable, and fatal disease with glucose monitoring and tight glycaemic control vital for effective illness prevention and management. Hydrogel-based HSs serve as a low-cost and label-free colorimetric sensing platform, which can be directly identified by the naked eye and spectroscopy for quantitative monitoring. Herein, a cost-effective and reusable holographic glucose sensor is developed *via* a single pulse UV-induced dual-photopolymerization of boronic acid functionalised hydrogels for POC diagnosis. Computational modelling of HSs response is conducted following Braggs law alongside the study of fabrication parameter optimisation and the effect on sensor swelling dynamics. The fabrication conditions, responsive and interference hydrogel compositions of the HSs are investigated to improve the response time, sensitivity in urine ($13.03 \text{ nm}/\text{mmol L}^{-1}$), the limit of detection (0.06 mmol L^{-1}), and reusability. Photolithographic patterning of hydrogel-based HSs permits the inscription of additional information into the sensors for qualitative measurement. Selectivity, reversibility, and continuous monitoring of urine samples are conducted over a range of physiological glucose concentrations ($0.0\text{-}9.4 \text{ mmol L}^{-1}$) to demonstrate the viability for indication of diabetic risk. The simple incorporation of the obtained glucose sensors in a reusable urinary analysis prototype was validated in human urine, showing the potential for POC to reduce patient dependency on invasive diabetic monitoring procedures.

5.1. Introduction

In modern life, minimal exercise and poor diet linked to sedentary work and processed food are expediting the pandemic growth of diabetes worldwide.^{1,2} It is estimated that 536.6 million people (10.5%) of the global population currently have diabetes with this figure expected to increase to 783.2 million (12.2%) by 2045.³ Healthcare providers have moved to a proactive approach in the prevention of diabetes launching the national health service (NHS) Diabetes Prevention Program to reduce extensive costs and detriment to patient health.⁴ These schemes utilise novel technologies to encourage patients at risk of diabetes to eat healthier, increase activity, and monitor for biomarkers of prediabetes.^{5,6} Diabetic patients suffer with variations in blood glucose concentration which can exceed normal levels (10 mmol L^{-1}), the kidneys are unable to reabsorb all of the glucose at these high levels which leads to excretion *via* the urine. A urinary glucose concentration greater than 0.8 mmol L^{-1} is defined as glucosuria and is a key indicator of kidney damage or type 1 and type 2 diabetes. To facilitate tight glycaemic control, patients utilise a small incision to the skin for invasive monitoring, which over time causes significant discomfort and a high risk of infection.⁷ The drive towards the non-invasive techniques explore the utilisation of readily available biofluids, such as saliva,⁸ tear fluid,⁹ sweat,¹⁰ and urine¹¹ removing the requirement for invasive procedures and reducing the burden of repetitive skin incisions. Saliva, tear fluid, and sweat contain significantly lower glucose concentrations and are prone to erroneous readings due to contamination and irritation during sampling.¹² Urinalysis provides the most easily-accessible, accurate biofluid with multiple daily reading opportunities to facilitate continuous monitoring and is regularly utilised by medical professionals to determine diabetic risk.

Commercialised glucose monitoring techniques utilise either electrochemical or colourimetric monitoring for glucose determination in urine.¹³ A range of urinary glucose monitoring platforms have been established such as electrochemical cells, fluorescent and

photonic sensors (Table 1).¹⁴ Electrochemical cells are highly valuable patient monitoring tools with rapid sensitive measurements. However, the reported electrochemical urinary glucose sensors are dominated by the use of enzymatic binding, which can be costly and prone to denaturing in harsh environments.¹³ In addition, electrochemical cells require a power source and are prone to signal drift over time, requiring patients to recharge or recalibrate their device to maintain accuracy, research is currently ongoing to mitigate these effects.¹⁵ Hence there is a desire to move towards optical systems for their power-free monitoring and minimal requirement for recalibration. Fluorescent sensors have been applied in biosensing,¹⁶ pharmacology,¹⁷ and environmental monitoring.¹⁸ However, fluorescent sensors are susceptible to photobleaching, irreversible measurements, require complex fabrication techniques, and UV excitation to stimulate emission, limiting their potential in POC devices.¹⁹ The current gold standard of urinary glucose analysis employs single-use dipsticks, which rely on enzymatic detection of glucose.²⁰ Dipsticks are single use and provide a semi-quantitative colourimetric result, comparable against a reference chart. Photonic sensors such as HSs provide a reusable platform for quantitative colourimetric analysis and are a focal point of research due to the simplicity of fabrication, readout, and removal of the requirement for complex analytical equipment.²¹

Name	Sampling Method	Development Stage	Analyte	Sensor Principle	Reference
Dexcom G6	Invasive	Commercialised	Blood	Electrochemical	22
FreeStyle Libre 3	Invasive	Commercialised	Blood	Electrochemical	23
FibreSense	Minimally Invasive	Clinical Trials	Blood	Fluorescence	24

N/A	Non-invasive	Clinical Trials	Blood	Near Infrared Spectroscopy	25
N/A	Non-invasive	<i>in vitro</i>	Aqueous/ serum solutions	Near Infrared Spectroscopy	26
N/A	Non-invasive	Proof of Concept	Urine	Photonic Crystal	27
N/A	Non-invasive	Proof of Concept	Tear fluid	Photonic Crystal	28
N/A	Non-invasive	Clinical Trials	Urine Testing	Photonic Crystal	29

Table 1: Comparison of current technologies being investigated and their research stage towards commercialisation.

HSs, comprise of a subwavelength variation in RI produces a tuneable Bragg mirror structure reflecting specific wavelengths dependent on the internal fringe spacing.³⁰ They can be developed into a reusable photonic sensing platform providing qualitative and quantitative analysis of urine glucose concentration. Due to their cost-effective, reversible, chemical functionalisation, resistance to electromagnetic interference, and real-time monitoring,³¹ HSs have been demonstrated for quantification of pressure,³² ions,³³ and drug molecules.³⁴ Conventional HSs are based on inorganic NPs are hindered from the toxicity of chemicals and tedious fabrication in POC biosensing applications.³⁵ Compared with NP based systems, DP HSs do not contain any inorganic nanoparticle components therefore present a beneficial advantage to the development of more biocompatible POC devices. NP systems rely upon continuous wave lasers were employed to record holographic gratings, requiring extended exposure times and potentially vibration diminishing equipment to ensure reliable recording.

The use of a pulsed single flash polymerisation simplifies the previously complex fabrication process into a simple 4-step procedure without the requirement for complex exposure set ups.

Herein, a single-flash UV dual photopolymerization was developed to produce a stable, NP-free, and reusable hydrogel-based holographic glucose sensor that can continuously, and quantitatively monitor glucose level in urine. Computational modelling was applied to inform the optimisation of sensor recording and provide an approximation of sensor swelling characteristics. DP HS consists of periodically alternating layers of two polymeric hydrogel materials with different RIs, named as the RM and IL. The formulations of the RM hydrogel with low RI and the IL hydrogel with high RI were optimised to improve the peak stability and device sensitivity. Experiments investigate the fabrication parameters influence sensor performance, to expand the current understanding of doubly UV photopolymerised HS characteristics. Demonstrations of sensor photopatterning with text define sensors applicability to label-free sensing. Sensor properties such as angular dependency, response time, reversibility, and sensitivity were characterised. Glucose sensors are validated for POC analysis in artificial urine (AU) over a physiological glucose range, and a simple device is demonstrated in human urine for a reusable urinalysis apparatus.

5.2. Results and Discussion

5.2.1. Fabrication of holographic glucose sensor

In this work, RM is a low crosslinking density hydrogel prepared by photo crosslinking of AM and glucose-specific 3-AAPB with 1.0 mol% MBA as a crosslinker (Figure 32a-i). The IL contains a high crosslinking density hydrogel formed by polymerization of AM and 29 mol% BAP (Figure 32a-ii). By increasing crosslinking density in IL, the doubly polymerised fringe has a higher RI than RM, leading to the production of a Bragg reflector. Herein, the crosslinker is varied between the RM (MBA) and IL (BAP) were selected to permit higher crosslinking densities in the IL monomer solution, due to the low solubility of MBA. 3-AAPB was used to

functionalise the RM hydrogel, of which the boronic acid group permits covalent binding of glucose reversibly.³⁶ Boronic acids function as a Lewis acid and have been well documented across a range of glucose sensing platforms.^{37, 38} 3-AAPB is trigonal and can react with water to form an anionic tetrahedral boronate (Figure 32b).³⁹ 1,2 or 1,3 cis-diols of carbohydrates act as Lewis bases which can bind with boronic acid to form a 5 membered cyclic boronate ester.⁴⁰ Boronic acids can bind through the trigonal and tetrahedral form, determined through several factors such as pH, temperature, and concentration.³⁹

DP HSs were generated by a 4-step fabrication process (Figure 29c). 1) A hot washed RM film was saturated with aerated IL monomer solutions and allowed to soak 5 min before 2) being dried for 10 min and 3) exposed to a coherent UV laser pulse, and finally 4) washed to remove unpolymerized material. HSs were recorded utilising a single pulse from an Nd:YAG laser (355 nm, 5 ns, 30 μ s delay). Unreacted monomers were removed, and the hydrogel thickness pre-exposure was reduced *via* drying, upon hydration sensors expand producing a replay wavelength in the visible region. Hydrogels are positioned at an angle in exposure (5°) to separate holographic signal from specular reflection interference. The pulsed laser was used in replacement of a continuous wave laser that can reduce the exposure time to nanoseconds, removing requirements for vibration-diminishing equipment. Post-exposure immediately washing removes unpolymerized material (deionized water, 25°C, >2 hrs), producing the two distinct hydrogel regions of the sensor. The developed sensors vary volumetric expansion relative to glucose concentration, due to glucose association and dissociation with 3-AAPB forming a polymerised ionic charge. The simplification of fabrication offers potential for mass production. However, the high crosslinking density in IL is anticipated to stifle the expansion (Figure 32d). The diffraction of the DP hologram corresponds to Braggs law, where d corresponds to the grating period, n is an integer, λ is the reflected wavelength and θ is the angle of diffraction from the normal (Figure 32e). The boronic

acid-binding of glucose initiates a volumetric change of the hydrogel film, expanding the d spacing of the hologram and thus changing the reflection wavelength.

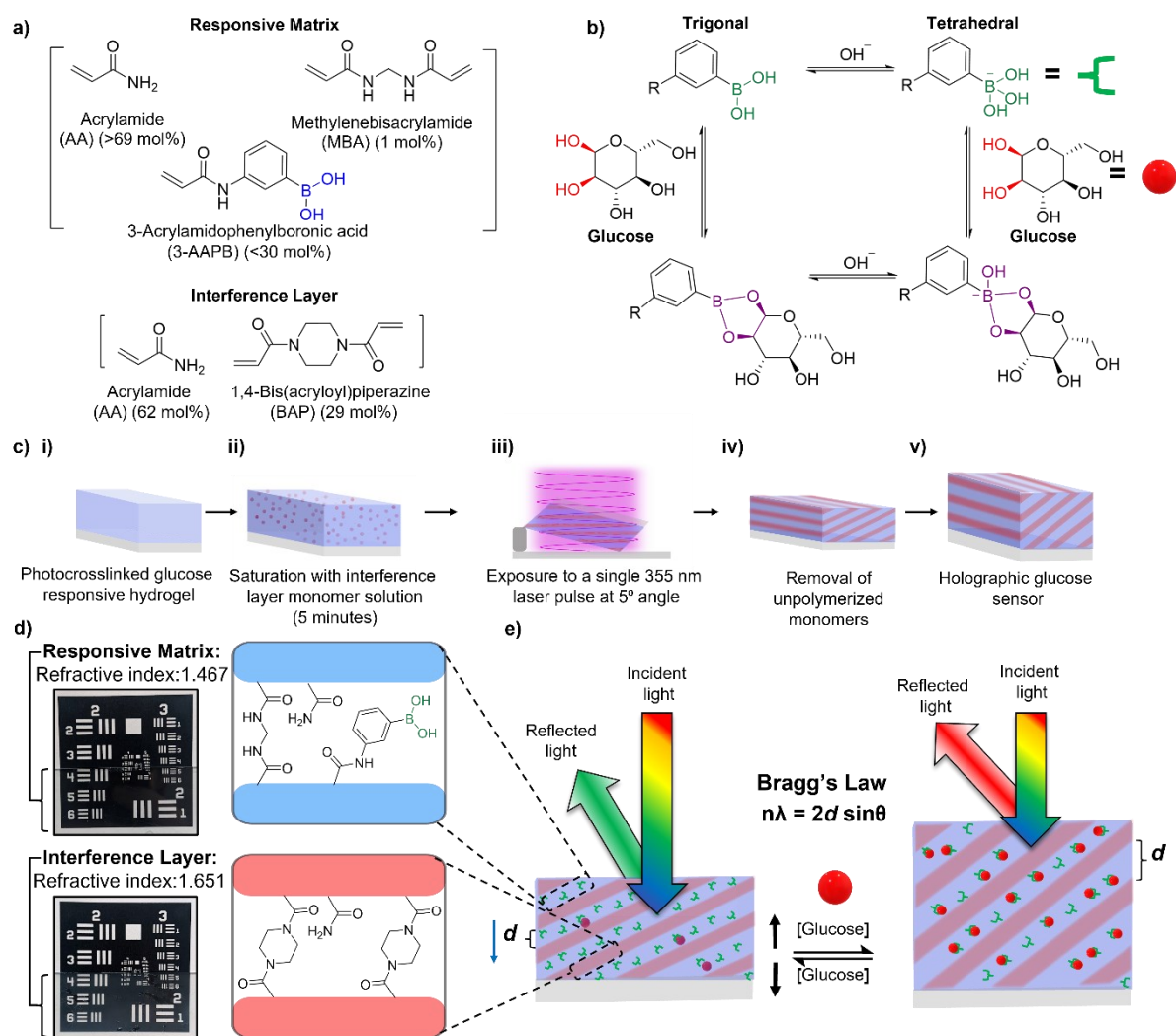


Figure 32. Schematic demonstrating the composition, fabrication, and functional mechanism of HS; a) Chemical structures of each component for the i) RM and the ii) IL; b) Reaction pathways for boronic acid binding of glucose in the trigonal and tetrahedral forms; c) Schematic highlighting 4 fabrication steps of DP HS, i) photopolymerisation of responsive hydrogel, ii) saturation of hydrogel with IL monomer solution and drying, iii) exposure to single 355 nm pulse, and iv) washing of hydrogel to remove unpolymerized material, v) completed holographic glucose sensor; d) Hydrogel compositions of RM and IL fringes and their respective RIs, inset photographs depict individual hydrogel covering a section of the USAF resolution target; e) Diagram of the working principle of HS upon exposure to glucose, expanding the internal fringe spacing d and changing the reflected wavelength by the Bragg's law.

5.2.2. Computational Modelling

Computational modelling is a vital component of HS research, permitting rationalisation, and visualisation of the fundamental mechanisms within HS. Previous techniques utilised silver NP photopatterning to produce a RI change, TEM imaging allowed visualisation of the internal swelling response of fringes.⁴¹ However, DP HS layers are difficult to be observed experimentally as RI variation is achieved by the hydrogel composition and crosslinking density variation. Computational models applied here have been designed to represent the general DP HS system, however RI variation between the IL has been elevated to correspond to expected RI variation in the AM glucose system. Herein, a study of the fabrication procedures affected on sensor response is conducted, examining the correlation between the layer number and the hydrogel thickness at the point of recording. Alongside this analysis of increasing layer number producing a reduction in sensor response is conducted to provide an approximation of the correlation between layer number and sensor performance.

The DP HSs were recorded through the constructive and destructive interference between incident and reflected beams of a coherent 355 nm laser exposure. Initial replay wavelength was determined by the constructive interference regions initiating polymerisation nodes correlating to the recoding wavelength. If the photosensitive hydrogel is swollen when exposed to the laser pulse, no expansion occurs upon hydration producing an initial replay wavelength comparable to the recording wavelength (355 nm), outside of the visible spectrum. Pre-exposure swelling was determined by IL solvents, humidity, and temperature. It is hypothesized that when the hydrogel thickness increases, more ILs are produced, therefore increasing crosslinking density across the hydrogel, and reducing the sensitivity. The developed modelling examines the variation of hydrogel thickness upon exposure, experimentally associated with pre-exposure drying. The holographic model employed was adapted from the COMSOL “single bit hologram.” The geometry demonstrated in Figure 33a-

i was designed to investigate the effect of photosensitive hydrogel thickness on the IL number utilising COMSOL. The external RI was set to 1.33, and the hydrogel matrix was set to 1.46 to correspond to RM. Counter propagating incident and reflected beams propagate from the two ports, with a physics-controlled trigonal meshing applied. The layer number was determined through the effective RI variation along the 2D cut line across the hydrogel. Thickness of layers at 6, 9, 12, 15, and 18 μm were examined with layer numbers at 51, 76, 101, 128, and 151 respectively (Figure 33b, S9), with the electric field density plots highlighted in (Figure 33c, S10).

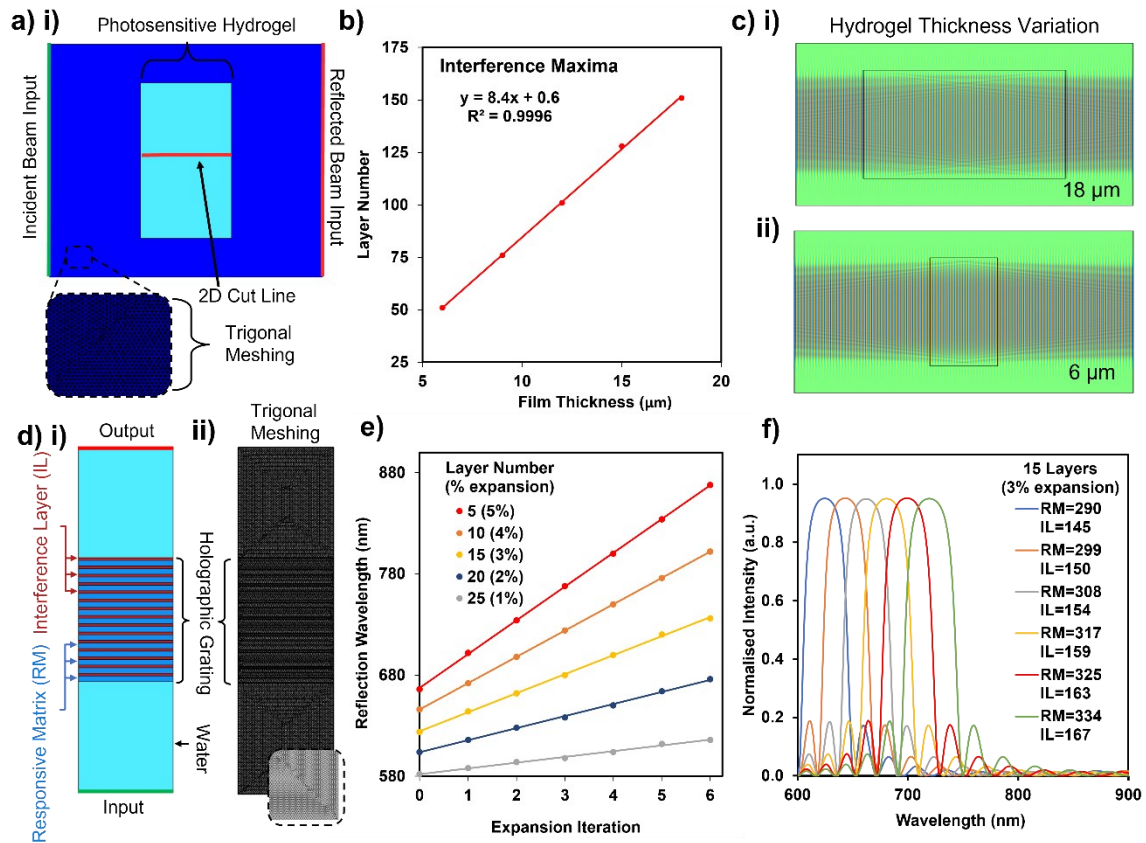


Figure 33. COMSOL modelling of HS response and recording parameter optimisation; a) i) Overview of geometry utilised to model HS recording with varying layer number; b) Graph of layer number recorded as a function of hydrogel thickness; c) Electric field intensity plots for i) 18 μm and ii) 6 μm of film thicknesses in exposure; d) Overview of the i) geometry designed to model the expansion and

response of HS, defining the specific regions such as RI and IL, and ii) demonstration of the trigonal meshing applied, with the magnified section demonstrating meshing geometry in more detail; e) Simulated reflection wavelength maxima obtained for hydrogel sensors with layer numbers of 5, 10, 15, 20, and 25 with expansion iterations of 5, 4, 3, 2, and 1% relative to the initial wavelengths; f) Simulated reflection spectra obtained from HS with 15 layers and an initial layer spacing of $RM=290$, $IL=145$.

Figure 30d depicts the COMSOL Multiphysics® computational model utilised to study fringe spacing variation corresponding to optimised sensor response.⁴² The model consists of 3 separate materials, the RM layer ($RI=1.40$), IL region ($RI=1.60$), and the exterior medium to represent the aqueous medium the hologram is situated in ($RI=1.33$). To simulate the illumination of the sensor under the broadband light, an input port is situated on the left of the model, and a receiving output port on the right. Parameter sweeps were conducted to examine the effect of layer thickness variation on the reflected wavelength. It is hypothesized that increasing the layer number recorded produces both a reduction in initial replay wavelength and diminishing sensitivity, experimentally correlated to film thickness prior to exposure. An investigation was designed to simulate this theory, whereby sensors with 5, 10, 15, 20, and 25 layers were given initial thicknesses at 310, 300, 290, 280, and 270 nm for RM, and 155, 150, 145, 140, and 135 nm for IL, respectively. Expansion iterations for both RM and IL were set to incremental increases of 5%, 4%, 3%, 2%, and 1% for both RM and IL relative to the initial thicknesses (Figure 33e, Table 2). The thickness ratio of RM:IL was set to 2:1 correlating with previously simulated DP HS modelling.⁴² The simulation conducted aims to simulate the restricted effect predicted to occur with a greater layer number. Layer numbers chosen here were to facilitate simple running of the computational model, values above 30 layers produced impractical simulation times. Simulated reflection spectra obtained permit an approximation of relative wavelength change expected with different degrees of expansion. Figure 33f shows the spectra obtained from 15 layers with a 3% expansion rate per iteration. As expected, the

lower the layer number the greater relative wavelength change observed, the simulation can provide an approximation of experimental sensor swelling from the relative wavelength shift.

	Layer	Expansion Iteration (nm)						
	Number	1	2	3	4	5	6	7
Responsive Matrix (RM)	5	310	326	341	357	372	388	403
	10	300	312	324	336	348	360	372
	15	290	299	307	316	325	334	342
	20	280	286	291	297	302	308	314
	25	270	273	275	278	281	284	286
Interference Layer (IL)	5	155	163	171	178	186	194	202
	10	150	156	162	168	174	180	186
	15	145	149	154	158	162	167	171
	20	140	143	146	148	151	154	157
	25	135	136	138	139	140	142	143

Table 2. Internal fringe spacing set for COMSOL modelling of thickness effect on sensor response.

5.2.3. Chemical optimisation of hydrogel formulations

A water bath optical setup integrated with a thermocouple as the temperature control was used for HS analysis (Figure 34a). Sensors were placed into a cuvette with a consistent analyte volume (1.5 mL) and illuminated with a broadband light source. Glucose concentration was altered *via* successive analyte removal and injection of a glucose solution (150 μ L, 20.0 mmol L^{-1}) producing glucose concentrations of 0.0, 2.0, 3.8, 5.4, 6.9, 8.2, and 9.4 mmol L^{-1} , and optical response was collected *via* fibre optics. The RM monomer solution was comprised of AM (82 mol%), 3-AAPB (15 mol%), MBA (1.0 mol%), and HMPP (1.3 mol%) with a dilution

of 0.67 g/mL (DMSO:H₂O, 1:1, v/v). IL monomer solutions were comprised of AM (61 mol%), BAP (29 mol%), and HMPP (10 mol%) with a dilution of 0.173 g mL⁻¹ (DMSO:H₂O, 1:1, v/v). A temperature range of 25–40°C was set to demonstrate sensor applicability to cover the physiological temperature. The rate of response was expedited as temperature increased from 25 to 40° C (Figure 34b). As the temperature increased, the initial replay wavelength increased marginally due to thermal expansion of the hydrogel, while the total wavelength shift remained constant in response to 2.0 mmol L⁻¹ of glucose. Figure 34c highlights wavelength changes after 20 minutes, with 40° C demonstrating the fastest response rate at 0.47 nm min⁻¹. 37°C as the physiological temperature in human body was employed for biosensors throughout the following experiments.

3-AAPB acts as a functional *co*-monomer in hydrogel materials, binding glucose to produce boronate anions, thus increasing Donnan osmotic pressure and stimulating swelling of hydrogels.⁴³ The hydrophilic/hydrophobic balance of the hydrogel can affect the swelling of HS. 3-AAPB contains a phenyl ring which increases the hydrophobicity of the hydrogel. Therefore, there is expected to be a point of optimum 3-AAPB concentration, where the number of ionisable groups and the hydrophilic/hydrophobic balance are achieved. To optimise the sensitivity to glucose, HSs were prepared by varying 3-AAPB concentrations (10, 15, 20, 25, and 30 mol%). Figure 34d-i demonstrates normalised Bragg reflection spectra for an optimised sensor with a 3-AAPB concentration at 20 mol% to physiological glucose concentrations (0.0-9.4 mmol L⁻¹). Figure 31d-ii demonstrates the optical response observed through a smartphone camera *via* the bifurcated receiving fibre to avoid interference from external light sources. The observed structural colour change improved qualitative identification of glucose concentrations. Figure 34e demonstrates the relative wavelength changes observed for each sensor as the glucose concentration varied from 0.0 to 9.4 mmol L⁻¹. The total shift was 36.0 nm for 20 mol% of 3-AAPB concentration, where the maximum

ionisable functional groups were incorporated whilst maintaining the hydrogel hydrophilicity (Figure 34f). Sensor shifting range has been significantly improved with 20 mol% sensors with a 36.0 nm wavelength shift in response to a glucose concentration of 9.4 mmol L⁻¹, demonstrating a 44% increase compared to 24.9 nm for 15 mol%.

The DP HSs are reliant on the high crosslinking density in IL to achieve the efficient RI variation in the Bragg reflector. However, due to an inability to obtain a monitorable grating with lower crosslinking densities in the IL, an investigation of the effect of IL monomer concentration was conducted to determine the correlation between IL dilution and sensitivity. The IL monomer solution contained AM (61 mol%), BAP (29 mol%), and HMPP (10 mol%). Increasing the crosslinking density in the film could reduce sensitivity, and reduction of the crosslinking density in the monomer solutions did not produce viable holograms for data collection. Therefore, IL monomer concentration (0.50, 0.60, 0.75, 1.00, and 1.50 mmol L⁻¹) was varied to observe the effect on glucose sensor performance (Figure 34g). The total wavelength shifts increase as IL concentration decreases (Figure 34h). The optimised IL concentration was determined to be 0.60 mmol L⁻¹. Total wavelength shift has been extended to 77.4 nm (± 1.5 nm) for 0.0-9.4 mmol L⁻¹, with an initial response of 23.4 nm to a 2.0 mmol L⁻¹ of glucose concentration. The sensors of 0.50 mmol L⁻¹ demonstrated a greater glucose sensitivity (98.1 nm, [glucose]: 0.0-9.4 mmol L⁻¹). However, the error margin for measurements ± 5.2 nm rendered a relative low sensor reliability. The optical response range has been extended from green (~ 558 nm) to red (~ 636 nm), permitting a more easily distinguishable response amenable to quantitative monitoring.

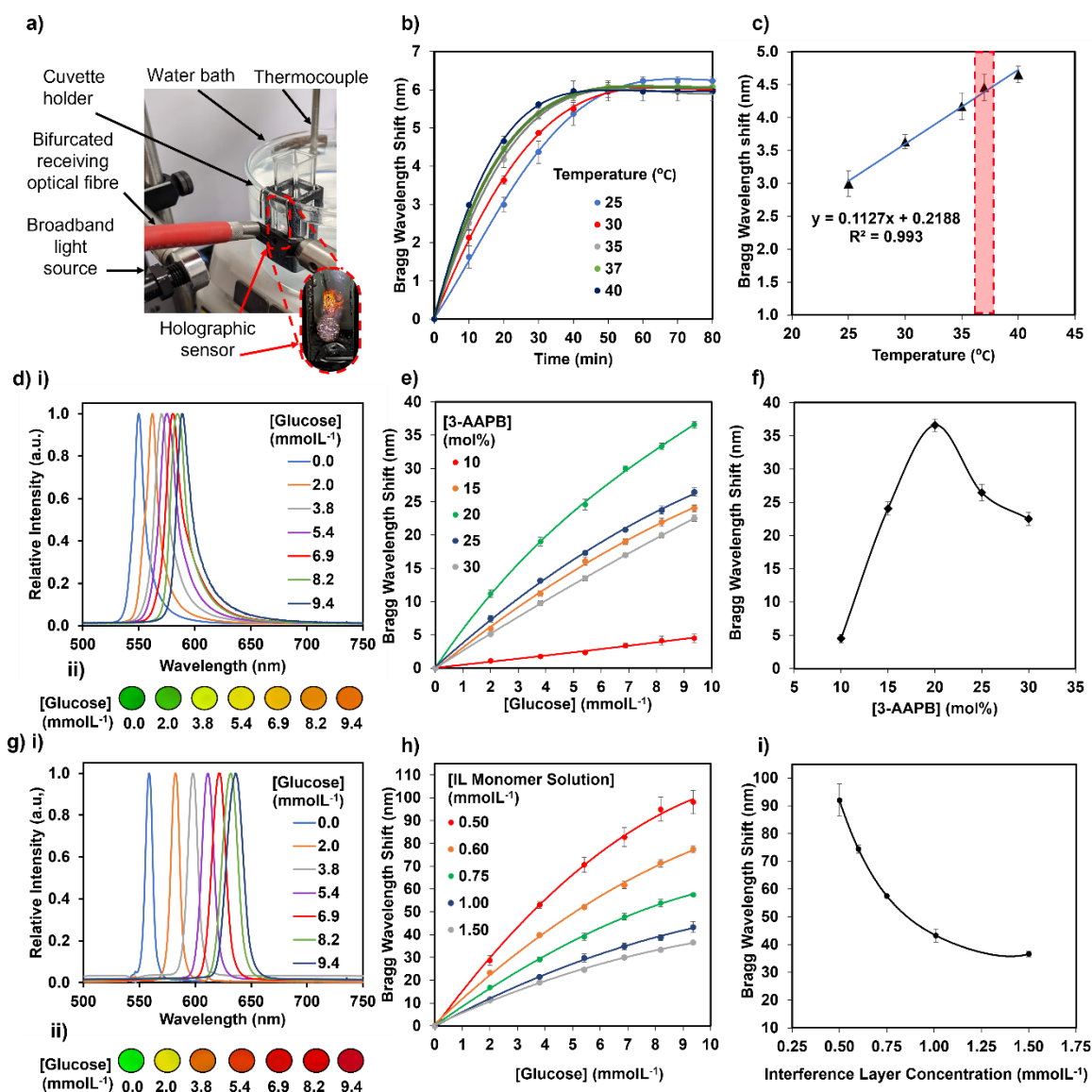


Figure 34. Optimisation of temperature response and chemical compositions of the DP holographic glucose sensors, with all error bars calculated through the standard deviation of three separate measurements; a) Photographs of the water bath-based optical set up utilised to conduct measurements at controlled temperatures, magnified image highlights the optical response of the HS; b) The effect of temperature variation on the HS response time (25-40 °C); c) Bragg wavelength shift over a 20 minute period at varying temperature (25-40 °C), with the area shaded in red demonstrating the physiological range; d) i) Bragg wavelength reflection spectra with 3-AAPB concentration (20 mol%) of RM at 37 °C with ii) the corresponding colour changes; e) The effect of 3-AAPB variations (10-30 mol%) on the response of HS to varying glucose concentrations (0.0-9.4 mmol L⁻¹); f) The total wavelength shift for 3-AAPB concentration variations (10-30 mol%) on HS responses to glucose concentrations of 9.4 mmol

L^{-1} ; g) i) Bragg wavelength reflection spectra of IL concentration optimised the HS (0.5 mmol L^{-1}) with ii) the corresponding colour changes; h) The effect on sensitivity of the HS from varying the IL monomer solution concentrations ($0.5\text{-}1.5 \text{ mmol L}^{-1}$); i) The total wavelength shift for varying IL monomer concentrations ($0.5\text{-}1.5 \text{ mmol L}^{-1}$) on the HS response to glucose concentrations of 9.4 mmol L^{-1} .

5.2.4. Physical optimisation of sensor fabrication

Film thickness has been proved to effectively alter response time and diffraction efficiency of DP HS.⁴⁴ Controlling the volume of RM monomer solution from 25, 50, 75, 100 to 125 μL effectively could vary film thickness from 6.0, 7.5, 11.0, 13.0 to 18.5 μm (Figure 36a). The optical changes of HS were examined at 1-min intervals after immersing in glucose solution (2.0 mmol L^{-1} in phosphate buffered saline (PBS)) (Figure 36b). As shown in Figure 32c, reducing film thickness showed a minor improvement in response times ($6.0 \mu\text{m} = 21.0 \text{ min}$ vs $18.5 \mu\text{m} = 25.0 \text{ min}$). The minor reduction in response time indicates that the rate-determining step is glucose binding to the boronic acid moiety. An unexpected effect of decreasing the sensor thickness was an increase in sensitivity, with films of 6 μm demonstrating a 21% increase wavelength shift (29.3 nm) compared to 18.5 μm (24.1 nm) to a 2.0 mmol L^{-1} glucose concentration. The sensitivity of the 6 μm sensor increased, with a 90 nm wavelength shift in response to a 9.4 mmol L^{-1} glucose concentration (Figure 36c-inset). Theoretically, the sensitivity is not affected by the thickness change when all variables, such as monomer solutions, drying time, and pulsed laser were maintained. It was hypothesised that the drying process of IL monomer solutions in RM was more effective in thinner films when exposing to the hot-air flow at the same time (Figure 35).

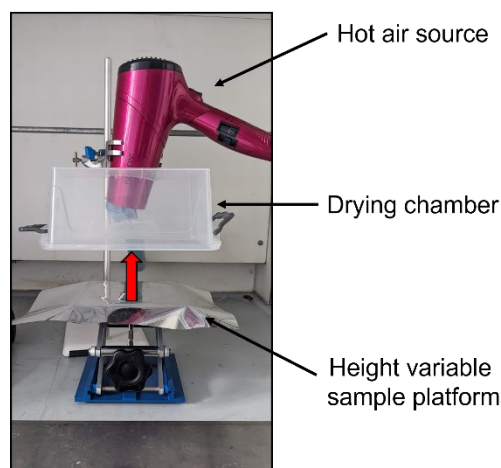


Figure 35. Hot air-drying apparatus utilised initially to dry holographic samples prior to exposure.

The IL monomers were dissolved in the mixture of H₂O and DMSO, with boiling points of 100 °C and 189 °C respectively, selected to ensure monomers solvation. To achieve improved drying control, the RM saturated in IL monomer solutions was transferred to an oven at 110 °C for the removal of water. It is well documented that water and DMSO exhibit a eutectic behaviour which reduces the melting point of mixtures,⁴⁵ however the effect on the individual boiling points of these mixtures is less well examined. Therefore it is possible that the drying behaviour is more complex than removing the water with full retention of DMSO. Sensors were prepared by producing a 6 µm thick film with the oven drying time varied from 2 to 10 min to examine the sensitivity by injection of a glucose solution at 2.0 mmol L⁻¹ (Figure 36d). As the extent of drying increased, the initial replay wavelength shifted to a longer wavelength from blue to red alongside an increase of total wavelength shift (Figure 36e/S12). Longer drying time leads to the removal of more solvent, reducing film thickness before pulsed laser exposure. Polymerisation was induced by high intensity constructive interference between recording incident and reflected beams. The spacing of these regions is independent of the film thickness, therefore the number of polymerised fringes is increased for thicker films. Consequently, the more ILs recorded within the sensor could increase overall crosslinking density. Initial replay wavelength is set by the degree of hydrogel expansion from recording thickness, with initial recorded fringe spacing considered to be $\sim \lambda/2$, as the hydrogel expands

the RM fringe spacing increases producing a red shifting of hologram reflection. Sensor initial replay wavelength can be directly correlated to sensitivity. A potential explanation could be due to the number of IL fringes recorded upon exposure, in agreement with computational modelling previously demonstrated (Figure 33a-c). This phenomenon is seldomly reported, hence the detailed investigations on the drying effect strengthen the understanding of DP HS and offer a simple way to adjust the sensor performance. An optimum 8-min drying time was selected due to the comparable sensitivity to 10 min, however improved visual differentiation between 0.0-2.0 mmol L⁻¹ permits qualitative measurement of glucose levels correlated with glycosuria (Figure 36f). Data collected experimentally here confirmed the computational modelling that by increasing the layer number recorded, an overall decrease in sensitivity was observed. Further examination of this effect should be conducted in future to determine the exact level of restriction caused by IL polymerisation.

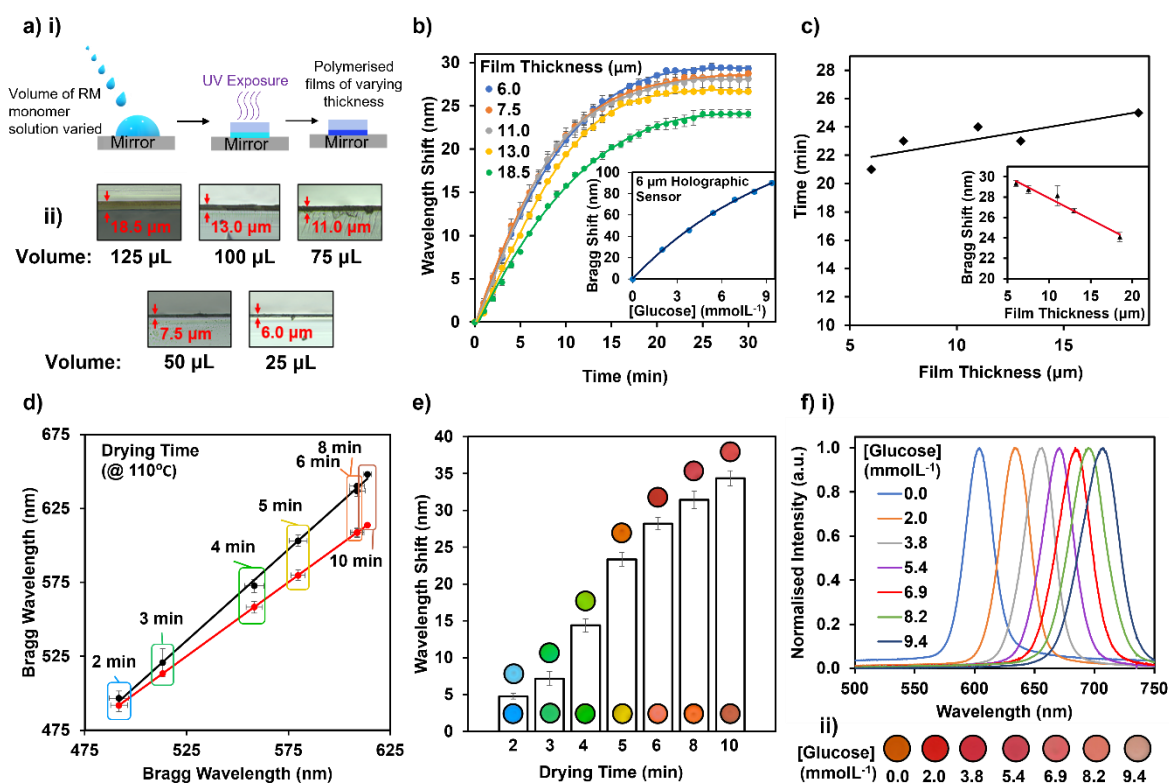


Figure 36. Physical optimisations in the fabrication of holographic glucose sensors, with all error bars calculated through the standard deviation of three separate measurements; a) Variation of film

thickness through the volume change of RM monomer solutions, i) Schematic illustration of varying thickness process, ii) optical microscope images for the measurement of hydrogel thickness; b) The effect of film thickness on HS response time and shift, inset, Bragg wavelength shift of 6.0 μm sensor to glucose variation (0.0-9.4 mmol L^{-1}); c) Comparison of response time for sensors with varying thickness, inset, comparison of wavelength shift for different film thicknesses; d) The initial Bragg wavelength change for sensors prepared with drying times of 2-10 min and the sensitivity to a 2 mmol L^{-1} of glucose; e) Comparison of relative Bragg wavelength shift observed 2 mmol L^{-1} of glucose and the corresponding colour changes; f) i) Normalised HS (8 min) in a reflection spectrum, ii) corresponding photos of HS for glucose concentration variations (0.0-9.4 mmol L^{-1}).

5.2.5. Holographic patterning and angle dependency

To facilitate simple understanding of the HS application, photopatterning of secondary information can be recorded onto the hologram such as lettering or images, with a measured diffraction efficiency of 31% (Figure 37a). Figure 37b highlights the photolithographic patterning procedure using a mirrored macroscale “Glu” characters to communicate sensor purpose to patients. Shaping the mirrored section limits the areas capable of reflecting incident laser light, and therefore the regions are capable to produce the constructive and destructive interference required for holographic recording. For sensors to be multiplexed in a device, several holograms functionalised to alternative biomarkers are required in close proximity, inscription of simple information will reduce the risk of human error. By controlling drying time, initial replay wavelength can be tuned to achieve a qualitative or quantitative sensor dependent on the specific requirements of treatment. Respective sensors were immersed in glucose free PBS solutions, after equilibration the analyte was changed to a 10 mmol L^{-1} glucose solution to observe the response. As can be seen from Figure 37c sensors demonstrate a clear colourimetric change to the presence of glucose, with the sensor dried for a shorter time (2 min) presenting a green to orange change and the sensor dried to a longer time (6 min)

demonstrating a yellow to deep red colour change. Therefore by varying the drying time the replay wavelength can be tuned, however reducing the drying time reduces the shifting range, therefore a balance needs to be made between quantitative and qualitative sensitivity of the device. As previously described, the HS was recorded at an angle relative to the laser interference planes to facilitate the separation of the Bragg reflection from specular reflection. As the sensor swells, the angle of the reflection can vary requiring a reorientation of the receiving fibre. Therefore, to examine the extent of angle change, an automated goniometer optical set-up was used to determine the angle of reflection variations (Figure 37d). Glucose concentrations were varied from 0.0 to 9.4 mmol L⁻¹, achieved via successive glucose additions, produce an overall angle change of 5° (Figure 37e/S14). All concentrations present a homogenous peak with a singular maximum and minimal hysteresis.

Minimal variation in reflection angle has permitted receiving fibres to be fixed throughout experimental monitoring. Fixation of the viewing angle validates sensors applicability to devices, where user expertise to align the collection angle is not required. The results demonstrate the ability to tune sensor characteristics, such as replay wavelength, recorded information, and collection angle to facilitate device development.

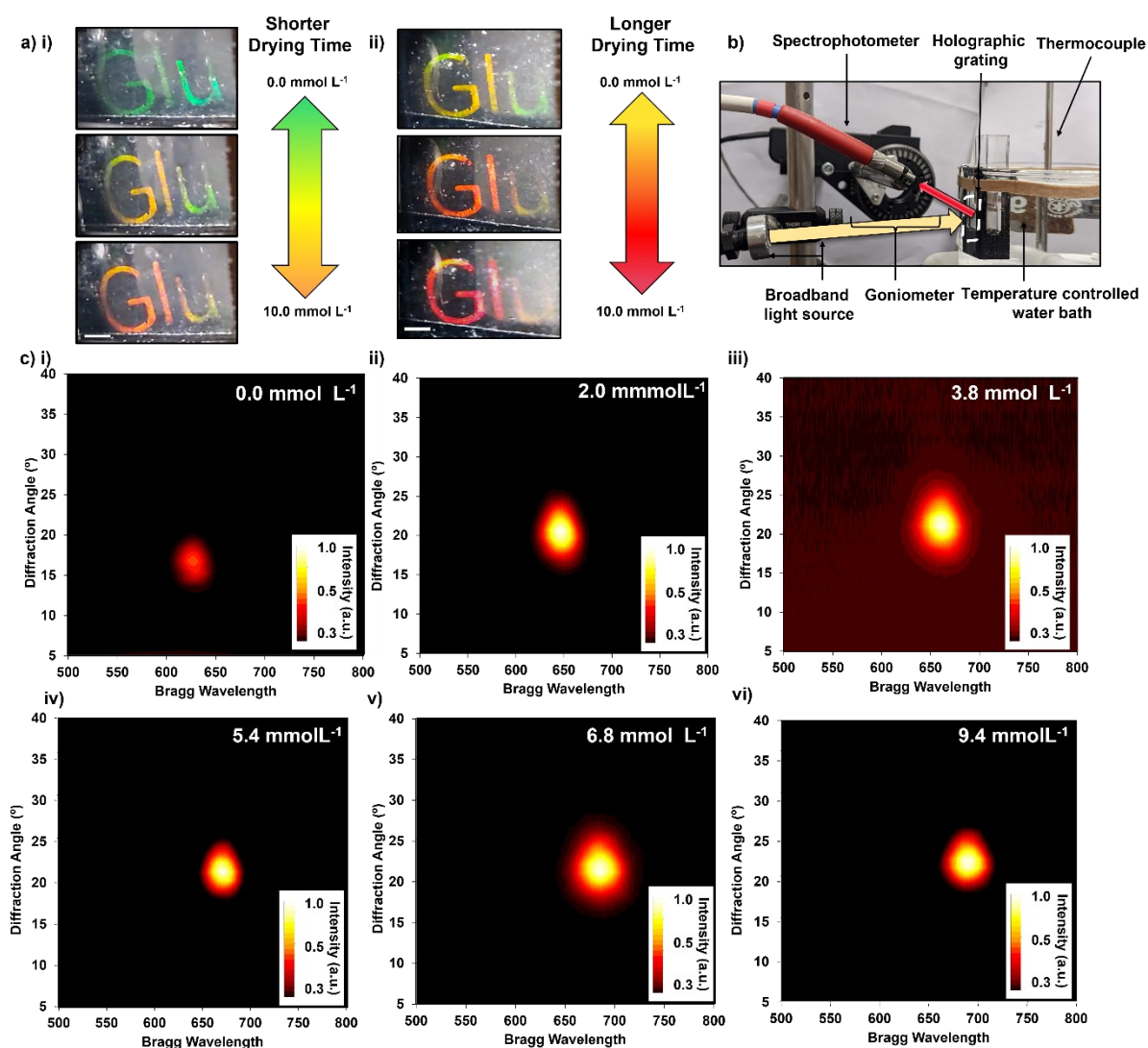


Figure 37. Characterisation of optimised sensor properties. a) Optical set up utilised in the diffraction efficiency measurements of holographic glucose sensors. b) Fabrication technique developed to inscribe HSs with lettering to reduce erroneous patient monitoring. c) Images of two HS inscribed with 'Glu' characters were prepared with i) shorter (4 min) and ii) longer (8 min) drying time (scale bar = 5 mm); d) Image displaying the vertical goniometer optical set utilised to examine HS angular dependency; e) Heat map plot of HS reflection angle dependency at previously measured glucose concentrations of i) 0.0, ii) 2.0, iii) 3.8, iv) 5.4, v) 6.8 and vi) 9.4 mmol L⁻¹.

5.2.6. Doubly photopolymerised holographic sensor urinalysis

Urine samples contain an array of compounds which can cause interference in glucose monitoring. To determine sensor selectivity to common interference compounds, glucose,

citrate, creatinine, fructose, lactate, sucrose, urea, and uric acid at physiological concentrations in PBS solutions were prepared respectively, and the pH value was adjusted to 7.6 (Table 3, Figure 38a).

Compound	Molar Mass (gmol ⁻¹)	Molarity (mmol L ⁻¹)
Uric Acid	168.11	1.49
Citrate	192.12	2.45
Creatinine	131.12	7.79
Urea	60.056	249.75
Fructose	180.16	0.04
Lactate	90.08	0.25
Sucrose	342.3	2.00
Ascorbic Acid	176.12	2.20

Table 3. Concentrations utilised in the testing of HSs reliability and selectivity against contaminant compounds.⁴⁶⁻⁴⁸

Fructose and lactate demonstrate minor shifts, 3.0 nm, and 0.9 nm respectively, while glucose shows a shift of 27.6 nm. Although fructose and lactate bare cis-diol groups capable of binding with the boronic acid groups, their low concentrations in urine produce a minor response. Sucrose is a disaccharide of glucose and fructose where the cis diols have been covalently bonded, hence the minor wavelength shift was observed (0.93 nm). A negligible shift observed for uric acid and creatinine are waste products from respiration excreted through the urine in low concentrations. Urea as a Lewis base is the most concentrated interference compound present in urine, a wavelength shift of 13.47 nm is observed. The attraction between Lewis acid and base could produce a dative covalent bond between the boronic acid and urea,⁴⁹ forming a weakly bound ionic charge within the hydrogel and therefore inducing Donnan osmotic

pressure swelling. Citrate shows a wavelength shift of 13.5 nm at 2.45 mmol L⁻¹, possibly due to the interaction between citrate and boronic acids.⁵⁰ The two carboxylic acid groups paired with the tertiary alcohol group present a potential binding site. Glucose response in the presence of each contaminant was investigated as interfering compounds could be competing with glucose to bind within the hydrogel. HSs were equilibrated in a PBS solution and secondary analyte solutions were prepared containing physiological concentrations of interference compounds and glucose (2.0 mmol L⁻¹, pH 7.6) (Figure 38b). A comparable trend of sensor sensitivity is observed with signal shift increasing by the previous interference compounds respective shifts. The developed sensors show suitable selectivity for the determination of urinary glucose concentration, and further improvements could be made *via* the use of diboronic acid *co*-monomers.⁵¹

The advantages of the HSs over other sensing platforms for urinalysis are their ability to determine glucose concentrations reversibly and accurately over an extended period. To demonstrate this reliability and reversibility, sensors were successively immersed between PBS solutions of glucose concentrations of 0.0 mmol L⁻¹ and 2.0 mmol L⁻¹ at 37°C (Figure 38c). After 15 cycles sensors showed minimal hysteresis between initial and final values. Accurate determination of concentrations greater than 2.0 mmol L⁻¹ is imperative for patient urinary monitoring. Sensor response was monitored at set intervals (2 min) to hypothetical daily urinary glucose variations (Figure 38d). The HSs demonstrate a quick response time irrespective of glucose concentration (10-12 min). The response is slower (30 min) when moving to glucose-free conditions, with minimal hysteresis observed at the baseline value ($\pm$ 2.06 nm). The reduced equilibration time is a minor issue for urinary monitoring as intervals between measurements will often exceed 30 min. This high reversibility demonstrates the applicability of sensors to POC, where devices can be reused multiple times daily by patients, reducing both waste and costs incurred through single-use techniques.

Compound	Molar Mass (gmol ⁻¹)	Molarity (mmol L ⁻¹)
Na ₂ SO ₄	142.04	11.97
C ₅ H ₄ N ₄ O ₃	168.11	1.49
Citric Acid	192.12	2.45
C ₄ H ₇ N ₃ O	131.12	7.79
CH ₄ N ₂ O	60.056	249.75
KCl	74.55	30.95
NaCl	58.44	30.05
CaCl ₂	110.98	1.663
NH ₄ Cl	53.49	23.67
K ₂ C ₂ O ₄ .H ₂ O	184.23	0.19
MgSO ₄ .7H ₂ O	246.47	4.39
NaH ₂ PO ₄ .2H ₂ O	119.98	18.67
Na ₂ H ₂ PO ₄ .H ₂ O	141.98	4.67

Table 4. Composition utilised for the preparation of artificial urine for HS testing prior to correction of pH to 7.6 with 1M HCl.⁴⁸

To establish sensor applicability to urinary glucose monitoring in patients, artificial urine (AU) was prepared according to the composition described by Sarigul *et al.* (Table 4) with pH corrected to 7.6.⁴⁸ Optimised sensors were immersed in glucose-free AU and allowed to equilibrate (1.5 mL, 1 hr). The glucose concentration was varied through successive removals of glucose-free AU (150 µL), followed by the addition of glucose-containing AU (150 µL, 20 mmol L⁻¹), maintaining analyte volume at 1.5 mL. The sensors displayed a wavelength shift of 95.4 nm for 0.0-9.4 mmol L⁻¹ of glucose, comparable to PBS testing (Figure

38e). Although the sensitivity showed no hysteresis between AU and PBS, a red shift was observable (0.0 mmol L^{-1} , AU = 628.2 nm, PBS = 601.1 nm) (Figure 38e). Figure 38f demonstrated the dynamic linear calibration curves for HS response in AU. A linear relationship is achieved between glucose concentration and wavelength change over the range of $0.0\text{-}5.4 \text{ mmol L}^{-1}$ with a sensitivity value of $13.03 \text{ nm/mmol L}^{-1}$, with sensor limit of detection determined to be 0.06 mmol L^{-1} . The red shifting visible in the colour changes (Figure 38f-inset) can be attributed to the presence of interference compounds such as citrate and urea. A simple POC device was developed, whereby a HS was fitted with a small amount of epoxy adhesive to a suitably sized urine receptacle with a colourimetric reference chart and illuminated with a broadband light source (Figure 38g i-ii). Glucose presence within urine identifies patients with diabetes or prediabetes to medical professionals. Figure 38g-iii demonstrates the devices response to glucose-free urine which was donated from a healthy non-diabetic 27-year-old male, validated utilising Roche dipstick testing. The pH value of the glucose-free urine was adjusted to 7.6 and spiked with glucose to validate sensor response to urinary glucose concentrations. Figure 38g-iv shows colour changes for healthy (glucose free), and patient at-risk urinary glucose concentrations ($0.0, 2.0, \text{ and } 5.4 \text{ mmol L}^{-1}$, 37°C) after a 15-min incubation time with sensors washed between measurements in deionised water. Glucose concentrations remain constant within urine after collection, therefore permitting temperature is maintained, response time and shifting will be consistent. Herein, temperature was maintained within a water bath to ensure a consistent response time however, in future POC devices an insulated receptacle could ensure temperature is maintained throughout the equilibration time. A qualitative red shifting of the signal is observable to the user; however, a minor interference can be observed due to the yellow colour of the sample. The human eye is less sensitive to colour variation in the red region of the visible spectrum. Quantitative analysis of the diffracted light wavelength could be achieved *via* a smartphone app with a background

subtraction to ensure reliable analysis. Here, the HSs have been proven to be viable in biological equivalent solutions and a simple device has been demonstrated in a POC application.

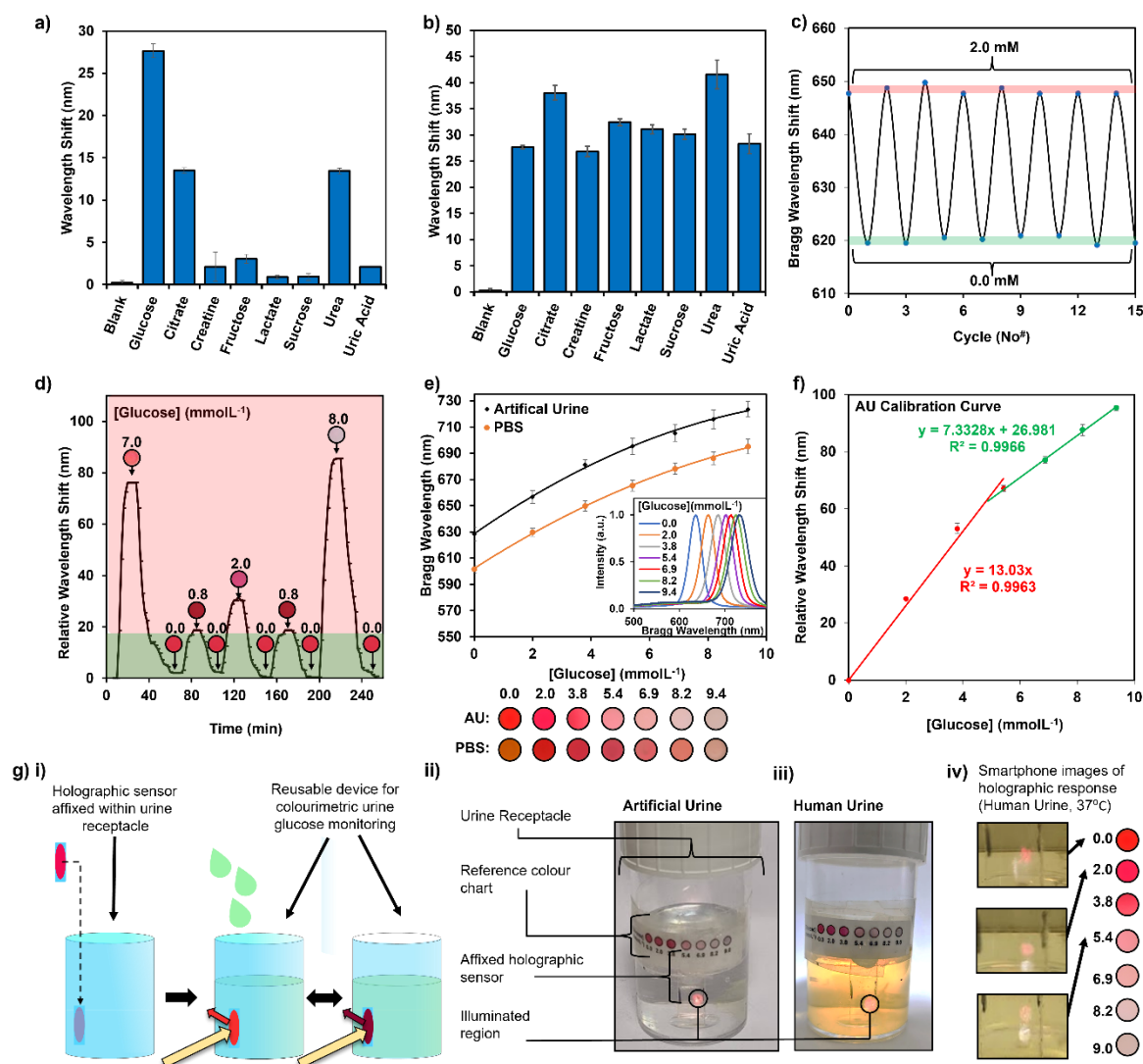


Figure 38. Establishment of optimised glucose sensor selectivity, performance in biomimetic solutions, and the construction of a urinalysis diagnostic device. Testing of the sensor wavelength shift to the addition of a) common interference compound in PBS solutions (pH 7.6) at physiological concentrations (Table 3); b) both interference compounds and 2.0 mmol L⁻¹ of glucose; c) Cycling of HS between 0.0 mmol L⁻¹ and 2.0 mmol L⁻¹ glucose concentrations for sensor reliability over 15 cycles; d) The sensor response to glucose at 2 minute intervals, green and red shaded areas highlight normal and at risk glucose concentrations respectively; e) Comparisons of the HS in response to glucose between AU and PBS, inset spectra obtained from monitoring glucose variation in AU over a range of

0.0-9.4 mmol L⁻¹; f) Biphasic calibration curves of HS response to glucose in AU over a range of 0.0-5.4 mmol L⁻¹ and 5.4-9.4 mmol L⁻¹; g) i) Schematic to demonstrate the preparation technique and analysis method for a simple HS, (20 min, 37°C). ii) Images display the HS fabricated and the components of the device, iii) Demonstration of HS performance in human urine, iv) Smartphone images of reusable device performance in human urine samples of varying glucose concentration.

5.3. Conclusion

In this chapter, comprehension of DP holographic fabrication techniques has been expanded with development of viable, colourimetric, reusable, NP-free, glucose sensitive devices with improved sensitivity and response stability. This approach reduces the cost incurred from raw materials and complex syntheses but also minimises waste produced in urinary glucose monitoring. Sensors provide patients with reliable quantification of their urinary glucose concentration and identify their risk of diabetes. Simulation of HS swelling provides an approximation of the reduction of device sensitivity with increasing hydrogel thickness when the direct experimental measurement is not applicable at the nanoscale. The developed HSs are efficient in determining glucose concentration over a physiological range (0.0-9.4 mmol L⁻¹) via both qualitative and quantitative colourimetric measurement. Optimisation of both the boronic acid functionalised RM and the high RI IL monomer solutions alongside the fabrication techniques permits a significant expansion of the DP HS for complex analytes. Optimised sensors achieve a 13.03 nm/mmol L⁻¹ sensitivity, a 397% increase compared to initially prepared HS and a limit of detection of 0.06 mmol L⁻¹. Investigation of the effect of drying time on sensor performance has expanded current understanding of the fundamental fabrication principles of the DP HS, informing future research of parameters that determine device sensitivity. Further investigations in the structure-performance relationships could fundamentally bring DP HS to the forefront as an optical sensing platform. Photolithographic patterning of text permits users to qualitatively assign sensor purpose in future multiplexed

devices minimising human error. Sensor angular dependence has been quantified with minor variations over sensor range, future works using spherical mirrors or rough surface mirrors as a recording substrate may assist in the improvement of this factor. Sensor performance has been studied in biomimetic and human urine validating sensor viability with minimal interference from common biological contaminants. Moreover, the functionalisation with more specialised boronic acid moieties could facilitate a more selective sensor for monitoring more complex biological analytes such as blood.⁵² Alongside this, the integration of a smartphone app to directly monitor the structural colourimetric changes would facilitate the POC urinary analysis.⁵³ This work demonstrates the progression of simple, adjustable and biocompatible DP HS is highly promising to produce a wide and powerful bioanalytical platform for advanced healthcare diagnostics.

5.4. References

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Chapter 6. Conclusions and Recommendations

6.1. General Conclusions

DP HS demonstrate a low-cost, reversible, colourimetric platform with clear potential in the development of POC devices. This body of work set out to expand the understanding of the DP HS system. Herein, investigations of polymer formulation and sensor fabrication parameters correlation with device performance have been exhibited. The DP HS developed are amenable to scale up with a simple and robust fabrication technique, offering potential for commercialisation of the technology. The biocompatible polymers used in tandem with the removal of NP components, previously required for HS RI variation, offer the opportunity for application in *in vivo* monitoring of biomarkers.

Computational modelling has provided an insight into the swelling dynamics of DP HS. Data presented informs readers of optimum experimental hydrogel properties and fabrication parameters, including recording wavelength, layer number/thickness, RM/IL RI, and recording thickness prior to experimental testing. Theoretically, reducing both financial and time investment required in future sensor design. Holographic pH sensors were incorporated into a device for wound pH monitoring in an *ex vivo* skin model and verified in biological solutions. The devices fabricated demonstrate utilisation of the same crosslinker in the RM and IL to produce a HS solely through crosslinking density variation. Research undertaken throughout pH sensor development facilitated the optimisation of parameters vital to sensor performance, including hydrogel thickness, functional *co*-monomer concentration, and crosslinking density. Prior DP UV HS published literature had not produced chemically functionalised sensors *via* the incorporation of a functional *co*-monomer, representing a significant advancement for the DP system. Sensors perform over the whole visible range permitting patients to colourimetrically determine variations in wound pH of 0.25 units qualitatively. Holographic

glucose sensors were developed, demonstrating an expansion of the DP HS field. Urinary glucose concentrations were determined reliably, reversibly, colourimetrically, and continuously in AU. The DP HS system was further advanced by uncovering a relationship between initial hydrogel thickness upon exposure and device sensitivity, improving sensor reliability and reproducibility. A simple POC device was proposed to demonstrate the potential of the platform in a patient setting. The research highlighted throughout this thesis has endeavoured to outline DP UV HSs and produce a platform for future developments to be taken forward from. The DP platform outlined throughout this thesis offers an opportunity in future to produce a low cost POC devices to facilitate an improvement in medical care providers approach to biomarker monitoring.

6.2. Limitations

The sensors described throughout this thesis present the first chemically functionalised UV photopolymerised HS. Although they are viable in their current state, there are some limitations which would need to be addressed. This thesis hypothesizes that the increased crosslinking density in IL fringes generates a restrictive force in hydrogel expansion. However, this is difficult to quantify experimentally. Precise determination of the IL fringe structure within a DP hologram would further expand the scientific community's comprehension of the platform, potentially achievable using polymerizable dyes. Currently DP HS have been developed for detection of alcohol, pH, and glucose. However, with advances in chemistry highlighting a range of potential hydrogels responsive to biomarkers such as potassium, sodium, drug molecules, ionic strength, and proteins, the further development of DP HS for applications in these fields is yet to be explored. For DP HS to be applied in a range of medical settings including exposure to open wounds, there is a requirement for extensive testing both *in vitro* and *in vivo* to ensure patient safety. Throughout this thesis, quantitative analysis has been achieved using a spectrophotometer. To facilitate widespread use of the DP HS platform,

utilisation of readily available technology such as a mobile phone camera needs to be developed to ensure sensors can achieve their full potential.

6.3. Future Works

DP sensors highlighted herein show a reduced sensitivity to their AgNP predecessor. The use of crosslinking density has been employed to achieve RI variation between distinct RM and IL layers, which can affect sensor performance limiting device sensitivity. Additionally, investigation into alternative techniques could present new avenues. Polymerisation of high RI polymers with low crosslinking density could achieve the required RI variation between distinct layers. Investigations have produced a framework to permit future development, nevertheless, future research will be required to facilitate the DP systems application to medical devices. Once sensors have been developed for more analytes, multiplexing HSs offer opportunity for a reusable colourimetric ‘lab-on-chip’ device for monitoring patient health simply and continuously. DP HS could be improved by expanding the potential viewing angle of the sensor using a spherical recording mirror, reducing the requirement for patients to align the receiving spectrophotometer. Although glucose sensors in the research conducted here are designed for urinary glucose monitoring, the sensing range is also applicable to blood glucose monitoring. Once a minimally invasive platform amenable to HS has been developed, the DP platform described here would be applicable as a drop-in sensing mechanism for *in vivo* monitoring. Additionally, to permit patients to quantitatively monitor some visually indistinguishable changes in holographic signal, the construction of a smartphone app is required. Several similar apps have previously been published, however adjustment to the DP system would be necessary.