Demonstration Of An Intelligent Hydrogel Based Diffraction Grating

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ABSTRACT

We report the fabrication of a diffraction grating cast into a responsive hydrogel using a silicone rubber intermediate cast from an engraved glass master grating. The aim to investigate if changes in the swelling of this gel in response to changes in the concentration of a specific analyte led to changes in the line spacing, and hence diffraction pattern, of the grating.

The protocol for casting gratings was initially developed using a composite carboxymethyl dextran/bovine serum albumin gel produced using carbodiimide chemistry to assess the optimum gel properties required. Examination under a light microscope showed that, formed under appropriate synthesis conditions, CM-dextran-BSA hydrogels retained the grating structure and appeared to have similar optical properties to the silicon rubber sub-master used for casting.

For a facile initial evaluation of the detection principle a number of similar gels were produced using cross-linked alginic acid. In this case excess carboxylic groups remaining after cross-linking were able to form additional ionic cross-links in the presence of divalent cations (Ca^{2+}). Test experiments with these gels showed that both the size and position of the reflected and refracted spots obtained from illumination with a Helium-Neon laser changed as gel swelling changed with calcium ion concentration i.e. the size of both diffraction and reflection spots increased as the alginate hydrogel shrank in response to changes in environmental Ca^{2+}.

The utility of the alginate based gels for the detection of cations, together with evidence that dextran protein gels can retain grating structures, suggest that this assay procedure should applicable to any hydrogel where the response is based on protein-ligand interactions. The key requirement is that the cross-linking interactions constraining gel swelling can be quantitatively displaced by the analyte acting as a specific competitor.

Keywords: diffraction grating, hydrogel, carboxymethyl dextran

1. INTRODUCTION

Biosensors are commonly used for analysis of biomolecular interactions and can give detailed information on binding affinity, and in many cases also on binding kinetics[1].

Biosensors generally consist of two components: a highly specific recognition element (receptor) and a transducer that converts the molecular recognition event into a quantifiable signal [2]. The receptor is always a biological element such as an enzyme, an antibody, or a nucleic acid. Signal transduction has been accomplished using a range of approaches including: electrode coupled reactions, field-effect transistors, and optical absorption, fluorescence and interferometric devices.

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In the past decade, optical biosensors that exploit surface plasmon resonance, waveguides and resonant mirrors have been used widely to analyse biomolecular interactions [3]. These sensors allow the determination of the affinity and kinetics of a wide variety of molecular interactions in real time, without the need for a molecular tag or label.

The successful commercialization of real-time optical biosensors based on surface plasmon resonance (SPR) has provided a powerful new tool for the research community and to the pharmaceutical industry in particular [4]. Commercial SPR devices detect alterations in the optical evanescent waves that result from small changes in refractive index at the interface between the sample and the device, which are caused by, for example, an antibody binding to an antigen. The advantage of these label-free biosensors is that they detect a binding event directly by monitoring the change in a physical property.

Recently, with the advent of sophisticated advanced microfabrication facilities and new material technologies, diffractive and miniaturized optical gratings have been widely used in optical systems. Production techniques, including mechanical processes, holographic interference, phase mask methods, photolithography, and electron-beam lithography, have been applied to manufacture diffraction gratings. These fabrication processes can be divided into two main steps. The first involves the fabrication of the grating structure on a photoresist thin film. The second step involves the transfer of the grating surface relief structure to the substrate by etching. Two-step patterning and transferring processes are routine procedures in the conventional microfabrication on photoresist-based materials. They are typically complicated in the etching process and this is especially true for grey-level surface relief structures.

Until recently, the use of intelligent hydrogels as sensor materials has been limited by the lack of accurate techniques to gauge their volume change in response to an external stimulus. A number of workers have developed methodologies to solve this problem. For example, , hydrogels have been fabricated with encapsulated colloidal crystals within the gel or by engineering surface patterns on the gel. The optical properties of these hydrogels have been shown to respond directly to small environmental stimuli [5].

The Asher group in Pittsburg University (U.S.A.) fabricated a novel sensing hydrogel material that reports on analyte concentrations via diffraction of visible light from a polymerized crystalline colloidal array (PCCA). The PCCA is a mesoscopically periodic crystalline colloidal array (CCA) of spherical polystyrene colloids polymerized within a thin, 'intelligent' polymer hydrogel film. CCAs are brightly colored, and they efficiently diffract visible light meeting the Bragg condition. The intelligent hydrogel incorporates chemical molecular recognition agents that cause the gel to swell in response to the concentration of particular analytes; the gel volume is a function of the analyte concentration. The color diffracted from the hydrogel film is, thus, a function of analyte concentration: the swelling of the gel changes the periodicity of the CCA, which results in a shift in the diffracted wavelength [6-7].

Zhibing Hu in University of North Texas (U.S.A) used sputter deposition to imprint the surface of a hydrogel with a square array of gold thin films. The periodicity of the array can be continuously varied as a function of temperature or electric field which alter the gel's volume, and so such an array might serve as an optical grating for sensor applications [8-9].

Everhart patented a optical diffracting sensing device, which comprises one or more hydrogels coated onto patterned self-assembling monolayers onto some substrates and the diffraction pattern can change upon exposure to external stimuli [10].This study demonstrates potential applications of modified gels in display and biosensor technology. However the fabrication procedures reported are significantly more complex than those needed to produce an intelligent hydrogel with an array structure cast directly in its surface. By producing gels incorporating reversible affinity or ionic cross-links based on coupled ligands and receptors which are displaced in response to an external stimulus it is possible to make materials that undergo swelling in response to a specific chemical trigger.

We have developed methods to fabricate directly intelligent hydrogels with a surface diffraction grating (IHDG). IHDG's swell or shrink in response to the concentration of specific analytes as internal cross-links are displaced. As the gel volume is a function of the analyte concentration, the diffraction and reflection of an incident light beam change as a function of analyte concentration. The swelling of the IHDG changes the periodicity of IHDG surface, which results in a change in the diffraction grating distance. Thus, the brightness or irradiance or position of a diffraction or reflection spot is related to the statistical average physical shape, size, and refractive index of the gel and the wavelength of the laser.

Previously we reported on the synthesis of differently functioning dextran hydrogels, which demonstrated that the release of proteins (insulin, lysozyme, or BSA) from these hydrogels could be regulated by varying D-glucose concentration, NAD concentration or pH [11-13]. This paper investigates the structural properties of these materials with a view to developing novel biosensor applications. Specifically the development of diffraction gratings based on responsive hydrogels which change their diffractive properties in response to specific changes in environmental conditions.

2. MATERIALS AND METHOD

2.1 Materials

Dextran and bovine serum albumin (BSA) were obtained from Sigma-Aldrich, UK. All other chemicals were of reagent grade and obtained from Lancaster Synthesis Ltd., UK. Precursors used in the formation of siloxane polymers were: ESSIL 291 resin (RE Z00728, Axson), ESSIL 291 catalyser (REF Z000729, Axson)

2.2 Methods

2.2.1 Submaster preparation of silicone rubber

The diffraction gratings are made using a soft lithography approach. Gratings are first cast in a siloxane polymer from an original etched master. This soft secondary master is used as a template for the formation of a hydrogel grating. 100 g ESSIL 291 resin was weighed and mixed with 10 g catalyser using a stirrer. The mixture was degassed using a vacuum pump for 20 minutes. The mixture was then poured onto glass or metal with diffraction grating on its surface and degassed for a further 25 minutes. The mixture was kept in an oven at 40 ^{o}C until set.

2.2.2 Fabrication of intelligent hydrogel with diffraction grating (IHDG)

Hydrogels in this study were prepared from polysaccharide with a natural or a modified carboxyl acid group and then intercrosslinked using carbodiimide chemistry as described in previous report [13]. Polysaccharide with carboxyl acid or modified carboxymethyl groups (for example alginate or CM-dextran - the preparation method of carboxymethyl (CM)-dextran can be found in a previous report [14].) can be inter-crosslinked by exposure to EDC/NHS.

Briefly, 0.5 g CM-dextran was weighed and dissolved using 2.5 ml distilled water while stirring thoroughly. 160 mg EDC and 25 mg NHS were weighed and dissolved in 1 ml distilled water. If no bubbles were found in the CM-dextran solution, 1 ml of the EDC/NHS mixture was added and stirred for 30 minutes. And then 50 mg BSA dissolved in 1ml distilled water was added into the mixture with stirring for another 5 minutes. The solution could be cast onto the silicon rubber diffraction grating master until gelation.

In the case of alginate hydrogel, 5-g 4% alginate solution (W%W) was inter-cross-linked using 2ml EDC/NHS(140mg/20mg). This solution waspoured onto the diffraction grating master and allowed to set.

Polysaccharide polymer is mixed with cross-linking reagents and poured onto the siloxane master or directly onto the original metal master. The polymer is then allowed to react until a stable hydrogel is formed. Once gelation has occurred the gel and master are left to air-dry whereupon a slight residual shrinkage of the hydrogel allows separation of it from the master with minimal surface damage. To aid visualization of the pattern formed the IHDG was coloured using Cibacron Blue before using.

2.2.3 Sensor testing

The test flow cell layout is shown in Figure 1. The IHDG is mounted over a flow channel and covered by a glass slide to keep constant humidity above the grating during the experiment. The arrows denote the direction of fluid flow. A Helium-Neon laser is directed at the surface of the IHGD and a digital camcorder employed to record the effect of

grating changes on the reflection and diffraction spots on a detection screen. The bulk solution containing analyte was pumped through the flow channel from the solution reservoir using a peristaltic pump.

2.2.4 The optical theory of IHDG

Refraction theory is summarised in Figures 2 and 3. When monochromatic light is incident on a grating surface, it is diffracted in discrete directions. Each grating groove can be pictured as a very small, slit-shaped source of diffracted light. The light diffracted by each groove combines to form a diffracted wave-front. The usefulness of a grating depends on the fact that there exists a unique set of discrete angles along which, for a given spacing (λ) between grooves, the diffracted light from each facet is in phase with the light diffracted from any other facet, so they combine constructively.

Diffraction by a grating can be visualized from the geometry in Figure 2, which shows a light ray of wavelength l incident at an angle a and diffracted by a grating (of groove spacing λ) along angles β_m. These angles are measured from a line normal to the grating (shown as the dashed line perpendicular to the grating surface at its centre). When a laser is used to illuminate the hydrogel diffraction grating with reflecting (or transmitting) elements separated by a distance comparable to the wavelength of light under study[15], the diffraction effect results from an electromagnetic wave incident on a grating, having its field amplitude, or phase, or both, modified in a predictable manner after diffraction[16]. When the groove distance λ between grating lines changes, the diffraction effect also changes. In the case of IHDG, this change will be observed by the size and shape of the diffraction or reflection spot which will increase when the grating area shrinks in response to an environmental trigger as seen in Figure 3. This effect is reversible and consistent with swelling changes in the hydrogel.

2.2.5 Morphology of the hydrogel

The morphology of the IHDG was examined using a Jeol 6310 SEM equipped with a cryo-stage and energy-dispersive X-ray (EDX). A sample of the hydrogel was clamped between two pieces of metal sheet and rapidly frozen in liquid nitrogen. It was then introduced into the SEM-chamber pre-cooled to a temperature of ca. –160 ^{o}C. The stage was then heated to a temperature of ca-80 ^{o}C to sublimate the surface water. After cooling to –160 ^{o}C, the sample was gold sputtered for 3 minutes. The sample surface with the diffraction grating was scanned.

In the case of the alginate hydrogel, one piece of hydrogel was split to two halves; one was kept in Tris buffer of 20 mM, another in Tris buffer of 20 mM with 0.1 M $CaCl_2$ solution to cause it to shrink before SEM detection.

3. RESULTS

3.1 Images from of intelligent hydrogel with a diffraction grating

IHDG fabrication was based on a microcontact printing technique for forming patterns on the hydrogel's surface with micron and submicron lateral dimensions. This method can offer experimental simplicity and flexibility in forming certain types of patterns from a master ruled grating. As showed in Figure 4 the pattern image of IHDG (Figure 4B) was apparently replicated from the metal (Figure 4A).

3.2 Morphology of the IHDG

Figure 5 show the surface array of a swollen hydrogel surface with diffraction grating imaged usingan optical microscope(Nikon Eclipse E400). The hydrogel diffraction grating was replicated from metal master with a specific pattern as seen in Figure 5B. It is noticeable from these images that the hydrogel holds the groove profiles and replicated them from the master seen in Figure 6. More importantly, the SEM structure of the hydrogel layer with a diffraction grating demonstrates the periodic array in Figure 6B, rather then a random distribution like a normal hydrogel cross section structure seed in Figure 6C. Obviously, this array can change with the whole structure of the hydrogel in response to an external stimulus. Although the surface arrays discussed here have an organized structure, many other

structural arrays can be created by using different masters. These include strip, rectangular, hexagonal, and even non-periodic surface arrays. The periodicity of the surface array is clearly demonstrated by both optical and diffraction observations.

The specific structural changes generated by an applied analyte will lead to changes in the diffraction grating, which will be observable from changes in the diffraction pattern obtained when a laser is diffracted from the gel's surface. Here, an alginate hydrogel with simple pattern was fabricated to make an IHDG. As Ca^{2+} ions cause the formation of additional ionic cross-links, thus it was anticipated that this hydrogel would shrink in response to Ca^{2+} ions.

3.3 Diffraction gratings testing

Diffraction experiments were performed by placing the hydrogel samples in the test cell shown in Figure 1. The changes of diffraction or the reflection spots on the screen was recorded using a digital camera when the IHDG surface was illumined the by He-Ne laser. The response of the diffraction grating master is shown in s in Figure 7A. The response of the hydrogel grating is shown in Figure 7B and it is apparent that the diffraction and reflection spots are less well defined indicating some loss of resolution during the casting step. To investigate changes in the optical properties of the IHDG in response to swelling changes it was mounted in the detector cell, and 0.1 M $CaCl_2$ solution was pumped into the flow channel; the alginate hydrogel contracted as the calcium ions diffused into the gel as can be seen from the chane in the size of the reflection spot (Figure 8A). The shape of the reflection spot was recorded against time during the diffusion of Ca^{2+} into hydrogel,the spot area appears to dramatically enlarge after 30 minutes, however, the brightness of the spot declined. To track the relationship between reflection shapes with time, the relative area ratio (*RA*) was calculated based on the area of these shapes. The absolute area of these shapes was determined by overlaying the images on a reference grid. The RA value was calculated using the relationship $RA = \frac{A_t - A_0}{A_0}$, where A_t is the spot area at time t and A_0 is the area at time. The curve in Figure 8B demonstrated that, as expected from diffusion limitations, RA shows an initially rapid increase and then tails off as equilibrium between internal and external calcium ion concentration is approached.

4. DISCUSSION

This study was carried out as a proof of concept for the analytical potential of an intelligent hydrogel with a diffraction grating. The groove pattern of a silicone rubber grating is easily replicated from a submaster onto the hydrogel. Because of the silicone rubbers' hydrophobic properties, the hydrophilic hydrogel can be easily separated from the submaster with minimal damage. The response of hydrogel with specifically patterned diffraction gratings can be observed from color variations using incident white light as the structure of grating changes in response to specific environmental stimuli such as temperature, pH, or specific analyte

Compared with alternative approaches the method of hydrogel fabrication used here offers a simplified manufacturing procedure that allows the diffraction grating to be directly replicated from a suitable submaster grating. The carboxyl groups in carboxymethyl-tailored or natural carboxyl acid polysaccharide are modified so as to give reactive ester functions by treatment with EDC/NHS. This allows the facile coupling of ligands and receptors containing amine groups including proteins and peptides.

By coupling a receptor protein and its complimentary ligand, affinity cross-links can be introduced into the gel such that swelling results from their competitive displacement by an externally applied analyte. By embossing a grating pattern on gel sheets containing regions of different functionality it should be possible to produce a micro-array capable of detecting a range of analytes.. This meets the two crucial requirements of an analytical micro array: (1) coupling chemistry which allows immobilization of proteins while retaining biological activity (2) providing a practical means of measuring the response to changes in analyte concentration with a suitable sensitivity and of operation[17] .

5. CONCLUSION

A novel material with an embossed diffraction grating has been fabricated. The hydrogel optical characteristics have been evaluated using a monochromatic laser. The potential application of this functional hydrogel for a range of analytical applications has been demonstrated.

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Figure Captions

Figure 1. Diffraction grating test cell showing diffraction gel, analyte flow path and positions of laser and detector. .

Figure 2: Diffraction by a plane reflection grating: the incident and diffracted rays lie on the same side of the grating. A beam of monochromatic light of wavelength l is incident on a grating and diffracted along several discrete paths. The triangular grooves come out of the page; the rays lie in the plane of the page. The sign convention for the angles α and β is shown by the + and – signs on either side of the grating normal.

Figure 3. Effect of changes in gel swelling on the refraction properties of a hydrogel with diffraction grating (IHDG).

Figure 4. Comparison of the refraction properties of a master (A- Metal,) and BSA-CM-dextran hydrogel with the same different grating patter when exposed to incident white light.

Figure 5. Comparison of the surface pattern of metal master (A) and hydrogel (B) with the same diffraction grating patter visualised by electron microscopy.

Figure 6. Comparison of the structure of metal (A) , diffraction grating alginate hydrogel (B) with the same diffraction grating pattern and hydrogel cross section (C) using SEM.

Figure 7. Comparison of the refraction properties obtained from the rubber master and a hydrogel cast from it.

Figure 8.

(A) The reflection spot area of an alginate hydrogel with diffraction grating as it changes time when exposed to 0.1 M $CaCl_2$ solution.

(B) The relative area ratio of the reflection spot as a function of time.

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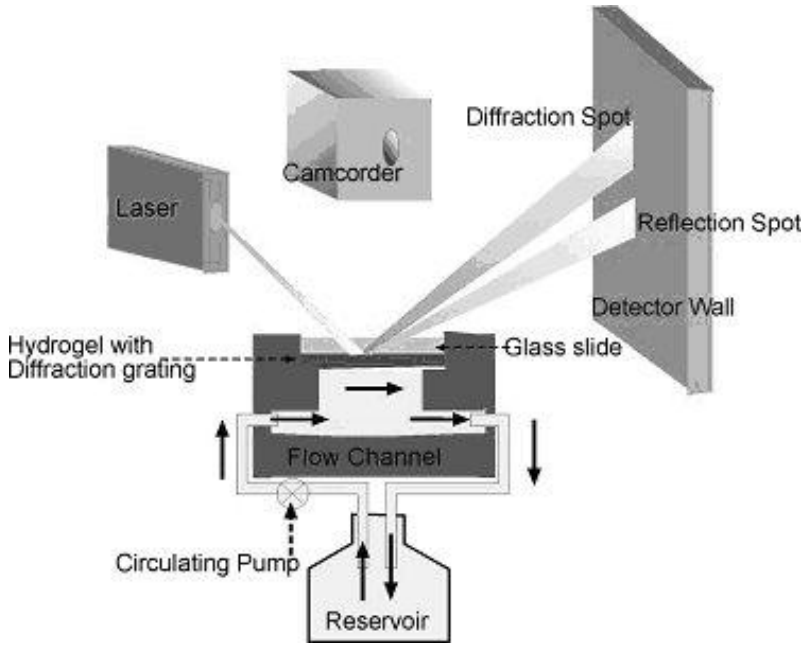
Figure 1



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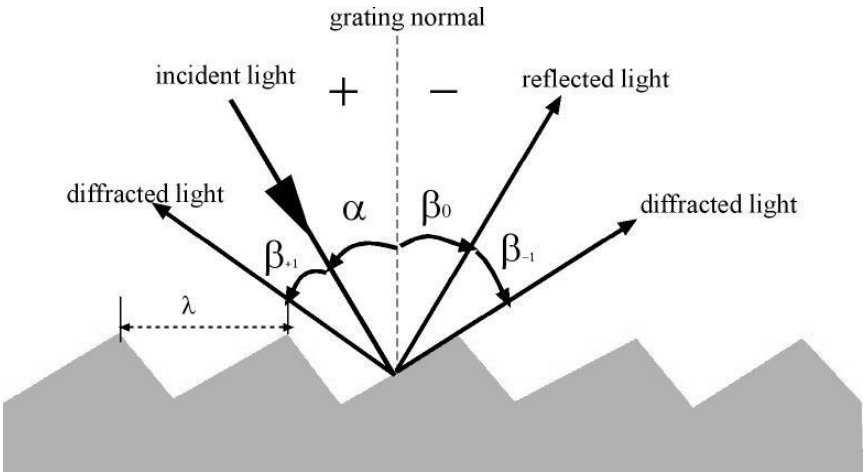

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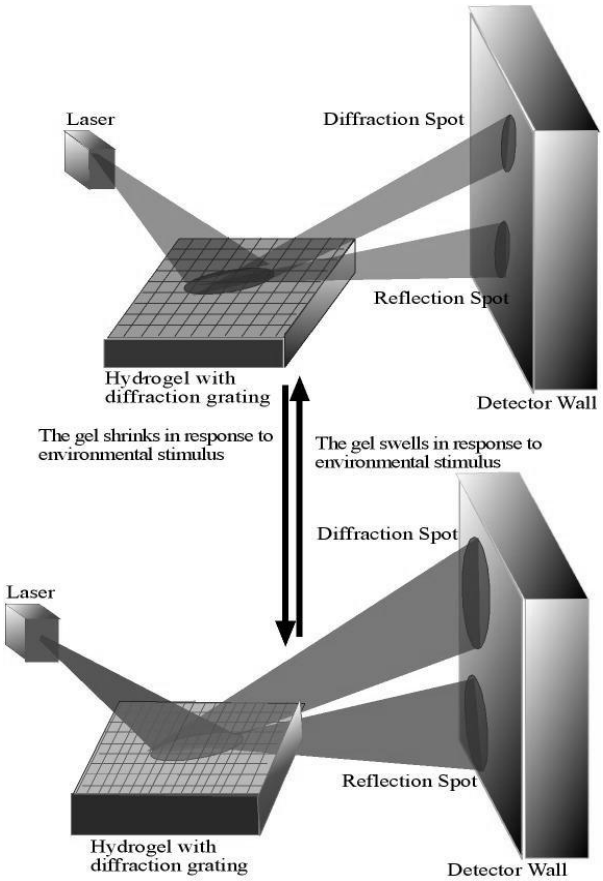

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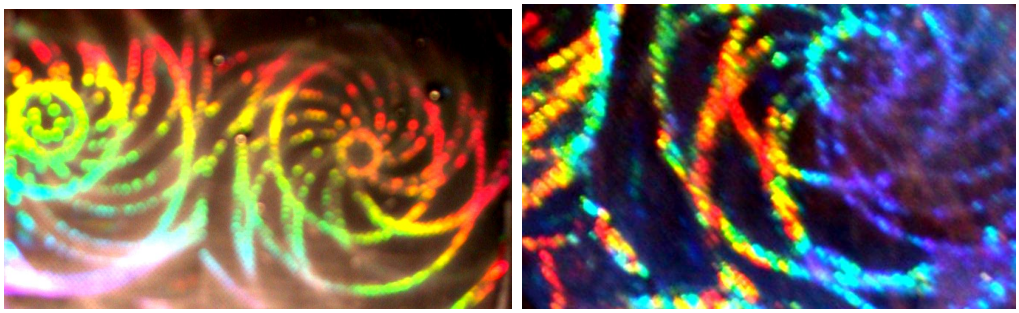

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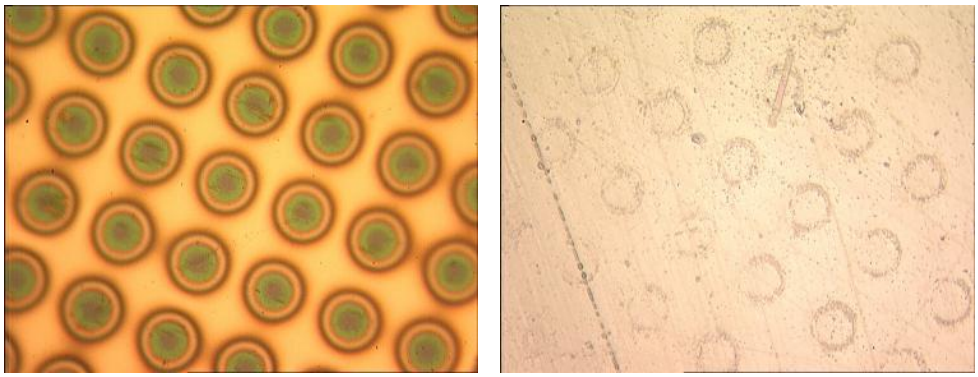

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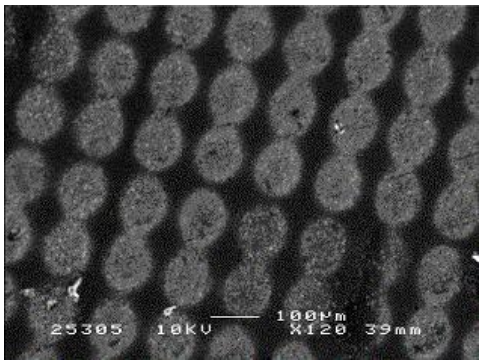

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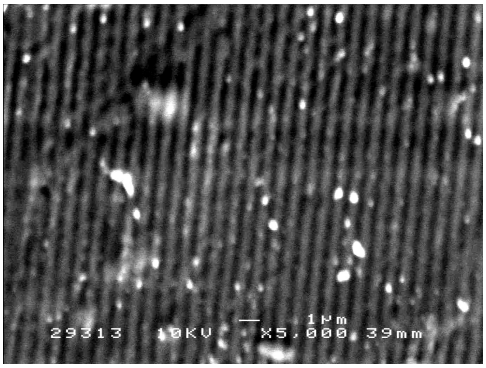

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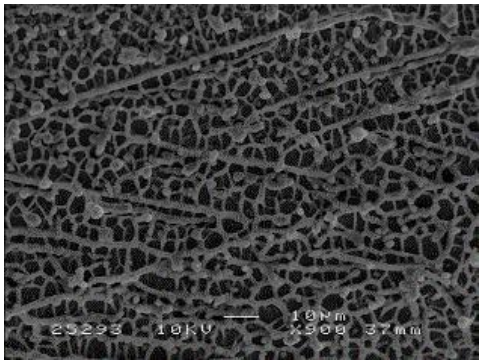

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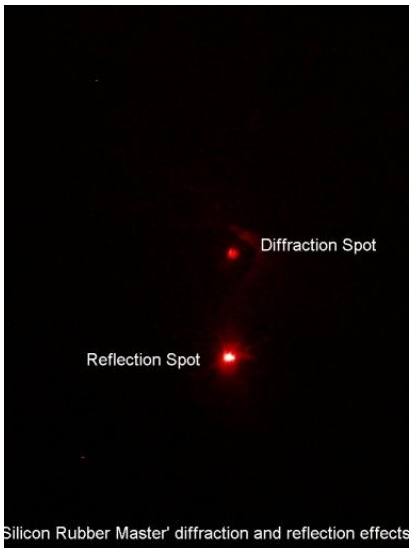
Figure 7



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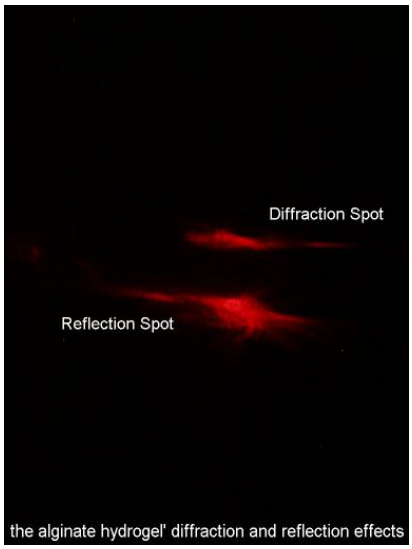

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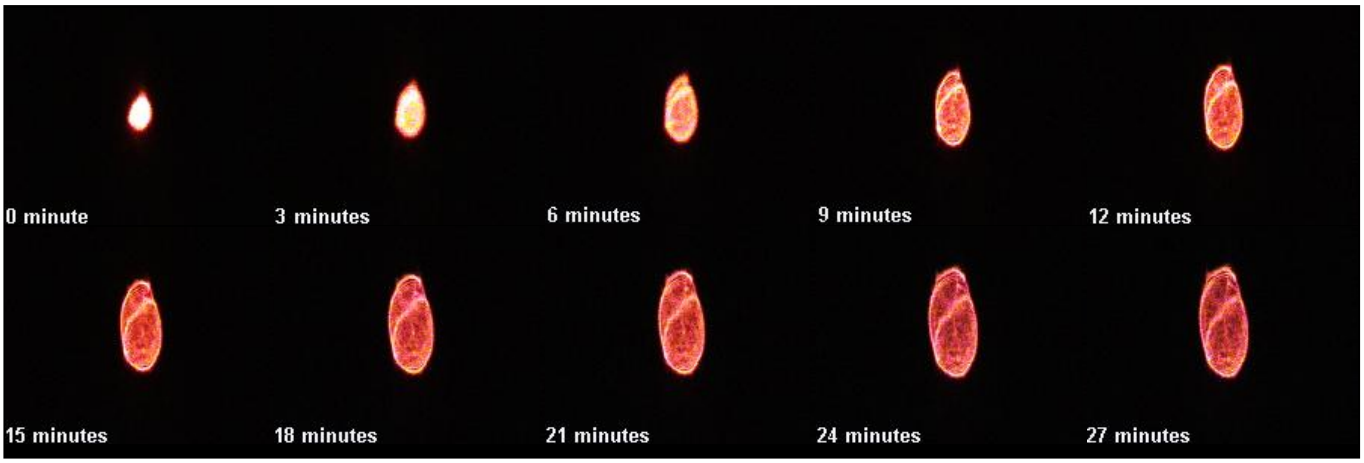

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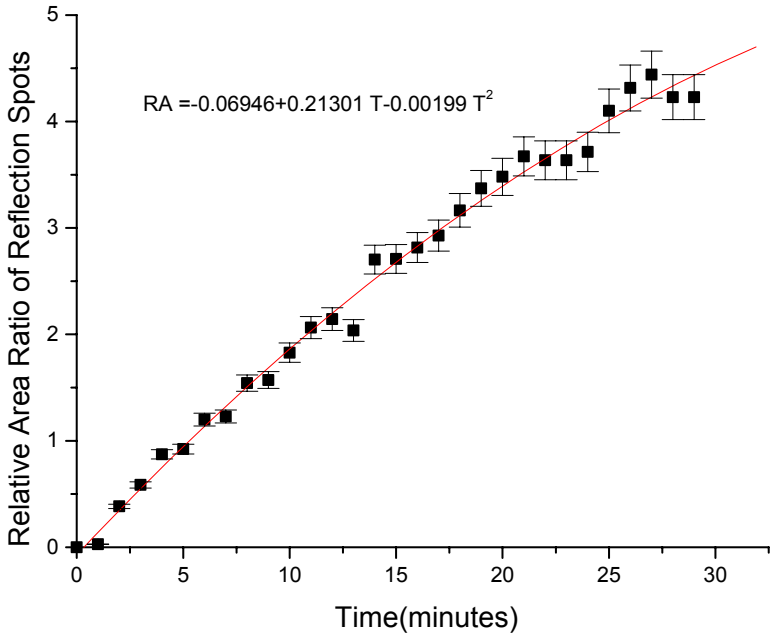

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