Detection of Clinical Biomarkers Using a Novel Parabolic Microchip Diagnostic Platform

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ABSTRACT

In this paper we present a novel biosensor platform, designed to enhance the collection efficiency of fluorescence from surface bound assay labels while simultaneously eliminating background signals from the bulk fluid on the chip. The chip consists of nine paraboloid elements moulded into a single piece of polymer, enabling a cheap, mass producible, efficient method of biomarker detection. Herein we demonstrate the detection of the clinically relevant biomarker C-reactive protein (CRP) using a sandwich assay adapted from a traditional ELISA platform. The platform demonstrates significantly enhanced capture of signal from the fluorescent labels leading to higher analytical performance.

1 INTRODUCTION

Detection of biomarkers indicating diseases and conditions such as heart failure [1] has led to the development of increasingly sensitive and accurate assays and biosensors. Typically, clinically relevant biomarkers are isolated and tested in a laboratory-based *in-vitro* setting, but recent years have seen a push towards increased point of care testing, driving the development of cheap, mass producible and reliable test platforms with easy user interfaces [2]. Common transduction methods developed to interface these biomarkers range from optical to electrochemical techniques. Of the optical techniques, the use of fluorescent-based systems are the most common, in which fluorescent labels such as cyanine dyes and quantum dots are attached to antibodies which in turn bind to the analyte of interest.

The goal of this work is to combine the trend towards use of low-cost polymer biochips and integrating this with an optical structure that facilitates the efficient the capture of fluorescence from an assay label. Modelling of an oscillating dipole close to the interface between a lower-index superstrate and higher-index substrate shows that the light does not emit isotropically, but in fact predominantly into the substrate (Fig 1), and of that the majority is emitted into supercritical modes [3]. By capturing only this Supercritical Angle Fluorescence (or SAF) emission, background from the bulk can be eliminated. Additionally the biochip is designed to excite the fluorophores through evanescent wave excitation using Total Internal Reflection Fluorescence (TIRF) to also limit the excitation volume.

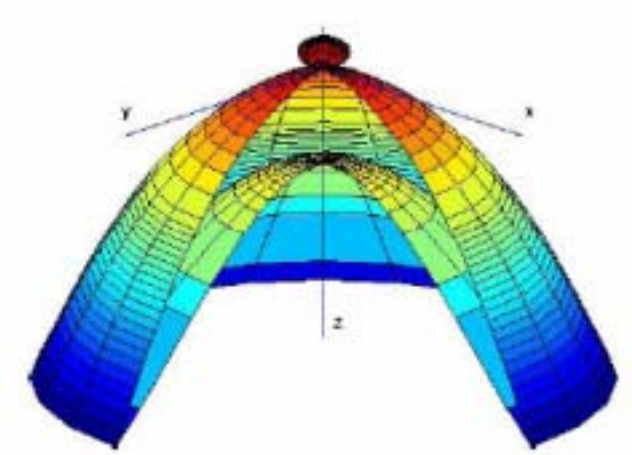

Fig 1: SAF emission from a low index superstrate to high index substrate, with dipole on interface in the x-y plane.

2 MATERIALS AND METHODS

2.1 Optical System

The key element of the biosensor is a platform of paraboloid elements (Fig 2), which is manufactured from a low auto-fluorescence zeonex® polymer, and is designed to collect collimated light from below and reflect it onto a focal point at the upper planar surface from a truncated parabolic element. The incident light is totally internally reflected and so excitation of the fluorophores occurs due to the evanescent wave, which decays exponentially with distance from the surface, providing good surface selection.

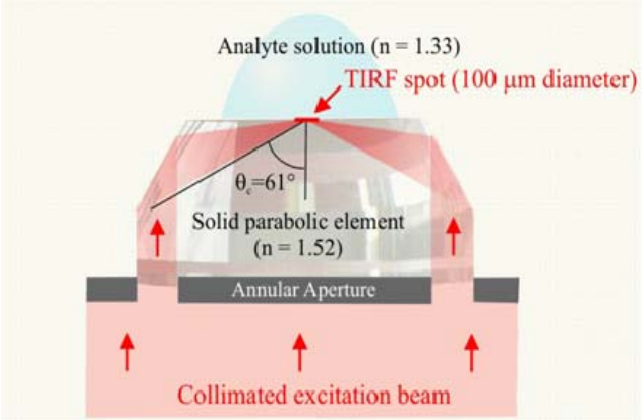

Fig 2: Parabolic element showing TIRF excitation.

The SAF emission that radiates into the substrate is then collected, again by the paraboloid edges (Fig 3), reflecting this into a loss-free collimated beam towards a detector. The magnitude of the detected light is indicative of the amount of fluorophore on the surface and is thus linked to the concentration of the analyte. An annular mask is placed below the paraboloid element to both eliminate excitation light passing vertically through the upper surface and also block non-SAF emission from the bulk liquid.

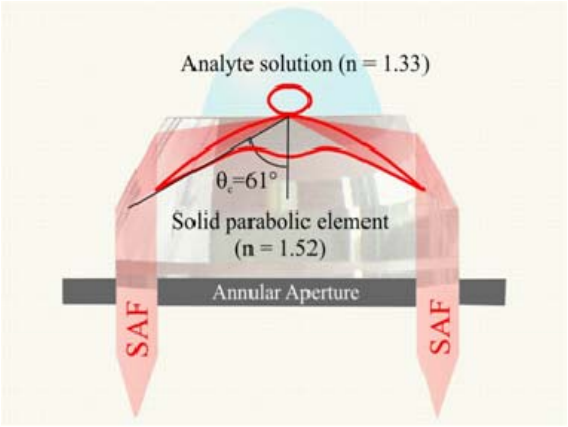

Fig 3: Collection of SAF light by same parabolic element.

The biochip platform consists of a 3x3 array, allowing nine surface reactions to be monitored in real time. The biochips were analysed with a custom designed optical system (Fig 4). A collimated beam of light from a laser diode of wavelength 635nm was reflected via a dichroic mirror onto the biochip, and the emitted fluorescence from the biochip parabolic elements was imaged onto a USB-controlled CCD camera. The system was linked to a computer with custom software developed for image capture and analysis, and a readout could be produced in under 5 seconds.

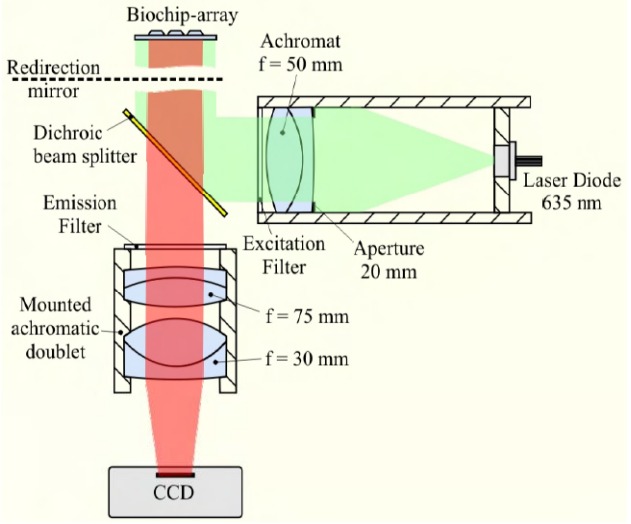

Fig 4: Custom optical setup for illumination and collection

2.2 C-Reactive Protein

C reactive protein is a marker of inflammation and infection produced by the liver and is thought to be strongly linked to the hardening of blood vessels seen in coronary artherogenesis [4] thus allowing it to be used as a prognostic indicator of future cardiac events in both healthy patients and those with a history of acute coronary syndrome [5] As CRP levels are useful in the prediction of thrombotic events such as coagulation of the blood in the heart, arteries, veins or capillaries, they may also offer an alternative to cholesterol measurements for monitoring wellness [6].

2.3 Antibody production and immobilization

The chosen antibody for this assay was an anti-CRP polyclonal antibody, produced through the immunization of a female while New Zealand White rabbit. The polyclonal IgG antibody fraction was purified from the rabbit serum using protein G affinity chromatography. The surface of the chip was functionalised using an amino-dextran coating, as antibodies have amine and carboxyl groups available for attachment. After surface functionalisation, the antibodies were deposited using a non-contact sciFLEXARRAYER™ (Scienion AG, Germany) piezo dispensing system.

2.4 Assay Details

The chips were functionalised and spotted with capture antibody as described in section 2.3. They were then washed in a phosphate buffer solution (PBS) and then blocked in a dilute (5% w/v) solution of milk powder overnight at 4^0C. After another washing step, different concentrations of CRP, again in PBS and additionally

different concentrations of CRP in depleted serum were placed on the tops of the chips, covering the paraboloid elements and left to incubate at room temperature for 2 hours, and agitated at 1 hour to remove concentration gradients. After an additional wash step CRP antibody labelled with DyLight™ 647 (Pierce Biotechnology Inc., Rockford, IL) was deposited onto the tops of the paraboloids and allowed to incubate for a further 2 hours at high humidity to stop evaporation of the droplets. The optimum dilution (1/400) of the labelled antibodies was determined empirically. After a final wash step, the chips were then analysed in a GMS 418 array scanner (Genetic Microsystems) to ensure good spot location and quality and then finally the fluorescence measurements were taken in the custom biochip reader.

3 RESULTS AND DISCUSSION

The collimated light from the chip was imaged onto the CCD camera and showed clearly the fluorescence rings (Fig 5). The outer rings appear distorted due to spherical aberrations and additionally are dimmer than the central ring due to the Gaussian beam profile from the laser. The Gaussian profile was accounted for by taking a reading of the illumination intensity through each of the annular rings and normalising to the central ring. The signal was integrated for each ring, giving a total fluorescence reading for that paraboloid, and then the noise background was subtracted from the final value.

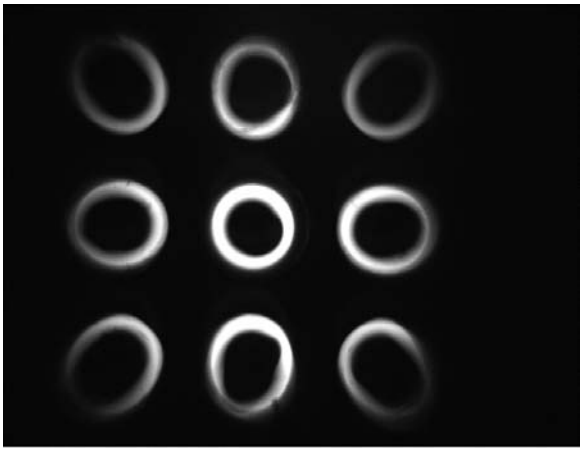

Fig 5: Image of the collimated fluorescent light.

3.1 Surface Coating Optimization

The optimal surface coating was determined by running a limited assay with different surface coating concentrations of capture antibody, ranging from 50µg/mL of antibody to a maximum of 300µg/mL (Fig 6). As the surface coating concentration increases, the slope of the graph increases up to 200µg/mL at which point the surface coating saturates and no further increase is apparent.

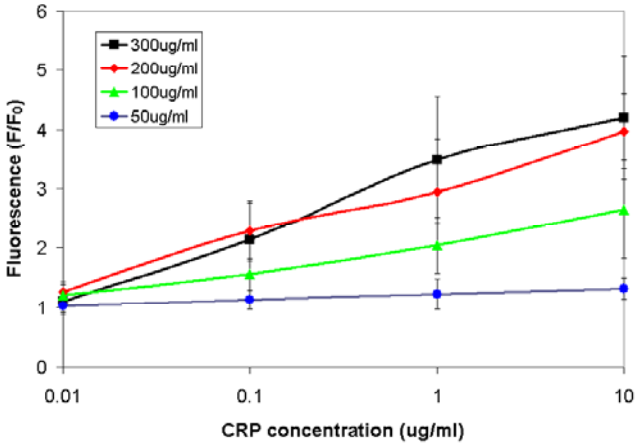

Fig 6: Normalised Fluorescence for multiple surface coating concentrations

This point was chosen as the optimal concentration for maximizing the slope while using the minimal amount of capture antibody. All results are normalized to a control chip which had no CRP added. This surface coating concentration correlated well with an additional experiment in which a fluorescently labeled antibody was deposited directly onto the surface and measured, in this case the fluorescence saturated at around 200µg/mL.

3.2 CRP Assay

The CRP assay was repeated three times at the optimal surface coating concentration of 200µg/mL outlined above, with each chip having one concentration of CRP, and hence nine data points. The CRP range covered the clinically relevant range 0.01 to 10µg/ml. For each assay the result was averaged over the nine parabolas, and then averaged over the three assays giving the final readings shown in figure 7.

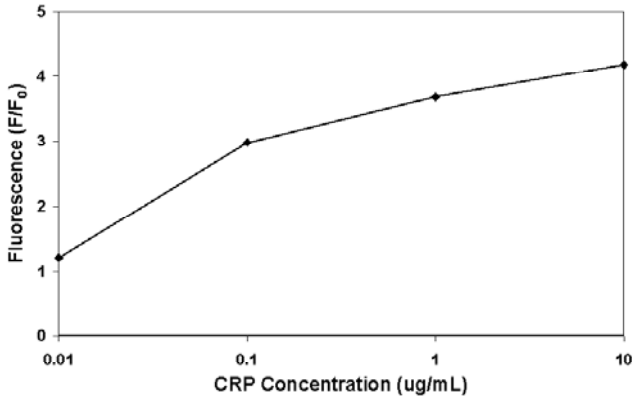

Fig 7: Fluorescence signal versus CRP in buffer assays

The assay was also conducted in duplicate using CRP-depleted human serum (Fig 8) spiked with different concentrations of CRP to verify the sandwich assay performance in a real sample matrix.

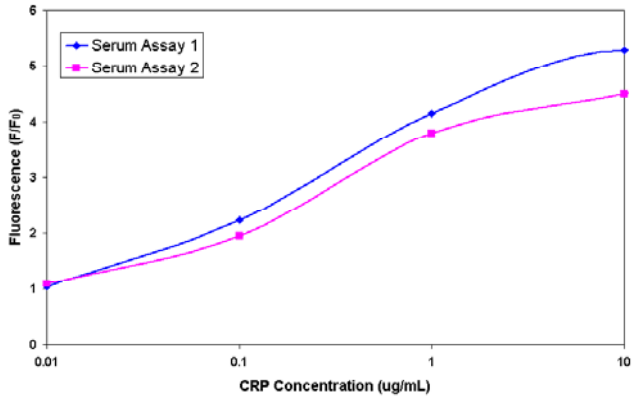

Fig 8: Assays conducted with CRP in blood serum

3.3 Surface Discrimination

In order to demonstrate surface discrimination the central part of four of the annular rings was removed to allow excitation light to pass up through the centre of the parabolic elements. Reaction vessels were then made from perspex and fixed to the top of the chip, with one vessel upon each parabolic element which were then filled with a solution of DyLight 647 dye- labeled antibody and placed on the reader. In this case, there is both direct illumination through the bulk (in addition to the TIRF illumination) and collection of non- SAF light from both the surface and the bulk. Figure 9 shows the resultant fluorescence detected. The four elements to the left have the central mask removed and show additional fluorescence generated from the bulk solution above each parabolic platform. The remaining five parabolas however show only the light from the TIRF excitation and SAF collection, with the optical system blocking and minimizing bulk fluorescence.

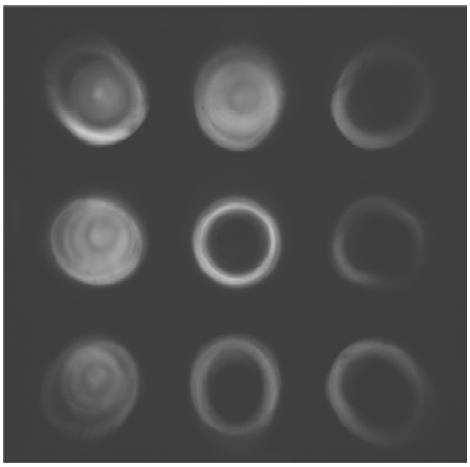

Fig 9: Bulk fluorescence in left 4 "open" annular rings. Bulk fluorescence is eliminated from the remaining 5 platforms due to the parabolic facilitating TIRF excitation.

The design of the system clearly eliminates unwanted signal from the bulk solution and only probes the region of interest on the surface of the parabola.

4 CONCLUSION

A novel biochip platform, which is designed to enhance the collection of fluorescence from biological assays and significantly reduce the signal from non-bound fluorescent labels present in the bulk liquid above the solution was demonstrated. The design of the system is such that it provides a practical method of performing multi-analyte studies in a single measurement, and allows the elimination of background noise. This biosensor represents a significant step towards disposable platforms for the conduction of sensitive and accurate assays for point-of-care applications.

Acknowledgements

This material is based upon works supported by the Science Foundation Ireland under Grant No. 05/CE3/B754".

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