Microbioreactor with Photodiode Detection for Monitoring Intracellular GFPUV Expression in E. *coli*

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Abstract: A prototype membrane aerated polydimethylsiloxane (PDMS) microbioreactor integrated with a photodiode detector was developed for monitoring intracellular green fluorescent protein ultraviolet (GFPUV) expression in *Escherichia coli*. The developed system is compact, simple and inexpensive and ideal for economical on chip detection of fluorophores in aqueous solution and intracellular GFPUV expression. The detection limit for cell free GFPUV was found to be 4.8×10^{-8} M. Intracellular GFPUV expression in *E. coli* cells was monitored for 12 h and the lowest detectable signal was recorded from a cell concentration of 2.7×10^{7} cells/mL with signal to noise ratio (SNR) value of 3. The performance of the photodiode detector was benchmarked with a CCD spectrophotometer and results showed favorable comparison. A miniaturized microbioreactor for monitoring intracellular dynamics in real time is demonstrated.

Keywords— *photodiode, fluorescence, microbioreactor, intracellular GFPUV*

1. Introduction

Green fluorescent protein (GFP) is a protein which occurs naturally in the jellyfish *Aequorea victoria* and other coelenterates. Since its discovery, GFP and its variants are widely used as fluorescent tags of single proteins, whole organisms, cells and complex protein structures [1]. The discovery of GFPs have enabled biologists to examine processes in living cells and study their dynamics aiding developments in neurobiology, and cell biology [2]. During the last decade, GFP's have been used in a variety of applications including transgenic fluorescent animals (mice, fish, chickens, mice, dogs, pigs, cats and marmosets) and decorative transgenic plants have been reported [3, 4].

GFP monitoring in biological processes is affected by a number of factors including: the requirements of molecular oxygen for fluorescence to occur, the amount of GFP produced and inner filter effect from intracellular light absorption and scattering by cell particles. To overcome these limitations there has been a drive by researchers to develop detectors that have high sensitivity, fast response times and relatively cheap and easy integration with lab-on-a-chip (LOC) devices, unlike conventional equipment such as spectrofluorimeters and fluorescence microscopes which are bulky and most expensive. These systems require relatively large sample volumes and data processing tools to analyse the fluorescence information gathered [5]. Key efforts for the detection of GFP expression in intracellular environments was made by Eichhorn *et al* [6] who reported an on-line sensor for measuring real time GFP in high optical density cultures of *E. coli* cells. Also, the group developed an improved low cost, highly sensitive sensor for monitoring of GFP production in a standard flow through cuvette which was coupled to a conventional bioreactor [7]. The sensor consisted of a LED induced excitation source and a pin photodiode as the detector. The dynamic range of the sensor was $7.4 \times 10^{-9} – 3.7 \times 10^{-5}$ M with limit of detection 7.0×10^{-9} M for GFP. Jóskowiak *et al* [5] developed a microscale GFP detection system using a PIN thin film amorphous silicon light sensitive layer microfabricated on a glass substrate integrated with an amorphous silicon carbon alloy absorption filter and an ultra-thin PDMS sheet to detect intracellular expression of GFP in *E.coli*. GFP was both detected in aqueous solution in nano molar concentration and intracellularly for 1×10^{6} cells/mL range.

Several optical sensors and sensor systems have been described in the literature, but research on their application for biotechnological purposes, especially cultivation and protein expression monitoring under real process conditions in LOC devices have been less well studied. Monitoring changes in fluorescence intensity of intracellular fluorophores, and hence changes in their concentration, is a powerful tool in many areas of bioengineering, ranging from bioprocess monitoring [8], drug testing to cancer research [9]. Here, we report on the development of a disposable membrane aerated PDMS microbioreactor integrated with a photodiode based detector and demonstrate the detection of both GFPUV in aqueous solution and intracellular expression in *Escherichia. coli* (pWTZ594) cells that constitutively expresses green fluorescent protein ultraviolet (GFPUV). The developed *in situ* fluorimeter was capable of providing continuous data on intracellular GFPUV expression and cell density. Also the developed system is highly sensitive, inexpensive, and suitable for both off-line and on-line

measurements. The device proposed in this research is the first step towards the development of a low cost system for monitoring intracellular events.

2. Materials and methods

2.1 Fabrication of the microbioreactor

Standard soft lithographic techniques [10] using polydimethylsiloxane (PDMS) were employed to fabricate the device (Figure 1). The microbioreactor chip was cast from a master mould that was created by direct micromilling using PMMA. Integration of the optical fibre into the microfluidic system, was achieved by using a polished aluminium wire with the same length and diameter as the optical fibre tip, and was glued ~ 2 mm away from the growth chamber on the PMMA master mould. This was to allow the creation of a space that could be used for insertion of the optical fibre for optimised excitation of fluorophores in the microfluidic chamber. The PDMS base and the curing agent (Down Corning Sylgard 184, USA) were mixed thoroughly in a ratio of 10:1(wt/wt), poured over the PMMA mould, degassed for about 30 min, and allowed to cure at 60 ºC for 70 min. The PDMS replica was cut and removed from surface of the master, and inlet and outlet holes were punched. The microbioreactor wells were cored out using a 6 mm biopsy punch (Kai, Europe GmbH) resulting in a hole that was 6.3 mm in diameter. The cored PDMS structure was bonded to a partially cured flat piece of PDMS layer to close the channels. A thin PDMS membrane (100 ± 10 µm), was used to close the microbioreactor chamber and to provide bubble free oxygen to the cells and the channels were for inoculation. The bottom layer was held by surface contact to a PMMA slide (25 mm × 75 mm) as a support base for the microbioreactor.

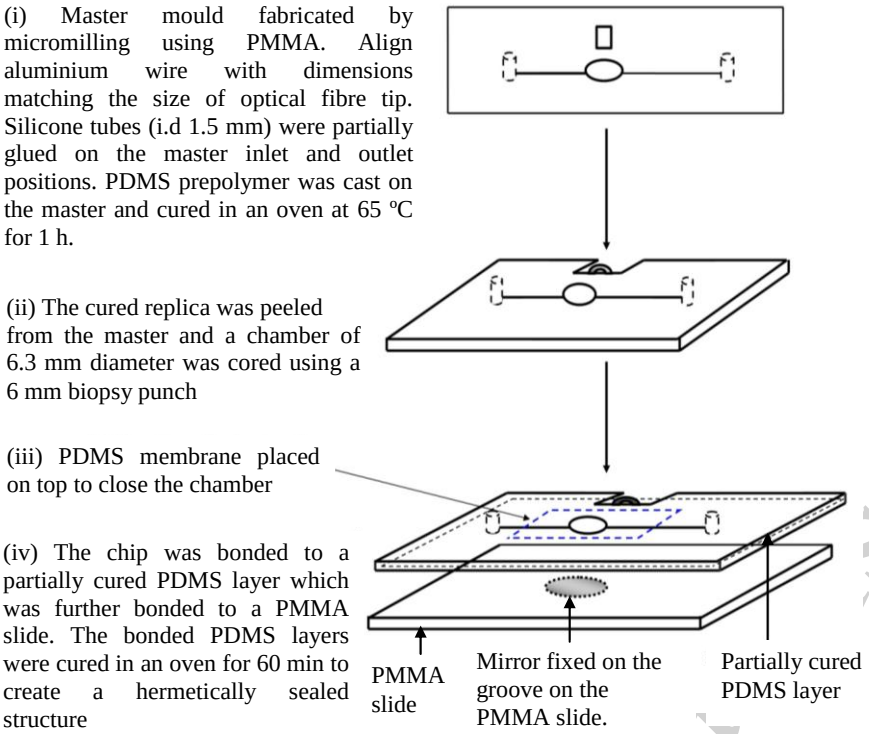

Figure 1: Step by step fabrication of the PDMS microbioreactor.

2.2 System setup

The schematic of the optical set up is shown in Figure 2. It consisted of a UV-LED excitation source with peak wavelength 395 nm (Roithner, Austria), a photodiode (PDA36 EC, Thorlabs UK) as the detector, lock in amplifier (405 DSP, Scitec Instruments, UK), a PC with LABVIEW 8.6 software and a data acquisition board USB 6221, (National Instruments, UK) for acquiring analog signals and converting them to digital signals, LED driver and a single chamber microfluidic bioreactor (Figure 2). The excitation light from the UV-LED was modulated at 1 kHz using a LED driver developed in house, which provided a fixed current source of 600 mA. The excitation light from the UV LED (395 ±10 nm) was not filtered as its spectrum was extremely narrow [10 nm Full-Width Half-Maximum (FWH)]. The excitation light from the UV–LED was coupled through a custom made optical fibre (core diam, 1200 µm, BFL371000 CUSTOM, Thorlabs UK) inserted on the slot created on the replicated chip at 0^{o} incidences, for exciting the fluorophore. The isotropic fluorescence emission photons from the sample was passed through a Kodak wratten 2 optical filter (Edmund Optics, USA) to block light of wavelengths <500 nm. The filter is also used to prevent excitation light from

the source reaching the detector. A mirror, with a higher reflective surface was placed in a grooved position on the PMMA slide, underneath the chip to facilitate the alignment of the sensitive area of the photodiode with the growth chamber of the microbioreactor and to reflect light. The output electrical signals from the photodetector were coupled on to a DSP lock in amplifier (DSPLA) which was under the control of a web programme.

In the DSPLA the fluorescence signals were passed through second order, high pass electrical filter with a cut off frequency of 1 kHz and further amplified and integrated with a time constant of 2 s. The integrated and filtered signals were collected from the DSPLA and channelled to the DAQ-USB 6221 which was linked to a computer via LabVIEW for data acquisition. The setup was evaluated by conducting a series of experiments using standard solutions of FITC and GFPUV.

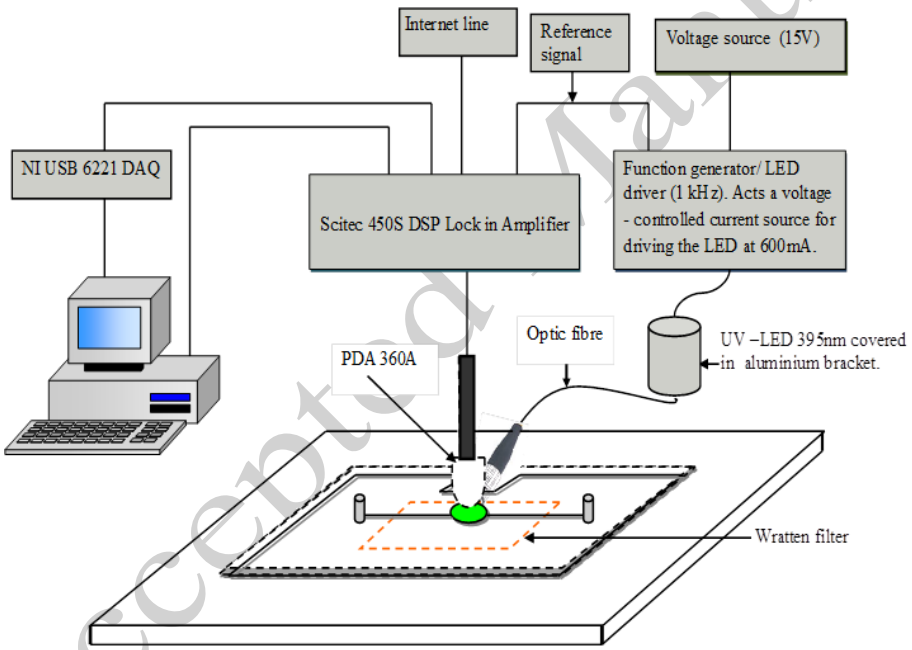

Figure 2: Schematic of the experimental set up.

2.2 Cultivation of microorganism

Detection of intracellular GFPUV were performed with transformed *E. coli* (MC1060/pWTZ594) strain containing the plasmid pWTZ594 that constitutively expresses green fluorescent protein GFPUV (E_x 395 nm, E_m 509 nm) (*E. coli* Genetic Centre, USA). A single colony of was initially subcultured on tryptone soya agar (TSA) (Oxoid, UK)

supplemented with ampicillin and 0.15 mM IPTG, (Sigma Aldrich, UK) for the induction of GFPUV expression and incubated at 37 ºC for 24 h. The inoculated TSA surfaces were illuminated on a UV lamp and the bright green fluorescent single colonies were picked and used to inoculate 50 mL of sterile modified terrific broth (MTB) (tryptone 10 g/L, sodium chloride 10 g/L and yeast extract 1 g/L) supplemented with ampicillin (100 µg/mL) (Sigma Aldrich, UK) in 250 mL Erlenmeyer flask, then incubated at 37 ºC for 2-4 h with shaking (150 rpm). Aliquots of the bacterial suspension were used to inoculate the microbioreactor and shake flask respectively. Sterility in the microbioreactor was achieved by rinsing with 70% ethanol as other methods of sterilisation such as autoclaving were not feasible. The addition of ampicillin to the culture medium was sufficient to suppress any contamination in the chip and hence maintaining sterility in the device. The microbioreactor was placed in a temperature controlled chamber developed in-house. The fluorescence signals from the induced *E. coli* cells were measured as a function of incubation time and results compared with shake flask experiments. Samples for shake flask (SF) experiments were diluted and their fluorescence measured on a F2000 Fluorimeter (Hitachi, UK). GFPUV expression in the microbioreactor was monitored by photodetection measurements of the fluorescence signals after every 2 h up to 12 h so as to allow sufficient production of GFPUV by the organism.

Estimates of cell concentration from the microbioreactor and the shake-flask were obtained by making direct viable bacterial cell counts using the standard technique of plating serial dilutions [11]. Samples from the microbioreactor were obtained by sacrificing the entire volume (50 µL) after measurements of GFPUV expression were made, so as to provide sufficient volume for serial dilutions. Thirty microliters (30 µL) of culture suspension from the microbioreactor at each specified time interval were suspended in 970 µL of 0.1% peptone water and serially diluted. Samples of 5 µL from each dilution were plated in triplicate on tryptone soya agar plates containing 100 µg/mL of ampicillin and 1 mM IPTG and, incubated at 37ºC for 18-24 h, followed by enumerating the number of green fluorescent colonies formed. The process was repeated until data was obtained for six time points in triplicate. Similarly, samples of 0.5 mL of the bacterial suspension from the shake flask were taken off-line every 2 h up to 12 h and serially diluted with 4.5 mL of 0.1% peptone water and plated as described for the microbioreactor. Cells grown under the same physiological conditions but

with no IPTG were used as a reference to assess the effect of basal GFPUV production and light scattering by the cells [5].

2.3 Preparation of cell free GFPUV and FITC standard solutions

GFPUV was extracted from the *E. coli* cells (MC1060/pWTZ594) cultivated in a flask shake. The cells were disrupted by sonication and pelleted by centrifugation [12]. The final GFP solution was filtered on a 0.2 µm syringe filter (Nalgene, UK), standardised and kept in phosphate buffer (pH 7.4) solution. Working solutions were made from the GFPUV (5 µM) and FITC (20 µM) standard stocks. The standard solutions were then used to evaluate the responsiveness of the sensor to increasing fluorophore concentrations. FITC was excited with a blue LED with peak emission wavelength of 460nm while cell free GFPUV was excited with a UV-LED with peak emission wavelength of 395 nm. Before measurements were made, the microbioreactor was filled with deionised water and measurements for background signals were recorded.

3.0 RESULTS AND DISCUSSION

3.1 Measurement of standard fluorophore solutions

The initial steps of evaluating the performance of the system was evaluated by measuring the fluorescence signals generated from known concentration of standard fluorophore solutions of FITC and cell free GFPUV. Initially deionised water was flowed in the microbioreactor chamber and the fluorescence intensity dropped significantly. When standard fluorophore solutions were introduced a clear increase in sensor output signals was observed demonstrating the ability of the photodetector to acquire the fluorescence of GFPUV in aqueous solution. The sensor output signals (V) as a measure of fluorescence emission were plotted as a function of the concentration of the standard solutions (Figures 3a-b). The results in both cases showed excellent linearity with regression correlation coefficients of R^2 = 0.986 and 0.989 for GFPUV and FITC respectively. The lowest detectable concentration of GFPUV and FITC on chip was found to be 4.8×10^{-8} M and 1.2×10^{-6} M respectively. The LOD was calculated by using the mathematical expression described in (1).

$$LOD = 3.3\sigma + S_B \quad (1)$$

Where $\sigma$the standard deviation of the response and SB is is the background signal.

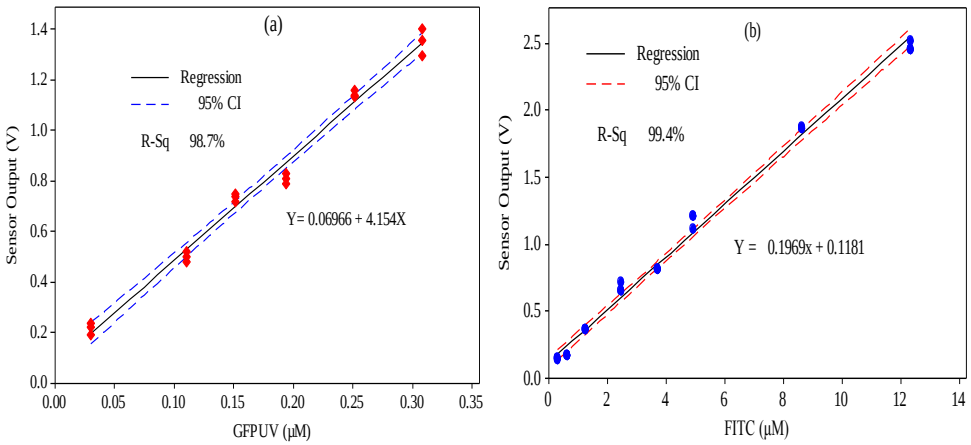

Figure 3: Detection of standard fluorophore solutions (a) Linear dependence of the fluorescence signals (V) with increasing GFPUV. (b) FITC standard solution concentration.

The difference in the LOD between GFPUV and FITC could be explained in terms of the stoke shift values between the two analytes. GFPUV has a higher stoke shift (218 nm) than FITC (28 nm). Fluorescence dyes with a very small stokes shift value such as FITC are susceptible to self-quenching due to the overlapping between absorption and emission spectra. This behaviour of self-reabsorption losses could lead to non-linear emission at high dye concentrations and consequently, affect the LOD of the analytes [13]. Additionally, the discrepancy could be due to the difference between the quantum yield values for GFPUV and FITC. Previous works have shown that quantum yield of fluorescent dyes decreases with concentration due to the formation of dye aggregates such as dimers and trimers [14,15]. This phenomenon could lead to the reduction of radiative energy efficiency due to a competing Forster–type energy transfer between molecules.

The performance of the detector on FITC and GFPUV was bench marked with a CCD Ocean Optics spectrometer (Figure 4). The results showed a favourable comparison between the two systems with limit of detection of 2.8×10^{-8} M for GFPUV and 1.4×10^{-6} M for FITC. In all the measurements, two baselines namely the dark voltage (response of the photodiode to

dark conditions *i.e.* when there is no illumination on the detector) and the background voltage (response of the photodiode with no fluorophore present in the sample volume), were considered. The background voltage could be due to the photodiode dark voltage, autofluorescence from PDMS material and stray light leaking from the excitation source.

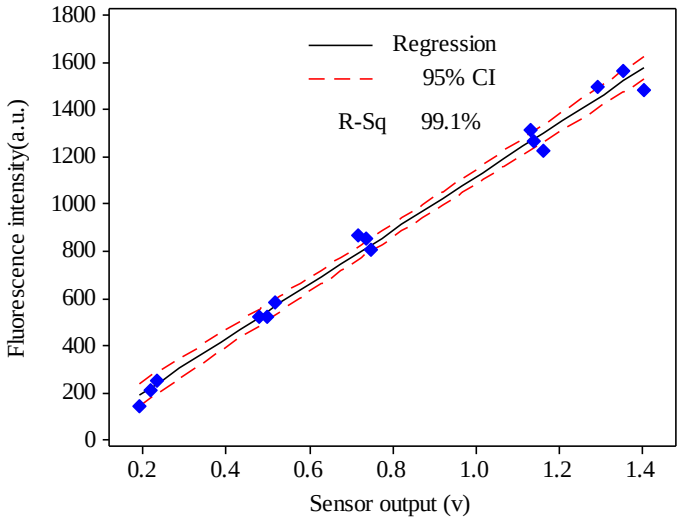

Figure 4: Comparisons of CCD spectrometer with photodiode detector to increasing concentration of GFPUV standard solutions. The linear relationship was quite strong between the two detectors (R^2 = 0.991). Data shown are for three independent experiments with a 95% confidence interval.

3.2 Detection of intracellular GFPUV expression in *E. coli*

The utility of the developed system was demonstrated by measuring the production of GFPUV expressed intracellularly by the transformed *E. coli*. In this study, the genetically modified *E. coli* cells harbouring the GFPUV reporter gene controlled by the lacZ promoter in pBluescript KS+ were cultured in the microbioreactor for 12 h and monitored for production of GFPUV. Cell growth was measured by recording the fluorescence signals arising from the production of GFPUV by the dividing cells and the results compared with conventional shake flask operations. Cell concentration was measured by serially diluting the samples withdrawn from the shake flask and the sacrificed microbioreactors and then estimated by plating methods (Figure 5a) as described in the method section.

Figure 5a shows a steady increase in the amount of GFPUV fluorescence intensity with time in both the microbioreactor and shake flask. The results demonstrated the suitability of the

microbioreactor system to sustain cell growth and allowed the efficient expression of GFPUV by the dividing cells and, the overall sensitivity of the sensor to increasing fluorescence signals. The increase in the fluorescence signals can be attributed to the expression of GFPUV by the dividing cells when induced by IPTG. IPTG is a synthetic equivalent of lactose that removes the blocking action of the *lac* repressor protein over the promoter [5]. The advantage of IPTG is that it is not metabolised by the cells as lactose and this allows the maintenance of high GFP expression levels during cell division. The disparity between the shake flask and the microbioreactor could be due to the inner filter effect arising from the shake flask experiments as a result of higher optical density values or turbidity of the bacterial suspension. The reduced sensitivity in the microbioreactor could be explained by the intrinsic autofluorescence arising from the PDMS material used in fabricating the microbioreactor chip.

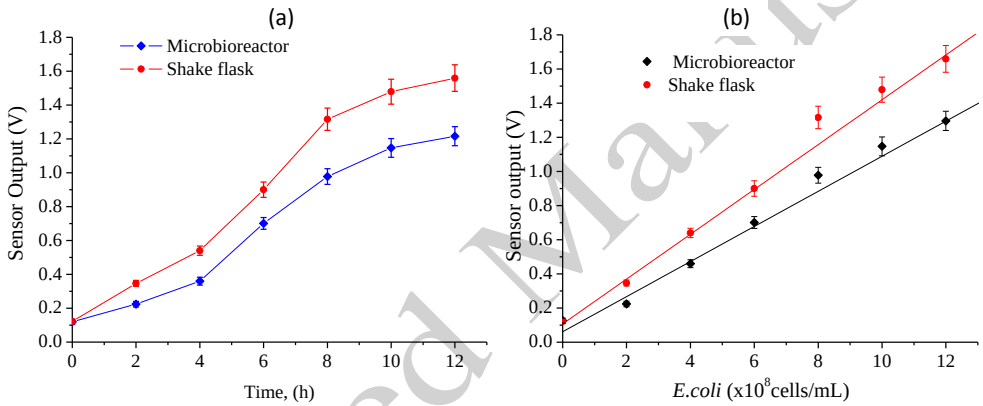

Figure 5 (a) Expression of GFPUV by ***E. coli*** *(MC1060/pWTZ594)* as a function of growth time in the microbioreactor and shake flask. (b) Relationship between fluorescence intensity and cell concentration. Data shown are an average of three separate experiments and the error bars represent the plus or minus standard deviation (±SD) between the measurements at each point.

Figure 5b shows a plot of the response of the photodetector (sensor output) versus cell concentration. The graph showed a linear dependence of the photovoltage signals arising from the intracellular GFPUV and the cell concentration. The linear relationship between the culture fluorescence intensity and the number of cells strongly suggested that GFPUV fluorescence measurement can be used to monitor cell growth in bioprocessing systems. The developed fluorescence sensor was capable of detecting GFPUV expression from an estimated lower cell concentration value of 2.7×10^7 cells/mL with an estimated signal to

noise ratio of >2. The results obtained in this study, were in close agreement with previous studies [5, 7]. From figure 5b, it is possible to quantify the *E. coli* cell concentration from a given suspension when the photodetector has been calibrated.

3.3 Comparison of GFP sensing with previous studies

The results on the performance of the photodiode detector were compared with previous GFP detection measurements (Table 1) in terms of the technique, LOD for standard fluorophores and intracellular GFP expression. A previous study by Jóskowiak *et al* developed a micrometer scale detection system in which a PIN thin-film amorphous silicon light sensitive layer microfabricated on a glass substrate was integrated with an amorphous silicon carbon alloy absorption filter and an ultra-thin PDMS sheet to detect intracellular expression of GFP [5]. Measurements of GFP expression were made by immobilising 5 µL of *E. coli* cell suspension cultured in the shake flask on the photodetector and recording the response of the expressed protein. The best LOD values obtained were 18.5 nM for pure GFP in aqueous solution and 2.58×10^6 cells/mL as the minimum concentration that gave a signal for GFP expression. The LOD value for the intracellular GFP was approximately ten times higher than the current study (2.7×10^7 cells/mL). The disparity between these values could be due to the difference in the manner the measurements were made, the sensitivity of the photodetector and the type of the fluorophore used. In this work, *E. coli* cells were cultured in the microbioreactor device and fluorescence measurements were made non-invasively through the PDMS membrane (100 µm). The reduced sensitivity could be due to the intrinsic fluorescence arising from the PDMS material [16] used in fabricating the microbioreactor chip, sensor type and the type of fluorophore.

Kostov *et al* made measurements for intracellular GFP expression on *E. coli* W3110 cells transformed with [pGFPUV-CAT] and Trc-HIS plasmid in a 1 cm quartz cuvette, where a large area PIN photodiode was used to detect the expressed GFP [7]. *E. coli* cells were cultured in a bench scale bioreactor, and culture medium containing the transformed cells was continuously pumped into the 1 cm quartz cuvette and detected for GFP expression. LOD values of 7 nM and 19 nM were obtained for pure GFP standard solutions excited with a UV and blue light respectively. The response of the sensor to intracellular GFP signals was calibrated against off-line spectrofluorimeters measurements taken during fermentation and

results showed a linear correlation (R^2 = 0.989 for UV and 0.998 for blue light). The sensor was capable of measuring GFP concentrations in cell suspension with higher optical density up to 100. The LOD value for UV excitation was 4 times lower than our results. Furthermore, the experimental set was more expensive and sophisticated than the current study.

Zanzotto *et al* demonstrated methods for in situ measurements of bioluminescence and fluorescence from bacterial cultures grown in 50 µL instrumented membrane aerated microbioreactor [17]. Experiments were conducted with reporter strains of *Escherichia coli* expressing GFP. GFP expression was measured by exciting *E. coli* cells with a blue LED (465 nm) which was passed through a collimating lens a band pass filter and a collecting lens. The emitted fluorescence was detected by a photomultiplier tube and the results showed that fluorescence signals in shake flask and the microbioreactor increased with bacterial growth. However, the LOD values for pure and intracellular GFP were not reported. Although the setup was very sensitive, it was more expensive (arising from the cost of a photomultiplier tube) and complex than the current study. As an example, the system required more time to align the different optical elements (filters and lens).

Based on the above comparisons on the detection of GFP, it would appear that the present study has made some significant improvements over the previous GFP sensors. This has been achieved with a setup which is relatively simpler, straight forward and cheaper.

Table 1: Comparison of GFPUV measurements with literature reports

Photodetector	Scale	Analyte	LOD	Excitation	Source
CCD spectrometer(Ocean Optics)	On chip	FITC	1.4×10^{-6} M	Blue LED (λ_{exc} = 470 nm)	This work
		Pure GFPUV	2.8×10^{-8} M	UV LED (λ_{exc} = 395nm)	
(13 mm^2) PIN photodiode PDA 360EC	On chip	FITC	1.2×10^{-6} M	Blue LED (λ_{exc} = 470 nm)	This work
		Pure GFPUV	4.8×10^{-8} M	UV LED (λ_{exc} = 395 nm)	
		Intracellular GFPUV	2.7×10^{7} cells/mL		
(13mm^2) PIN photodiode 1223-01	A standard 1cm quartz cuvette	Pure GFP	7.0×10^{-9} M	UV LED (λ_{exc} = 370 nm)	[7]
		Intracellular GFP	-		
200 × 200µm thin film pin a Si: H photo sensor	Immobilised on the sensor	Pure GFP	1.85×10^{-8} M	Blue LED (λ_{exc} = 480 nm)	[5]
		Intracellular GFP	2.58×10^{6} cells/mL		
Photomultiplier tube	On chip	Pure GFP	-	Blue LED (λ_{exc} = 465 nm)	[17]
		Intracellular GFP	-		

3.4 Factors affecting the detection of GFPUV

The detection of intracellular GFPUV could be affected by a number of factors including the background fluorescence signals coming from the medium, as its emission was in the same range as the GFPUV fluorophore. Many biological molecules autofluorescence at the same wavelength as GFP, making background noise a significant concern especially when expression of the gene of interest is very low [18, 19]. Simple experiments using different

media recipes were conducted where, the terrific broth medium was modified to MTB and found to support the growth of *E. coli* and the subsequent expression of GFPUV. The GFPUV fluorophore used in this work had a large amount of fluorescence approximated to 18 times brighter than the wild type GFP [20] and this made it easy to detect intracellular protein expression from a low cell concentration (2.7×10^7 cells/mL) and pure GFPUV solutions at (4.8×10^{-8} M) with the photodetector. During each fermentation run, the microbioreactor was inoculated with non-fluorescing *E. coli* at the same physiological state but not induced with IPTG to offset the influence of light scattering by the cells and the background voltage. The background and dark voltage for each measurement were subtracted from the overall photodiode response. The dark noise of the PIN photodiode PDA36 EC was very low typical of photo devices made of silicon (400-1000 nm) [21]. The transimpedance amplifier integrated on the photodiode also controlled the dark current flowing out and this minimised the effects of dark current present in the system. Alternatively, the dark noise of the sensor can be reduced by using a powerful light source, but a compromise has to be reached because too much light may photobleach the GFP or kill the cells.

The geometrical arrangement of the setup and positioning of all elements such as the photodetector, microfluidic chip, excitation light, and filters had direct influences on the measurements obtained. The reflecting mirror used for aligning the system contributed to the improved collection efficiency of the sensor as fluorescent emission light was reflected backwards, thus increasing the intensity of light reaching the detector. The photodetector was positioned directly above the microbioreactor growth chamber to maximise the detection of the emitted fluorescence.

The amount of excitation light reaching the GFPUV fluorophore trapped in the cell was maximised by placing the optic fibre tip in close proximity (2 mm) of the growth chamber of the microbioreactor (Figure 2). This optical arrangement facilitated the coupling of approximately 95% of the excitation light to excite the fluorophore without much loss through scattering and open space coupling of the light, and resulted in higher fluorescence signals. Stray light emanating from external sources and the excitation LED light of wavelengths <500 nm were efficiently filtered from reaching the detector by incorporating a thin Kodak wratten 2 optical filter (Edmund Optics, USA) between the microbioreactor chamber and the PD. This enabled the maximum isotropic fluorescence emission of GFPUV photons from the

sample to be recorded without any contribution from the excitation source. To ensure that the emitted fluorescence light was the only light incident upon the detector, ambient light was prevented from leaking into the photodiode by shielding the entire setup into a box painted black. This arrangement had the effect of reducing the electrical noise from nearby electrical components such as computers. The sensitivity of the sensor was also found to be dependent on the number of fluorescing bacterial cells, where the lowest detectable amount of GFPUV was produced by cell concentration of 2.7×10^7 cells/mL. The limit of detection was improved by coupling a lock in amplifier to the system. Moreover, the reduced path length of the microbioreactor chip also contributed to the diminished inner filter effect (IFE) in the system and maximised the excitation.

The PDMS microbioreactor was fabricated by micromilling and this had the advantage of creating a high aspect ratio structure unlike in photolithography where the height of the cell culture chamber would be restricted to the thickness of the photoresist, generally between 50-250 µm. The low aspect ratio structures would limit the volume of medium held within the device for cell viability particularly in batch fermentation. Moreover, the coring of the microbioreactor chamber, to create an open structure, allowed access to the cell culture chamber for sampling and making off-line analysis. *E.coli* cells in the growth chamber of the microbioreactor were oxygenated through the permeable PDMS membrane. The reported diffusivity of O_2 in PDMS is 3.4×10^{-5} cm^2/s [23]. Due to the high surface area to volume ratio of the LOC device large interfacial area over which O_2 diffusion can occur, was created and this was considered adequate to meet the O_2 requirements of the *E. coli* cells. Furthermore, the diameter of the microbioreactor culture well was 6.3 mm providing a volume of ~50 µL and the surface area of 28 mm^2 was large enough to accommodate a significant number of cells.

4. CONCLUSIONS

A simple integrated fluorescence detector system for microfluidic applications has been developed in several stages. The developed system was capable of detecting the concentration of pure GFPUV solutions down to 48nM, while for intracellular GFPUV the lowest detectable cell density that would give a minimum detectable fluorescence signal was 2.7×10^7 cfu/mL with a SNR = 3. The developed system allowed cell growth to be monitored non-invasively and key biological information about cellular developments such as protein expression to be

obtained in real time and using low cost techniques. The developed proof of concept fluorescence detection system could be useful in a variety of applications, including environmental, biomedical studies, drug development as well as for on-line fluorescence monitoring of reporter gene expression dynamics and cell culture studies such as in cancer cell progression. Although the system is viewed as highly sensitive, further improvements could be made by incorporating a photodiode with high spectral sensitivity such as avalanche photodiode [23] and a high power laser for exciting the fluorophore. Future work will focus on studying the chromophore cyclisation dynamics of GFPUV and optimise the production process.

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