Oligofluorene truxene laser sensor: towards bacteria growth detection

Eilidh Johnston*†, Anne-Marie Haughey*, Mark Scullion*, Alexander L Kanibolotsky‡
Peter J Skabara‡ and Nicolas Laurand†
*Fraunhofer Centre for Applied Photonics, Glasgow, UK
†Institute of Photonics, Dept of Physics, SUPA, University of Strathclyde, Glasgow, UK
‡ WestCHEM, School of Chemistry, University of Glasgow, Glasgow, UK
Email: eilidh.johnston@fraunhofer.co.uk

***Abstract*— Work toward the utilisation of an organic laser as a bacterial growth detector is presented here. The sensor used is an optically excited 2nd order DFB (distributed feedback laser) made of oligofluorene truxene. In the drive towards a practical bacterial growth detector, temperature stability and the optimum growth conditions of bacteria are challenges to be overcome. The resultant DFB laser exhibits a sensitivity of 9 nm/RIU.**

I. INTRODUCTION

Antimicrobial resistance (AMR), a growing global problem deemed critical by government and the public alike, is the focus of the 2014 Longitude Prize [1]–[3]. The problem arises from the self modification of bacteria or microbes in a manner such that current medicines used to treat them (antimicrobials) become ineffective and can no longer fight the infection. The overuse of antimicrobials fuels the rate at which AMR is occurring meaning that society will run out of effective antimicrobials faster. To consider only the human cost, deaths as a result of AMR are predicted to rise from 700, 000 to 10 million per year. One way of improving our chances in the fight against AMR is to improve the antiquated diagnostic techniques which result in uninformed antibiotic prescribing [1]. The key testing characteristics to target for improvement are reduced time and increased specificity (i.e. which antibiotic works on this specific strain of bacteria).

One promising area for updating medical diagnostics is through optical means, optical sensors already provide non-invasive, bio-compatible, high-resolution and specific solutions to various medical diagnostics with the potential to provide further impact in the field [4]–[8].

Organic materials for lasing can prove advantageous in this domain [9]–[11]. Those formed of polymers based on π-conjugated structures have low band gap energies so can be utilised in the ongoing push for high efficiency lasing [12], [13]. In addition to low thresholds, organic lasers can be made in thin films through techniques such as soft nano-patterning resulting in low cost fabrication and the construction of the polymer structure can tune the emission wavelength. When considering optically-pumped DFB lasers, as in this paper, organic materials can be easily fabricated to alter the layer thickness or grating periodicity, which again allows emission wavelength control. Their alignment can be non-critical making them easily integrated in sensor systems and have higher refractive indices than dye-doped materials which increases the chances that light will be confined within the gain layer and is advantageous for sensitivity [12]–[15]. An example of an oligofluorene truxene (T3) laser sensor has been shown by Haughey et al. [14] to detect the specific binding of avidin to a biotin functionalised surface by monitoring the wavelength (the wavelength changes when analytes binds or are in close proximity to the laser surface).

This paper looks at the potential and challenges of using an T3 DFB laser sensor to detect the growth of bacteria. Early results are reported, in particular, the stability of the emission wavelength over time and as a function of temperature when the laser is immersed in different media, including those suited for bacteria growth, is studied. These early results are critical for the future demonstration of bacteria growth. They also demonstrate the potential capability of organic lasers for other applications such as micro-thermometry in integrated optofluidic systems.

II. EXPERIMENTAL MATERIALS AND METHODOLOGY

A. Materials

To construct the DFB lasers, T3 is used as the gain layer. The laser substrate is made from an optical epoxy, Norland 65 from Norland Products. To create the grating structure, a silica master grating, originally fabricated to our specifications from MC2, Chalmers University, Sweden is imprinted into the Norland substrate. The DI water used experimentally was purified using a Milli-Q (Millipore) system. The biological related experiments utilised Lysogeny broth (L-Broth), a nutrient rich growth media for bacteria comprising of peptides, peptones, vitamins and trace elements which was supplied in liquid form. For initial testing, a non-pathogenic strain of Escherichia coli (E. coli) K-12 was also used. All chemicals were used as received, however the K-12 strain was incubated and diluted accordingly.

An in-house custom-made heated metal-well plate was used throughout measurements to accommodate the laser sensors as explained further.

B. DFB principles

The T3 DFB laser fabricated following the procedure in section II-C results in a structure consisting of three distinct layers (two of which share a grating structure), the resultant laser cross section can be seen in figure 1. The three layers

are (from top to bottom) the substrate, composed of the Norland 65; the gain layer, the T3 organic semiconductor; and the superstrate, the outward facing surface in contact with a chosen media.

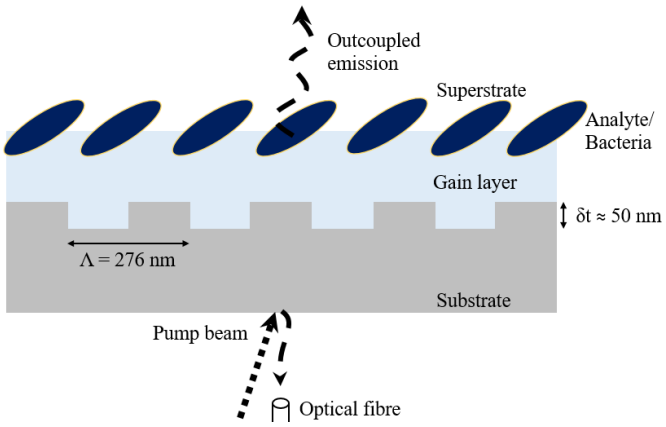

Fig. 1: A cross-sectional illustration of the 3 layers of the T3 DFB laser. Showing from top to bottom, the substrate, T3 gain layer and the superstrate (sensing) layer. The grating layer and dimensions are specified, with the pump beam entering from below and the emission output exiting in the same direction.

T3 exhibits some interesting optical properties that are utilised in our experiments. It experiences low waveguide losses when used in a film, it has an absorbance peak at 370 nm while our sensor is optically pumped within the material peak at 355 nm (pulsed). The output wavelength of our T3 laser configuration is in the region of 430 nm, T3 has a refractive index of 1.81, making it higher than both surrounding layers [9], [15]. The refractive index change between the layers provides a mechanism for total internal reflection to occur, thus creating a means of light confinement within the T3 gain layer. The grating nanostructure between the substrate and gain layers provides feedback at the second diffracted order (creating the resonance for laser oscillation) and out-coupling at the first order (emission) The lasing output from the DFB is governed by the Bragg equation, Eq.1.

$$\lambda = n_{eff}\Lambda \tag{1}$$

Our grating structure has a periodicity of 276 nm (Λ) and λ is the emission wavelength. This leaves n_{eff}, which is the effective refractive index of the laser mode. This means that any change to the refractive index of the superstrate will result in a shift in the emission wavelength, hence this is the transducing mechanism for our sensor. A red-shifted spectrum corresponds to an increased n_{eff}, while the contrary is true for a blue-shifted spectrum. Thereby changes at or very near the surface of the lasers (such as happens if an analyte binds) can be detected.

C. T3 DFB laser fabrication

The T3 lasers were fabricated by first imprinting a diffraction grating into the laser substrate [9]. The silica master grating provided the diffraction grating, this was drop-coated with Norland 65 optical epoxy and covered by a 0.1 mm thick acetate sheet cut to 15 mm x 15 mm. A UV lamp centred at 370 nm is used for 50 s to photo-cure the diffraction grating into the Norland 65 (this provides an exposure dose ≈ 300 J cm^{-2}). The cured Norland 65 on acetate was then removed from the master grating and photocured for a further 60 min. The grating substrate is then spin-coated for 90 s at 3.2 krpm with a solution of toluene and T3 (20 mg mL^{-1}) to produce a thin layer of gain medium. The gain layer thickness, influences the emission wavelength, the number of modes which can be supported in the laser and the wavelength shift per refractive index unit, The conditions stated above result in a T3 layer of ≈ 70 ± 10.0 nm thickness with >95 % of devices exhibiting an emission of a single transverse mode (TE_0) [9].

D. Experimental set-up

Figure 2 shows the optical bench-top laser set-up used to excite the DFB and collect the resultant emission. The excitation source is a Nd:YAG laser that is Q-switched and frequency-tripled, giving an output at 355 nm of 5 ns pulses at 10 Hz. The laser is mounted on the underside of the heated metal well-plate using kapton tape with the laser gain layer facing upwards, it is excited from below (i.e. approximately perpendicular to the surface) and emits in the same vertical direction. It should be noted that the alignment of the pump laser to the DFB is non-critical as long as it resides within the 5 mm^2 grating active area. The laser is photopumped by a spot size of ≈ 120 µm by ≈ 150 µm with the output emission coupled back through the substrate layer (separated from the pump path via a dichroic mirror) and collected by a fibre with a 50 µm core. The grating coupled CCD spectrometer was connected via this fibre and the selected channel provided a resolution of 0.13 nm.

E. Measurements

Using the set-up as described in section II-D, various investigations were run with differing experimental conditions to determine the output emission characteristics of the T3 DFB. Experiments were either run by considering the output spectra with respect to time or temperature. The pump energy was kept constant at ≈ 3 times the threshold. Since the laser is attached such that it seals a well, this well can be filled with the required substance under test (see figure 3). Tests were run with the laser in contact with air; DI water; L-broth and L-broth spiked with K-12 E. coli. The last main variable investigated was the activation of the heater, to provide a heated condition within the well, the heater was either considered as 'on' or 'off'. At each time/temperature interval, a full spectral output as collected by the spectrometer was recorded. Once all data was collected, it is analysed through a MATLAB script to output the spectra and identify the location of the peak and thus determine any shift in wavelength over the operating conditions.

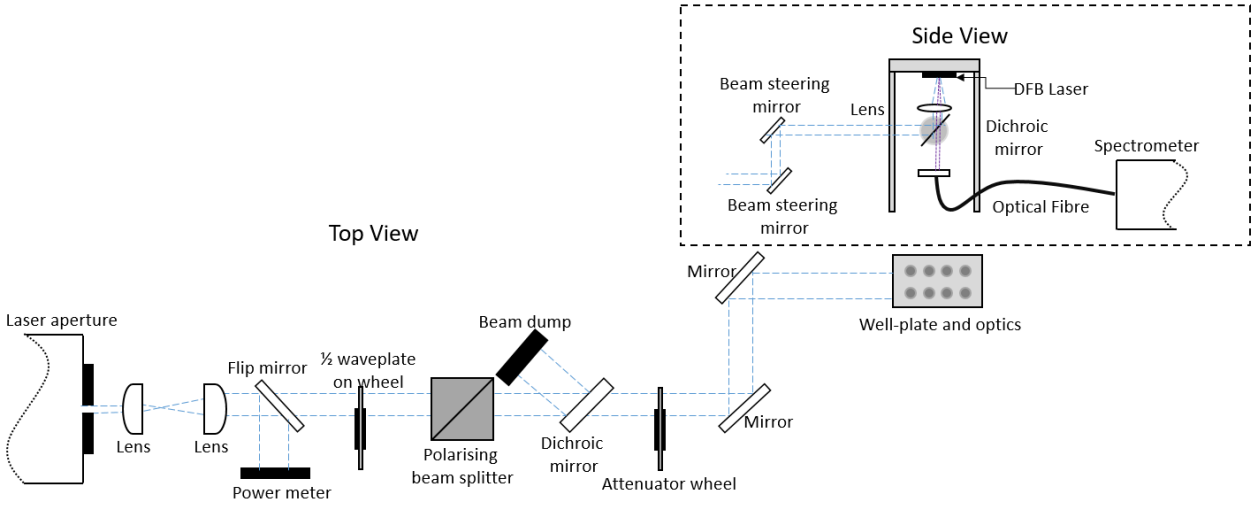

Fig. 2: Schematic view of excitation laser set-up, both top and side views where appropriate. The pump beam is a 355 nm pulsed source with a 10 Hz repetition rate and 5 ns pulse duration. There are two methods to attenuate the beam, either by an ND filter in attenuation wheel or a via 1/2 waveplate. The beam is then directed across an optical bench to a tower where the DFB laser is excited and emission collected (by a grating coupled channel on a spectrometer with 0.13 nm resolution) in the same direction.

III. RESULTS AND DISCUSSIONS

A. Basic threshold and spectra

The spectral output of one of the T3 lasers at room temperature with air as the superstrate is shown in figure 4. This is representative of most of the DFBs in air, however the peak wavelength can change by ≈ 10 nm across the face of the 5 mm^2 active region, with some locations allowing multiple laser modes to oscillate. This is due to imperfections in the spin coating/potential defects on the grating. By improving the fabrication process undertaken in section II-C, such changes across the laser will be minimised in future work. As shown in figure 2, the set-up allows for attenuation of the pump laser, through this feature, the lasing threshold of the T3 DFBs has been determined to be ≈44 nJ, which corresponds to a fluence of ≈ 77 μJ cm^{-2}.

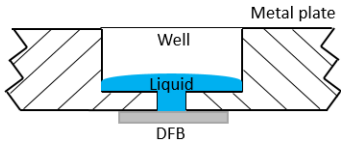

Fig. 3: Cross section of well-plate set-up showing the location of the DFB attached to the bottom surface and placement of the liquid in relation to it. Sensing occurs on the liquid interface with the DFB and the excitation and emission are both through the bottom substrate surface of the DFB.

B. Effects of temperature and L-broth immersion

Experiments, as mentioned in section II-E were undertaken to measure the T3 lasers' response to various superstrate conditions and thus establish whether the T3 laser can be utilised for bacteria growth detection. In order to aid bacterial growth, a growth media called L-broth was used to supply key nutrients to the bacteria. The presence of L-broth (see section II-A) was investigated over time to see if the L-broth affected the spectral output of the DFB. For

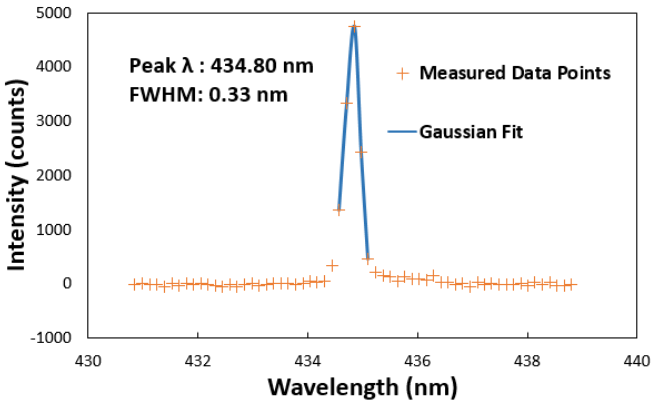

Fig. 4: T3 laser spectra recorded at room temperature with an air superstrate. The peak wavelength and FWHM are also shown.

each time interval, a full spectra was recorded, a Gaussian fit performed and the peak central wavelength determined. Thus, the wavelength shift over time of the DFB output in the presence of L-broth was calculated. One such plot for L-broth at room temperature is shown in figure 5a, where the emission wavelength is stable over time (spectrometer resolution of 0.13 nm).

During the experiments, the spectra was recorded prior to liquid addition to determine the wavelength shift between air and the liquid, the sample was given >10 min to reach the temperature of the metal well before any measurements taken. A clear demonstration of the resulting wavelength shift is shown in figure 5b. An initial red-shift of 3 nm occurs due to the immersion in L-broth, this is equivalent to a refractive index change of ≈ 0.33, thus the sensor has a bulk sensitivity in the region of 9 nm/RIU (refractive index unit) in that range. This red-shift is followed by a slight blue-shift while the liquid reaches the temperature of the well, which is then followed by a stable emission wavelength - a stable output

for the growth media is preferential to facilitate bacterial growth detection from the background.

To improve the growth conditions for the bacteria, it is beneficial to have the growth media at 37 °C, or as close as possible, for this reason a heater was installed to the metal well-plate. Experiments were repeated with the heater switched on (the temperature of the well measured 32 °C), the results are shown in figure 5c. The trend of the output wavelength is a clear blue-shift. Further experiments were then undertaken to understand the temperature response of the DFB, Figure 5d shows one such result of the wavelength shift seen as the temperature increases (air superstrate). The temperature sensitivity of the lasing mode ≈ -0.04 nm/K is caused by the thermo-optical coefficient of the gain material, T3.

The same tests were carried out with DI water, it was noted that larger blue-shifts occurred in heated liquids than with an air superstrate. The exact origin of the additional blue-shift (engineering or physical issues) are still under investigation, for example the liquid heating up considerably more than the surrounding metal, or loss of gain material through 'washing' of the top surface (this would also result in a blue-shift). The latter reason has been largely ruled out due to drying and re-lasing of the DFB to obtain emission similar to the original central peak wavelength.

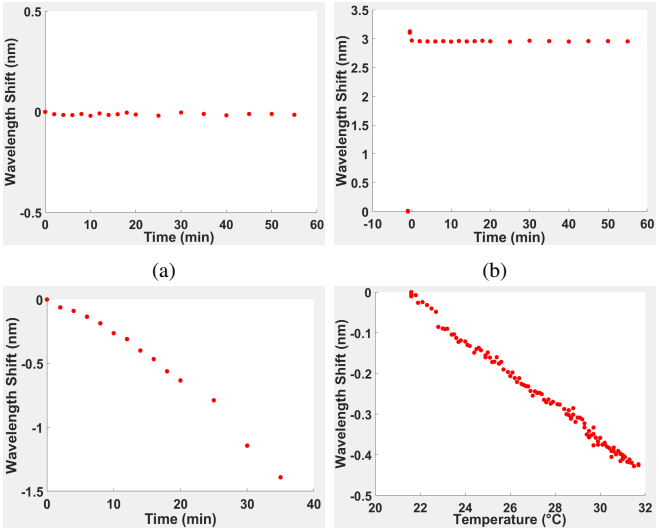

Fig. 5: The emission wavelength shift against time/temperature for the T3 laser for a substrate of a) L-broth at room temperature, b) L-broth at room temperature where the addition of L-broth can be seen and then the L-broth stabilising to the metal well temperature, c) L-broth in the presence of the heater (≈ 32 °C) and d) air during the process of the metal well-plate heating up.

IV. CONCLUSIONS

A sensitivity of 9 nm/RIU has been achieved by a T3 DFB laser, with stable operation when immersed in L-broth at room temperature. The system has a temperature dependence of the lasing mode of ≈ -0.04 nm/K due to the thermo-optical coefficient of the T3 gain layer. It is clear from initial results that there are numerous challenges to overcome in the development of an oligofluorene truxene laser sensor for the detection of bacteria growth, as to be expected. Currently any red-shift as a result of bacteria growth would be masked by the as yet unexplained blue-shifting response due to the heated liquids. Further challenges will be faced when ensuring adhesion and growth of the bacteria on the T3 laser surface. While bacterial growth has yet to be detected, the work performed here shows promising results for future detection and highlights areas to continue investigations.

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Supporting data (dataset) can be found at: https://doi.org/10.15129/d3d48a4a-c040-4e18-b19f-95c1108eb8b3

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