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DOI:

[10.1109/TIM.2021.3082276](https://doi.org/10.1109/TIM.2021.3082276)

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Document Version

Peer reviewed version

Citation for published version (Harvard):

Goddard, NJ & Gupta, R 2021, '3-D printed analytical platform for automation of fluid manipulation applied to leaky waveguide biosensors', *IEEE Transactions on Instrumentation and Measurement*, vol. 70, 7502612 .
<https://doi.org/10.1109/TIM.2021.3082276>

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3D Printed Analytical Platform for Automation of Fluid Manipulation Applied to Leaky Waveguide Biosensors

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Keywords: Automation, 3D printing, additive manufacturing, autosampler, peristaltic pump, leaky waveguide.

Abstract

Automation of fluid manipulation is needed to increase throughput and reproducibility of experiments while reducing the time spent by researchers on performing repetitive tasks. Current solutions are however bulky and expensive, and hence not suited to enable automation in academic laboratories. This work therefore reports a low cost, modular and programmable analytical platform comprising of 3D printed autosampler and peristaltic pump with footprints of 248 mm by 243 mm and 104 mm by 112 mm, respectively. The autosampler consisted of four samples probes/needles that can be driven independently to any of the available solution vials placed along the circumference of a circle. The 3D printed autosampler developed in this work can access higher number of solution vials independently and is lower cost, but accommodates fewer number of total solution vials, than commercial devices. The autosampler and peristaltic pump were applied to determine (1) refractive index sensitivity, (2) porosity, and (3) sensing capability of leaky waveguides (LWs) where the read-out instrument was also 3D printed. We demonstrated that the automation of fluids enabled by the analytical platform improved the accuracy of results by separating the effect of temperature drifts from analyte solutions from the output of LWs. This work shows that 3D printed instrumentation can provide results comparable with standard laboratory versions at much lower cost, making it ideal for use in resource-limited settings, and with the possibility of customization as experimental needs change.

I. Introduction

Most academic laboratories are currently heavily reliant on manual manipulation of fluids. A limited number of variables can therefore be tested in selected timeframes, which in turn slows down experimental progress. The situation has been worsened by the current SARS-CoV-2 outbreak, where social distancing measures mean that far fewer number of researchers can work simultaneously in laboratories than before the pandemic [1]. In addition, operations performed manually are subject to operator error and fatigue, which in turn reduces experimental accuracy and reproducibility. The repetitive sequences of steps involved in fluid manipulation (i.e., change fluid vials, start/stop pumps) often take up a large majority of researchers' time. As a result, researchers are left with minimal time for critical thinking and performing high-skilled tasks. Automation of fluid manipulation can therefore significantly improve throughput and reproducibility of

experiments, and allow researchers to focus on more complex thinking tasks [2, 3]. Large high-tech robotic solutions are, however, not appropriate for automation in academic laboratories, which often operate under constrained financial and spatial resources [4]. This implies low-cost, modular, and customisable solutions are required to enable automation in academic laboratories. The realisation of such solutions has now been made feasible with the widespread availability of 3D printing (or additive manufacturing) methods [5, 6]. 3D printing provides benefits for constructing analytical instrumentation, but also has significant drawbacks. The benefits of additive manufacturing methods are that arbitrary 3D shapes can be fabricated, which would be difficult or impossible to produce by subtractive methods, new designs can be fabricated and tested very rapidly, and 3D printers are reducing in cost and increasing in functionality very rapidly. Drawbacks of 3D printing are that fused filament fabrication (FFF), as used in this work, has limited resolution and a relatively limited range of materials. In addition, as shown by our experience, the use of thermoplastics can significantly limit the maximum working temperature of the instrumentation. Other 3D printing techniques can overcome these limitations, but generally are much more expensive. For example, metal parts can be fabricated by selective laser sintering, but the printers are considerably more expensive than FFF printers. Stereolithography has significant potential for fabricating plastic parts from photopolymers, but low-cost printers are very limited in their build volume and, for example, would not be able to fabricate the autosampler in one piece. The advantages of stereolithography are the high resolution (currently 37 μm using a 4K LCD panel) and the wider working temperature range of the resulting cross-linked photopolymers.

The key components of automated fluid manipulation systems are pumps and autosamplers. Pumps, particularly peristaltic pumps, are widely used in the development of biosensors and cell cultures because they allow recirculating flow of reagents and delivery of large volumes of buffers required for immobilisation and washing steps, respectively [7]. Peristaltic pumps also reduce the risk of cross contamination by non-contact delivery of solutions [8]. Peristaltic pumps typically consist of a motor-driven rotor with three (or more) arms each with a roller that compresses the pump tubing to drive fluids. The rotor typically is driven by a DC or stepper motor controlled by a microcontroller. The speed of the stepper motor and diameter of tubing used determine the flowrate [9]. Peristaltic pumps made of 3D printed gearboxes/spinners along with housings to accommodate electronic and mechanical components have been reported in literature [9, 10]. Some limitations of the 3D printed peristaltic pumps reported so far include rapid wear of polymeric gearboxes and highly pulsating flow particularly in case of three arm spinners.

So far, there have been no reports of 3D printed autosamplers. In many currently available autosamplers, each sample probe/needle is driven by three orthogonal linear motion stages to obtain translational freedom in all three dimensions [11]. Such autosamplers can be used to access large number of vials, but are expensive and have large footprints. Equally, current autosamplers have only up to two sample probes/needles, but these probes/needles are often not driven independently. As a result, the implementation of specific sequences of fluid manipulation may require operators to make careful decisions on the relative positions of vials. Equally, multiple vials containing same fluids may be needed which in turn can increase the volumes of sample/reagent solutions required to implement some sequences of fluid manipulation.

This work for the first time reports a 3D printed autosampler that provides four sampling needles that can be driven independently to any of the available solution vials placed along the circumference of a circle. Two versions of the autosampler have been constructed to accommodate different size vials and the basic design can easily be modified to change the number and size of vials. Table 1 gives the number and size of vials for each version of the 3D printed autosampler. Version 1 of the autosampler, which was used in this work, can accommodate 2×100 mL, 13×7.5-25 mL, and 1×2.5-10 mL vials simultaneously. The control system has eight stepper motor drivers, five of which are used to control the autosampler, leaving three to drive external 3D printed peristaltic (or syringe) pumps.

Version	Vial size		
	100 mL, 56 mm diameter	7.5 - 25 mL, 23 mm diameter	2.5 - 10 mL, 17 mm diameter
1	2	13	1
2	2	5	11

Table 1: Number and size of vials for the two versions of the autosampler

A comparison of the 3D printed autosampler reported herein and existing, but not 3D printed, autosamplers is provided in Table 2. As shown in Table 2, the primary benefit of the autosampler reported in this work is its low cost, making it more widely available for use in resource-limited settings. Towards this end, the reported 3D printed autosampler and peristaltic pump reported in this work have been made available as open source. Furthermore, the 3D printed autosampler developed in this work can access higher number of solution vials independently than commercial vials and hence is well-suited to carry out the complex sequences of operations required for the development of biosensors, for which commercial autosamplers are not an optimal solution.

Autosampler application	Type of motion	Typical footprint	Typical total number of 20 mL vials	Vials that can be accessed independently	Sampling type	Typical costs (£)
Chromatography and mass spectrometry [12, 13]	Sample vials or sample needles undertake linear movements in orthogonal directions	600 mm × 800 mm	200-300	2	Single shot, variable sample volume	Tens of thousands
Titration [14, 15]	Sample vials rotate around a circle. Sample needle moves radially or pivots around a point and up and down	250 mm × 390 mm	20-100	1	Single shot or continuous flow, variable sample volume	
This work – biosensors	Sample vials are fixed while sample needles rotate around a circle and move up and down	248 mm × 243 mm	18	4	Continuous flow, variable sample volume	<£1000

Table 2: Comparison of herein reported and existing autosamplers

Furthermore, this work demonstrates a complete analytical platform comprising of a 3D printed autosampler, peristaltic pump, and read-out instrument to determine two key parameters of leaky waveguide (LW) biosensors [16]. In LWs, light waves are partially confined in hydrogel films deposited on glass substrates. These light waves can sense changes in refractive index (RI) on/in hydrogel films, which can be correlated to the concentration of analytes. The minimum analyte concentration i.e., limit of detection (LOD) is determined by the refractive index sensitivity (RIS) of LWs and the porosity of the hydrogel films to analytes of different molecular weights [17, 18]. We therefore applied the 3D printed analytical platform to determine the RIS of LWs and porosity of hydrogel films. We also applied the 3D printed analytical platform to demonstrate sensing of exemplar analytes, streptavidin, biotin protein A and immunoglobulin G (IgG), using LWs. Beyond this, the 3D printed autosampler and peristaltic pump developed in this work can be applied to automate fluid manipulation of for instance, microfluidic cell culture systems [19, 20].

II. Experimental

Chemicals and materials: Ethanol, 1 M acetic acid, methanol, 25% (v:v) glutaraldehyde (GA), poly(ethylene glycol) (PEG) (Mw: 10, 100 and 300 kDa), (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES), 1 M sodium hydroxide (NaOH), anhydrous dimethyl sulfoxide (DMSO), biotin protein A (BPA), immunoglobulin G (IgG), and 10 mM phosphate buffered saline (PBS) of pH 7.4 were bought from Sigma-Aldrich (Gillingham, UK). Decon 90, glycerol (Mw: 92) and chitosan (Mw: 100,000-300,000, 90% deacetylated) were purchased from Fisher Scientific (Loughborough, UK). Streptavidin (2-0203-100-IBA) was purchased from Stratech Scientific (Ely, UK). Microscope glass slides and NHS-(PEG)₁₂-Biotin were bought from VWR (Leicestershire, UK). Finally, the bootlace ferrules (211-4252) that formed the fluidic inlet/outlet were purchased from RS Components (Corby, UK) and O-rings (01-08-01901, 19 mm internal diameter, 1 mm wall thickness) made of nitrile rubber were bought from Ashton Seals (Barnsley, UK).

Fabrication of leaky waveguides (LWs) and microfluidic flowcells: Glass slides were cut into ~25.4×25.4 mm squares, and cleaned in Decon 90, de-ionised water and ethanol for 30 minutes each. Chitosan was purified using the procedure described previously [21], dissolved in 0.1 M acetic acid with magnetic stirring for 18 h to form 1% (w:v) solution. ~100 µl of the chitosan solution was dispensed onto a glass square and spun at a speed of 900 rpm with an acceleration of 100 rpm/s for 30 s. The slide was placed in an incubator at humidity of 75-80% and temperature of 25 °C for 3 minutes. 0.03125% (v:v) GA solution was prepared by diluting 25 µl of 25% (v:v) GA stock solution to 20 ml using 100 mM HEPES buffer, pH 7.4. Subsequently, the slides were immersed in a 0.03125% (v:v) GA solution in 100 mM HEPES buffer, pH 7.4 for 10 minutes to crosslink the chitosan film. The slides were washed in 100 mM HEPES buffer, pH 7.4. Chitosan films were stored wet in 100 mM HEPES buffer, pH 7.4 until being used.

A flowcell was made by CNC machining 3 mm thick black PMMA forming a recessed circular cavity of diameter of 18 mm and 0.2 mm deep surrounded by a groove 1 mm wide and 0.75 mm deep in which an O-ring was mounted. The plate was placed on the waveguide and held in place using a 3D printed fixture.

Alternatively, a two-channel flowcell was used to do the work on protein sensing. The two-channel flowcell was also made by CNC machining 3 mm thick black PMMA forming 8.3 mm long, 1.9 mm wide, and 0.2 mm deep recessed cavities. Each cavity was surrounded by a groove 1 mm wide and 0.75 mm deep in which an O-ring was mounted, and the two channels were separated by 2 mm.

Read-out instrument: The read-out instrument for LWs has been described previously [22] and consisted of structures and case made by 3D printing of carbon fibre-filled-polylactic acid (CF-PLA) and polylactic acid (PLA), respectively. The structures made of CF-PLA were used to hold optical, mechanical, and electronic components. Figure 1 shows the optical components present in the instrument and their respective positions.

Figure 1: 3D printed read-out instrument for LWs (adapted from [22])

The light produced by a LED with peak wavelength of 650 nm was collimated, transverse electric (TE)- polarised, and ultimately passed through cylindrical lens to obtain a wedge-shaped beam with an apex angle (marked as α in Figure 1) of 12.8° and lowest angle of incidence (marked as θ in Figure 1) of 58.6° . The equilateral prism had square faces of $38.1 \text{ mm} \times 38.1 \text{ mm}$ and was used to couple light into the LW. The light reflected from each point along the width of the channel on the flowcell mounted on the LW was recorded on a camera. 2D reflectivity curves where y-axis and z-axis represented angle of incidence and channel width, respectively were therefore obtained. The camera can be moved in the z-direction by $\pm 4 \text{ mm}$ and had an imaging area of $6.440 \text{ mm} \times 4.616 \text{ mm}$ with the area of each pixel being $1.67 \mu\text{m} \times 1.67 \mu\text{m}$. The path of the reflected light was folded using a mirror that can be rotated so that the range of angles falling on the camera can be selected. The LED, an assembly of gear and motor attached to the rotatable mirror, and a linear motor attached to the camera were controlled using a PCB present in the instrument, and the camera was interfaced with computer using USB2. The PCB was computer controlled by a software written using C++ builder. The software was also used for capturing and real-time processing of 2D reflectivity curves to display a chart of shift in resonance angle of the selected region of the channel of the flowcell mounted on the LW *versus* time.

III. Methodology

Design and construction of peristaltic pump and autosampler: The 3D printed peristaltic pump and autosampler developed in this work are discussed below.

Peristaltic pump: The peristaltic pump was assembled from four 3D printed parts: the main body, the upper and lower halves of the rotor and the arm to compress the tubing onto the rotor as shown in Figure 2 (a). The main body was printed from black PLA using a Flashforge Creator Pro (Zhejiang Flashforge 3D Technology Co. Ltd, Jinhua, China) 3D printer, while the rotor and arm were printed in clear resin (Anycubic Clear, Anycubic, Shenzhen, China) using an Anycubic Photon 3D printer. To increase the lifetime of the rotor assembly, the part contacting the tubing was the stainless-steel exterior circumference of ball bearings of 12 mm outside diameter, 4 mm inside diameter and race width of 4 mm (S604ZZ, RobotDigg, Shanghai, China). Two bearings were stacked to form a roller 8 mm high, held in place by an M3 thread slotted pan head shoulder screw (SFH-M3-8-A1, Accu Ltd, Huddersfield, UK). Ten of these rollers were sandwiched between the upper and lower rotor halves using the shoulder screws to hold together the complete assembly. The motor consisted of a NEMA 17 stepper motor with an attached 14:1 planetary gearbox (17HS3001-CF14X, Robotdigg). The main body was held on the motor using M3 screws, after which the rotor assembly was attached to the motor spindle using an M4 grub screw. The arm was attached to the main body using an M6 cap head screw as a pivot and used an M6 cap head screw and nut to allow the tubing to be compressed against the rollers.

Figure 2: 3D printed (a) peristaltic pump and (b) autosampler

The speed of the motor and hence the fluidic flow rate was controlled by the stepper motor drivers located on the control board, which was placed at the back of the autosampler. The stepper motor was driven using 16× microstepping to generate very smooth rotation of the pump rotor. The mass flow rate was determined by using pump at a selected step rate to transport water from a vial to a weighing scale (Accuweight 255) and measuring the mass of de-ionised water collected after 10 min of pump operation. The mass was then divided by the density of de-ionised water to determine the corresponding volumetric flow rate.

The relationship between volumetric flow rate (V , $\mu\text{l min}^{-1}$) and pump microstepping rate (P , Hz) is given by $V = 0.257P$ with $r^2 = 0.996$. To maintain compatibility of flow rates with previous work, the step rates used were considerably lower than the theoretical maximum of $\sim 16,000$ microsteps s^{-1} ($\sim 4 \text{ mL min}^{-1}$). A commercial laboratory peristaltic pump (Gilson Minipuls 3) has a rotor rotation rate of between 0.01 and 48 rpm, whereas the pump described here has a rotation rate between 0.02 and ~ 43.7 rpm. The lower bound on the rotation rate is governed by the timers on the microcontroller while the upper bound is governed by the maximum step rate of the stepper motor.

Autosampler: For a two-channel optical biosensor, we require a sampling needle for each channel, plus a return needle to allow recirculation of expensive materials such as antibodies. This means that we need four independently controlled needles plus at least a two-channel peristaltic pump. Initially, we considered orthogonal linear motion, with four linear stages on one axis to allow independent sampling, but this meant that only vials on that axis could be sampled independently. As with conventional autosamplers, this would mean that in some cases samples would have to be duplicated. To overcome this issue, rotary motion of the sampling needles was chosen. As shown in Figure 2 (b), each sample needle was attached to a 3D printed arm, which in turn was driven by a pancake stepper motor (36HM12-0504, Robotdigg). Four stepper motor/arm assemblies were stacked vertically at 25 mm spacing and attached to a plate that could be moved along the Z axis by another stepper motor with a directly attached 8 mm pitch leadscrew (17HS3001-150N, Robotdigg). Optical interrupters (Optek OPB390T51Z) were used to determine when the arms and Z axis were at a safe home position. Two versions of the arms were fabricated. The first version used 1.5 mm OD, 0.5 mm ID stainless steel tubing (ChenXi Shop on Amazon.co.uk) for the needles with 2.0 mm OD, 0.5 mm ID silicone rubber tubing (Sourcing Map shop on Amazon.co.uk) attached to act as the peristaltic pump tubing. The second version used 2 mm OD, 1.6 mm ID stainless steel tubing (TuToy shop on Amazon.co.uk) for the needle with 1.5 mm OD, 0.5 mm ID silicone rubber tubing (Sourcing Map shop on Amazon.co.uk) threaded through to the end of the stainless-steel tubing. This allowed a single run of tubing from the autosampler through the pump to the flowcell to avoid connections that could introduce bubbles.

A control board with an ARM microcontroller (STM32F091VCT6, ST Microelectronics, Geneva, Switzerland), eight microstepping stepper motor drivers (DRV8886PWP, Texas Instruments Inc, Texas, USA), eight optical interrupter inputs, a USB 2.0 interface (FT230XS, FTDI Ltd, Glasgow, UK), and analogue and digital interfaces to a Gilson Minipuls peristaltic pump was designed and fabricated. A block diagram of the electronics on the 3D printed autosampler is provided in Figure 3. Five of the stepper motor drivers and optical interrupters were used to control the movement of the four sampling arms and the Z axis, and determine their safe home position, respectively. The remaining three stepper motor drivers were used to control peristaltic or syringe pumps. The Gilson Minipuls digital interface was not used for the work described herein. The Gilson analogue interface provided two open collector transistor outputs to stop/start and control the pump direction with an analogue output from a digital-to-analogue converter on the microcontroller to set the pump rotation rate. The Gilson digital interface used an RS-485 driver to provide asynchronous serial control of the pump. The step rate of the stepper motor drivers was set using a separate 16-bit timer for each driver, clocked at 1

MHz to allow (micro)step rates between 1 MHz and 15.26 Hz. The DRV8886PWP driver allows full stepping as well as half, quarter, eighth and sixteenth step microstepping. For the autosampler, sixteenth microstepping was selected. The driver also allowed the stepper current to be reduced to half its full value to maintain the stepper position but reduce the power dissipation in the motor to one quarter of the full value. This reduced motor heating, and was activated whenever the motor was stationary. This was necessary when using PLA for the arms, as continuous full power resulted in the motor temperature rising above the glass transition temperature of PLA (58 °C [23]) causing the arms to distort. The optical interrupter LEDs were controlled by a driver chip (TLC5922DAP, Texas Instruments Inc.) that allowed the LED current to be set to any value between 0 and 20 mA with $\sim 80 \mu\text{A}$ resolution. This facility permitted compensation for variations in the current transfer ratio of the optical interrupters. The optical interrupter phototransistor output current was converted to a voltage using 3.3 k Ω resistors and digitized to 12-bit resolution using the analogue-to-digital converter (ADC) of the microcontroller. The autosampler and peristaltic pump were powered by a 24 V, 3 A external power supply. The instantaneous power consumption of the entire system varied considerably depending on whether the stepper motors were active or not. The stepper motors driving the arms have a peak power consumption when stepping of 6W, which reduces to 1.5 W in reduced torque mode. The Z-stage stepper motor has a power consumption when stepping of 7.6 W, reducing to 1.9 W when stationary. The peristaltic pump stepper motor has a continuous power consumption of 3.4 W and never enters low torque mode. The measured power consumption was 23.04 W when moving the Z stage with the arm stepper motors in high torque mode and a single peristaltic pump connected, 6.72 W in low torque mode and the peristaltic pump disabled and 11.52 W in low torque mode with the pump stepping at 500 steps per second. Typical sample delivery times are in the range 600 – 1800 seconds, while movement to a new sample takes about 10 seconds, so the arms and Z-stepper are in reduced torque mode for most of the time.

Figure 3: Block diagram of electronics on 3D printed autosampler

Software: The code for the autosampler and pump was written in C using the freely downloadable ST Microelectronics STM32CubeIDE integrated development environment. This package provides drivers and generates initialisation code for all the peripherals used in a project. Because of the simplicity of the software, no real-time operating system (RTOS) was used; interrupt service routines for the timers were used to

determine if a step was required for the associated stepper motor. Direct memory access was used to transfer ADC conversions to memory, after which an end of transfer interrupt service routine unpacked the ADC data for each interrupter and applied a threshold to determine if the interrupter was blocked or not.

Code for the PC controlling the autosampler and pump was written in C++ using the free C++Builder integrated development environment community edition (Embarcadero Inc, Texas, USA). The Visual Components Library was used to create the autosampler control window. The autosampler program consists of up to 100 steps, each of which has a set time, a set position for each arm and an enable, direction, and speed for each of the five possible peristaltic pumps. The autosampler program can be aborted at any time, which causes all the pumps immediately to stop and the Z stage to retract to the safe position. Autosampler programs could be saved and recalled as text files.

The code for controlling the 3D printed LW read-out instrument has been described previously [22]. The software was used to control the position of the camera/mirror and LED current. The software also allowed time capturing of images from the camera and real-time processing of images to determine the position of the resonance angles, which were displayed in a chart recorder-like graph and saved on a disk in comma separated values (CSV) format.

IV. Results and Discussion

Peristaltic pump and autosampler: Table 3 provide the volumetric flow rate (V) in $\mu\text{l min}^{-1}$ for different pump step rate (P) in Hz and rotor rotation rate in rpm. The measurement of the volumetric flow rates was repeated 3 times, and as shown in Table 3, the errors were $< 3.6\%$.

The relationship between volumetric flow rate (V , $\mu\text{l min}^{-1}$) and pump microstepping rate (P , Hz) was given by $V = 0.247P$ with $r^2 = 0.9992$ and $V = 0.264P$ with $r^2 = 1$ for the bottom and top channels, respectively. To maintain compatibility of flow rates with previous work, the step rates used were considerably lower than the theoretical maximum of $\sim 16,000$ microsteps s^{-1} (~ 4 mL min^{-1}). A commercial laboratory peristaltic pump (Gilson Minipuls 3) has a rotor rotation rate of between 0.01 and 48 rpm, whereas the pump described here has a rotation rate between 0.02 and ~ 43.7 rpm. The lower bound on the rotation rate is governed by the timers on the microcontroller while the upper bound is governed by the maximum step rate of the stepper motor.

Pump step rate, P (Hz)	Rotor rotation rate (rpm)	Volumetric flow rate, V ($\mu\text{l min}^{-1}$)	
		Bottom channel	Top channel
0	0	0.0	0.0
100	0.137	25.4 ± 2.8	26.0 ± 0.5
200	0.273	50.8 ± 0.8	53.1 ± 0.9
300	0.410	77.1 ± 2.8	79.3 ± 1.0
400	0.546	100.5 ± 3.6	105.7 ± 0.5
500	0.683	120.0 ± 1.6	131.4 ± 0.6

Table 3: Measured volumetric flow rate for different step rates and rotation rate of the rotor of the 3D printed peristaltic pump (where the measurements were repeated 3 times)

Typical experiments required continuous use of the 3D printed autosampler and peristaltic pump for 12-16 h, but for most of this time the autosampler was not moving so was in its low power consumption state.

Experiments were performed during daytime and overnight in a room with typical temperatures between 17 and 22 °C. The experiments were performed over several consecutive days and no difference in the performance of the 3D printed autosampler and peristaltic pump were observed over two months (i.e., the total duration of this work). The forced failure was performed by running all the four stepper motors driving the arms of the autosampler at a peak power consumption of 6W per motor for 4-5 h. We observed that the overheating of the stepper motors driving the sampling arms caused the stepper motor supports and arms to soften and deform to the point at which they could no longer move. The softening of the motor supports, and arms was because the glass transition temperature of the material used to make the 3D printed parts i.e., PLA is 58 °C [23]. Similarly, the 3D printed instrumentation could not be used at very high ambient temperatures because of the low glass transition temperature of PLA. Nevertheless, the instrumentation will work over a much wider range of temperatures, pressures, and humidities than the human operators can tolerate. Naturally, if a printed part fails it is very simple to print a replacement.

Application of the analytical platform for LW studies: Figure 4 shows a picture of the complete analytical platform where the footprints of the 3D printed autosampler and peristaltic pump developed in this work were 248 mm by 243 mm and 104 mm by 112 mm, respectively.

Figure 4: Analytical platform comprising of 3D printed autosampler, peristaltic pump, and LW read-out instrument

Figure 5 (a) shows a typical 2D reflectivity profile of LWs with chitosan films deposited on glass substrates where the angular positions of total internal reflection (TIR) and resonance are marked. The corresponding 1D reflectivity profile, which plots reflectivity at a selected distance *versus* angle of incidence, is provided in Figure 5 (b). As discussed previously [24], exponentially decaying interference fringes are observed at resonance angles of LWs with waveguide layer made of low refractive index (RI) materials, and can be seen in the 2D and 1D reflectivity curves. This approach of observing resonance angles of LWs as

exponentially decaying interference fringes avoids the need to attach dye molecules to hydrogel waveguides [25] and/or deposition of metal (typically, titanium and gold) layers in between hydrogel waveguides and glass substrates [26, 27], respectively. While the use of dye molecules increases non-specific adsorption of interferents in samples, the fabrication of metal layers requires the use of a vacuum deposition system.

As shown in Figures 5 (a) and (b), the TIR and resonance angles (based on the position of the dip in the reflectivity curves) were observed at 62.354° and 62.596° , respectively. The smaller the separation between the TIR and resonance angles, the lower the RI of the chitosan film. The position of the resonance angle was $62.579 \pm 0.033^\circ$ over the full camera width of 4.616 mm (see Figure 5 (a)), suggesting that the thickness of chitosan films was reasonably uniform.

Figure 5: (a) 2D and (b) 1D reflectivity profiles of a LW with chitosan waveguide on glass substrate, and (c) shift in resonance angle for different concentrations of glycerol solutions after correcting for drift in the baseline (inset is a plot of resonance angle *versus* RI of glycerol solutions and error bars represent variations over a distance of 4.616 mm)

Refractive index sensitivity (RIS): The position of resonance angles is affected by the RI of solutions on/in hydrogel (in this case, chitosan) films. A summary of RI, which was measured using RFM900-T refractometer (Bellingham and Stanley, Kent, UK) with an accuracy of $\pm 1 \times 10^{-5}$, of different concentrations of glycerol solutions is provided in Table 4.

Glycerol concentration % (v:v)	Refractive index
0.125	1.335605
0.250	1.335787
0.500	1.336120
1.000	1.336768

Table 4: Refractive index of different concentrations of glycerol solutions prepared in PBS

The 3D printed autosampler and pump were programmed sequentially to introduce different concentrations of glycerol solutions with intermediate de-ionised water in the flowcell mounted on top of the

LW. The arm of the autosampler was programmed to spend 10 min in each vial and the total duration of the programme was 180 min. As different solutions were sequentially introduced, shifts in resonance angle were determined by monitoring the position of the dip in the reflectivity curve *via* the 3D printed read-out instrument (see Figure 5 (c)). The analytical platform manipulated the solutions and recorded shifts in resonance angles overnight to confirm “lights-out” operation. Subsequently, as shown in the inset in Figure 5 (c), the shift in resonance angle *versus* refractive index was plotted. The equations for best lines for shifts in resonance angle ($\Delta\theta_{R,Gly}$) and refractive index (n_{Gly}) when high to low and low to high concentrations of glycerol solutions were pumped are given by $\Delta\theta_{R,Gly} = 172.3 \times n_{Gly} - 230.0$ and $\Delta\theta_{R,Gly} = 171.9 \times n_{Gly} - 229.5$, respectively. This implies RIS of chitosan LW tested in this work, which is equal to the slope of the best fit line, for the cases when high to low and low to high concentrations of glycerol solutions were pumped were 172.3 ± 4.2 ° RIU⁻¹ and 171.9 ± 3.4 ° RIU⁻¹, respectively.

Like all RI sensors, resonance angles of LWs shifts because of drifts in ambient temperature over the duration of experiments. It is therefore essential to separate shifts in resonance angles because of drifts in temperature and for instance, glycerol solutions. This in turn requires that de-ionised water/buffer is introduced in the flowcell on top of LWs at regular intervals during the duration of experiments and record the corresponding shifts in resonance angles. In cases when solution vials were changed manually, de-ionised water/buffer was only introduced at the start and end of experiments, which made it difficult to accurately determine the trend of the drift (e.g., linear *versus* exponential) and the corresponding best fit curve. By automating experiments with the use of the analytical platform reported in this work, intermediate water samples were introduced between each glycerol sample, allowing the baseline change to be more accurately determined. Figure 6 shows the baseline drift for daytime and overnight autosampler runs. The daytime run shows a negative trend while the overnight run shows a positive trend in the resonance angles. Since there was no temperature control in these experiments, the overnight trend is associated with decreasing temperature and the daytime trend is a result of increasing temperature.

Figure 6: Shift in resonance angle for de-ionised water over the duration of two experiments (where scatter shows the experimental data and lines show the best fit curves)

Exemplar protein sensing: We subsequently demonstrated the applicability of the analytical platform for sensing exemplar proteins, streptavidin, biotin-protein A (BPA) and immunoglobulin G (IgG).

A two-channel flowcell was used to perform protein sensing where one of the channels acted as sample and the other as reference. A Gilson Minipuls 3 was used to demonstrate that the autosampler can be used to control the peristaltic pump's flow rate and direction using the analogue interface. The autosampler was programmed to control the order and duration for which solutions are injected in channels of the flowcell mounted on the LW (see Table 5 for details).

Step number	Solution in sample channel	Solution in reference channel	Duration (min)
1	100 mM PBS, pH 7.4		10
2	20 μ g/ml NHS-(PEG) ₁₂ -Biotin in PBS and 0.2% (v:v) DMSO	100 mM PBS, pH 7.4	30
3	100 mM PBS, pH 7.4		10
4	220 μ g/ml streptavidin in PBS		10
5	100 mM PBS, pH 7.4		10
6	90 μ g/ml BPA in PBS		10
7	100 mM PBS, pH 7.4		10
8	208 μ g/ml IgG in PBS		10

Table 5: Program used by the autosampler to control the order and duration of solutions introduced in sample and reference channels

As shown in Table 5, same solutions were introduced in sample and reference channels except in step 2 where 20 μ g/ml NHS-(PEG)₁₂-Biotin in PBS and 0.2% (v:v) DMSO was only introduced in the sample channel. To implement step 2, one of the arms of the autosampler was immersed in a vial containing NHS-(PEG)₁₂-Biotin solution and another arm was immersed in PBS, demonstrating that the arms of the autosampler can be controlled independently. The NHS group of NHS-(PEG)₁₂-Biotin reacted with the amine groups in chitosan films, attaching biotin molecules to chitosan films underneath the sample channel. As shown in Figure 7 (a), these biotin molecules can then bind to streptavidin. The streptavidin has four binding sites for biotin; some of which were used up to bind to NHS-(PEG)₁₂-Biotin and the remaining to bind to the biotin in BPA. Finally, protein A in BPA can bind to IgG.

The camera in the LW read-out instrument was rotated by 90° so that the 2D reflectivity profiles of sensor and reference channels can be observed simultaneously as shown in Figure 7 (b). Shifts in resonance angles in sample and reference channels can therefore be monitored simultaneously. Shifts in resonance angles in sensor and reference channels were recorded by the LW read-out instrument as solutions were introduced sequentially, and the resulting plot is provided in Figure 7 (c). The difference in the resonance angles of sensor and references channels is plotted in Figure 7 (d). By taking the difference in the resonance angles of sensor and reference channels, the effect of changes in sample composition, non-specific adsorption, and temperature drifts can be eliminated, while being able to study the binding of proteins [28].

Figure 7: (a) Our approach for sensing exemplar proteins, (b) 2D reflectivity profile of LW with two channel flow-cell after completion of protein binding studies, (c) absolute and (d) differential shifts in resonance angle for PBS, NHS-(PEG)₁₂-Biotin, streptavidin, biotin protein A (BPA) and IgG solutions

As NHS-(PEG)₁₂-Biotin solution was introduced, the resonance angle corresponding to the sample channel increased, which was primarily because of the presence of 0.2% (v:v) DMSO in PBS. 100% DMSO has a RI of 1.4768, which is much higher than the RI of de-ionised water [29]. After a PBS wash, the difference in the resonance angles of the sensors and reference channels was ~0.5 mdeg, because of the binding of NHS-(PEG)₁₂-Biotin in the sensor channel. The difference in the resonance angles of the sensors and reference channels was small because the molecular weight of NHS-(PEG)₁₂-Biotin linker is low (~941 Da *versus* 150 kDa for IgG). Similarly, as summarised in Table 5, the differences in the resonance angles of the sensors and reference channels because of streptavidin and BPA were 135.1 and 61.7 mdeg, respectively. Figure 7 (d) clearly shows that the differences in the resonance angles of the sensors and reference channels was increased because of IgG, but did not reach an equilibrium value in 10 min, suggesting that the rate of binding of IgG is slower than streptavidin and BPA (see Table 6 for details). The rate of binding of IgG is expected to be slower than streptavidin and BPA because the molecular weight of IgG is the highest and thus the diffusion coefficient and porosity of the hydrogel to IgG is lowest. Equally, the dissociation constant between IgG and BPA is at least 10⁵ times higher than streptavidin and biotin [30, 31].

Species	Molecular weight (Da)	Concentration		Resonance angle difference at equilibrium (mdeg)	Initial rate of binding (mdeg min ⁻¹)
		µg/ml	µM		
NHS-(PEG) ₁₂ -Biotin	~941	20	~21.3	0.63 ± 0.1	Not applicable – initial increase was because of 0.2% (v:v) DMSO
Streptavidin	~53,000	220	~4.2	135.1 ± 0.4	70.6 ± 0.8
BPA	~42,000	90	~2.1	61.7 ± 1.4	27.5 ± 0.5

IgG	~150,000	208	~1.4	Had not reached equilibrium	6.1 ± 0.1
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Table 6: Summary of proteins used, resonance angle difference and rate of binding

Porosity studies: To study the effect of protein binding on the porosity of chitosan films, 0.5% solutions of PEGs of molecular weight 300, 100 and 10 kDa prepared in PBS were introduced using the autosampler both before and after binding of streptavidin (see Figure 8).

Figure 8: Resonance angle shifts observed for 0.5% solutions of PEGs of molecular weight 300, 100 and 10 kDa before and after streptavidin binding

Table 7 summarizes the resonance angle shifts and the percentage porosity of chitosan, which was calculated using Equation 1, for PEGs with respect to glycerol.

PEG solutions	Refractive index, n_{PEG}	Observed shift before streptavidin (degrees)	Observed shift after streptavidin (degrees)	% Porosity before streptavidin	% Porosity after streptavidin
0.5% (v:v) 300k PEG	1.335970	0.1331 ± 0.0064	0.1421 ± 0.0094	59.9 ± 3.3	63.9 ± 4.6
0.5% (v:v) 100k PEG	1.335860	0.1504 ± 0.0072	0.1582 ± 0.0097	73.9 ± 4.1	77.8 ± 5.2
0.5% (v:v) 10k PEG	1.335680	0.1634 ± 0.0070	0.1782 ± 0.0110	94.7 ± 4.8	103.3 ± 7.0

Table 7: Refractive index, observed resonance angle shifts and percentage porosity with respect to glycerol before and after streptavidin binding of different PEG solutions (error bars were calculated using $n=8$ and represent area-to-area variations for a LW)

$$\%P = 100 \frac{\Delta\theta_{R,PEG}}{\Delta\theta_{R,Gly}} \quad (1)$$

Where, %P is percentage porosity, $\Delta\theta_{R,PEG}$ and $\Delta\theta_{R,Gly}$ are shifts in resonance angle (degrees) for PEG and glycerol solutions, respectively of same refractive index. $\Delta\theta_{R,Gly}$ was calculated using the best fit line (i.e., $\Delta\theta_{R,Gly} = 171.9 \times n_{Gly} - 229.5$) for the case when $n_{gly} = n_{PEG}$ and n_{PEG} are provided in Table 6. $\Delta\theta_{R,Gly}$ was selected as the expected shift in resonance angle at a selected refractive index because glycerol is a very small hydrophilic molecule, to which chitosan should be fully porous. Table 7 clearly shows that the resonance angle shifts increased after streptavidin binding for all three PEGs, indicating that streptavidin is increasing the apparent porosity of chitosan, possibly because its binding causes the chitosan to swell somewhat.

V. Conclusions

This work is an unprecedented report of a 3D printed autosampler for automation of fluid manipulation. The autosampler was made of low-cost materials/components and had a footprint of 248 mm by 243 mm. The autosampler was therefore well-suited to enable automation of fluid manipulation in (academic) laboratories with limited financial resources and space. The autosampler comprised of four independent sample arms/needles and hence can be used to perform operations such as injection of two different solutions and their re-circulation, which is often required for the development of biosensors. The autosampler was used to control either a 3D printed or Gilson Minipuls peristaltic pumps and combined with 3D printed leaky waveguide (LW) read-out instrument to realise an analytical platform suitable for determining refractive index sensitivity, protein sensing capability, and porosity of a type of biosensor, the Leaky Waveguide. The work described here has shown that it is possible to create an autosampler and peristaltic pump by 3D printing and use these instruments, along with a previously described 3D printed optical biosensor instrument, to create a fully automated bioanalytical platform capable of “lights out” experimentation. The performance of this all-3D printed platform shows that it can rival the performance of standard laboratory instrumentation. The main advantages of the instrumentation described in this work are that it is low cost but provides functionality comparable with much more expensive devices, it can be modified very quickly as needed and it does not need very expensive equipment to fabricate the printed parts. This means that these analytical techniques can be made more widely available for use in more resource-limited situations. The main disadvantages are that some of the components cannot be 3D printed (such as cameras, lenses, mirrors, stepper motors, electronics, bearings), and the limited range of materials suitable for 3D printing means that the temperature range over which the instrumentation can work is limited.

Acknowledgements

The authors acknowledge the funding support from the Engineering and Physical Sciences Research Council (EPSRC) (Grant EP/N02074X/2. The authors also thank Ms Hazel J. Dixon for fabricating chitosan films.

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