Electronic supplementary information

Microfabrication

The absorbance chips were manufactured in six stages. Firstly, two blocks (120 × 60 mm) were cut from a 10 mm thick black cast acrylic sheet (PMMA). This was sufficient for three devices, with the tops having laser cut through-holes for the photo diodes and fluidic interfaces using a Trotec SP500 CO_2 laser engraving/cutting system (Trotec, Australia) Each piece was over size in order to allow for clearance for clamping within the Datron CNC's vice. See Figure S1(I) below.

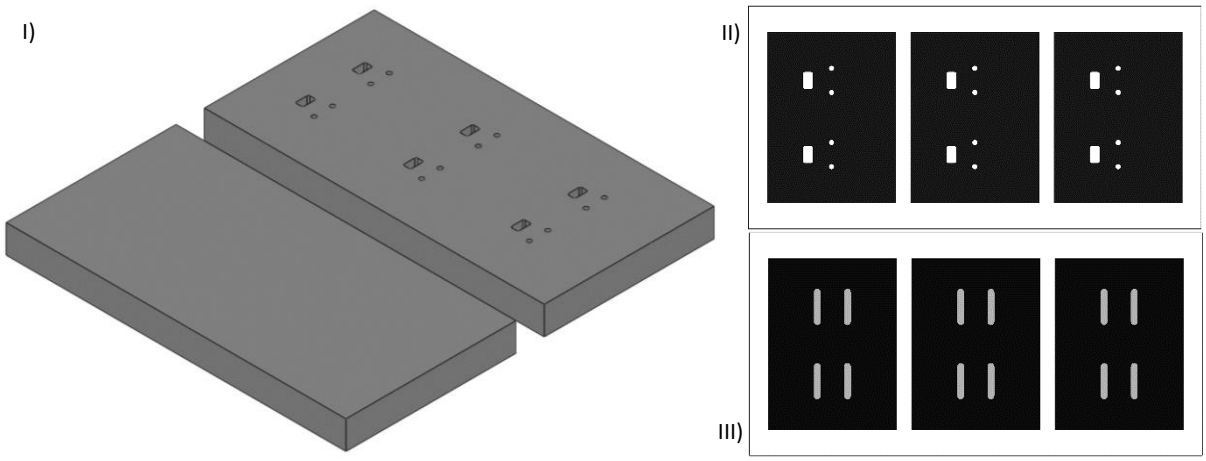

Figure S1. Laser cut blanks (I), the first CNC operation for the fabrication of the top plate (II), and the first CNC operation for the fabrication of the bottom plate (III).

The outer dimensions for each chip were machined to 46 × 34 mm using a CNC micro milling machine (Datron AG, Germany). A centre rectangle probing method was utilised in order to maintain the positional accuracy of the through-holes within the top plate to account for the width of the laser's cutting beam (See Figure S1 (II)). In order to allow the light to pass through the chip efficiently, four slots (6 × 2 × 10 mm) were also machined into each of the base plates

Figure S1(III)), each of these housed a clear PMMA window laser cut from 2 mm thick cast PMMA each 8 × 10 mm in size. To insert and seal the windows, a hermetic seal was created using chloroform to soften the PMMA (as described by Mohammed *et al*.,[1]) before being pressed into place as shown in Figure S2.

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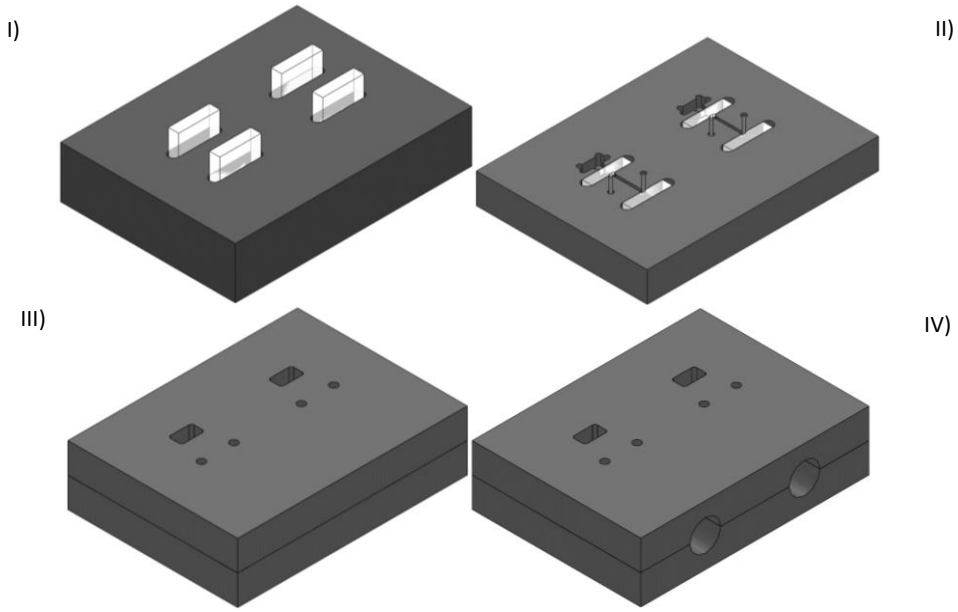

Figure S2. The final stages of the manufacturing process for the absorbance chip post hermetically sealed window placement: (I) transparent PMMA windows inserted and solvent treatment, (II) faced off bottom plate, (III) microfluidic device sealed and finally (IV) milling of the 5 mm LED pockets.

Both plates were then faced flat before the fluid network and photodiode pocket were machined into the base as shown in Figure S2(II). The chip was then assembled using a double-sided adhesive tape (9088FL, 3M, USA) as shown in Figure S2(III), before the final machining of the two pockets for the LEDs as shown in Figure S2(IV). Following this, the electronic components were placed within the device and capillary tubes were glued into place using a two-part 5-minute epoxy (Araldite, Australia) completing the fabrication process of the absorbance chip, as shown in Figure S3.

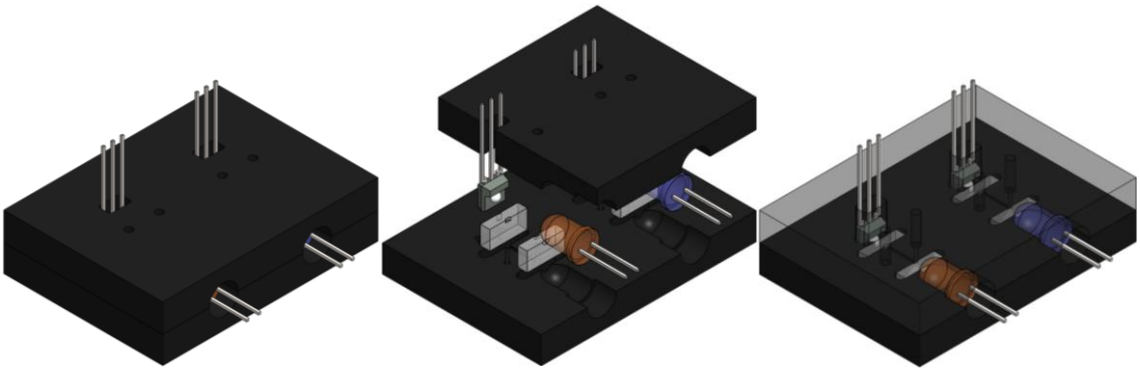
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Figure S3. The assembled chip (left) and an exploded view showing internal geometry and components (centre), and the assembled chip with transparent top plate (right) (capillary tubes not shown).

Electronics

A circuit was constructed (see schematic in Figure S4) allowing the brightness of each LED to be adjusted using a variable resistor. Each LED was interfaced with a TAOS TSL257 integrated light to voltage converter, which contains an integrated detector, amplifier and lens. The output from the sensor (SIG_OUT) was connected to the data recorder using a screw terminal to male BNC (Bayonet Neill–Concelman) connector. Power for the two LED's and two light detectors (+5V) was sourced from the USB (universal serial bus) port of a computer using a recycled USB cable. It should be noted that the LED used in this project are excessively bright for the path length and detector used – the LED current had to be limited to a low level in order to avoid detector saturation.

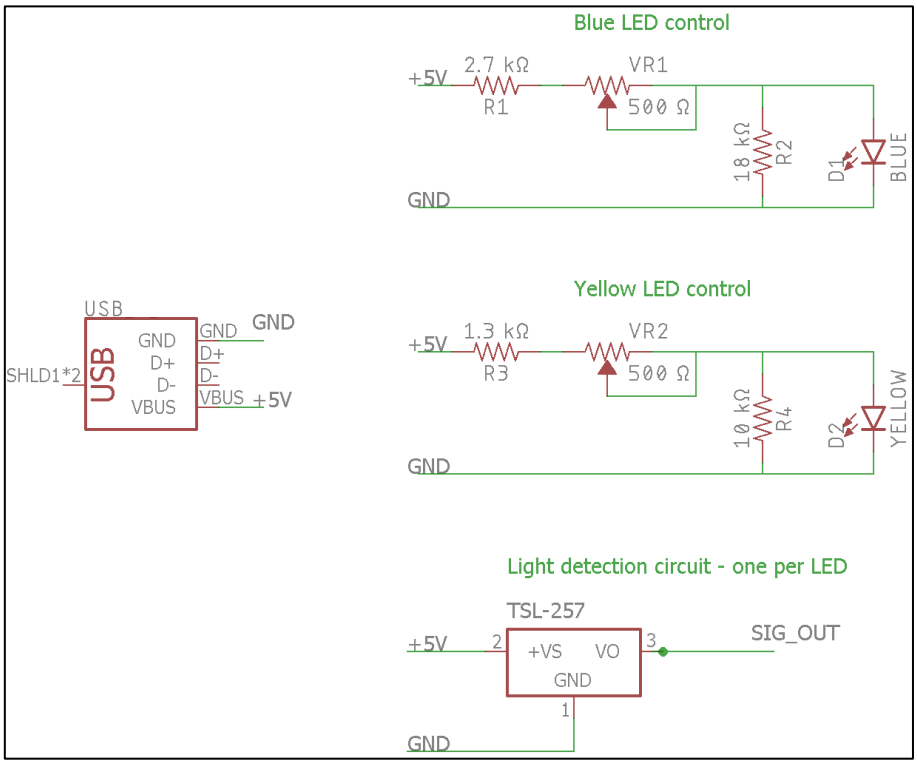

Figure S4. Circuit schematic.

Parts list:

Component	Value / Description	Part # (RS Components or manufacturer)
R1	2.7 kΩ ¼ watt resistor	739-7496 (RS)
R2	18 kΩ ¼ watt resistor	739-7550 (RS)
R3	1.3 kΩ ¼ watt resistor	739-7465 (RS)
R4	10 kΩ ¼ watt resistor	707-7745 (RS)
VR1, VR2	500 Ω 25 turn variable resistor	522-0388 (RS)
TSL-257 (x2)	Visible light photodetector & amplifier	642-4430 (RS)
D1	Multicomp high brightness 465 nm LED	MCL053UBLC (manufacturer)
D2	Optek high brightness 595 nm LED	OVLGY0C9B9 (manufacturer)
Connector – SIG_OUT	Screw terminal to BNC, male	124-2521 (RS)
Connector – USB	USB type A plug, male	674-2268 (RS)

LED emission spectra

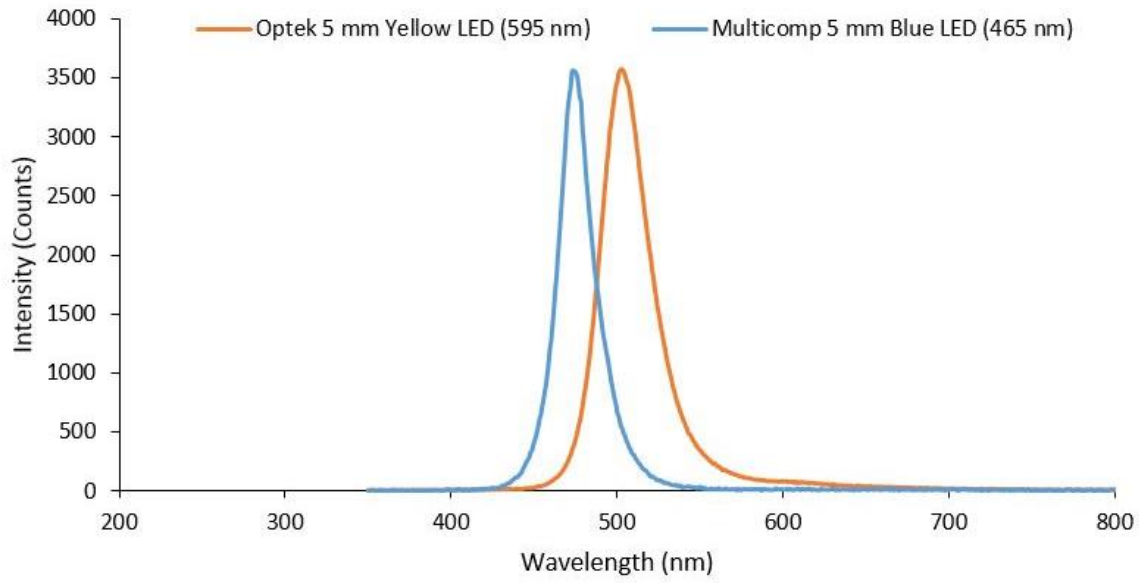

Figure S5. LED emission spectra were determined using a charged coupled device spectrometer (Ocean Optics, U.S.A), with the following parameters; open slit, acquisition time of 1 s, electric dark correction applied.

Additional results

The overall data are shown below in Figure S6 and S7.

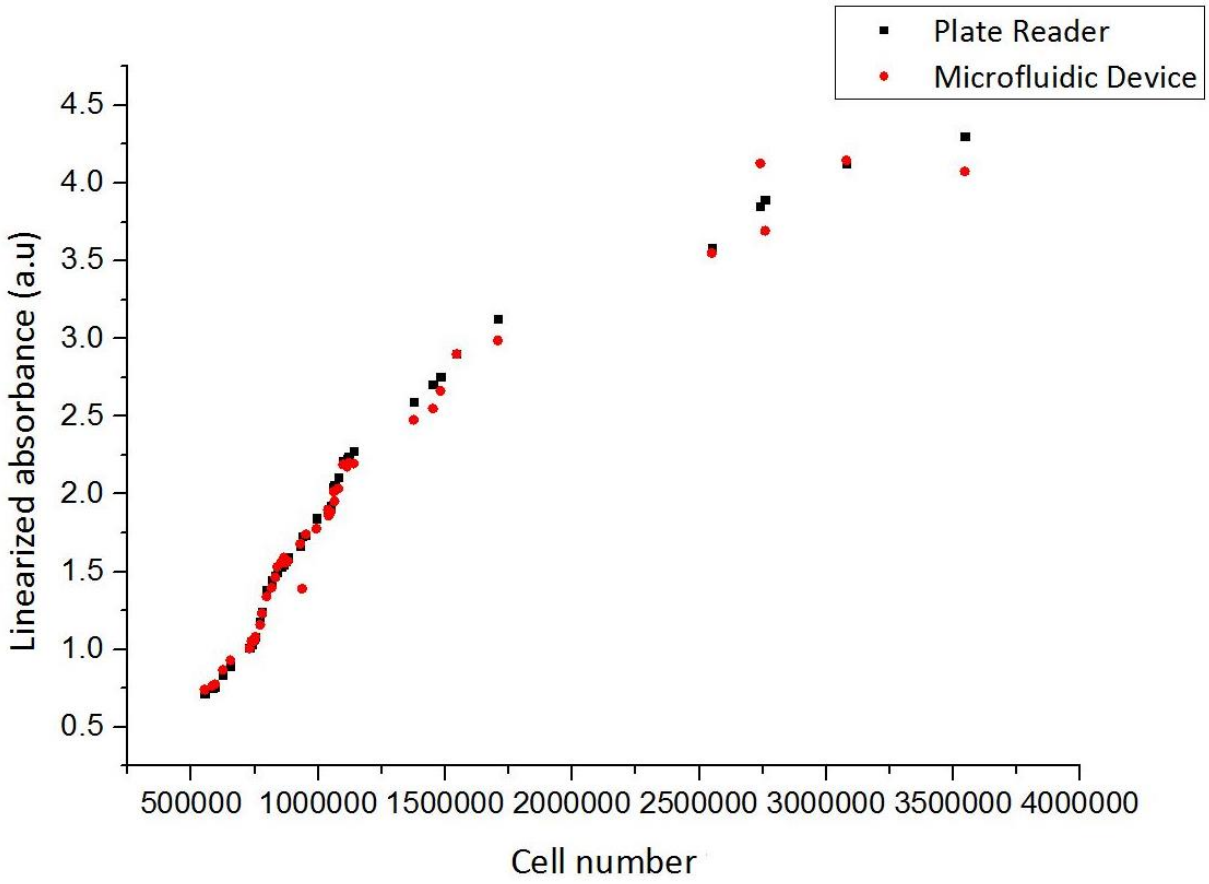

Figure S6. A graph depicting the linearised absorbance against cell number for the entire data set.

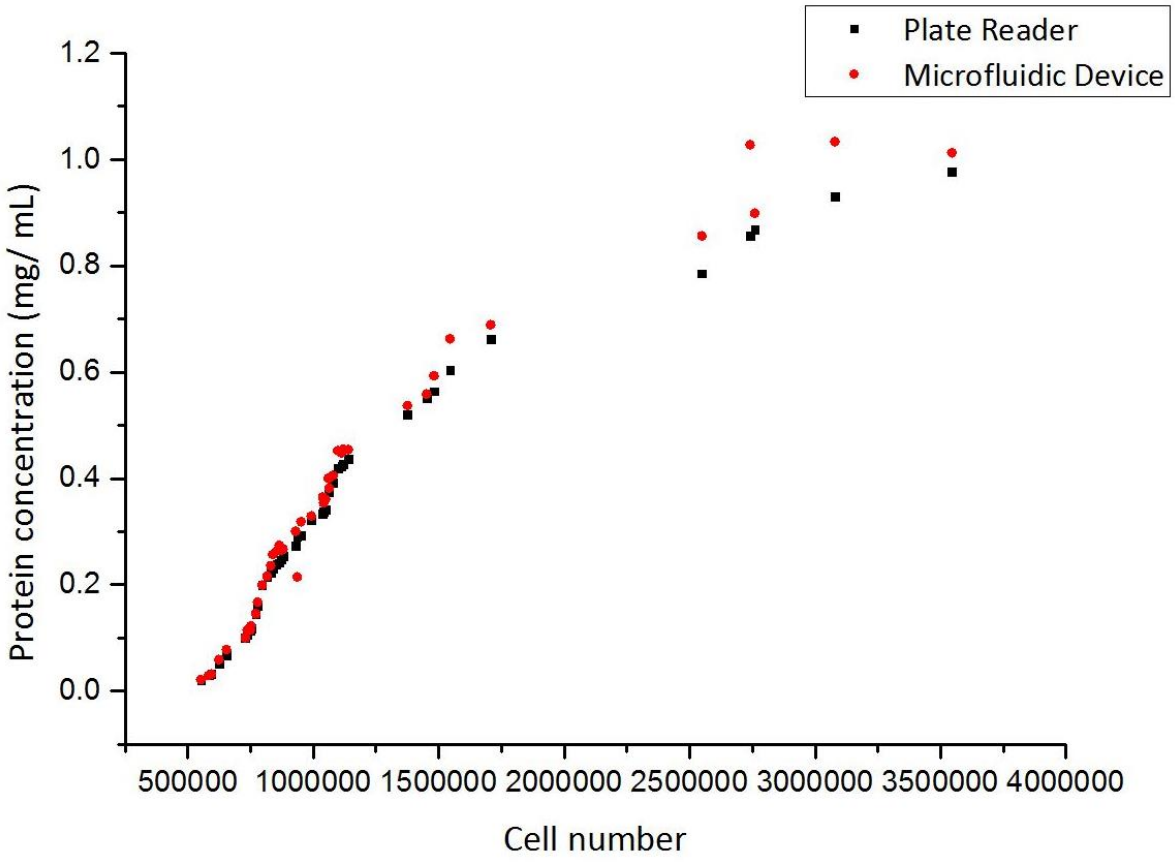

Figure S7. A graph depicting the total protein concentration plotted against cell number for the entire data set. The linear range was plotted within the paper.

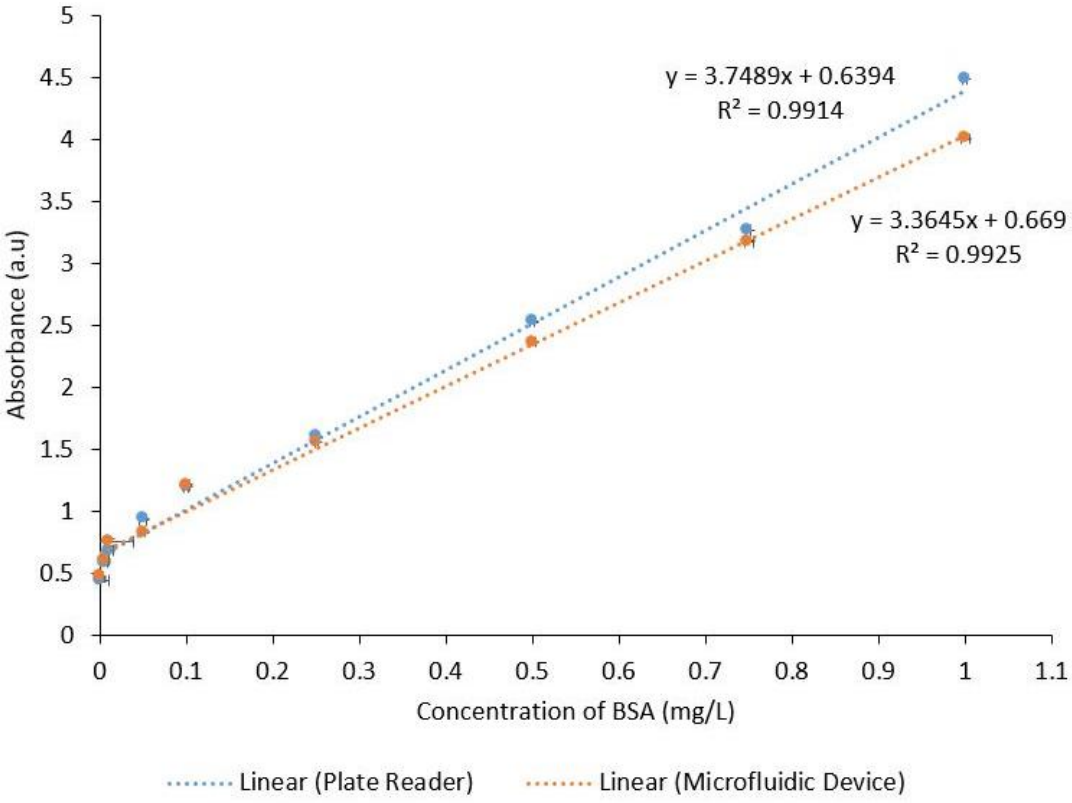

Figure S8. A graph depicting the calibrations for both plate reader and the microfluidic device using linearised absorbance measurements of the Bradford's reagent assay.

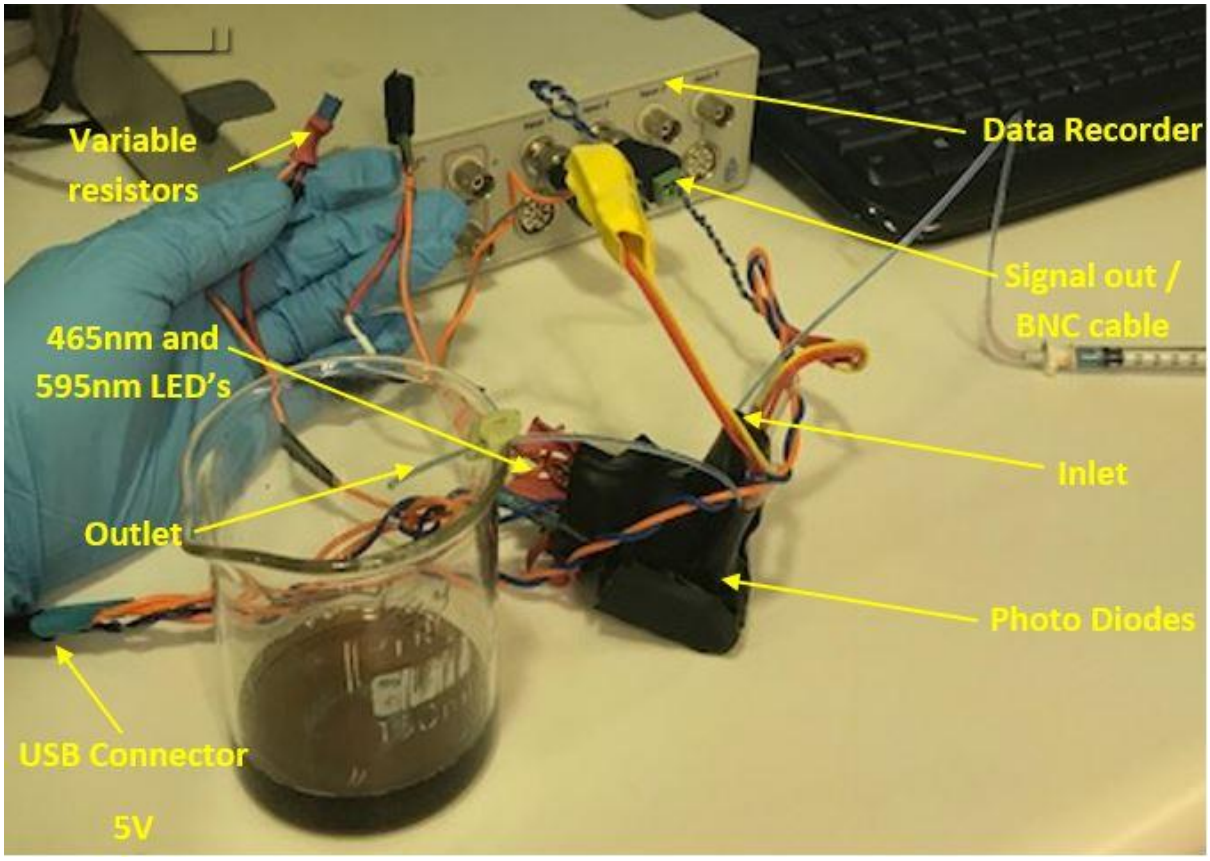

Figure S9. An image showing the set up utilised within this research for the quantification of cell number *via* protein from lysed buccal swab samples.

1. Mohammed, M. I.; Quayle, K.; Alexander, R.; Doeven, E.; Nai, R.; Haswell, S. J.; Kouzani, A. Z.; Gibson, I., Improved Manufacturing Quality and Bonding of Laser Machined Microfluidic Systems. *Procedia Technology* **2015**, *20*, 219-224.