Electronic Supplementary Material (ESI) for Lab on a Chip

Programmable large area digital microfluidic array with integrated droplet sensing for bioassays - ESI

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Operation of the AM-EWOD TFT electronics backplane

The operation of the TFT electronics is explained with reference to the schematic of figure 1, main text.

To write an electrowetting voltage to each array element, the digital data to be written is input via a single serial input line. The EWOD row and column driver circuits then organise this data and write it to the appropriate array element, so each array element in turn is written. In typical operation a frame of data may be written in 20ms. Once written, an SRAM memory cell within each array element stores the programmed data until such a time as it is re-written. The programmed state of the SRAM memory then configures the state of two analogue switches so as to determine which one of the externally supplied voltage signals V1 or V2 (see figure 1, main text) are connected through to the ITO electrode. Voltage signal V2 is also connected to the electrode of the counter substrate. The AM-EWOD may be operated in either DC or AC mode according to the choice of driver signals for V1 and V2. For DC actuation, V1 is set to +10V and V2 to -10V, so that for an actuated array element a voltage difference of V1-V2=20V is developed between the array element electrode and the electrode of the counter substrate, whilst for a non-actuated array element this voltage difference is zero. For AC actuation, V1 and V2 are both comprised of square wave waveforms of high level +10V and low level -10V, where V2 is the logical complement (inverse) of V1. For an actuated array element, the voltage difference between the element electrode and the counter substrate electrode now alternates between +20V and -20V, whilst for a non-actuated array element this voltage difference remains zero. Whilst EWOD actuation is possible with either DC or AC actuation, in practise AC is found to be preferable to avoid voltage shift and reliability problems due to the trapping of charge in dielectric insulator layers.[1,2]

Droplet sensing is implemented by comparing the load impedance, due to the presence or absence of a droplet, with a reference capacitance. The measured voltage from each array element is read out through sensor column amplifiers, which are based on a similar design to that used by CMOS image sensors.[3] Finally the sensor signal is converted to a serial analogue output and read-out through a single output line. The ESI video shows droplet manipulation and the corresponding impedance sensor images.

Considerations for on-chip enzymatic assays

Oxygen depletion

Many biomedical assays rely on enzymatic signal enhancement. Often the enzymatic pathway requires oxygen as an additional substrate, as is the case for the assay used in this study, which contains the enzyme Glucose Oxidase (GOx). It is of critical importance to maintain an excess of oxygen compared with the target to avoid oxygen limiting the rate of enzyme turnover and thus becoming the substance that is inadvertently measured. When run as an open, bench-top assay (e.g. using a microtiter plate) oxygen diffuses across the aqueous/air interface; oxygen depletion is therefore rarely a problem. Within the confines of micro and nanofluidic devices, where the exposure of aqueous solutions to external sources of oxygen may be limited, the amount of oxygen present must be considered in the assay design.

Figure S1. The effects of oxygen depletion on oxygen-dependant enzyme assays. The inset shows an assay droplet and highlights two areas analysed in more detail, the mean absorbance of these areas is tracked (main graph) where clear divergence is observed after 40 s when oxygen becomes rate limiting. Enzyme turnover continues within the droplet near the interface with the surrounding oil as this is a source of oxygen.

The effects of oxygen depletion in the confined environment of the droplet assay can be illustrated using the colourimetric glucose assay (Figure S1). Aqueous reagent droplets, 10 μL volume, containing a high concentration of glucose (20 mM) were sandwiched between two ITO-coated glass slides and surrounded by dodecane. Initially the rate of absorbance increase (which represents the rate of GOx turnover) was constant and uniform across the droplet. After one minute a disparity in absorbance was observed between the edge and the bulk of the droplet; the absorbance was found to continue to increase in

areas close to the oil. However, in the droplet centre the reaction was halted due to oxygen depletion. As the photograph, inset in Figure S1 shows, the region around the small air bubble has the same absorbance as the edge of the droplet.

Two solutions are proposed to this issue

- Smaller droplets can be used, increasing the surface area to volume ratio, and thus the net flux of oxygen into the droplet from the surrounding environment (oil). From the image in Figure S1 it is apparent a droplet with a diameter smaller than 2 mm would be less affected by oxygen depletion (for this glucose concentration). Higher concentrations of glucose would necessitate further reductions in droplet size.
- Samples can be diluted prior to mixing with enzymatic reagents on device. Dilution reduces the rate of enzymatic turnover and thus the rate of oxygen depletion. Dilution should ensure that the rate of oxygen consumption is below the rate of oxygen flux into the droplet.

Human serum typically contains glucose levels far greater than the limit of detection for this assay performed on the AM-EWOD devices (typically 4 – 6 mM, compared with a LoD of 33 µM). All the analysed samples were diluted by a factor of 25-fold prior to loading onto the chip so that supply of oxygen was not rate limiting.

Surfactant considerations

Surfactant is generally added to solutions processed by electrowetting devices to reduce surface tension at the boundary layer and thus aid droplet movement. When developing the glucose assay the widely used surfactant Tween®-20 (Polyoxyethylene (20) sorbitan monolaurate) was added for this purpose. However, Tween®-20 was found to be incompatible with the enzymatic reagents, resulting in a false positive signal in the absence of glucose. As GOx is highly specific for cyclic D-glucose (of the correct conformation) it is not clear why the Tween®-20 is disrupting the assay and leading to the creation of a coloured product.

Figure S2. The effects of different surfactants on the enzymatic reagents (in the absence of glucose), Tween® 20 and IGEPAL® clearly result in a false positive signal

Other surfactants were evaluated for their compatibility with the enzymes and false positive signals. Three different surfactants were mixed with the enzymatic reagents at 1% v/v and the absorbance measured as a function of time (Figure S2). The experiments indicated that Tween®-20 gave the highest false positive signal, whilst the surfactant IGEPAL® CA-630 (octylphenoxypolyethoxyethanol) also produced a (reduced) false positive. GAENE (glycolic acid ethoxylate 4-nonylphenyl ether), did not give any measureable absorbance increase, and had no discernible effect on the turnover of glucose by GOx. GAENE is less soluble in aqueous buffer than the other detergents. For the experiment, one droplet contained detergent at 2% v/v, which is above the critical micelle concentration (CMC) and appeared opaque. This was mixed (diluted) with an equal volume of enzymatic reagent which then led to a decrease in absorbance as the surfactant solution became transparent (Figure S2). This surfactant was therefore used (at the lower concentration of 0.2% v/v) in all the assays.

Explanation of glucose assay data analysis

Figure S3. The distribution of droplets immediately prior to the running of a glucose assay on an AM-EWOD array. The column on the left contains droplets of the sample (970 µM glucose), in the middle are droplets containing enzymatic reagent and on the right droplets of buffer. Region A indicates where droplet brightness would be measured and region B where the brightness of the array would be calculated as an average brightness within a droplet annulus.

The raw data from the experiments were in the form of 12-bit greyscale image. Custom Labview programs were used to measure droplet size and absorbance. For each assay, images were taken of droplets before mixing (e.g. Figure S3) and these were used to measure initial droplet size and brightness. This data was used to calculate droplet absorbance immediately prior to the assay (A_0).

Droplet volume, v_D – A threshold was used to select the bright contour of a droplet (from the reflected light) and convert to a binary image. Image processing was used to remove noise and small defects; the area (and therefore volume) of the droplet (v_D) is given by the number of pixels.

Droplet absorbance, A_D –The background signal and noise (I_B) from the camera was estimated by imaging the device in the absence of droplets and without transmitted light. Droplet absorbance (A_D) was calculated using the formula

$$A_D = -Log_{10}\left(\frac{I_D - I_B}{I_{BG} - I_B}\right)$$

where I_D is droplet brightness measured within the boundary indicated (Figure S3, region A) and I_{BG} the brightness of the array (background) within the immediate vicinity of the droplet as measured by an annulus around the droplet (600 μm in thickness) (Figure S3, region B).

Figure S4. Images of a glucose assay run on an AM-EWOD array. a) taken at initiation (upon droplet merging and mixing) at t = 0 min, and b) at t= 20 min. End-point absorbance changes are calculated using data obtained from these images.

A_0 was calculated from the absorbance of the sample droplet (or in the case of blank, the buffer droplet) (D1) and reagent droplets (D2), after adjusting for droplet volumes.

$$A_0 = \frac{(A_{D1}v_{D1}) + (A_{D2}v_{D2})}{v_{D1} + v_{D2}}$$

After droplet merging and mixing, images were acquired at 5 second intervals for a total of 20 minutes. These images were analysed to obtain the change in absorbance of droplets with time (Figure S4 shows images of the assay taken at t = 0 min and 20 min after mixing).

According to the assay protocol, sample and reagents droplets were merged in a 1:2 volume ratio. The absorbance (A_x) of the droplets (3 samples and 1 blank) as measured from the images at $t = x$ min is normalised to account for variation in droplet volume ($\tilde{A}_x$).

$$\tilde{A}_x = A_x\left(\frac{v_{D1} + v_{D2}}{3v_{D1}}\right)$$

The end-point change in absorbance (ΔA) is defined as the measured change in absorbance of the combined sample and reagent droplets (D_{sample}) minus the measured change in absorbance of the combined buffer and reagent droplets (D_{blank}) at the end-point of the reaction (t = 20 min after initiation), see Figure S5.

$$\Delta A = (\tilde{A}_{20} - \tilde{A}_0)_{\,D_{sample}} - (\tilde{A}_{20} - \tilde{A}_0)_{\,D_{blank}}$$

Figure S5. Glucose assay time course data with annotation illustrating the origin of end-point absorbance values

References

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