ISFET sensor system for real-time detection of extracellular pH oscillations in slime mould

B. Nemeth, S. Tsuda, C. Busche, L. Cronin and D.R.S. Cumming

An ion sensitive field effect transistor (ISFET) array for the direct observation of physiochemical activity in a living biological specimen of a slime mould is demonstrated. The chip comprises a 64 × 64 pixel array of 10.2 × 10.2 μm sensors. The device was fabricated in a standard 0.35 μm foundry CMOS process. Using this device it was possible to observe periodic variations in pH consistent with the biological activity of slime mould with oscillatory amplitudes of 450 μV ± 70 μV peak-to-peak, equating to 0.022 pH units at 20 mV/pH sensitivity, when taking data at a rate of 400 μs per pixel-sample. These oscillations arise from intracellular Ca^{2+} variations giving rise to an extracellular pH change.

Introduction: Ion-sensitive field effect transistor sensors [1] have been developed into arrays to investigate the activity of living cells in an aqueous environment. Particular attention has been paid recently to demonstrating the ionic transport and the local ionic concentration fluctuations of cells in culture [2–4]. The sensor membrane used in these studies was Si_3N_4 that is sensitive, to varying degrees, to several ionic species including sodium ions and protons. Thus, it is challenging to identify and separate the responses of the respective ion channels. Slime mould has a well-known characteristic, intracellular time-dependent Ca^2+ oscillation, that leads to an extracellular variation in pH [5]. These characteristics mean that slime mould is an excellent biological exemplar for exploration using ISFET technology, since the physiochemical behaviour exhibited by the system is well-defined. A slime mould is a large single-cellular multi-nuclear organism that periodically changes its interior state every 150–180 seconds [6]. Our objective, therefore, was to show that we could directly observe this characteristic using ISFET arrays, thus opening up a field of investigation of exploring complex biological systems.

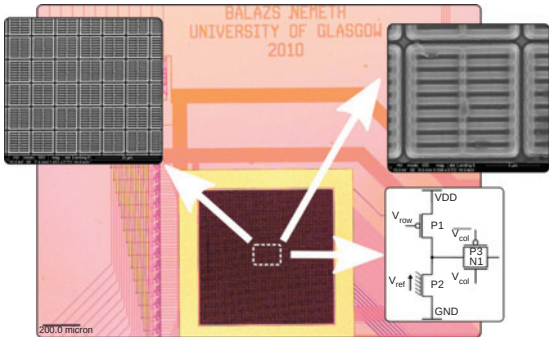

Fig. 1 *Structure of pixels and micrographs of fabricated sensors and system*

Sensor system description: The structure of a pixel, the basic unit of the integrated array of pH sensors, and the fabricated device is shown in Fig. 1. The array, containing 4096 sensor devices on silicon, each with dimensions of 10.2 × 10.2 μm, occupied an area that was 715.8 × 715.8 μm. The chip was fabricated using an unmodified 0.35 μm CMOS process from *austriamicrosystems*. Each pixel contains four transistors as shown in Fig. 1. Transistor P1 is used for row addressing and sources a drain current of 16 μA into transistor P2 that is the pixel's PMOS ISFET sensor. The transmission gate circuit formed by transistors P3 and N1 enables exclusive access to the ISFET sensor in each pixel, thus avoiding potential charge sharing between pixels on the same output rail. Possible crosstalk between activated and deactivated lines was efficiently reduced by connecting the inactive nodes to either the ground or power rails. Because of the large number of pixels and the requirement for an adequately high sampling rate, 16 pixels were sampled simultaneously and the data readout was pipelined and parallelised on to 16 analogue output channels. Real-time data acquisition was performed using two NI PXIe-6358 measurement cards, each providing the pixel addresses and data conversion of the analogue samples. The experiments were controlled using LabVIEW Real-time on one PC. The maximum data acquisition rate is 40 μs per pixel, hence 10.2 ms per sensor array frame, resulting in a data rate of 1.56 Mbyte/s that is streamed to a second PC that is running LabVIEW in order to store the data to disc.

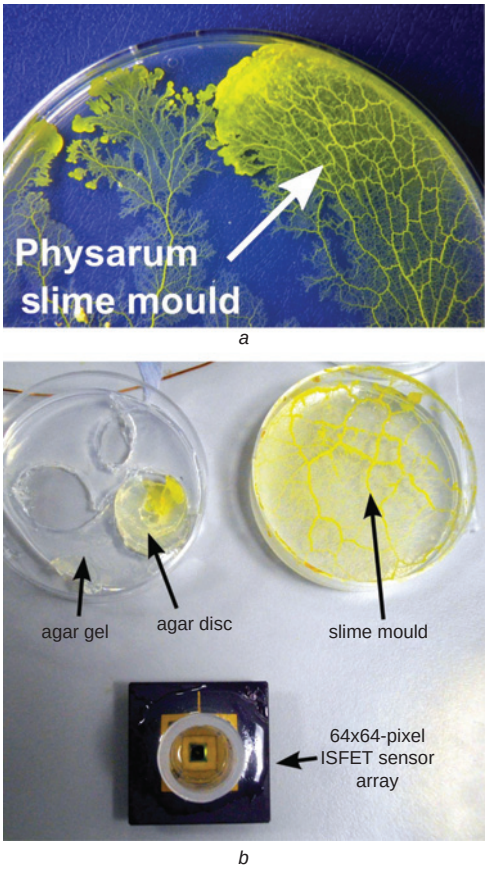

Fig. 2 *Physarum slime mould growing on 1.5% agar gel (Fig. 2a) (slime mould shape consists of flat area in growing fronts and tubular structures in interior side of cell); slime mould was placed into plastic chamber on top of chip carrier (Fig. 2b) (agar gel deposited over cell to maintain moist environment)*

Slime mould and experimental setup: A plasmodium of true slime mould *Physarum* Polycephalum is a single-cellular multi-nuclear organism as shown in Fig. 2*a*. The cell thickness of this amoeba-shaped amorphous organism has a spontaneous and regular variation at time period of 1.5 to 3.5 minutes. The activity of the cell is due to internal actin-myosin contraction-relaxation cycles, similar to muscle cells [6]. Under no-stimulus condition, the internal Ca^{2+} oscillations, as well as pH oscillations on the cell surface [5], are spatially synchronised throughout the cell which is observed as propagating waves similar to peristaltic movements. The oscillations have been detected by injecting calcium-sensitive photoprotein aequorin into the cell or freezing it by liquid nitrogen [7]. These methods, however, can damage or kill the cell and thus it was necessary to develop an alternative method to observe oscillations of intact slime mould in situ. We therefore applied our sensor array system as a tool to observe the intracellular [Ca^2+] fluctuation induced extracellular pH oscillations in a non-invasive manner. The *Physarum* cells used for the experiments were cultured on a 1.5% agar gel Petri dish in darkness at ambient temperature. Commercial oat flakes were provided once a day as nourishment to keep the *Physarum* cells alive. The components of the measurement system along with its preparation method are shown in Fig. 2*b*. Pieces of *Physarum* cells were cut from the Petri dish and placed onto the surface of the array. An agar gel disc was then placed in the polypropylene chamber on the surface of the ceramic chip carrier. The agar gel was used to prevent the cells from drying out. A hole was formed in the middle of the agar disc, over the chip, to supply fresh air to the slime mould. Slime mould was then deposited over the sensor array, and on to the top of the agar. Several depositions of slime mould were needed to achieve this. A reference electrode was placed in contact with the slime mould on top of the agar. The completed assembly was left for 30 minutes to allow the cell to finish merging and resume

its natural biochemical oscillations. Signals were acquired at 400 μs per pixel, 102.4 ms per sensor frame. Samples were collected over a 20-minute-long period and further processed in Matlab. Several such cycles of data acquisition were completed.

Results: Oscillating signals of 150–180 seconds period in time and around 2 mV in amplitude as a consequence of 0.1 pH changes were expected, corresponding to the activity of the slime mould. The results shown in Fig. 3*a* display the measured periodic signals that were observed consistently across the sensor array. The same extracellular behaviour across the complete slime mould cell was observed by investigating the responses of spatially distant pixels of the array. The time domain signals of five pixels (one from each corner and one pixel from the middle) of the array were evaluated. A portion of the slime mould was present over each of the pixels concerned. The responses showed continuous oscillations in pH, at the surface of the silicon nitride, with a 150 s ± 10 s second period. The average pH was approximately 7 and the amplitude of pixel peak-to-peak output voltage signal was 450 μV ± 70 μV as indicated in Fig. 3*b*. The equivalent peak-to-peak change in measured pH fluctuation as a result of the inner activity of the slime mould cell was estimated using:

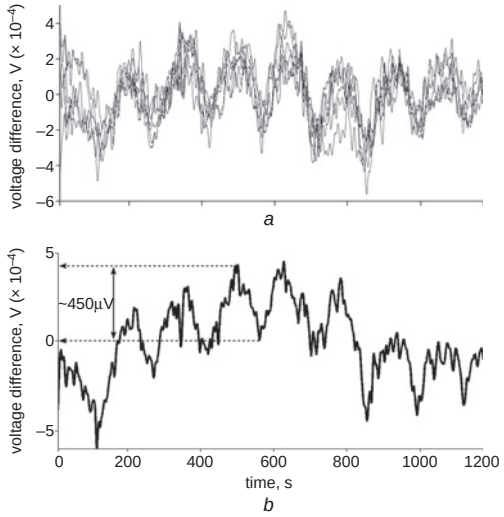

Fig. 3 *Response of five pixels shows excellent correlation (Fig. 3a) (pixels selected from four corners and one from middle of array); amplitude of pixel output voltage signals (Fig. 3b) (response of pixel located in middle of array displayed)*

$$\Delta\text{pH} \cong \frac{\Delta V}{\text{pH sensitivity}} = \frac{450\mu\text{V}}{20\dfrac{\text{mV}}{\text{pH}}} = 0.022\text{pH}$$

The linear pH sensitivity of 20 mV/pH was determined by making measurements against test solutions with pH in the range 4–10, with DI water rinses between each measurement. The measurement resolution of the system is therefore better than 0.022 pH resolution. This is to be compared with a resolution of 0.02 pH points for DNA sequencing chips that are coated with Ta_2O_5 [8]. In the present work, the high resolution is obtained using the unmodified silicon nitride passivation layer deposited on to the CMOS chip in the standard foundry process.

Conclusions: A 64 × 64-pixel ISFET sensor array system is presented providing maximum real-time 40 μs per pixel, 10.2 ms per sensor frame data acquisition speed. The system was fabricated in an unmodified CMOS process and showed a pH sensitivity of 20 mV/pH. The array showed excellent consistency in the measurements obtained from pixels that were spatially distant from one another. The use of slime mould allowed measurement of low frequency, 450 μV ± 70 μV peak-to-peak amplitude, H+ signals. The measured 0.022 pH fluctuations show how relatively easy it is to obtain foundry silicon devices that may be used for integrated ion sensitive lab-on-a-chip applications. The technology may prove to be widely applicable to a range of biological studies. In this Letter, we have clearly demonstrated that pH oscillations on the surface of a single cell organism can be directly detected with high resolution. The array device enables measurement of oscillations both temporally and spatially. The work therefore demonstrates that mainstream ISFET electronics technology may be adapted to allow the study of living cells in culture, including re-organisation of spatial oscillation patterns in response to external environmental stimuli. In future work we will extend this to producing dual-sensitive ISFET arrays that can simultaneously detect more than one type of ionic species at a time, allowing the spatial and temporal measurement of a range of ion-fluxes.

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One or more of the Figures in this Letter are available in colour online.

B. Nemeth and D.R.S. Cumming (*Electronics Design Centre, University of Glasgow, Glasgow G12 8LT, United Kingdom*)

E-mail: david.cumming.2@glasgow.ac.uk

S. Tsuda (*Japan Science and Technology Agency, Exploratory Research for Advanced Technology (ERATO), Yamadaoka 1-5, Suita, Osaka 565-0871, Japan*)

C. Busche and L. Cronin (*School of Chemistry, University of Glasgow, Glasgow G12 8QQ, United Kingdom*)

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