Supplementary Material

A CMOS-Based Multi-Omics Detection Platform for Simultaneous Quantification of Proteolytically Active Prostate Specific Antigen and Glutamate in Urine

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1. Data Processing

In this section, additional details about data processing are discussed and the main parts of the custom data processing script are reported. Data was processed using a custom MATLAB script. Here we use dataset for glutamate assay in buffer with initial glutamate concentration of 250 µM and a recording duration of 5 minutes as demonstrative dataset.

1.1. Visual Inspection

Data from all the pixels of the array are inspected before any data processing. Pixels showing abnormal artifacts are excluded for further computations. MATLAB code for performing these instructions is reported below (Code S.1). Fig. S.1(a) shows raw data from each pixel of the demonstrative dataset. The MATLAB custom function enables to graphically delete corrupted pixels.

Code S.1. Initial settings and visual inspection.

```
% Visual inspection section
dark = 0; start_after_x_seconds = 0; Niir = 8; seconds = 10; Fst = 0.05; Fs = fps; SP1 = 0; SP2 = 0; SP3 = 0; SP4 = 0; ch1 = 1:16*16;
pix = 1:256; data_label = [pix', data]; title_graph = ' Channel 1 '; ch1 = deletepixels(data_label, ch1,title_graph);
function ch1 = deletepixels(data_label,ch1,title_graph)
    figure(99); plot(data_label(ch1,:)'); plotedit on; title(title_graph); xlim = [2,size(data_label,2)]; pause();
   figure(99); a = get(gca,'Children'); ydata = get(a, 'YData'); close all; ch1_default = ch1; clear ch1;
   for i=1:size(ydata,1)
      temp = ydata{i,1}; ch1(i) = temp(1);
   end
end
```

1.2. Data Processing

After visual inspection, raw data is converted into voltage and the average over the array is calculated (see Code S.2). The average is then plotted so that the user can manually select the starting point of the reaction. The user input provides a reference point for further processing. Data from each pixel is cropped from the starting point of the reaction and is filtered using a moving average method with a time window of 30 seconds. The time vector is also normalized to shift the starting point of the reaction to zero seconds. Fig. S.1(b) shows a typical data out at this stage of processing.

The data is then down-sampled to 1 sample per second by averaging data in non-overlapping 60 second time windows (Code S.3). The resulting signal is then fitted using MATLAB built-in fitting functions and showing in Fig. S.1(c).

Code S.2. Average, filtering and data cropping.

```
data1 = data(ch1,:)*3.3/2^16-dark;                    % Convert and normalise
medio1 = mean(data1); figure(); plot(good_time, medio1);   % Calculate spatial average
% Select starting point for channels
figure(); title('select sample insertion time'); plot(medio1); hold on; plot(medio1,'or'); title('Channel 1'); zoom on;
waitfor(gcf, 'CurrentCharacter', char(13)); zoom reset; zoom off; [x1,y1] = ginput(1);
x = round(x1); data1_cut = data1(:,x:end); filtered = data1_cut; % Cut and filter data - pixel by pixel
for i = 1:size(filtered,1)
    filtered(i,:) = movmean(filtered(i,:),round(fps*30));
end
medio = mean(filtered,1);                             % Spatial average of filtered data
time = good_time(x:end)-good_time(x);                 % Handle time vector
```

Code S.3. Downsampling, fitting and data representation.

```
% Downsampling
X1 = time;Y1 = medio; step = round(fps); j = 1;
for i=1:step:length(X1)-step-1
    X2(j) = mean(X1(i:i+step)); Y2(j) = mean(Y1(i:i+step)); j = j+1;
end
% Fitting and data rappresentation
[xData, yData] = prepareCurveData( X2, Y2 ); ft = fittype( 'exp2' ); opts = fitoptions( 'Method', 'NonlinearLeastSquares' );
opts.Display = 'Off'; [fitresult, gof] = fit( xData, yData, ft, opts ); X3 = X2; Y3 = feval(fitresult,X3); figure(); hold on;
plot(X2, Y2-Y2(1)); plot(X3, Y3-Y3(1)); pause ();
```

1.3. Reaction Rates Calculations

The initial reaction rate is calculated over 5 different fixed time windows with lengths of 30, 60, 90, 120 and 300 seconds (see Code S.4). For each time window, data is fitted using a linear model using MATLAB built-in functions. The reaction rate is then calculated using the derivate of the linear model. Data is also represented for each time window (see Fig. S1(d-h)).

As expected, reducing the length of the computation window yields to an increased initial reaction rate estimation (See Fig. S1 (i)). However, reducing the length of the computation window also increases variability over different recordings. We have experimentally observed that using a time window of 120 seconds yields to more stable and reproducible results for both glutamate and aPSA assays. Therefore, in this paper a time window of 120 seconds was used to extract the final estimation of the initial reaction rate. Data from the photodiodes are in Volts. Therefore, the voltage drop (ΔV) over time which is directly related to the reaction rate. Thus, reaction rates are expressed in mV/min

Code S.4. Calculation of the reaction rate.

```
% Rates in fixed time windows
time_windows = [30, 60, 90, 120, 300]; figure(); hold on;
for i = 1:size(time_windows,2)
    t = find(X2>=time_windows(i));  tt = t(1); X_temp = X2(1:tt); Y_temp = Y2(1:tt); P = polyfit(X_temp,Y_temp,1);
    yfit = P(1)*X_temp+P(2); Linearised_rates(i) = abs(P(1)*1000*60); %expressed as mV/min
    plot(X_temp, Y_temp,'o'); plot(X_temp, yfit); pause(); clear X_temp; clear Y_temp; clear P;
end
```

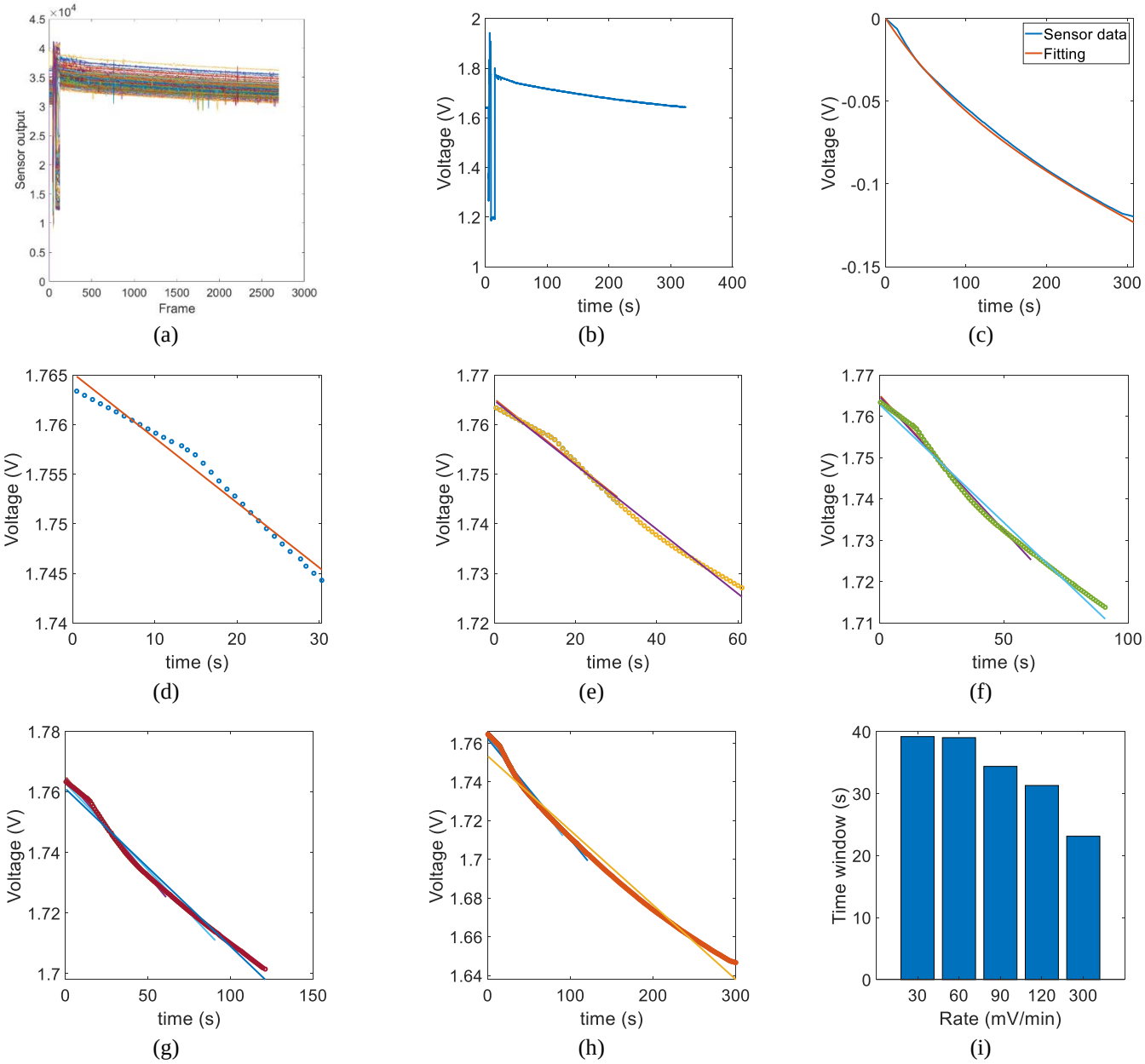

Fig. S.1. (a) Pixel-by-pixel data representation. (b) Average over the sensor array. (c) Processed data and fitting. (d) Data (circular markers) and linear fit (solid line) over 30 seconds. (e) Data (circular markers) and linear fit (solid line) over 60 seconds. (f) Data (circular markers) and linear fit (solid line) over 90 seconds. (g) Data (circular markers) and linear fit (solid line) over 120 seconds. (h) Data (circular markers) and linear fit (solid line) over 300 seconds. (i) Comparison of reactions rates calculated over different time windows.

2. Synthetic Urine Sample Preparation

In this section, additional details concerning the preparation of the synthetic urine samples are discussed. Synthetic urine samples were prepared according to Table S.1. Quantities illustrated in Table S.1 are for the preparation of samples with final volume of 50 µL. Samples were freshly prepared and immediately introduced on to the testing device. The devices used for measuring the samples were stored at room temperature in an enclosed container. The protocol we used was adopted to mimic a point-of-care test where the sample is freshly collected while the testing device is typically stored at room temperature. We have not investigated the performance of the platform when using samples stored in various conditions. The fabrication of a new

testing device from the bare CMOS chip takes about two days. As explained in the main text, devices were cleaned and reused due to limited resources. We have not observed obvious data variations related to storage time of the testing device.

Table. S.1. Preparation of synthetic urine samples.

Sample #	DI water (µl)	Synthtetic urine (µl)	Glutamate 5mM solution (µl)	aPSA 12.5 µg/ml solution (µl)	Total Volume (µL)	Glutamate concentration (µM)	aPSA concentration (ng/ml)
1	3.6	46.4	0	0	50	0	0
2	3.42	46.4	0.1	0.08	50	10	20
3	3.24	46.4	0.2	0.16	50	20	40
4	2.4	46.4	0.6	0.6	50	60	150
5	2.2	46.4	1	0.4	50	100	100
6	0	46.4	2	1.6	50	200	400
7	2.81	46.4	0.75	0.04	50	75	10

3. Test Time

For both aPSA and glutamate assays, reaction rates in buffer were calculated over different computation time windows (30, 60, 90, 120, 300 and 600 seconds) as described in Supplementary Section 1. Average and relative standard deviations were calculated for all the concentrations in each time window for both aPSA (see Table S.2) and glutamate (see Table S.3). The resulting precision for each time window was calculated as the average over the relative standard deviations of the dataset. We observed that for the aPSA assay, the best precision was achieved when rates were calculated over 2 minutes. For the glutamate assay, the best precision was achieved when rates were calculated over 1 minute. Since this work aims at measuring both aPSA and glutamate at the same time, we selected a test duration of 2 minutes that best relative precision for the aPSA (22.86%) and still ensures an acceptable precision for glutamate (20.3%).

Table. S.2. Average reaction rate and standard deviation of aPSA assay in buffer calculated using different time windows.

Time window (s)	30		60		90		120		300		600	
aPSA(ng/mL)	µ	σ	µ	σ	µ	σ	µ	σ	µ	σ	µ	σ
0	4.34	68.14	1.60	54.01	1.13	48.65	1.26	25.22	1.45	9.19	1.38	11.08
3.13	2.00	23.45	3.53	4.43	3.14	24.40	3.28	31.47	2.64	27.18	1.95	30.12
6.25	4.65	54.85	3.80	38.02	3.07	28.36	4.24	30.91	2.84	21.23	2.18	27.01
12.5	6.64	11.37	7.42	16.02	6.44	14.09	5.38	9.33	3.69	44.50	3.43	59.09
25	18.70	42.27	11.86	30.13	8.08	29.39	6.54	25.40	3.12	18.59	2.13	13.02
50	15.13	28.20	15.65	16.84	12.41	21.09	10.27	21.54	6.13	47.92	5.36	62.26
125	24.48	46.88	22.60	37.55	20.88	30.78	19.96	26.78	15.19	10.57	10.25	13.58
250	54.38	51.25	53.72	36.25	47.76	21.52	43.13	12.21	28.32	4.58	17.28	14.25
Precision (%)		**40.80**		**29.16**		**27.29**		**22.86**		**22.97**		**28.80**

µ: average reaction rate (mV/min); σ: relative standard deviation (%); Precision: average of the relative standard deviation for a given dataset.

Table. S.2. Average reaction rate and standard deviation of aPSA assay in buffer calculated using different time windows.

z	**30**		**60**		**90**		**120**		**300**	
Glutamate (µM)	**µ**	**σ**	**µ**	**σ**	**µ**	**σ**	**µ**	**σ**	**µ**	**σ**
0	6.45	25.10	5.38	19.45	3.21	55.14	3.46	21.29	4.55	71.73
20	21.92	43.73	18.42	43.68	15.43	46.89	13.32	50.12	6.95	48.30
50	24.69	30.50	22.57	15.53	19.99	11.09	17.87	7.58	9.51	11.11
100	26.62	24.04	26.54	28.06	24.38	29.80	22.87	31.38	16.11	28.68
250	46.17	10.44	46.61	11.89	43.05	15.70	40.28	17.97	30.45	21.07
500	51.44	7.98	60.46	9.71	61.41	9.17	60.95	7.63	50.20	5.14
1000	71.46	10.99	81.75	7.79	81.32	6.53	79.10	6.11	62.44	6.01
Precision (%)		**21.83**		**19.45**		**24.90**		**20.30**		**27.43**
µ: average reaction rate (mV/min); σ: relative standard deviation (%); Precision: average of the relative standard deviation for a given dataset.										