Supplementary Information

A multivariate biosensor for non-invasive glucose and urea monitoring via saliva

Amit Kumar Singh [a,b], Shweta Panwar [b], and Sandeep Kumar Jha[b, c]

[a] Department of Biomedical Engineering, Vignan's Foundation for Science Technology & Research, Vadlamudi, Guntur, Andhra Pradesh, 522213, India.

[b] Centre for Biomedical Engineering, Indian Institute of Technology Delhi, New Delhi 110016, India

[c] Department of Biomedical Engineering, All India Institute of Medical Sciences, New Delhi 110029, India

Corresponding author: sandeepjha@cbme.iitd.ac.in

Supplement Eq. 1:

LII = Light Intensity Integration

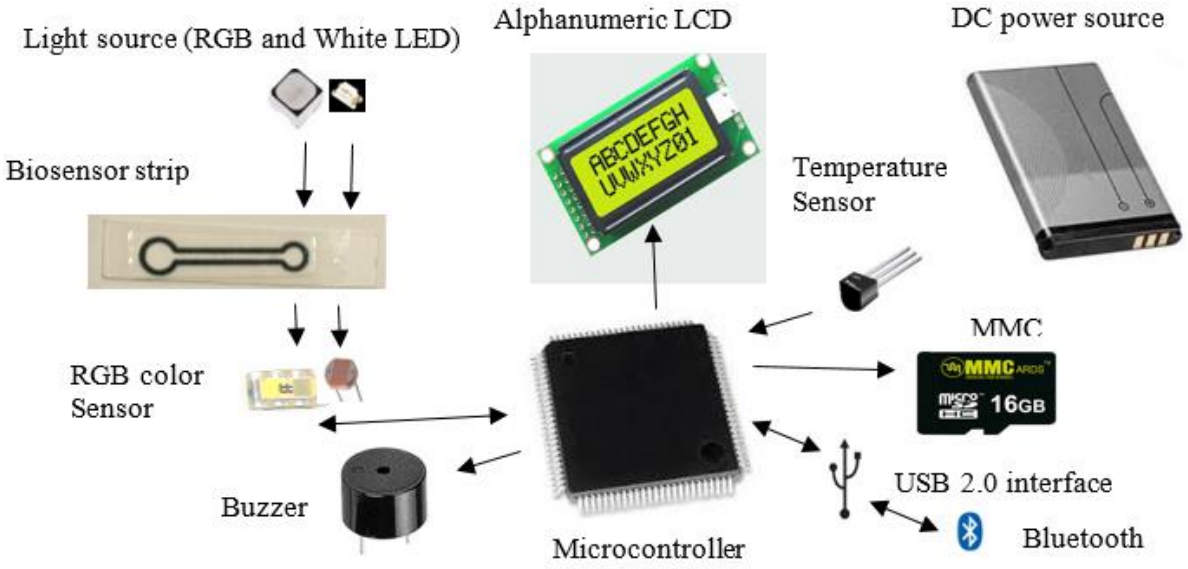
$$\text{Glucose concentration (mg/dL)} = 75.4 + (0.3374 \times \text{LII}) - (2.308 \times \text{Temp}) + (0.008108 \times \text{LII} \times \text{Temp}) + (0.03349 \times \text{Temp}^2) \quad \text{(Eq: S1)}$$

Supplement Eq. 2:

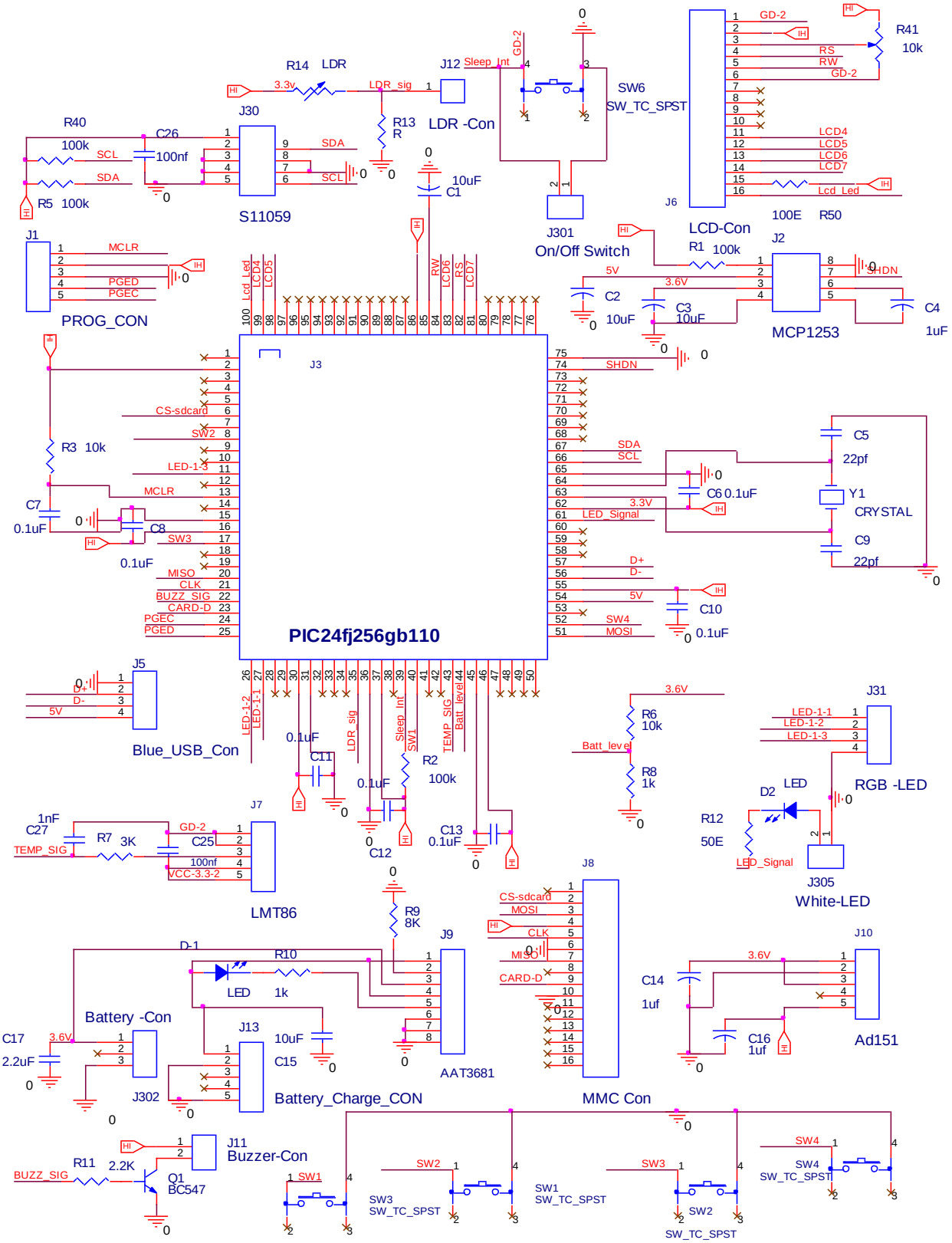
LII = Light Intensity Integration

Urea conc. (mg/dL) = 1350-30.76×LII+189.5×Temp-0.06291×LII^2+0.176×LII × Temp-9.685×$Temp^2$-0.0001276×LII^3+ 0.006515×LII^2×Temp-0.2086× LII×$Temp^2$+0.2133×$Temp^3$-2.018e-07×LII^4 +5.382e-06× LII^3× Temp-0.000229× LII^2× $Temp^2$+0.00449× LII× $Temp^3$-0.00172× $Temp^4$+ 3.056e-11× LII^5+ 4.192e-09×LII^4×Temp-8.476e-08×LII^3×$Temp^3$+2.483e-06×LII^2× $Temp^3$- 3.551e-05× LII×$Temp^4$ ……..(Eq: S2)

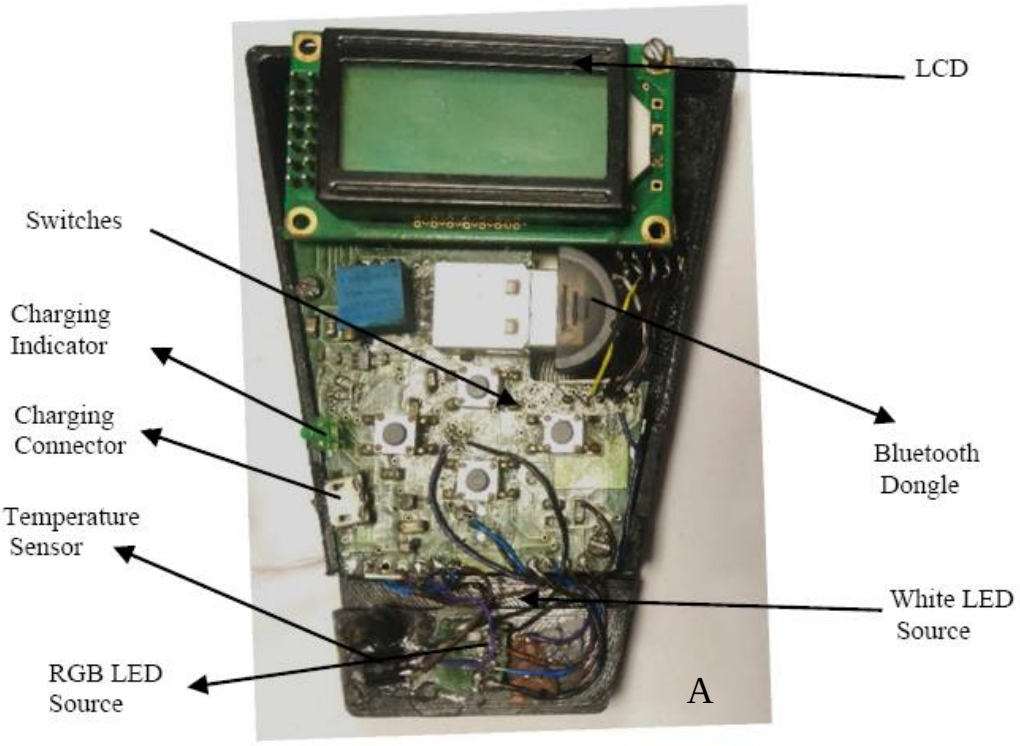
Supplement Fig. S1. Block diagram of the instrument and its components.

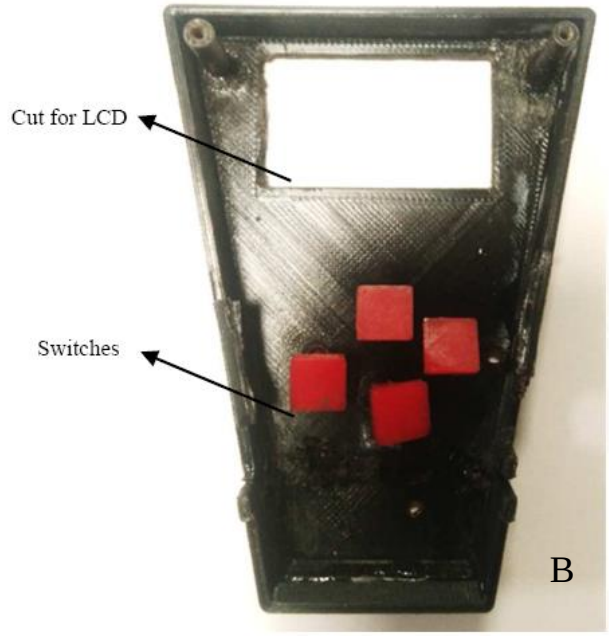


Supplement Fig. S2. Circuit diagram of the multi-analyzer instrument

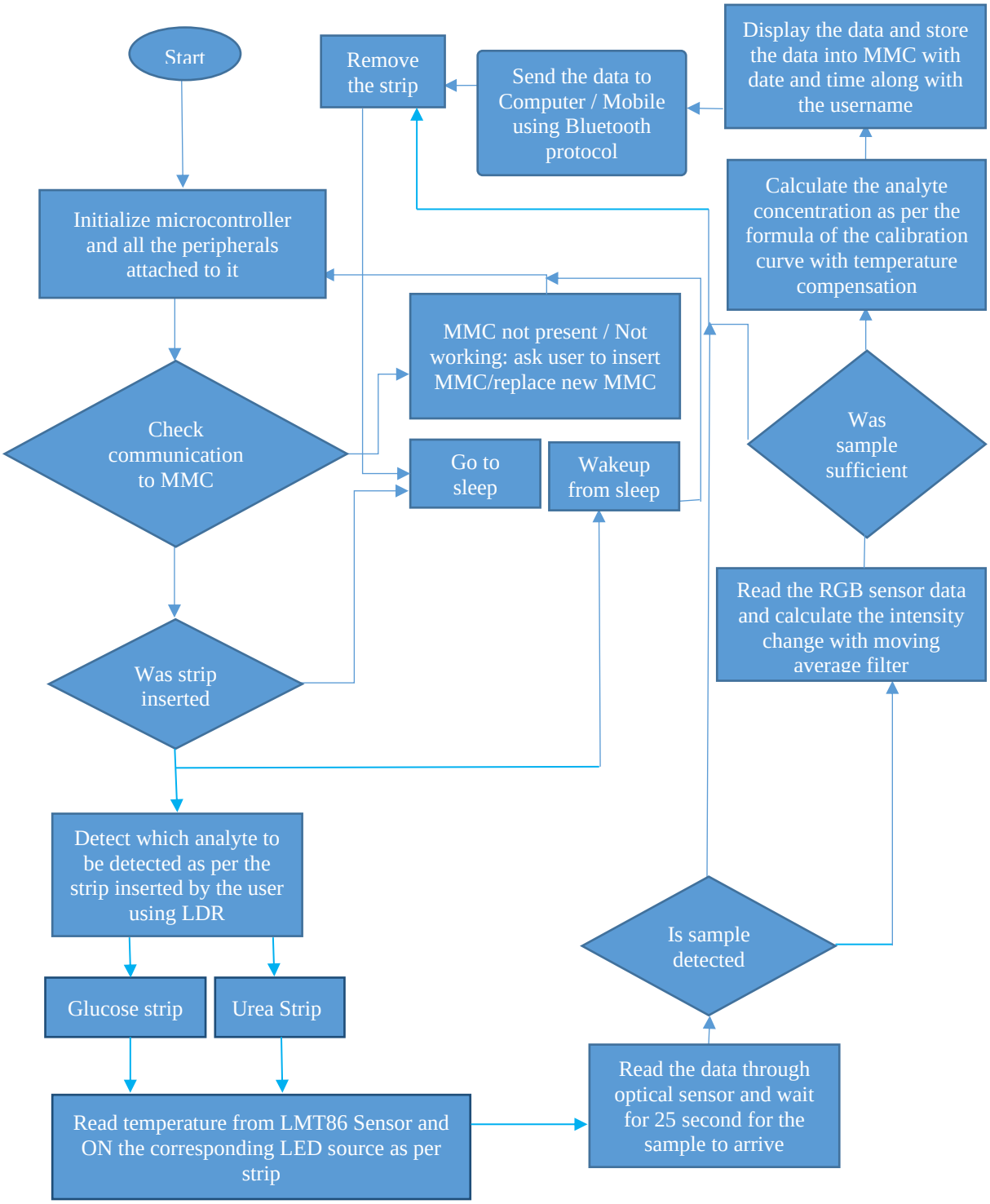


Supplement Fig. S3. Internal view of the developed instrument (A) showing the bottom half of the instrument containing all the electronic part and (B) inverted top cover of the instrument.

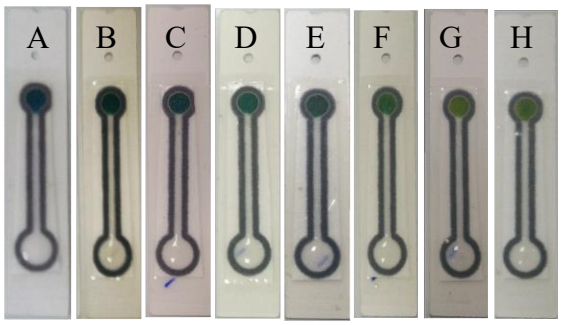




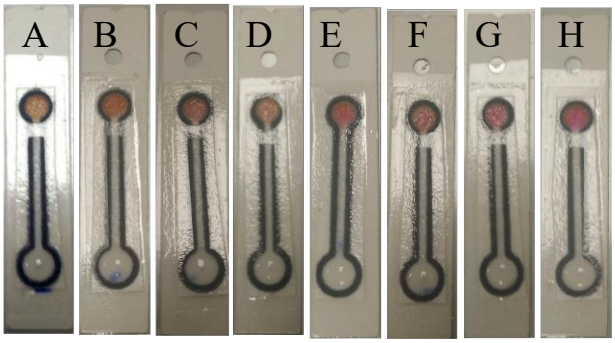
Supplement Fig. S4: Flow chart of the Microcontroller program for the operation of the instrument.



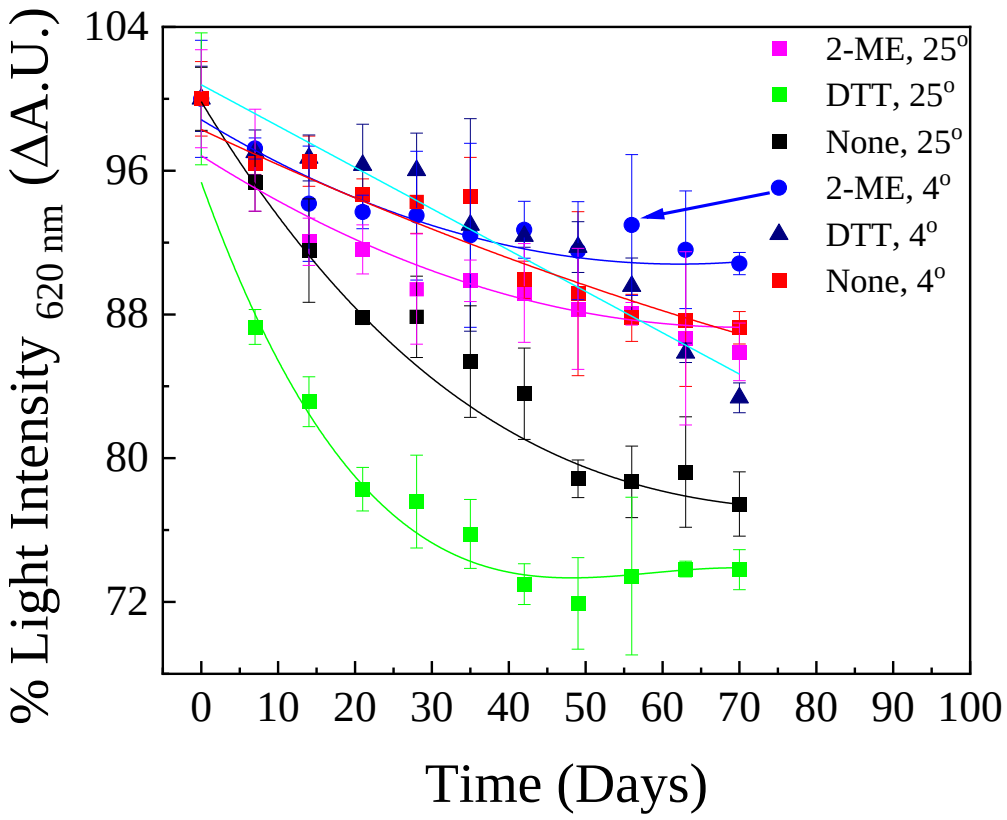
Supplement Fig. S5. Glucose biosensor strip's front view: color change after reaction with different glucose concentrations spiked in saliva. Values ranging between A-H: 0, 12, 37, 62, 112, 162, 262, 362 mg/dL (intrinsic saliva glucose concentration plus spiked value) respectively tested at 25°C with 2-ME.



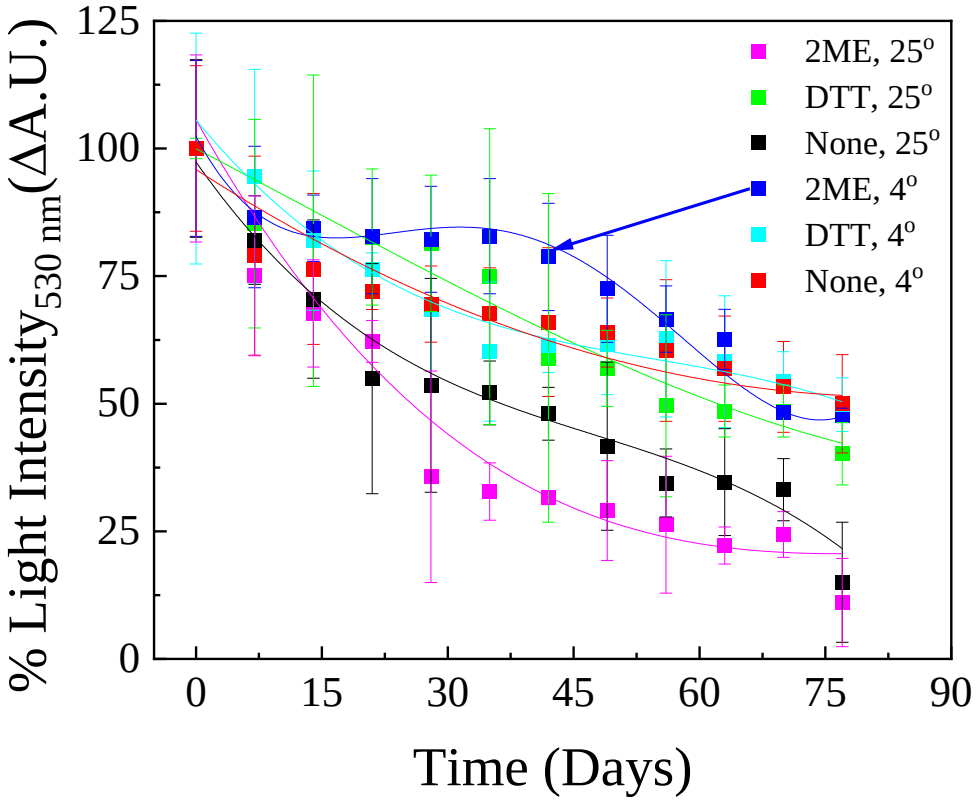
Supplement Fig. S6. Urea biosensor strip's front view: color change after reaction with different urea concentration spiked in saliva. Values ranging between A-H: 0, 5, 20, 30, 45, 65, 90 and 120 mg/dL (intrinsic saliva urea concentration plus spiked value) respectively tested at 25°C with 2-ME.



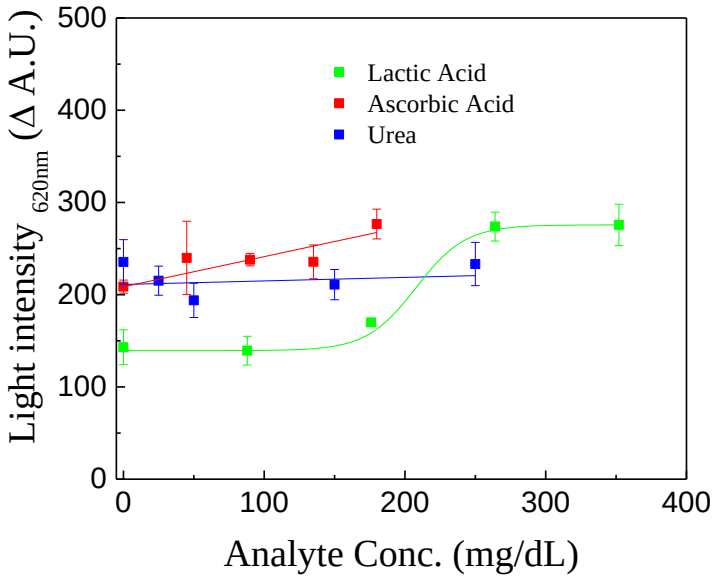
Supplement Figure S7: The glucose sensor strip stability: sensor readings in saliva sample spiked with 100 mg/dL glucose each time with strips fabricated with different stabilizing agents and stored at different temperatures. The different conditions used for the tests were: (A) GO_x +2-ME, (B) GO_x +DTT, (C) GO_x without stabilizer stored at 25°C respectively; (D) GO_x +2-ME, (E) GO_x +DTT, and (F) GO_x without stabilizer, stored at 4°C, respectively.



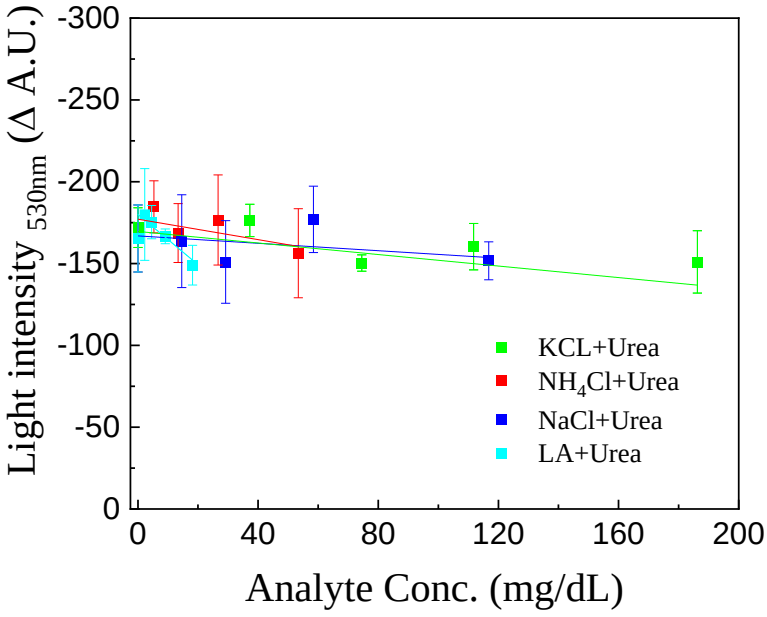
Supplement Figure S8: The urea sensor strip stability: sensor readings in saliva sample spiked with 30 mg/dL urea each time with strips fabricated with different stabilizing agents and stored at different temperatures. The different conditions used for the tests were: (A) urease +2-ME, (B) urease +DTT, (C) urease without stabilizer stored at 25°C respectively; (D) urease +2-ME, (E) urease +DTT, and (F) urease without stabilizer, stored at 4°C, respectively.



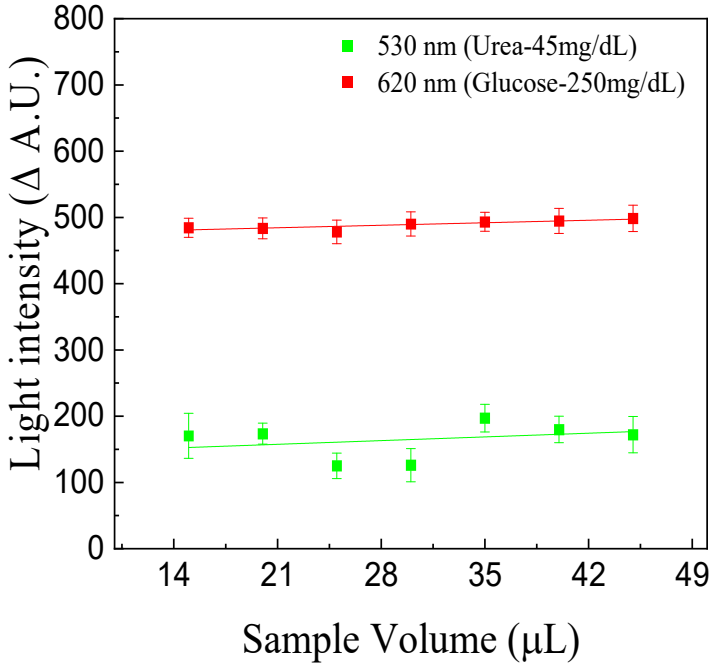
Supplement Fig. S9. Interference tests for glucose strip in lactic acid, ascorbic acid and urea with different glucose concentrations spiked in saliva.



Supplement Fig. S10. Interference tests for urea strip in LA, KCl, NaCl, and NH_4Cl with different urea concentrations spiked in saliva.



Supplement Fig. S11. Sample volume variation effect tested for glucose and urea sensor strips.



Supplement Table S1 Analytical performances of some non-invasive multi-variate biosensors/ instruments including commercializable ones developed so far in comparison to our developed biosensor.

Sensor Techniques	Analytes Detected	Linearity	Detection Range	Response time	Sample involved/ body site	Author/ Product Name
Optical	(1) Glucose (2) Ascorbic acid (3) Protein	Non-linear	As per the medical test standard	Minutes	Urine	Hong et al. [1]
Optical	(1) Glucose (2) Lactate (3) Pyruvate	Non-linear	As per the medical test standard	40 s	Blood, Saliva, and Urine	Srinivasan et al. [2]
Optical	(1) Creatinine (2) eAG (3) HbA1c	Linear	As per the medical test standard	180 s	Blood, Urine	SD Biosensor [3]
Electrochemical	(1) Glucose (2) Lactate (3) Oxygen	Linear	(1) 10-450 mg/dL (2) 1-15 mg/dL (3) 0-18 mg/L	90 s	Blood	Dutta et. al. [4]
Microwave	(1) Fat (2) Calcium	Non-linear	As per the medical test standard	Minutes	Blood	Jamal et. al. [5]

Amperometric	(1) Glucose (2) Lactate (3) Sodium	Non-linear	(1) 0-200 µM (2) 0-30 mM (3) 10-160 mM	Minutes	Sweat	Gao *et al.*[6]
Optical (Developed Instrument/ Sensor)	(1) Glucose (2) Urea	Linear at 25°C	(1) 8-375 mg/dL (2) 5-120 mg/dL	Seconds	Saliva	Singh et. al./Saliva analyzer [7]

References

[1] J. Il Hong and B.-Y. Chang, "Development of the smartphone-based colorimetry for multi-analyte sensing arrays.," *Lab Chip*, vol. 14, no. 10, pp. 1725–1732, May 2014

[2] V. Srinivasan, V. Pamula, M. Pollack, and R. Fair, "A digital microfluidic biosensor for multianalyte detection," in *The Sixteenth Annual International Conference on Micro Electro Mechanical Systems, 2003. MEMS-03 Kyoto. IEEE*, 2003, pp. 327–330

[3] V. Srinivasan, V. K. Pamula, M. G. Pollack, and R. B. Fair, "Clinical diagnostics on human whole blood, plasma, serum, urine, saliva, sweat, and tears on a digital microfluidic platform," in *7th International Conference on Miniaturized Chemical and Biochemical Analysis Systems, California USA*, 2003.

[4] M. Dutta *et al.*, "Multi-analyte detection handheld analyzer for point-of-care application with disposable biochips," in *SENSORS, 2003 IEEE*, 2003, vol. 1, pp. 617-621 Vol.1, doi: 10.1109/ICSENS.2003.1279011.

[5] F. I. Jamal, S. Guha, M. H. Eissa, S. Vehring, D. Kissinger, and C. Meliani, "K-Band BiCMOS based near-field biomedical dielectric sensor for Detection of fat and calcium in blood," in *2015 European Microwave Conference (EuMC)*, 2015, pp. 821–824

[6] W. Gao *et al.*, "Fully integrated wearable sensor arrays for multiplexed in situ perspiration analysis," *Nature*, vol. 529, no. 7587, pp. 509–514, 2016

[7] S. K. Jha and A. K. Singh, "A biosensor for detecting multi analyte in oral fluid," Indian patent no. PCT/IN2019/050443, 2019.

Product Demonstration Video can be downloaded here

SGL 31, 33, 39 mg/dL Av. 34.33 mg/dL SD 4.1 mg/dL