Electronic Supplementary Information (ESI)

Lipidest: A Lipid Profile Screening Test at Extreme Point of Care Settings using a Portable Spinning Disc and an Office Scanner

Victor Pakira[a], Rahul Agarwal[b], Subhamoy Chatterjee[c], Arghya Mukherjee[b], Suman Chakraborty[b*]

[a] Advanced Technology Development Centre, Indian Institute of Technology Kharagpur, Kharagpur – 721302, India.
[b] Department of Mechanical Engineering, Indian Institute of Technology Kharagpur, Kharagpur – 721302, India.
[c] Department of Electronics and Electrical Communication Engineering, Indian Institute of Technology Kharagpur, Kharagpur – 721302, India.

Corresponding Author:

Suman Chakraborty

Email: suman@mech.iitkgp.ac.in

Phone: +91-3222-282990

S1. Dimensions of the layers of Compact Disc (CD)

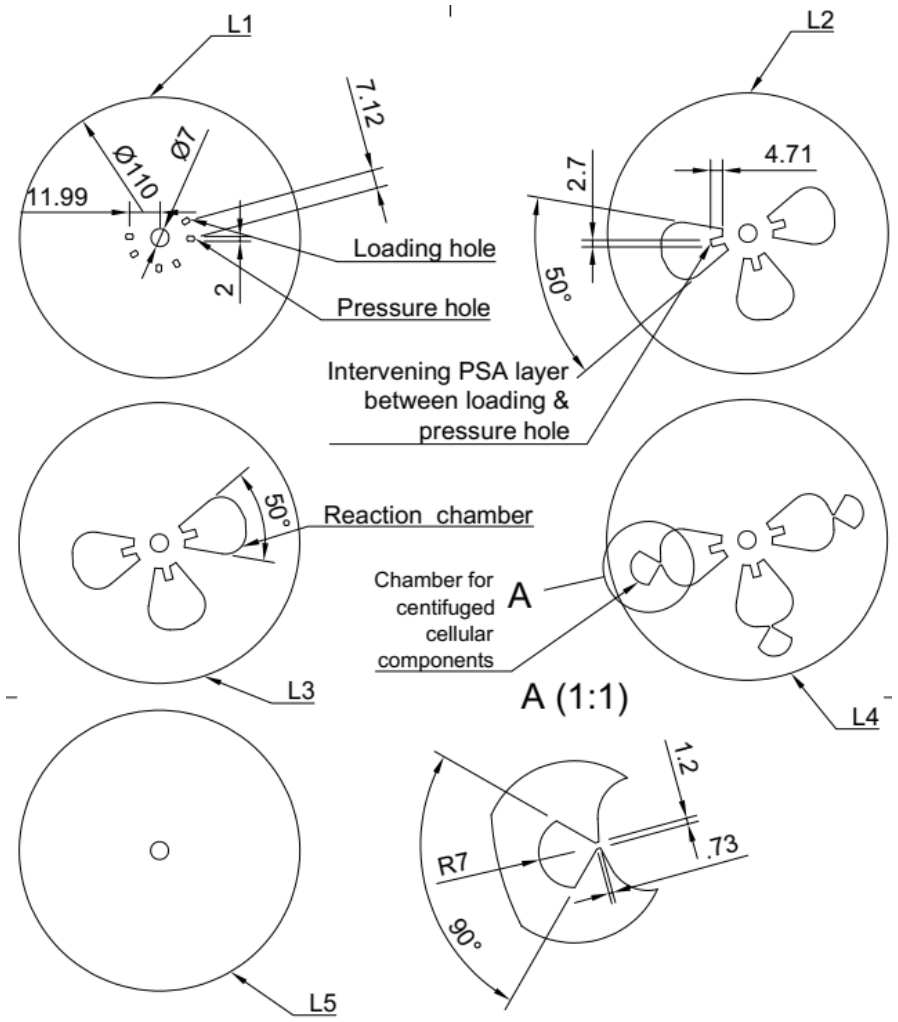

Figure S1 Detailed schematic diagram of the component features of the layers of the disk, from top to bottom – L1, L2, L3, L4 AND L5. L1-topmost layer with loading and pressure holes; L2- second layer containing the intervening PSA strip separating the loading and pressure holes; L3- with the rection chamber; L4-containing the chamber for centrifuged blood separated from the reaction chamber, via a small channel of 1.2mm x 0.73mm; L5-bottommost supporting layer.

The loading hole and the pressure hole are equiradially placed at a distance of 12 mm from the center, and separated by a linear dimension of 7.12 mm.

The second PSA layer has a thin intervening strip of 4.7mm x 2.7mm separating the loading and the pressure hole. During the tine of fluid loading through the loading hole this strip prevents the spreading meniscus of the fluid from reaching the pressure hole. The pressure hole if blocked would not allow the chamber to be filled with the fluid.

The reaction chamber in the third layer is surrounded by edges radiaally tapered at an angle of 50 degree towards the center of the disk. The tapered chamber allows any form of bubble that may be intrduced during fluid handling, to be centrally pushed towards the pressure holes, during rotation, thus allowing the trapped air to escape.

Table S1 Composition and volumes of the reagents used for the quantification of lipid profile

Reagent	Chemical Composition: Active ingredients	Volume (μL)
HDL-Reagent-1 (L1)	Cholesterol Oxidase 10000 U/L; Peroxidase 15000 U/L; Goods Buffer 30 mM/L; Detergent 1; Surfactant and Preservatives.	100
HDL-Reagent-2 (L2)	4-Aminoantipyrine 3 mM/L; Cholesterol Esterase 6000 U/L; Goods Buffer 30 mM/L; Detergent 2; Surfactant and Preservatives.	100
LDL-Reagent-1 (L1)	Goods Buffer 50mM/L; Cholesterol esterase 4800 U/L; Peroxidase 4800 U/L; Cholesterol oxidase 3600 U/L.	100
LDL-Reagent-2 (L2)	Goods Buffer 50mM/L; 4-Aminoantipurine 14mM/L; Detergent; Preservatives; Non-Reactive Stabilizers, Detergents and Preservatives.	100
Triglycerides-Reagent-1 (L1)	Lipoprotein Lipase (Microbial) > 3,000 U/L; Glycerol Kinase (Microbial) > 600 U/L; Glycerol phosphate oxidase (Microbial) > 6,000 U/L; ATP 2.5 mM/L; Mg2+ 2.5 mM/L.	100
Triglycerides-Reagent-2 (L2)	Peroxidase (Horseradish) > 2,000 U/L; 4-Aminoantipyrine 0.4 mM/L; 4-Chlorophenol 2 mM/L.	100

S2. Development of the Calibration Curve

Experiment outline

Pure calibrators of HDL cholesterol, LDL cholesterol and triglycerides (TG) were used for the calibration. The calibrators contain 38mg/dl of HDLc, 114mg/dl of LDLc and 200mg/dl of TG. Each of the solution were diluted with twice and thrice amount of DI water to give a resultant solution of 19mg/dl and 12.6mg/dl of HDLc, 57mg/dl and 38mg/dl of LDLc, and 100mg/dl and 66.6mg/dl of TG. 20µl of the pure calibrators were each dispensed on a separate chamber within the disk. the disk was rotated to send the transport the fluid to the radially distant end of the chamber. 100µl each of the respective reagents1 & 2 were added sequentially. Mixing was carried out on disk by rotating it in the mixing sequence (as given in 'Mixing' in 'Operational Steps' section). The readings were repeated three times separately for all the concentration range.

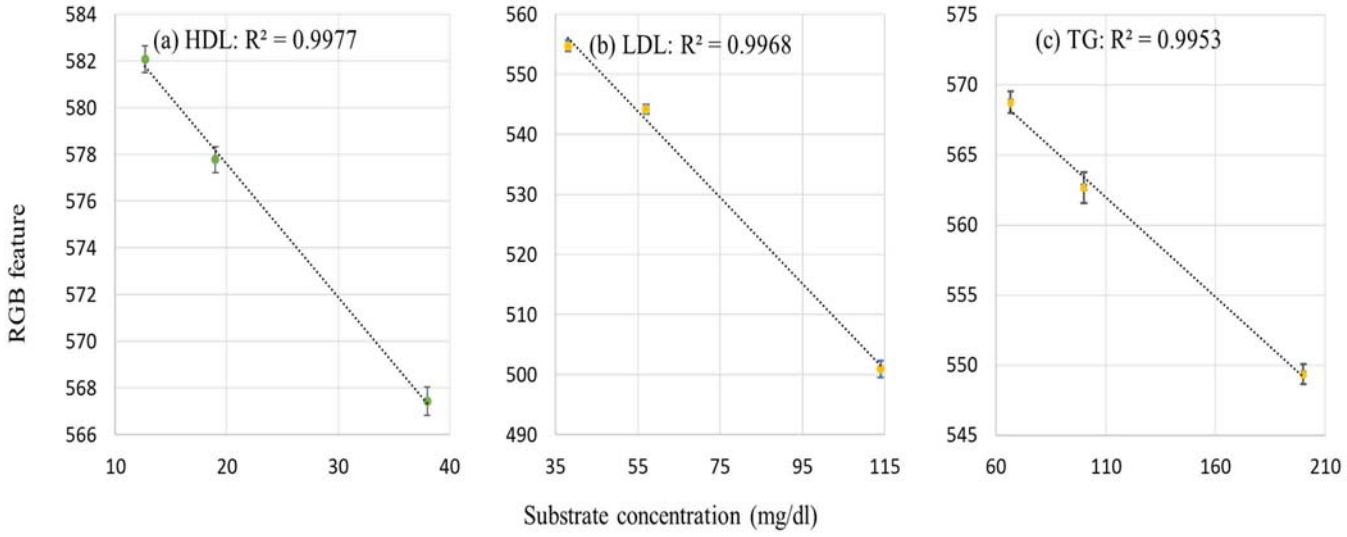

Figure S2 RGB features vs pure calibrator for HDL, LDL and TG show a strong correlation and discrimination ability of the RGB features for the different standard solution concentrations used for the in-vitro analysis. It indicates that the average values of the RGB components of the images are significantly correlated with the concentrations of the respective analytes. The features chosen from the standard sample solutions are then used to detect outliers before performing cross-validation on the training set to evaluate the algorithm's performance in a real-time scenario.

S3. Blood plasma separation and capture of the blood cells.

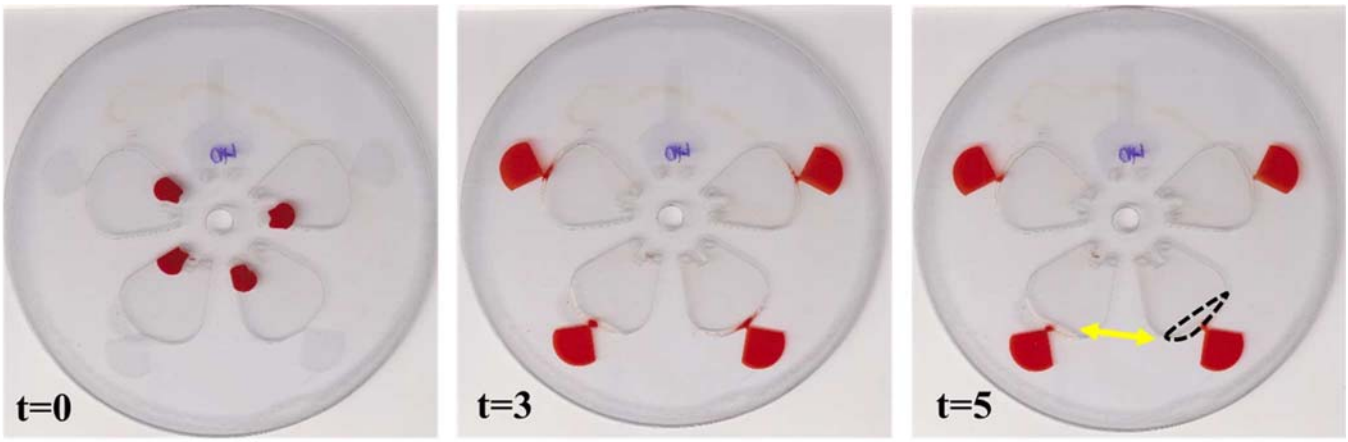

Figure S3 Blood plasma separation is carried out at 2000 rpm for 5 minutes. The images are taken at time t=0, t=3 and t=5 minutes. The sudden narrow constriction of the connecting channel of dimension of 1.2mm x 0.73mm prevents the free escape of the centrifuged blood cells into the chamber R2 as seen at t=5. A clear separation of plasma from aggregated cells is observed and, the backflow of cells into the plasma/reagent chamber is prohibited due to geometrical constraints.

The thickness of chamber that captures the cellular matter is ~50 µm, whereas the thickness of the chamber where the separated plasma is contained is ~500 µm. This discreapancy aids in controlling the direction of flow. During the spin in the plasma separation stage the cells are transported into the thin chamber. These are physically constrained in that chamber due to geometrical constraints. A adverse difference in height blocks the cells into this chamber thereby preventing any backflow into the plasma chamber. This also allows a massive reduction in colorimetric contamination of the end point detection results.

S4. Graphical User Interface (GUI)

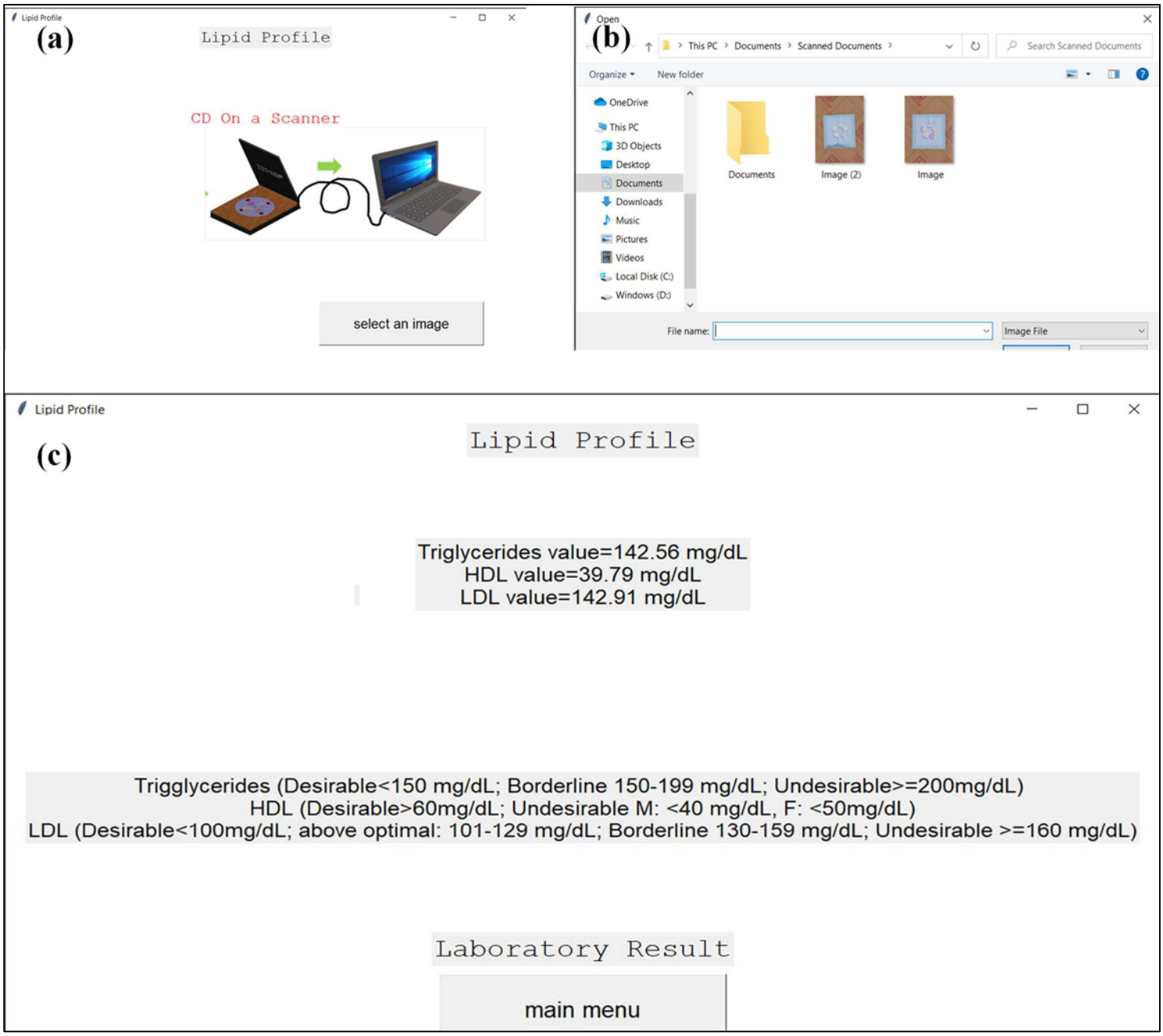

Figure S4 Steps involved in GUI. (a). Opening page; (b) Selection of an image; (c) Display of results.