SUPPLEMENTARY INFORMATION (ESI)

A Novel Point-of-Care Diagnostic Prototype System for the Simultaneous Electrochemiluminescent Sensing of Multiple Traumatic Brain Injury Biomarkers

Milica Jović,[a] Denis Prim,[a] Ophélie Righini,[a] David Tagan,[b] Mélanie Stäuble,[a] Marc Pignat,[b] Steve Gallay,[b] Martial Geiser[c] and Marc E. Pfeifer[a]*

[a] *Diagnostic Systems Research Group, Institute of Life Technologies, School of Engineering, University of Applied Sciences and Arts Western Switzerland (HES-SO Valais-Wallis), Rue de l'Industrie 23, 1950 Sion, Switzerland*

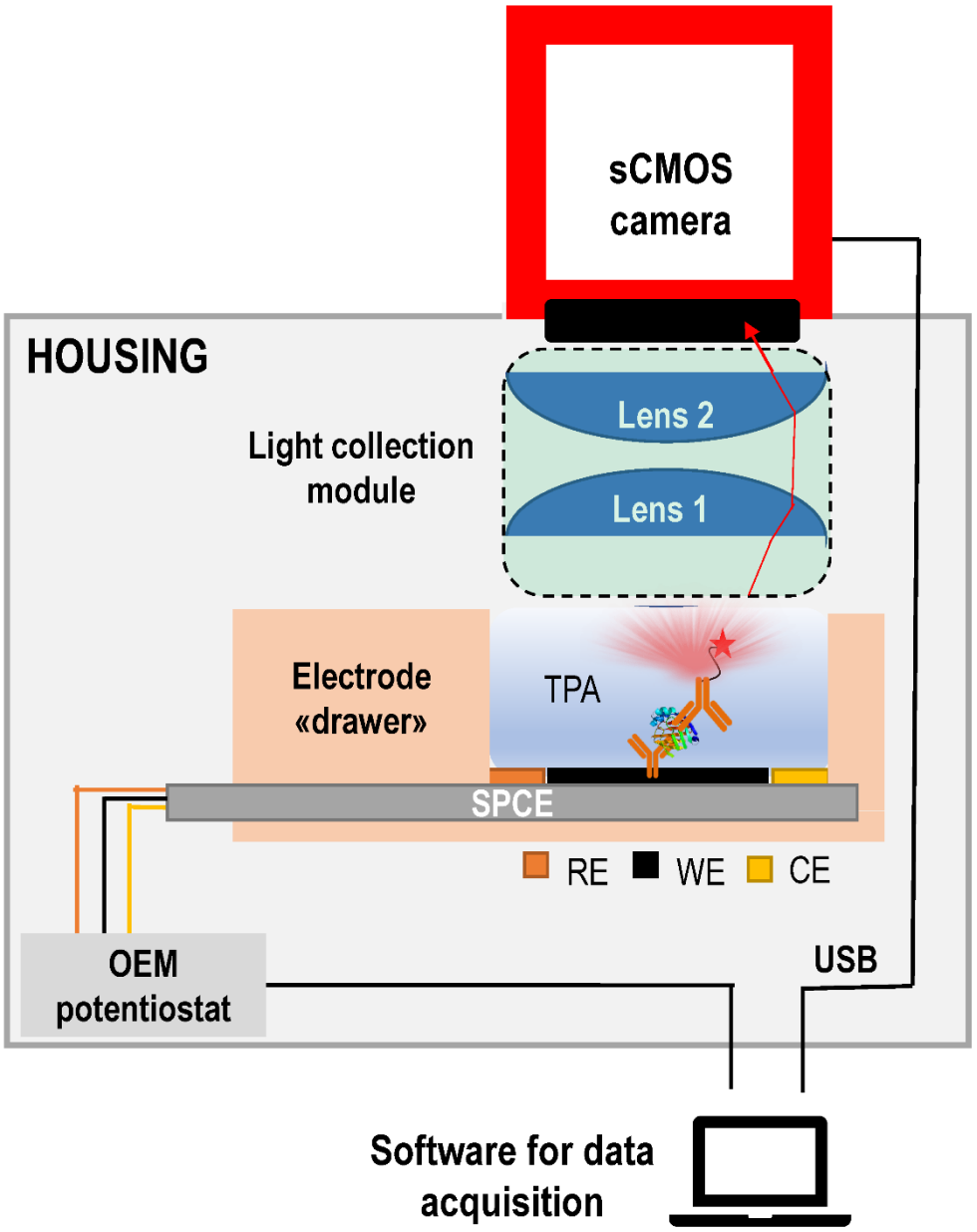
[b] *Institute of Systems Engineering, School of Engineering, University of Applied Sciences and Arts Western Switzerland (HES-SO Valais-Wallis), Rue de l'Industrie 23, 1950 Sion, Switzerland*

[c] *Y-YBAR, Rue de la Dixence 10, 1950 Sion, Switzerland*

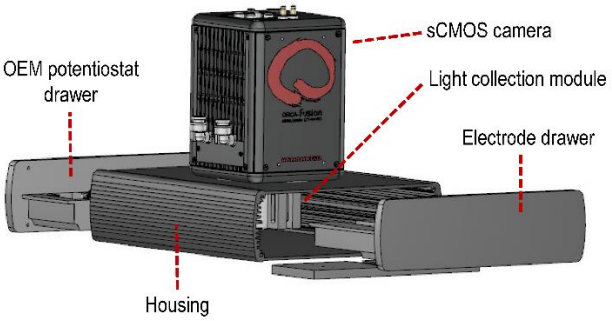
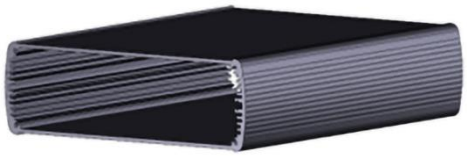
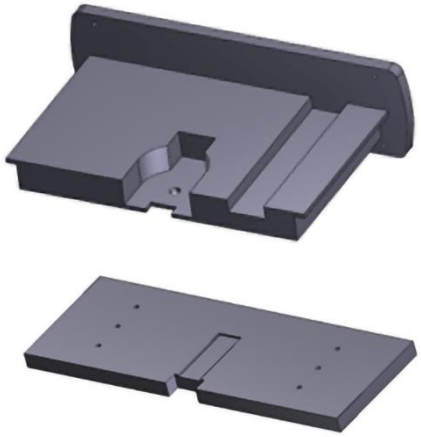
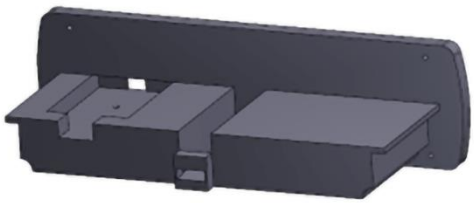
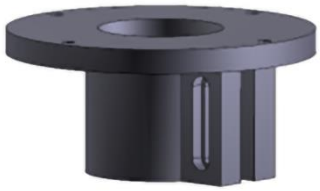
E-mail addresses: milica.jovic@hevs.ch (M. Jović); denis.prim@hevs.ch (D. Prim); ophelie.righini@hevs.ch (O. Righini); david.tagan@hevs.ch (D. Tagan); melanie.staeuble@master.hes-so.ch (M. Stäuble); marc.pignat@hevs.ch (M. Pignat); steve.gallay@hevs.ch (S. Gallay); martial.geiser@y-ybar.com (M. Geiser), marc.pfeifer@hevs.ch (M. Pfeifer).

** Corresponding author: marc.pfeifer@hevs.ch (M. Pfeifer); Tel.: +41-58-606-86-61*

SI-1: Schematic representation of the prototype design.

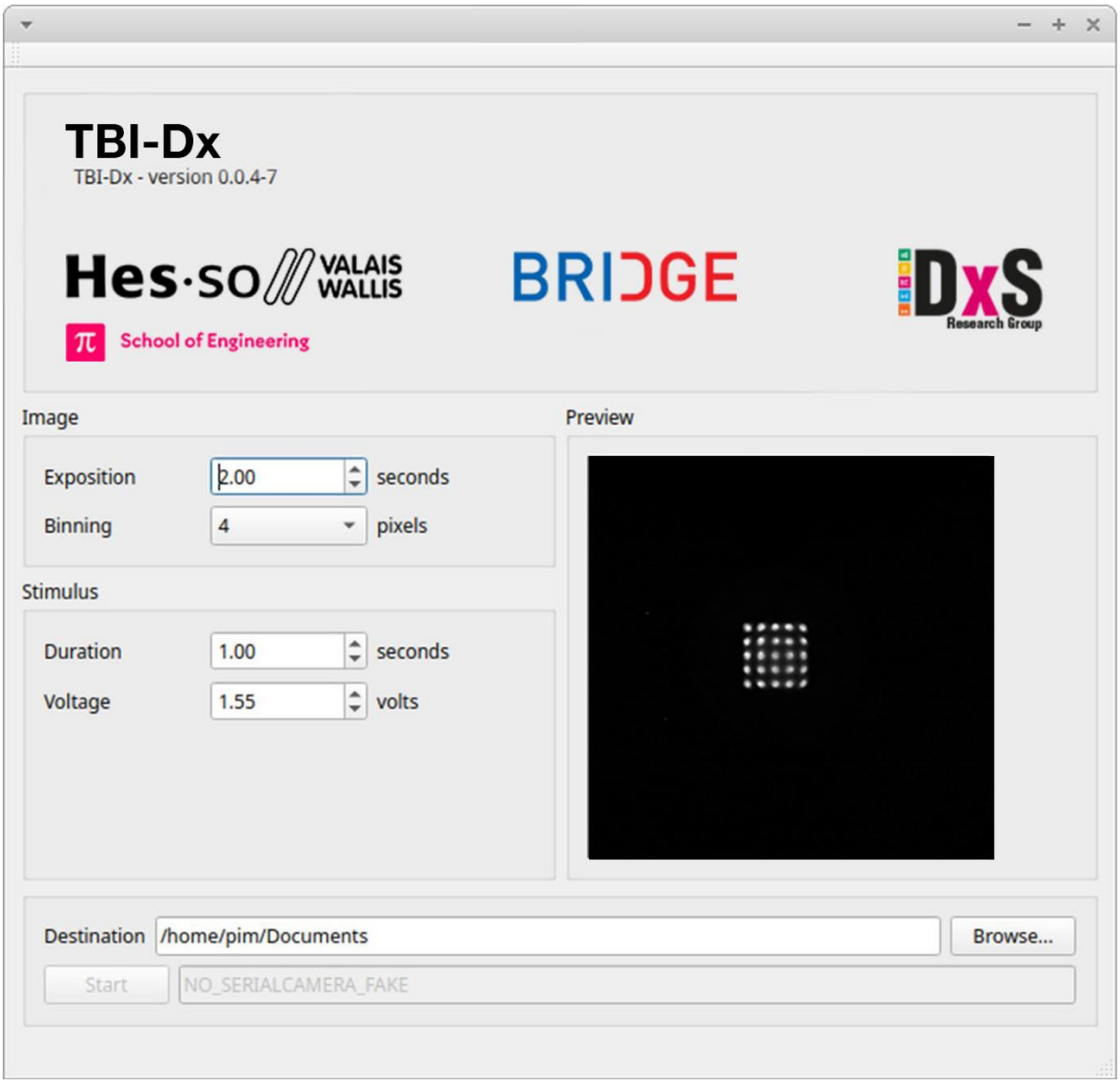


SI-2: CAD drawings of the prototype components.

Prototype	
Housing (Main article, **Fig. 1A**)	
Electrode compartment (Main article, **Fig. 1B**) *(top & bottom part)*	
Potentiostat compartment (Main article, **Fig. 1C**)	
Light collection module (Main article, **Fig. 1D**)	

CAD drawings of prototype components fabricated at the Institute of Systems Engineering, School of Engineering, University of Applied Sciences and Arts Western Switzerland (HES-SO Valais-Wallis). Design and copyrights HES-SO Valais-Wallis.

SI-3: Software interface.

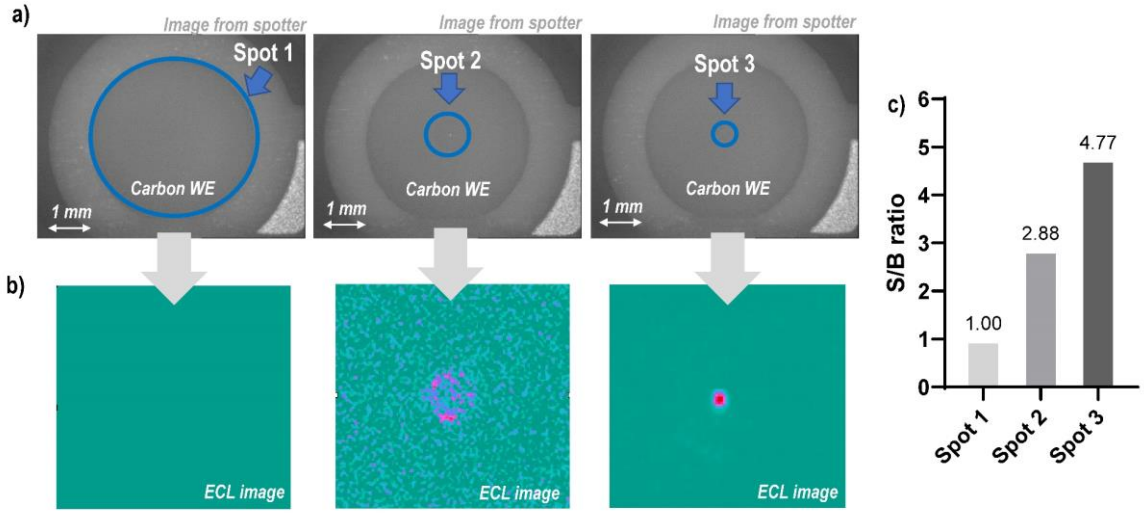


SI-4: Concept of the ECL microarray.

To conceptually show the advantage of the ECL microarray approach over conventional assays, an experiment was performed using a GFAP sandwich immunoassay (blank and 50 ng mL^{-1}) where the same amount of capture antibodies was spotted on three different spots on SPCE: Spot 1 (12.56 mm^2), Spot 2 (0.70 mm^2), and Spot 3 (0.15 mm^2). The amount of cAb in each spot was equal, while the cAb density/spot was increasing with the decreasing spot size.

The signal-to-blank (S/B) ratios were increasing with the increase of cAb density/spot (or decrease of the spot surface), in the order: Spot 1 > Spot 2 > Spot 3, confirming the advantage of the microarray approach over classical assays.

Spot No.	Spot 1	Spot 2	Spot 3
Surface area of the spot	12.56 mm^2	0.70 mm^2	0.15 mm^2
Spotted volume of cAb	30'000 nL	126 nL	14 nL
Number of drops	1	360 drops x 350 pL	40 drops x 350 pL
Spotted concentration of cAb	0.05 µg mL^{-1}	11.11 µg mL^{-1}	100.00 µg mL^{-1}
cAb amount/spot	1.40 ng	1.40 ng	1.40 ng
cAb density/spot	0.11 ng mm^{-2}	2.00 ng mm^{-2}	9.33 ng mm^{-2}



a) Photos of SPCEs with different spots of GFAP capture antibodies obtained with the camera from S3 contactless nano-spotting device Scienion AG. Blue circles represent the spot area on carbon working electrodes (WE); b) ECL images from SPCEs obtained for the GFAP sandwich immunoassay. Antigen concentrations were 0 and 50 ng mL^{-1}. ECL signals were obtained using chronoamperometry at 1.55 V, and acquisition time of 15 seconds. ECL image was processed with Ice palette in ImageJ for easier observation of details. c) Signal-to-blank (S/B) ratios obtained for the GFAP sandwich assay from Spot 1, Spot 2, and Spot 3.

SI-5: Immunoassay conditions.

CRP singleplex assay

cAb diluent	PBS 1X + 5% v/v glycerol
cAb concentrations	25 µg mL^{-1}
Blocking agent	PBS 1X + 1% BSA
Antigen diluent	TRIS (pH 8.6) 50 mM + 1% BSA + 1 mM $CaCl_2$
Wash buffer	PBS 1X + 0.06% Tween-20
dAb diluent	TRIS (pH 8.6) 50 mM + 1% BSA
dAb concentrations	5 µg mL^{-1}
Read buffer	MSD Read buffer T 2X

3-plex assay (GFAP, h-FABP, S100β)

cAb diluent	PBS 1X + 1 mM $CaCl_2$ + 5% v/v glycerol
cAb concentrations	100 µg mL^{-1}
Blocking agent	PBS 1X + 2% BSA + 1 mM $CaCl_2$
Antigen diluent	TRIS (pH 8.6) 50 mM + 0.1% BSA + 1 mM $CaCl_2$
Wash buffer	PBS 1X + 0.06% Tween-20
dAb diluent	TRIS (pH 8.6) 50 mM + 0.1% BSA + 1 mM $CaCl_2$
dAb concentrations	5 µg mL^{-1}
Read buffer	MSD Read buffer T 2X

SI-6: Brief overview of recent publications reported on miniaturized ECL systems/devices.

Note: Non-POC diagnostic type device publications were not considered.

Year	ECL system	Detector	Electrode	Analyte	LOD	Multianalyte detection	Ref.
2015	Luminol/H_2O_2 $Ru(bpy)_3^{2+}$/TPrA	CCD	BPE	TPrA H_2O_2	8.7 µM 46.6 µM	No	[1]
2016	Luminol/H_2O_2	Smartphone	BPE/GOD	H_2O_2 Glucose	1.75 µM 17 µM	No	[2]
2016	Luminol/H_2O_2 $Ru(bpy)_3^{2+}$/TPrA	CCD	BPE/GOD	TPrA H_2O_2 Glucose	1.265 µM 27 µM 32 µM	No	[3]
2016	Luminol/H_2O_2 $Ru(bpy)_3^{2+}$/TPrA	CCD	BPE/GOD	TPrA H_2O_2 Glucose	85 µM 24 µM 195 µM	No	[4]
2016	$Ru(bpy)_3^{2+}$/TPrA	CCD	Carbon	PSA PSMA PF-4	0.3 pg mL^{-1} 0.535 pg mL^{-1} 0.42 pg mL^{-1}	Yes	[5]
2017	Luminol/H_2O_2	CCD	BPE/GOD	H_2O_2 Glucose	24 µM 23 µM	No	[6]
2018	Luminol/H_2O_2	Smartphone	SEES ITO	H_2O_2	0.27 µM	No	[7]
2019	Luminol/H_2O_2	PMT	SEES	H_2O_2	0.26 µM	No	[8]
2021	$Ru(bpy)_3^{2+}$/TPrA Luminol/H_2O_2	Smartphone Photomultiplier tube	BPE	Dopamine Choline	2 µM 1.25 µM	No	[9]
2021	Luminol/H_2O_2	Smartphone	BPE/GOD	H_2O_2 Glucose	5.87 µM 0.138 µM	No	[10]
2021	Luminol/H_2O_2	Smartphone	Laser-induced graphene SE	H_2O_2 Glucose Xanthine Dopamine	1.71 µM 3.76 µM 1.25 µM 3.40 µM	No	[11]
2021	Luminol/H_2O_2	Smartphone	Laser-induced graphene BPE	H_2O_2 Glucose Choline Lactate	4.36 µM 2.51 µM 4.01 µM 5.32 µM	No	[12]
2021	Luminol/H_2O_2 Luminol/O_2	Smartphone	BPE	H_2O_2 O_2 CO_2 Glucose	0.069 µM 0.15 mg L^{-1} 0.45 mg L^{-1} 0.31 µM	No	[13]
2021	Luminol/H_2O_2	Smartphone	BPE	Vitamin B12 Vitamin C	0.109 µM* 0.96 µM*	Yes	[14]
2022	$Ru(bpy)_3^{2+}$/TPrA	Silicon PM module	ITO/SNM	Dopamine	0.0035 µM	No	[15]
2022	IrpiqSQ/Procell	PMT	SPCE	CRP	4.2 µM	No	[16]
2022	Luminol/H_2O_2	CMOS	SEES	Uric acid	26.09 µM	No	[17]
2022	Luminol/H_2O_2	PMT Smartphone	Graphene-based SE	Lactate	6.47 µM	No	[18]
2022	Luminol/H_2O_2	Smartphone	BPE	Glucose Choline	24 µM 10 µM	Yes	[19]
2023	Luminol/H_2O_2	Smartphone	Carbon black-doped PLA	Glucose	60 µM	No	[20]

2023	Luminol/H_2O_2	Smartphone	Graphite pencil-based SE	Cholesterol	15.71 µM	No	[21]
2023	$Ru(bpy)_3^{2+}$/TPrA	sCMOS	SPCE	h-FABP	237 pg mL^{-1}	Yes	This work
				GFAP	742 pg mL^{-1}		
				S100β	583 pg mL^{-1}		

Abbreviations: BPE – bipolar electrode; CRP – C reactive protein; GOD – glucose oxidase; IrpiqSQ - [Ir(piq)2(pt-TOxT-Sq)]Cl where piq = 2-phenyl-iso-quinoline, and pt-TOxT-Sq = a pyridyltriazole ligand with trioxatridecane chain and squarate amide ethyl ester); ITO – indium tin oxide; PM – photomultiplier; PMT – photomultiplier tube; PF-4 – platelet factor-4 ; PLA – polylactic acid electrodes; PSA – prostate specific antigen ; PSMA – prostate specific membrane antigen ; SE – single electrode; SEES – single electrode electrochemical system; SNM – silica nanoporous membrane; SPCE – screen printed carbon electrode. *LOD for individual detection.

References

1 R. Liu, C. Zhang and M. Liu, *Sensors and Actuators B: Chemical*, 2015, **216**, 255–262.
2 L. Chen, C. Zhang and D. Xing, *Sensors and Actuators B: Chemical*, 2016, **237**, 308–317.
3 W. Guan, M. Liu and C. Zhang, *Biosensors and Bioelectronics*, 2016, **75**, 247–253.
4 M. Liu, R. Liu, D. Wang, C. Liu and C. Zhang, *Lab Chip*, 2016, **16**, 2860–2870.
5 K. Kadimisetty, I. M. Mosa, S. Malla, J. E. Satterwhite-Warden, T. M. Kuhns, R. C. Faria, N. H. Lee and J. F. Rusling, *Biosensors and Bioelectronics*, 2016, **77**, 188–193.
6 M. Liu, D. Wang, C. Liu, R. Liu, H. Li and C. Zhang, *Sensors and Actuators B: Chemical*, 2017, **246**, 327–335.
7 W. Gao, K. Muzyka, X. Ma, B. Lou and G. Xu, *Chem. Sci.*, 2018, **9**, 3911–3916.
8 X. Ma, L. Qi, W. Gao, F. Yuan, Y. Xia, B. Lou and G. Xu, *Electrochimica Acta*, 2019, **308**, 20–24.
9 M. Bhaiyya, M. B. Kulkarni, P. K. Pattnaik and S. Goel, *Luminescence*, 2022, **37**, 357–365.
10 M. Bhaiyya, P. Rewatkar, M. Salve, P. K. Pattnaik and S. Goel, *IEEE Transactions on NanoBioscience*, 2021, **20**, 79–85.
11 M. L. Bhaiyya, P. K. Pattnaik and S. Goel, *IEEE Transactions on Instrumentation and Measurement*, 2021, **70**, 1–8.
12 M. Bhaiyya, P. K. Pattnaik and S. Goel, *IEEE Transactions on Electron Devices*, 2021, **68**, 2447–2454.
13 M. Salve, A. Mandal, K. Amreen, B. V. V. S. N. P. Rao, P. K. Pattnaik and S. Goel, *IEEE Transactions on Instrumentation and Measurement*, 2021, **70**, 1–10.
14 M. Bhaiyya, P. K. Pattnaik and S. Goel, *Sensors and Actuators A: Physical*, 2021, **331**, 112831.
15 L. Zhu, W. Fu, J. Chen, S. Li, X. Xie, Z. Zhang, J. Liu, L. Zhou, B. Su and X. Chen, *Sensors and Actuators B: Chemical*, 2022, **366**, 131972.
16 L. D'Alton, S. Carrara, G. J. Barbante, D. Hoxley, D. J. Hayne, P. S. Francis and C. F. Hogan, *Bioelectrochemistry*, 2022, **146**, 108107.
17 R. Abbasi, J. Liu, S. Suarasan and S. Wachsmann-Hogiu, *Lab Chip*, 2022, **22**, 994–1005.
18 M. Bhaiyya, P. Rewatkar, P. K. Pattnaik and S. Goel, *J. Micromech. Microeng.*, 2022, **33**, 024001.
19 M. Bhaiyya, P. K. Pattnaik and S. Goel, *Microchim Acta*, 2022, **189**, 79.
20 D. Calabria, E. Lazzarini, A. Pace, I. Trozzi, M. Zangheri, S. Cinti, M. Difonzo, G. Valenti, M. Guardigli, F. Paolucci and M. Mirasoli, *Biosensors and Bioelectronics*, 2023, **227**, 115146.
21 M. Bhaiyya, P. S. Kumar, P. K. Pattnaik, K. Shankar and S. Goel, *IEEE Sensors Journal*, 2023, **23**, 750–757.