“Single molecule bio-electronic smart system array for clinical testing”

Grant Agreement n° 824946

Deliverable 7.8 Administrative, Financial and Technical Final Report

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Release history

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1	21.01.2022	EI/EIC	1st version from Partners compiled materials
2	30.01.2022	CSGI	Validation
3	30.01.2022	L. Torsi	Review
4	31.01.2022	E Cantatore	Review
5	28.02.2023	CSGI	Redrafting after 3 Review meeting

1. EXPLANATION OF THE WORK CARRIED OUT BY THE BENEFICIARIES AND OVERVIEW OF THE PROGRESS

Summary: The general objective of the SiMBiT project is to develop a bio-electronic smart system that can perform single-molecule detection of both proteins and DNA bio-markers of pancreatic cancer precursors. Specifically, the SiMBiT activities have developed a lab-based device into a cost-effective portable multiplexing array prototype with extremely fast time-to-results. During the third reporting period, the Programme of Activities, described in the Annex 1 of the Grant Agreement, has been successfully carried out, in accordance with the plans, except only few minor deviations (some foreseen and some not foreseen) that have been successfully tackled. The deliverables and the milestones have been completed and submitted on time, according to Amendment 824946-20 (submitted in December 2022). Only in one case a small delay was incurred and justified. Overall, the SiMBiT Consortium has successfully reached the end of the third reporting period via a systematic cooperative work involving an intensive inter-sectorial research effort from academia and industrial partners, accomplishing all planned steps to develop the final SiMBiT cost-effective bio-electronic smart system array.

1.1 OBJECTIVES

The progress booked in the third period for each specific objective described in the submitted proposal, is detailed in the following section:

Objective 1.1 : Validate the already demonstrated single-molecule label-free sensing technology based on an electrolyte-gated thin-film transistor, EG-TFT (WP2 and WP3)
The bio-electronic Electrolyte Gated-TFT (EG-TFT) integrates a layer of recognition elements that are specific for each bio-marker to be analysed. It also exploits solution-processable semiconducting material that are stable in water. The bio-electronic device operates directly in real bio-fluids and can detect both genomic and protein markers.
Status of the objective at the end of the 3rd Period
WP2 in close collaboration with WP3, WP4, WP5 and WP6 has successfully accomplished the label-free and selective detection of MUC1, CD55 (proteins) as well as KRAS (DNA sequence) with ultra-high sensitivity with the 4x4 and 8x12 SiMBiT prototypes. The assessment of the SiMBiT prototype analytical performance has been carried out, demonstrating that the SiMBiT technology is the most cost-effective and fastest of all state of the art diagnostic procedures, namely NGS, ELISA and Simoa, as well as the only test allowing for simultaneous testing of protein and genomic markers using only one device. Importantly, a comprehensive analysis considering all three SiMBiT markers (MUC1, CD55, KRASG12D) based on machine learning approaches resulted in 100% sensitivity and specificity for the detection of mucin-producing neoplasm and for the detection of high-grade mucin-producing neoplasm in cyst fluids as well as blood plasma samples. Moreover, to benchmark the results obtained with the SiMBiT platform, all pancreatic cyst fluids and blood plasma samples from patients with pancreatic cystic lesions were analyzed by SiMoA technology (as reported in D6.2. and D6.3). The deliverable D2.7 (M48) reports the analysis performed with SiMBiT 4x4 as well as 8x12 prototypes of human blood serum and cysts' fluids at the single molecule detection limit. The final SiMBiT prototype is able to produce a reliable fully digital output, provided by a machine learning classifier, capable to reliably discriminate among high grade mucinous cysts, low grade cysts and negative samples directly in blood serum. The results on the benchmarking of the final SiMBiT prototype are reported in D2.8 submitted at M48.

Objective 1.2 : Develop at the pre-clinical level a set of four already identified genomic and protein biomarkers whose quantification conjunctly enable focused diagnostics of a specific disease (WP6 and WP8)
Pancreatic cancer through potential precursors such as cystic lesions will be tackled. Along with the biomarkers also their biological recognition elements (already identified as well) will be developed. The unmatched SiMBiT single-molecule sensitivity will eventually enable the development of new procedures for early and faster diagnostics of cancer through the analysis of markers in blood (WP6). The foreseen approach complies with ethical issues as described in WP8.
Status of the objective at the end of the 3rd Period
Ethical issues have been addressed in WP8 in a very early phase of the project before starting the prospective sample collection. Forms for patients' information and informed consent have been prepared and approved by the local ethic committees. Patients from whom samples were collected have signed the informed consent. The results have been reported in D8.1 and D8.2 reported submitted at M3. WP6 has defined four markers (three genomic markers and one protein marker) that enable diagnosis of mucin-producing PDAC precursors and possibly identify those at high risk of progression. At the end of the 3rd period, the three genomic markers (KRAS, TP53 and GNAS) have been tested in all cyst fluids (n=101) and in 29 blood samples by NGS. The protein marker MUC1 has been tested by ELISA in 59 cyst fluid samples. CD55 has been introduced as additional protein marker during the project and tested successfully by ELISA in 61 cyst fluids. The sample collection as well as the oligonucleotides and proteins/antibodies for biomarker identification have been made available to WP2 for the conduction of SiMBiT analysis, reported by WP2. The results have been reported in D6.1 (M42), 6.2 (M46) and 6.5 (M42).

Objective 1.3: Implement strategies to integrate a layer of stable and functional biological recognition elements into a set of EG-TFTs to enable selective detection of each bio-marker in a real biofluid (WP2 and WP3)
To this end, already proven advanced large-scale processes for bio-functionalisation of the TFT interfaces will be optimized.
Status of the objective at the end of the 3rd Period
The optimized functionalization protocol of the 8x12 SiMBiT 3D array of gates has been developed and validated. This activity involved mainly the task 2.3 (Bio-functionalization of the gate surface and molecular characterization of the bio-layers) and task 2.4 (Electronic detection at single-molecule level of biomarkers) of WP2. The functionalization process has been optimized taking into account the functionalization volume offered by the final SiMBiT prototype. Moreover, the biofunctionalization protocol for CD55 antibodies has been developed during the third reporting period. The 3D sensing gate array' gold surfaces functionalized with anti-MUC1, anti-C55 and KRAS, were tested with both the 4x4 and

8x12 SiMBiT protypes using plasma samples from healthy donors spiked with concentration ranging from 10 zM to 100 fM of the pancreatic cancer biomarkers. The optimized biofunctionalization protocol has been validated in D6.3 (M46), demonstrating that KRAS, CD55 and MUC1 markers are detected in plasma as well as in cyst fluid collected from patients (WP6) at the single molecule detection limit with the optimized SiMBiT prototype.
Moreover, an optimized Simoa SP-X assay for the detection and quantification of MUC 1 and CD55 proteins was developed and optimized. At the state of the art, Single-molecules-array (Simoa) technology, is the most sensitive technology to detect protein markers, indeed has been employed to measure proteins in different matrices reaching limit-of-detections (LODs) in the low femtomolar range. The elicited Simoa SP-X technology is based on the printing of high-density capturing antibodies on the bottom of the wells of a microtiter plate, followed by a standard sandwich-type immunometric chemiluminescent detection. SiMoA represents an already commercialized method that can be conveniently customized. At first a full factorial experimental design has been undertaken to optimize the assay, leading to a reduced experimental effort and an increased quality of the information obtained, compared to the traditional one-variable-at-a-time (OVAT) approach. After the cyst fluids and peripheral blood plasma samples were analyzed using the Simoa technology. The Simoa SP-X assay optimization for MUC1 and C55 detection has been performed within WP6 and WP2 (Task 6.5, Task 2.6). Those results are included in D6.2 (M46) and D2.8 (M48).

Objective 1.4: Fabricate via a cost-effective scalable solution processed manufacturing method, a bioelectronic sensing array system based on the developed single biosensors (WP4 and WP6)

Mass manufacturable and large-area compatible, scalable technologies such as organic electronics and 3D printing processes are efficiently combined. A 96-well plate format, which matches the golden-standard and worldwide-used enzyme-linked immunosorbent assay (ELISA) microplate for clinical testing, will be adopted for multiplexing assay in a clinical environment.

Status of the objective at the end of the 3rd Period

The Objective was met at the end of the 3rd Period. Starting from the processes developed in WP4 to realize single and 4x4 arrays of biosensors based on EG-TFTs, arrays of 8x12 biosensors were designed and fabricated. The 8x12 biosensors were fabricated by adopting large-area lithography on plastic foils for gold source, drain, lateral gates and interconnections. Subsequently, inkjet printing was adopted to pattern the semiconductor P3HT on channel areas, to pattern the insulator SU8 to protect metal lines from exposition to water, and to print Ag silver bridges over SU8, necessary for the complete routing towards the contact pads. In order to allow the use of the array in the final SiMBiT prototype, the contact pads were designed according to the connector selected in WP5 (to allow interconnection with the OTFT foil by FE), and the geometry of the array was re-optimized to reduce the total number of contacts. Such modifications were implemented with a close collaboration between WP4 (led by IIT) and WP5 (led by FE). Again in agreement with WP5, it was decided to bond the bottom-less ELISA plate on the biosensors array only after lamination of the latter into the final cartridge. The bonding of the ELISA plate was accomplished by adopting a commercial medical grade adhesive, prepatterned thanks to a laser process. The 8x12 3D gate plates were fabricated in WP4 according to the optimized design of WP3, thanks to a 3D printing process, followed by a blank deposition of a smoothing and adhesion layer (parylene C) and patterning of gold electrodes by evaporation through a 3D printed shadow mask. The final 8x12 biosensors array, complete with ELISA plate and 3D gate array were successfully validated. WP6 has completed the objective by performing multiplex analysis of clinical samples using the generated SiMBiT prototype. By the end of the 3rd period, 47 clinical samples (35 cyst fluids and 12 blood samples) have been analysed by SiMBiT taking into account the genomic marker KRASG12D and the two protein markers MUC1 and CD55. The results have been reported in D6.3 at M46.

Objective 1.5: Interface the signals of the EG-TFT bio-electronic sensor array by integrating at the periphery of the well plate organic TFT electronics on foil for addressing and conventional silicon IC technology for signal amplification (WP5)

This results in a low-cost and disposable smart biosensor module, which is read-out by multi-use plate reader electronics on PCB. The full SiMBiT bioelectronics smart system is cost–effective and modular, as the smart biosensor module can be disposed after each assay and interchanged to detect different samples. It provides a standard data interface (e.g. USB) to further processing systems. (WP5).

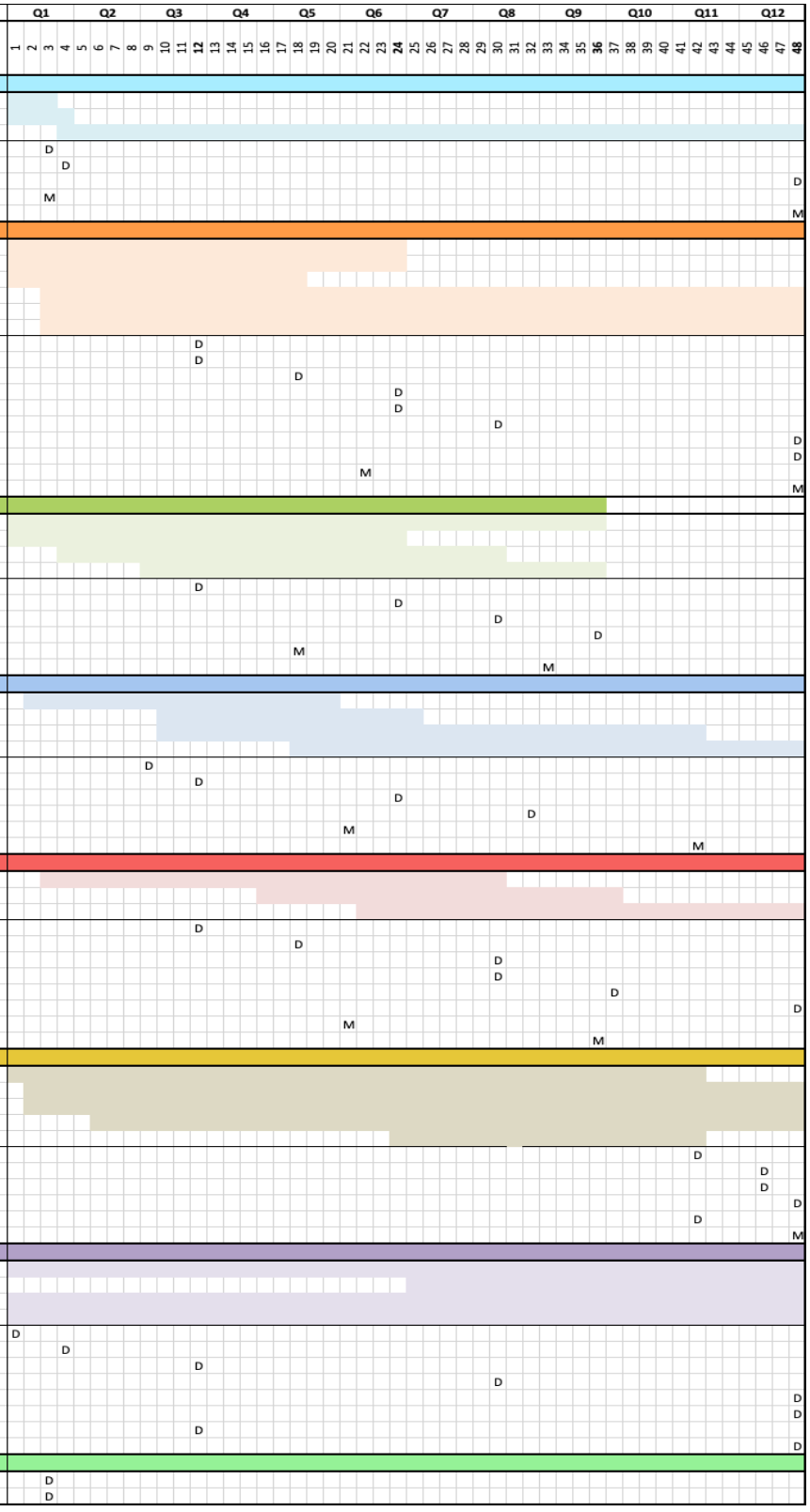
Status of the objective at the end of the 3rd Period

As part of WP5 the design, fabrication, and integration of the final prototype components: EG-TFT bio-electronics sensor array, organic TFT electronics on foil for addressing, PCB and silicon IC for signal amplification, have been successfully completed. Thus this objective has been achieved, and is reported in D5.6, which describes the results of the lab tests of the final prototype.

Objective 2: Validate a scalable cost-effective manufacturing process of a bio-electronic smart sensing array system through a portable prototype comprising a 96-well plate with the EG-TFTs sensing array and the bio-functionalized gates module along with addressing and front-end electronics and an electronic plate reader (WP1, WP5 and WP6).
The prototype will be validated in a clinical environment to prove at least two orders of magnitude better sensitivity and time-to-results shorter by at least a factor of 2 compared to state-of-the-art array bio-sensing platforms. The portable prototype is designed to work in point-of-care and low resource settings but can also be integrated into a walk-away fully automated machine. The final aim is to perform multiplexing testing of a set of biomarkers, including proteins and DNA, at the single-molecule level to enable non-invasive early diagnosis of life-threatening progressive diseases with high societal impacts such as the highly lethal pancreatic cancer.
Status of the objective at the end of the 3rd Period
During this reporting period, the final prototype was ready for testing and for sensing trials. Integration trials of all components of the final prototype were successful, including integration with a microfluidic handling system. Although we did not manage to get all 96 wells working yet, in an industrial manufacturing setting the yield of the EG-TFTs sensing devices would be significantly better. Therefore the present final prototype provides a proof of principle that it is possible to scale up the SiMBiT concept to a 96-well plate. This objective has thus been met and is reported on in detail in D5.6.
Management, dissemination, communication and outreach activities (WP7)
WP7 aims at ensuring the overall management and coordination of the project as well as at promoting the broad dissemination of the SiMBiT outcomes. The WP7 activities are carried out throughout the project duration with for main objective to enable an effective and sustainable dissemination of knowledge among and beyond the members of the SiMBiT consortium.
Status of the objective at the end of the 3rd Period
WP6 has provided a comparative analysis of the results of the state of the art technology (NGS + cytopathology), SiMoA and SiMBiT considering sensitivity, specificity and diagnostic accuracy, thereby showing the superiority of SiMBiT compared to both other methods. These very encouraging results have to be considered preliminary due to the small number of analysed samples. In addition, WP6 has provided a comparison of costs and time-to-result, which can be used in the planning of further exploitation activities related to SiMBiT. These results have been reported in D6.4 at M48 and in the milestone of WP6, MS11 (M48). SiMBiT final WP7 activities were carried out as committed with minor time deviation related to the latest technological developments. SiMBiT main achievements and outcomes demonstrated to significantly raise awareness through many dedicated communication and dissemination channels and related actions. As expected, SiMBiT also identified and analysed future valorisation and exploitation routes and initiated contacts with prospective stakeholders in this matter.

1.2 EXPLANATION OF THE WORK DONE PER WP IN THE 3RD REPORTING PERIOD

	Partner's Person/Months										
	CSGI	UDUS	UNIBS	ABO	IIT	TUE	FE	MASMEC	EI	FLE-TECH	TOTAL
WP1	3	1	1	0,34	3	3	2	10	1	0	24,34
1.1	3	1	1	0,34	3	3	2	2	1	0	16,34
1.2	0	0	0	0	0	0	0	4	0	0	4
1.3	0	0	0	0	0	0	0	4	0	0	4
D1.1								X			
D1.2								X			
D1.3								X			
MS1								X			
M2								X			
WP2	24	15	6	12	4	0	0	0	0	0	61
2.1	4	0	1	1	4	0	0	0	0	0	10
2.2	4	0	1	3	0	0	0	0	0	0	8
2.3	4	0	1	1	0	0	0	0	0	0	6
2.4	4	7	2	3	0	0	0	0	0	0	16
2.5	5	6	1	3	0	0	0	0	0	0	15
2.6	3	2	0	1	0	0	0	0	0	0	6
D2.1	X										
D2.2	X		X								
D2.3	X	X									
D2.4	X										
D2.5	X			X							
D2.6	X		X	X							
D2.7	X		X	X							
D2.8	X		X								
MS3	X										
MS4	X										
WP3	3	0	25	26,8	10	4	0	0	0	0	68,8
3.1	1	0	2	14,8	3	0	0	0	0	0	20,8
3.2	0	0	10	0	0	0	0	0	0	0	10
3.3	1	0	6	6	0	0	0	0	0	0	13
3.4	1	0	7	6	7	4	0	0	0	0	25
D3.1			X	X							
D3.2			X								
D3.3			X	X							
D3.4	X		X								
MS5			X								
MS6			X								
WP4	2	0	5	1	52	6	4	0	0	1	71
4.1	1	0	1	0	12	1		0	0	0	15
4.2	0	0	1	0	17	2	0	0	0	0	20
4.3	1	0	3	1	1	1	0	0	0	0	7
4.4	0	0	0	0	22	2	4	0	0	1	29
D4.1					X						
D4.2					X						
D4.3	X		X								
D4.4					X						
MS7					X						
MS8	X		X		X						
WP5	1	0	1	0	7	37,1	30,18	25	0	11,12	112,4
5.1	0	0	1	0	7	24	6	2		0	40
5.2	0	0	0	0	0	7,1	12,68	7		0	26,78
5.3	1	0	0	0	0	6	11,50	16		11,12	45,62
D5.1						X					
D5.2						X					
D5.3						X					
D5.4						X	X				
D5.5						X	X				
D5.6								X			
MS9						X	X	X			
MS10						X	X	X			
WP6	16	32	0	7	0	0	0	0	0	0	55
6.1	0	7	0	1	0	0	0	0	0	0	8
6.2	4	7	0	2	0	0	0	0	0	0	13
6.3	4	4	0	2	0	0	0	0	0	0	10
6.4	4	7	0	1	0	0	0	0	0	0	12
6.5	4	7	0	1						0	12
D6.1		X									
D6.2		X									
D6.3		X									
D6.4		X									
D6.5	X	X									
MS11		X									
WP7	7	3	1	1	3	1	2,8	1	21	2,9	43,7
7.1	3	3	1	1	3	1	0,8	1	3	2,9	19,7
7.2	1	0	0	0	0	0	2	0	6	0	9
7.3	1	0	0	0	0	0	0	0	6	0	7
7.4	2	0	0	0	0	0	0	0	6	0	8
D7.1	X								X		
D7.2	X								X		
D7.3	X								X		
D7.4	X								X		
D7.5	X								X		
D7.6	X								X		
D7.7	X								X		
D7.8	X								X		
WP8											
D8.1	X	X									
D8.2	X	X									



1.2.1 WORK PACKAGE 1 – STATUS AT THE END OF 3RD REPORTING PERIOD

WP1 title: "SiMBiT bio-electronic smart system specifications for users, validation and regulatory compliance"

WP Leader: MASMEC

Participant: CSGI, UDUS, UNIBS, ABO, IIT, TUE, FE, MASMEC, EI

Summary of progress in third reporting period: The WP1 consists of three tasks:

Task 1.1 Defining hardware and software specifications for the SiMBiT prototypes as a clinical point-of-care platform suitable for a fast application of the regulatory procedures by the International Organization for Standardization.

Task 1.2 Defining Quality Control and Quality Assessment (QC/QA) procedures that will be provided for a medical device certification.

Task 1.3 Monitoring that the activities carried out in SiMBiT comply with the technical specifications and procedures identified in WP1.

The activities in WP1 enabled to follow and trace all the documentation produced in the project period. This will be important to proceed further, after the project, towards the industrialization roadmap presented in the Exploitation Plan, in compliance with Standard Procedures and Quality Control Insurance.

All the tasks of the WP1 have been performed without delay and the related objectives were achieved without deviations.

All tasks of the WP1 have been concluded without delay and the related objectives were achieved without deviations.

1.2.2 WORK PACKAGE 2 – STATUS AT THE END OF 3RD REPORTING PERIOD

WP2 title: "Single-molecule label-free, selective bio electronic sensor"

WP Leader: CSGI

Participant: UDUS, UNIBS, ABO, IIT

Summary of progress in third reporting period: WP2, in close collaboration with WP3, WP4, WP5 and WP6 has validated the final SiMBiT prototype to assay both proteins, namely MUC1 and CD55, and genomic markers, KRAS of pancreatic cancer in samples collected from patients at UDUS. The SiMBiT assay has been accomplished both in cyst fluid as well as in blood plasma samples. The reliable and stable operation of the SiMBiT electrolyte gated – thin-film transistor (EG-TFT sensing system), as well as the optimized biofunctionalization protocol have been defined. Moreover, the analysis of the data collected with the SiMBiT prototype have been analysed with a machine learning approach, allowing to reliably discriminate among high-grade and low-grade mucinous cyst or non-mucinous/negative patients' samples. The SiMBiT technology has been benchmarked with the Quanterix SP-X System to assay both MUC1 and CD55 in blood plasma as well as in cyst fluid. However, due to lower sensitivity of the Simoa assay for CD55 and MUC1 proteins, the biological value of plasma remains limited as for NGS and ELISA.
The objective of the action has been successfully achieved in line with the Grant Agreement. To this end, CSGI, in close collaboration with ÅBO, UNIBS, Tu/e, IIT, MASMEC, FlexEnable, and UDUS produced 6 deliverables during the third reporting period of the SiMBiT project, namely D2.6 (First report on SiMBiT EG-TFT sensors for the detection of the target biomarkers at the single-molecule level– Leader CSGI), D2.7 (Final report on SiMBiT EG-TFT sensors for the detection of the target biomarkers at the single-molecule level– Leader CSGI), D2.8 (Benchmarking SiMoT technology with the Quanterix SP-X System– Leader CSGI), D6.2 (Analysis of clinical samples by conventional NGS and SIMOA– Leader UDUS), D6.3 (Analysis of clinical samples through SiMBiT technology– Leader UDUS), and D6.4 (Prospective validation of the results using clinical samples and/or data and method comparison– Leader UDUS).

1.2.3 WORK PACKAGE 3 – STATUS AT THE END OF 3RD REPORTING PERIOD

WP3 title: "Optimized bio-electronic EG-TFT"

WP Leader: UNIBS

Participant: CSGI, ABO, IIT, TUE

Summary of progress in third reporting period: During the third reporting period, WP3 developed optimized transistor structures with high stability, reliability, and bio-electronic amplification. Specifically, new physically based analytical and numerical models of bio-functionalized transistor structures operated as multi-modal sensors have been successfully proposed and validated with extensive measurements and considering a wide range of operating conditions and geometries. New bio-sensing structures have been developed based on electrolyte-gated transistors exploiting solution-processed semiconducting materials with stable and reliable operation in an aqueous environment. The bio-sensing transistor structure, bias conditions, and measurement strategies have been optimized to achieve high-performance system integration. We demonstrated bio-electronic and nano-structured materials combined into new high-performance bio-sensing transistor structures for smart sensing applications. The transistor structures are suitable for integration in the sensor array and fully compatible with the 96-well ELISA standard. These achievements are in full agreement with the objectives.

All the deliverables planned in WP3 have been submitted on time. The Deliverables 3.1, 3.2, and 3.3 have been accepted (reporting period 1 and 2) and Deliverable 3.4 has been submitted during this last reporting period (reporting period 3). The Deliverable 3.4 reports on the activities about optimization of the high-performance bio-sensing transistor structure.

In WP3 there are two milestones. The milestone MS3.1 "First optimized high-performance and reliable bio-sensing transistor structure for array integration (prototype 1)" was achieved in month 18 (June 2020). The milestone MS3.2 "Final optimized high-performance and reliable bio-sensing transistor structure for array integration (final prototype)" was achieved in month 34 (October 2021).

1.2.4 WORK PACKAGE 4 – STATUS AT THE END OF 3RD REPORTING PERIOD

WP4 title: "Cost-effective manufacturing of the SiMBiT bio-electronic smart sensing array"

WP Leader: IIT

Participant: CSGI, UNIBS, ABO, IIT, TUE, FlexEnable

Summary of progress in third reporting period: During the reporting period, WP4 demonstrated large arrays of 8x12 EGTFTs with high performance, reproducibility and stability. Furthermore, large-area, solution based, and scalable processes have been used and optimized so that the final device can be produced in a cheap, yet industrially and commercially viable way. The fabrication of multiple arrays provided for extensive electrical characterization, demonstrating the robustness and reliability of the process. The design of the final EGTFTs matrix is also compatible with 96-well ELISA plates, as well as for external electrical connection with the readout and addressing electronics specifically developed by WP5. These achievements are in full agreement with the objectives.

All the deliverables planned in WP4 have been submitted on time. Deliverable 4.4 "Report on planar 8x12 array of EG-FETs with lateral gate and stability" has been submitted during this last reporting period. The Deliverable 4.4 demonstrates the fabrication and characterization of the first array of 8x12 EGTFTs, with stability assessment in line with the specifications.

1.2.5 WORK PACKAGE 5 – STATUS AT THE END OF 3RD REPORTING PERIOD

WP5 title: "SiMBiT smart bio-electronic modular system prototype"

WP Leader: FE

Participant: CSGI, UNIBS, IIT, TUE, FE, MASMEC

Summary of progress in third reporting period: Within this reporting period, the design of the final SiMBiT prototype and all its components were finalized. A pipeline of first SiMBiT prototypes were fabricated and delivered for sensing trials at UNIBA/CSGI throughout this time period. The final prototype components (OTFT addressing foil, OTFT sensing array, Si-IC and PCB) were fabricated and electrically tested independently (M5.2 and D5.5). Integration trials of the final prototype including the cartridge were performed and sensing trials using the final prototype were performed as well as integration of the prototype into a microfluidic handling system (D5.6).

PROJECT PARTNER	ACTIVITIES IN WP5 DURING M31-M48
TUE	• Design of Si-IC for final prototype • Design of OTFT addressing foil for final prototype • Testing of Si-IC and OTFT addressing foil for final prototype • Integration of prototype for electrical verification and sensing trials, testing support • Deliverable report preparation.
FLEXENABLE	• WP5 management and support in deliverable report preparation. • Involvement in design of OTFT addressing foil • Fabrication and test of OTFT addressing foil • Input on cartridge design • Bonding and contact validation of components • Connector design for final prototype • Development and optimisation of prototype assembly
MASMEC	• PCB design and test for final prototype • Software interface for final prototype • Verification of testing protocols • Cartridge design • Final prototype integration and test support • Integration of final prototype with microfluidic handling system • Deliverable report preparation
IIT	• Design of lateral OTFT array for final prototype. • Fabrication and testing of lateral OTFT arrays for first and final prototypes • Perform part of the process to make 3D bio-gate array • Contributions to deliverable reports

UNIBS	• Design, fabrication and testing of 3D bio-gate array. • Contributions to deliverable reports
CSGI	• Testing of first and final prototype in sensing trials • Feedback on sensing trials to influence next steps • Perform part of the process to make 3D bio-gate array • Contributions to deliverable reports

Over the last reporting period, the final prototype was ready for testing and for sensing trials. Integration trials of all components of the final prototype were successful, including integration with a microfluidic handling system. Although we have not successfully got 100% of the 96 wells working, in a manufacturing setting the yield of OTFT sensing devices would be significantly better, therefore this works acts as a proof of principle that it is possible to successfully scale up to the SiMBiT prototype to a 96-well plate. This objective has thus been met and is reported in detail in Deliverable D5.6.

1.2.6 WORK PACKAGE 6 – STATUS AT THE END OF 3RD REPORTING PERIOD

WP6 title: "SiMBiT testing in clinical, point-of-care and low-resources settings"

WP Leader: UDUS

Participant: CSGI, ABO

Summary of progress in third reporting period: The work of WP6 enabled the validation of SiMBiT technology as a viable tool for the identification of mucin-producing cystic neoplasms and of high-grade mucin-producing neoplasms of the pancreas with optimal sensitivity and specificity in a clinical, point-of-care setting. It proved that SiMBiT technology could detect the presence of these neoplasms using both pancreatic cyst fluids and blood plasma samples and confirmed that SiMBiT technology enables a multiparametric analysis including protein and genomic biomarkers, which is time- and cost-effective at the same time. Due to its cost-effectiveness, SiMBiT is a promising technology for implementation in low-resource settings as well. Due to the technical issues in WP5, only a subset of samples could be analyzed by the SiMBiT 4x4 prototype (see D2.7), so these promising results should be seen as preliminary and should be validated in larger number and, possibly, in prospective cohorts.

1.2.7 WORK PACKAGE 7 – STATUS AT THE END OF 3RD REPORTING PERIOD

WP7 title: "Management, communication, dissemination and outreach activities"

WP Leader: CSGI

Participant: CSGI, UDUS, UNIBS, ABO, IIT, TUE, FlexEnable, MASMEC, EFFICIENT

Summary of progress in third reporting period: WP7 is dedicated to management, communication, dissemination and outreach activities. One of SiMBiT communication and dissemination objective is creating awareness, raising interest, fostering engagement and accelerating the market uptake. In those matters, SiMBiT achieved major quantitative and qualitative results as fully detailed in the related Deliverables submitted over the 3rd period and especially Deliverable 7.5 : *Final report on dissemination, communication and exploitation activities (M48) and Deliverable 7.6 : SiMBiT Exploitation Plan.(M48).*
Detailed description of progress: The tasks and objectives of WP7 for the third period were accomplished according to plans. The dissemination was carefully planned and carried out correctly, and several communication activities were implemented mostly through 31 events attended, 32 publications released, and over 100 K views over the different social networks used by the project.

DISSEMINATION ACTIVITIES

Following on from the first two years of the project, the whole consortium increased its effort to communicate about the project's activities through:

- **Social networks:** The LinkedIn profile and the project page were used to relay important information about the project (main publications, events in which the consortium was involved, progress reports). This information was relayed as well on the Twitter profile that was created following the first review and shortly after the dedicated Facebook and Instagram profiles. Those social media enabled all together to reach a quite diversified audience respectively 40 K views for LinkedIn, 38 K views for Twitter, 17K views for Facebook and at last 13 K views for Instagram.
- **Web site: The project web site has been continuously updated so as to present the SiMBiT main achievements, upcoming news and events. A latest major upgrading of the web site enabled to include as well videos and the latest scientific publications.**
- **Events:** Through the 3rd Reporting Period the attendance of 7 workshops, and over 10 international conferences and other relevant events, presented good opportunities to disseminate and discuss the SiMBiT project and goals with other members of the scientific community and industrials. This was greatly strengthened by the organisation of 2 SiMBiT related conferences. All those enabled to reach over 3 500 stakeholders, among those two third were industrials
- **Scientific publications:** the consortium has released a total of 43 publications throughout its latest reporting period. Among those, it is worth mentioning 24 articles in major scientific journals, 13 publications in conference proceedings/workshops, as well as 2 book Chapters and 4 Open reports.

OVERALL COORDINATION OF THE PROJECT

The SIMBIT project has been designed according to a transparent organization and management structure, based on coordinator's broad experience in leading international research projects. CSGI, as project coordinator carried out all the activities related to technical, financial and administrative management. The foreseen tasks in WP7 have been fulfilled also thanks to an active and fruitful collaboration among partners.

The following actions during the third period have been conducted:

- The monitoring of the progress of the activities was carried out through monthly meetings with all the partners. Inter-work package meetings were also organised for the organisation and the operational monitoring of tasks.
- Coordination and submission of due deliverables and milestones.
- In December 2022 an amendment to the Grant Agreement has been submitted. Below are detailed the three deviations which have been included in the procedure (the list of changes and their explanations are available on the EC portal):
  1. Extension of Work Package 5 "SiMBiT smart bio-electronic modular system prototype" and delay of deliverable D5.6 "Lab. test report of the final SiMBiT prototype "from M42 to M48.
  2. Change of the title of D7.6 from" Plan for use, dissemination and exploitation of foreground, including IPR manual" to "Final SimBiT exploitation plan"" and submission delay from M42 to M48.
  3. Termination of beneficiary n. 7 - FlexEnable Limited (PIC 991778053) and addition of FlexEnable Limited Technologies as new beneficiary n. 10 (PIC 884731375). The residual FlexEnable Limited's tasks, budget and personnel effort have been transferred to the new beneficiary.

1.3 IMPACT

The SiMBiT initial information provided in the Section 2.1 of the DoA remains relevant as far as the expected impacts is concerned. Nevertheless, additional information can still be shared to justify in greater details the prospective impact of SiMBiT related technologies and achieved prototype towards its targeted markets enabling personalised medicine:

- Impact of SiMBiT to enable early detection of pancreatic cancer biomarkers with single molecule limit of identification.
- Impact of the fast and cost effective SiMBiT platform to enable a reliable diagnosis of complex diseases.
- Impact of SiMBiT innovative technique to detect different biomarkers with a multiplexing approach.
- Impact of SiMBiT innovative technique to contribute to future European and worldwide standards for high sensitivity medical diagnostics.

As such the initial entry point of SiMBiT technologies remains unchanged and will then still focus on both research, and point-of-care use in primary and secondary care. SiMBiT versatility, cost effectiveness and short time of result demonstrate a strong competitive advantage, which shall support a faster and stronger societal impact, especially towards patients with different type of aggressive and deadly cancer. Better early diagnosis could then be life-saving tens of thousands of European patients, while significantly reducing by millions € the related financial burden towards both European patients' out-of-pocket

costs and their national health-care systems.

2. UPDATE OF THE PLAN FOR EXPLOITATION AND DISSEMINATION (IF APPLICABLE)

As due, SiMBiT Final exploitation plan has been updated so as to include the project latest achievements in terms of outcomes and related IPR. Of course, the related exploitation and commercialisation opportunities have been considered in view of the targeted markets prospective evolution over the coming years. The Final Exploitation and Dissemination Plan also details the new knowledge generated by the Partners and how they will manage it form an individual or joint valorisation including the sharing of future revenues through dedicated business models.

List of attended events in the frame of the third reporting period

Event	Topic	Type of events*	Estimated number of persons at the event	Estimated number of persons reached	Type of persons reached	Date	Entity	Partners attending
IEEE IFETC 2021	Pushing the Limits of Printed and Flexible Organic Electronics: Thin, Fast and Edible	Participation to a Conference	100		Scientific Community (Higher Education, Research)	10 August 2021	IIT	Mario Caironi
European Congress of Pathology	Pathology	Organisation of a Conference	virtual	400	Scientific Community (Higher Education, Research)	29th-31th August 2021	UDUS	Irene Esposito
CAD-TFT 2021	12th International Conference on Computer-Aided Design for thin Film Transistor Technologies	Participation to a Conference			Scientific Community (Higher Education, Research)	3rd september 2021	TUE	Eugenio Cantatore
Viszeral Medizin	internal medicine, gastroenterology, oncology	Participation to a Conference	8 000	200	scientific Community (Higher Education, Research)	17th September 2021	UDUS	Irene Esposito
UEGW	internal medicine, gastroenterology, oncology	Participation to a Conference	virtual	300	Scientific Community (Higher Education, Research)	3rd-5th October 2021	UDUS	Irene Esposito
MRS (Materials Research Society)		Participation to a Conference			Scientific Community (Higher Education, Research)	November 29-December 2 (2021)	IIT	Fabrizio Viola
GI Cancer day	internal medicine, gastroenterology, oncology	Participation to a Conference	3000	500	Scientific Community (Higher Education, Research)	4th December 2021	UDUS	Irene Esposito
Spring Meeting - Fun-OrgBio22	Fundamentals of Organic Bioelectronic Devices	Participation to a Conference				10th Mars 2022	ABO	Luisa Torsi, Eleonora Macchia
LOPEC	driving the future of printed electronics	Participation to a Conference			Industry	23-24th march 2022	CSGI / TUE	Luisa Torsi, Eugenio Cantatore

The pharmaceutical and biotechnological sectors in belgium and apulia - experiences, possibilities for cooperation	workshop on pharmaceutical and biotechnology	Participation to a Conference				4th April 2022	CSGI	Luisa Torsi
German congress of surgery, DCK	surgery, oncology	Participation to a Conference	7000	200	Scientific Community (Higher Education, Research)	6th-8th April 2022	UDUS	Irene Esposito
MRS Spring 2022	Materials Research Society spring event	Participation to a Conference	4000	50	Scientific Community (Higher Education, Research)	8-13 May 2022	IIT	Mario Caironi, Fabrizio Viola, Francesco Modena
Conference Wilhelm Exner Medals	award ceremony - honored selected researchers	Participation to an Event other than a Conference or a Workshop			Scientific Community (Higher Education, Research)	15-19 May 2022	CSGI	Luisa Torsi, Eleonora Macchia, Paolo Bolella
7th International Conference on Bio-Sensing Technology	**Biosensors**	Participation to a Conference	200	200	Scientific Community (Higher Education, Research)	22nd-25th May 2022	ABO	Kim Björkström
ISCAS	International Symposium on Circuits and Systems	Participation to a Conference	1000	200	Scientific Community (Higher Education, Research)	28 May - 1 June 2022	TUE	Marco Fattori
Workhop gruppo di chemiometria SCI	Recent analytical-based chemometric approaches	Participation to a Workshop	50		Scientific Community (Higher Education, Research)	30 May 2022 - 1st June 2022	CSGI / ABO	Eleonora Macchia, Cecilia Scandurra
German Congress of Pathology, DGP	pathology	Participation to a Conference	3000	400	scientific Community (Higher Education, Research)	8th-11th June 2022	UDUS	Irene Esposito
Gordon Research Conference on Electronic Processes in Organic Materials	Electronic processes in organic materials	Participation to a Conference	80		Scientific Community (Higher Education, Research)	26th June 2022 - 1st July 2022	CSGI	Luisa Torsi, Cecilia Scandurra

76th international workshop and 6th orbitaly	fundamental mechanisms to drive progresses in organic and large-area bioelectronics	Organisation of a Workshop			Scientific Community (Higher Education, Research)	3rd - 9th July 2022	CSGI / ABO / IIT	Luisa Torsi, Lucia Sarcina, Ronald Osterbacka, Mario Caironi
IEEE - fleps 2022	international conference on flexible printable sensors and systems	Participation to a Conference				10-13th July 2022	CSGI	Luisa Torsi
PRORISC	Program for Research on Integrated Systems and Circuits	Participation to a Conference	150	150	Scientific Community (Higher Education, Research)	14-15 July 2022	TUE	Enrico Genco-Eugenio Cantatore
ICSM	organic electronics	Participation to a Conference				17th -22nd July 2022	Abo	Ronald Osterbacka
SPIE Optics and Photonics 2022	multidisciplinary optical sciences and technology	Participation to a Conference			Scientific Community (Higher Education, Research)	21- 22th August 2022	CSGI / ABO	Eleonora Macchia
European Congress of Pathology	pathology	Organisation of a Conference	4000	800	Scientific Community (Higher Education, Research)	3rd-7th September 2022	UDUS	Irene Esposito, Lena Häberle
XXIX Congresso della Divisione di Chimica Analitica della Società Chimica Italiana	Analytical Chemistry for a green and sustainable future	Participation to a Conference	250		Scientific Community (Higher Education, Research)	11-15th September 2022	CSGI	Eleonora Macchia, Lucia Sarcina, Cecilia Scandurra
Viszeralmedizin 2022	internal medicine, oncology, gastroenterology	Participation to a Conference	10000	200	scientific Community (Higher Education, Research)	11th-16th Sept. 2022	UDUS	Irene Esposito
ITC	International Thin-Film Transistor Conference	Participation to a Conference	200	200	Scientific Community (Higher Education, Research)	14-16th September 2022	TUE	Eugenio Cantatore, Enrico Genco

MSCA-Staff Exchanges	opportunities for companies - promote international, cross-sectoral and interdisciplinary collaboration in research and innovation	Brokerage Event				13th October 2022	CSGI	Luisa Torsi
German Congress of Cancer, DKK	oncology	Participation to a Conference	10 000	80	Scientific Community (Higher Education, Research)	13th-16th November 2022	UDUS	Irene Esposito
ÅAU Sensor Seminar (online event)	workshop on sensors and biosensors for diagnosis procedures	Participation to a workshop	50			24/11/2022	ABO	Luisa Torsi, Eleonora Macchia
MRS Fall 2022	Materials Research Society fall event	Participation to a Conference	4000	50	Scientific Community (Higher Education, Research)	27 November, 2 December	IIT	Fabrizio Viola

3. UPDATE OF THE DATA MANAGEMENT PLAN (IF APPLICABLE)

Nothing new.

4. FOLLOW-UP OF RECOMMENDATIONS AND COMMENTS FROM PREVIOUS REVIEW(S)

The SiMBiT Consortium during the RP3 has carefully addressed the list of recommendation from the previous review. In the following section, the actions taken to address those recommendations are reported in an itemized fashion.

- **Testing on real samples should be performed asap**

47 real samples have been assayed with the SiMBiT technology (10 of which assayed in double blind): 35 cyst fluids and 12 blood plasma samples. SiMoA assay for MUC1 and CD55 has been performed on 74 real samples; 60 cyst fluids and 14 blood plasma samples.

- **Stability of the samples after various transfers and transportation should be verified**

The stability of the EG-TFT arrays has been tested and validated over 22 4X4 arrays and 4 8X12 arrays, showing that all matrixes after shipment are in specification. Moreover, it has been also proven that transportation and freezing/thawing of blood serum as well as cysts' fluid collected from patients at WP6 does not represent a hurdle.

- **Next to reliability and stability, reproducibility aspects between different prototypes and systems should be tackled in a more systematic way at this stage of the project.**

22 first prototype sensing arrays were delivered to CSGI for sensing trials. The sensing experiments have been performed using 4 different prototypes, each one used to perform 16 sensing experiments (despite the prototype being built as a single-use device). Operational lifetime shown to be >2 months and shelf life > 3 months. The final SiMBiT readout system passed tests for power stability, data transfer reliability and analogue performance as reported in D5.6.6 for fully assembled final prototypes completed. 4 final prototypes were delivered to CSGI to complete sensing trials including reliability testing. Both first and final SiMBiT prototypes performing well, meeting main specifications reported in D1.1.

- **The communication activities are also well organized; however, it was noted that the audience targeted is mainly focused on scientists and academic community, therefore more attention should be dedicated to address a larger and more diverse audience. Exploitation activities could benefit of more specificity, for example from an action/business plan with a roadmap and timelines indicating how the project results can be brought to the market and what is needed for that. The recommendations for improvements will be provided under the following sections.**

As justified in the dedicated WP7 section, the 3rd Reporting Period enabled the SiMBiT Partners to attend over 30 events, which contributed to reach an audience of more than 3 500 people. On the basis of the targeted audience of those attended events, either conference or workshop, one can estimate that over 2 600 industrials were reached only through this channel. Specific supports including videos have been prepared to better and easily communicate towards the industry ecosystem. Those have been widely disseminated through various channels including social media and the web site. The total of industrials reached out through the various posts, news and linked views sums up to close to 50 000.

The Final SiMBiT exploitation plan through its related Deliverable presents a personalised roadmap according to the SiMBiT final prototype prospective targeted markets. A detailed action plan has also been implemented to ensure that the development of the latest SiMBiT technology will be continue further, towards certification and industrialisation steps.

- **Revision is requested for D7.4 "Mid-term report on dissemination and communication activities": This deliverable should contain the Exploitation Plan which was provided apart. Furthermore, the exploitation plan should be revised and completed following these recommendations: Exploitation planification activities should be included (like a pre-agreement between partners depicting how commitments and benefits will be shared, it could be under the form of a table). The aim is to open the discussions between partners, and to plan future actions: how the IP will be shared, who will manufacture, who will pay the licences, who will commercialize, who will take in charge the CE-IVD marking, how will it be funded and when is it planned); Specific decisions have to be taken like who will manufacture, who will licence IP to others partners, how will be shared the investments and benefits? Timeline of development ending, and potential commercialisation phase should be defined. In D7.4, more specific details about targeted audiences should be included (e.g. what type of Industry is targeted and/or was reached).**

The revisions requested from the external Reviewers related to D7.4 have been implemented and submitted on December 20th, 2021. Specifically, D7.4 now includes an updated list of the events attended and publications released in the second reporting period by all partners, along with the identification of SiMBiT expected main outcomes and related individual exploitation plan, including a detailed roadmap to access market. Remarkably, due to the public dissemination level of D7.4, the table summarizing the new knowledge generated by the partners and know-how in their respective fields of expertise, which will be exploited at the end of the project, has been moved to the Exploitation Plan and included in the Second Periodic Technical Report, due to its confidentiality.

Additional activities to ensure the project results exploitation after its completion have also been identified to anticipate related time constraint and associated financial needs.

The Exploitation Plan has been implemented with the requested information on the planned activities and their timeline.

- **The risks established during the preparation of the project are still relevant. The mitigation actions which were set are subject to active monitoring and management. Besides, the consortium is actively tracking in timely manner unforeseen risks and propose appropriate measures for addressing them. However, risks associated with design, management, IP or future exploitation are not discussed. The risk analysis should contain not only technical risks.**

An additional Unforeseen Risk_U11 has been considered: "After project follow-up activities implementation and financing", and the following mitigation strategy has been proposed: "A SWOT analysis has been carried out to anticipate prospective risks in relation with the future exploitation of SiMBiT results, and foresee appropriate mitigation actions."

- **The Intellectual Property aspects are properly addressed, an IPR register was created to follow up on IP assets within the consortium. This includes an IPR tracking model to identify IP assets, project outcomes, and possible exploitation of the results. However, the exploitation planification should be more specific and presented more like an action/business plan with a roadmap and timelines. It should specifically address the project results valorisation, i.e. how they will be potentially exploited, shared and brought to the market in e.g. 3 or 5years after the project ends.**

A dedicated valorisation roadmap and its related action plan have been defined thanks to the input all the SiMBiT Partners and especially those with stronger interest towards the prospective industrial and commercial exploitation of the achieved results (See Deliverable 7.6).

- **The scientific quality of the results is high, however in order to assure a higher impact, more focus needs to be put on action/business-oriented exploitation plan containing more specific steps needed before commercialization. Also, the path to get from TRL5 targeted by the project to TRL7-8 needed for industrialization should be considered early, even if these steps might be achieved after this SiMBiT action ends.**

An innovation centre on "Single-Molecule Digital Assay" has been launched in May 2022 that involves UNIBA and UNIBS (Luisa Torsi, Eleonora Macchia, Gaetano Scamarcio, and Fabrizio Torricelli) and the oncological main hospital in Bari. The main goal of the Regional Centre is to bring the SiMoT (Single Molecule with a large Transistor) technology from TRL5 to TRL7 by performing 1000 clinical trials. The Center enables daily contact with clinicians and end users.

Please find attached to this document (*as Annex 1- RP2 Compliance Report*) the list of recommendations and comments from the previous review and the information on how they have been followed up. This document (as *SIMBIT RP2 Compliance report_201221*) has been uploaded to the EC Funding and Tender Opportunities Portal in December 2021.

5. DEVIATIONS FROM ANNEX 1 (IF APPLICABLE)

In December 2022 an amendment to the Grant Agreement has been submitted. Below the three deviations which have been included in the procedure (the list of changes and their explanations are available on the EC portal):

4. Extension of Work Package 5 “SiMBiT smart bio-electronic modular system prototype” and delay of deliverable D5.6 “Lab. test report of the final SiMBiT prototype “from M42 to M48.
5. Change of the title of D7.6 from” Plan for use, dissemination and exploitation of foreground, including IPR manual” to “Final Simbit exploitation plan”” and submission delay from M42 to M48 .
6. Termination of beneficiary n. 7 - Flexenable Limited (PIC 991778053) and addition of Flexenable Limited Technologies as new beneficiary n. 10 (PIC 884731375). The residual Flexenable Limited’s tasks, budget and personnel effort have been transferred to the new beneficiary.

5.1 TASKS

The project tasks of the third reporting period have been implemented, and the critical objectives fully achieved.

5.2 USE OF RESOURCES

The resources (budget and personnel effort) have been planned during the proposal preparation phase taking into consideration the activities and tasks which are performed by each institution. Beneficiaries have submitted their RP3 Financial Statements through the EU system “Funding and Tender Opportunities”. In the final technical report tables will be included: one summarizing the expenses claimed by each institution for the third period (July 2021 – December 2022) and for the whole project. The second table will summarise the person months spent per institution and work package. In general, the claimed costs and % of PMs are consistent with the planned WP activities. An explanation will be provided in case of minor deviations from the planned budget in terms of claimed expenses and personnel months. Dissemination and exploitation of results