

**ESA OSIP OPEN CHANNEL STUDIES EVALUATION SESSION 2020-02**  
**March 2021- Contract N° 4000132843 .**  
**EXECUTIVE SUMMARY REPORT**  
(to be released to public)

Company Biositech - France/ SD-Innovation, Frouard, France.  
[www.Biositech.com](http://www.Biositech.com) (SME DD).

**Industrial development of the first miniaturized incubator for long-term cell culture and real-time observation under multiple micro-environmental niche conditions**

## **MIBA : MIncro Incubator Biomedical Autonomous**

**Objective :** This project concerns the proof of concept, design and construction of the first micro-incubator to enable biologists to optimize cell and tissue culture conditions and to get as close as possible to real-life physiological and pathological conditions. There was no mobile and autonomous biomedical device allowing both incubation and observation under multiple conditions in real time. This development therefore concerns a miniaturized incubator containing all the functionalities to be easily set up and allowing great transport mobility, the maintenance of culture conditions by perfusion of fluids and the observation at any time and without physical contact of the samples it contains.

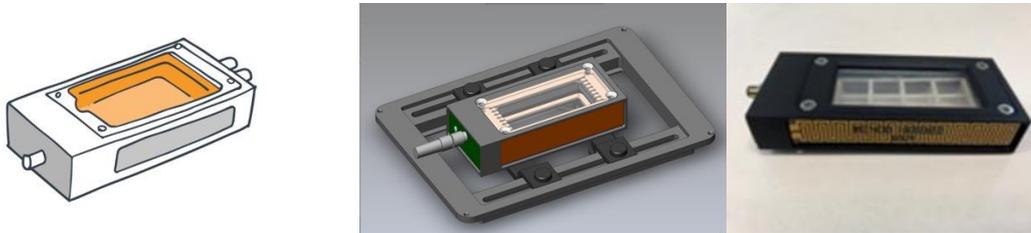
**Background :** Since 2012, we have developed a first prototype for the long-term monitoring of cell culture, which operated in a single channel, i.e. with a single culture condition. This project to develop a multi-channel device supported by an ESA fund was carried out between 2020 and 2022 by the young startup Biositech with the support of its partner SD-innovation (Frouard, France), which proposed to create a multi-way device to maintain and compare the different culture conditions in real time.

**Invention :** MIBA relates to a thermo-regulated enclosure which receives a chamber for depositing biological materials and an perfusion for the renewal of the maintenance media. This chamber is transparent to allow observation by various means without physical contact with the sample (microscopic through the bottom of the chamber, macroscopic from a distance). The most complex design part concerns the miniaturization of the MIBA insert and the integration of functional modules: a fluidic module for the management of media and gases (nitrogen, oxygen, and carbon dioxide), a thermal module mounted on each micro-incubator for temperature maintenance and other functionalities to come around the alternative energy supply.

### **Performances :**

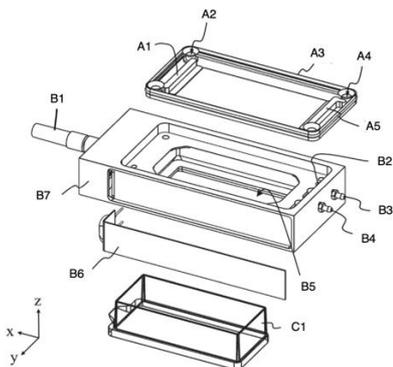
*Miniaturization and transport : MIBA is based on an independent insert sized for the samples (cells, tissues, etc.) and no longer built as a cage around the observation instruments, which implies the miniaturization of the metrological and control modules (heating, gas pressure, perfusion). This reduced size device allows to receive classical*

*culture chambers and adapted to the movement and transport of the insert in an independent way. It allows to transport biological material from one laboratory to another while guaranteeing the metrological conditions of culture.*



This thermoregulated device of reduced size (90mmx40mm) allows to receive classic culture chambers (5cmx3cm),

*Perfusion and change of culture media* : MIBA integrates a perfusion module by a pump with automatic culture medium change to access to very long kinetics with living cells (from a few hours to several weeks). The input-output have been studied to limit the vortices generated by the flow, the depressions in the chamber and the air bubbles at the top surface, for flow rates adjustable between 0 and 23 ML per minute.



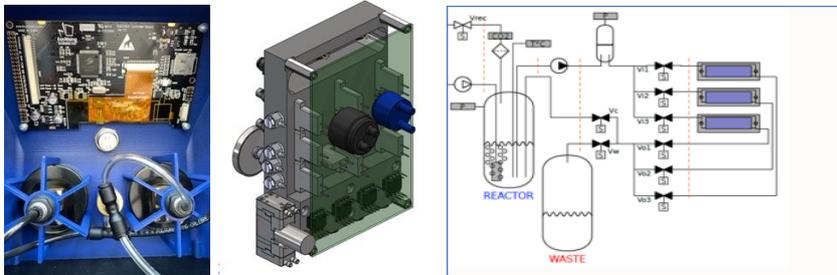
Representative of Micro-incubator. A1: Flow microchannel; A2, A4: fixation; A3: Cover ; A5: CoverGlass; B1: Connector; B2: Flow channel; B3: In Flow; B4: Out Flow; B5: Seal; B6: Heat device; B7: Insert, C1: chambered coverglass type (Labtek).

*Multichannels for multiple tests and monitoring* : A "multi-channel" stage support has been developed for different functions and can receive up to 3 MIBA inserts in parallel: magnetization to hold the inserts, insertion on the stage holder of a microscope, and automatic repositioning on the same cell with known coordinates on the stage of a microscope.



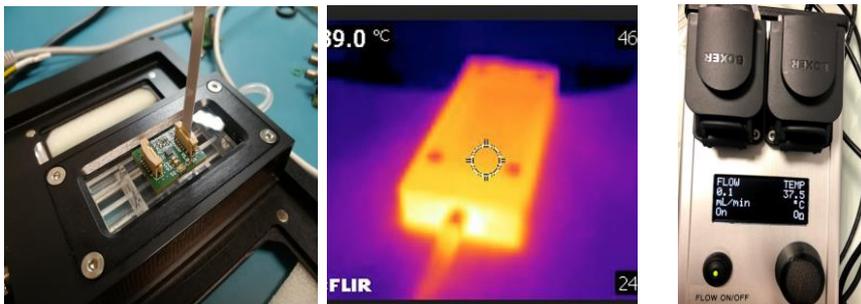
3 MIBA inserts in parallel with a perfusion module to renew the medium and observed in real time.

This “multi- channel” function makes it possible to compare in real time all the chambers and wells under different culture conditions. In addition, each way is identified (synoptic below) in waste monitoring, and allows biosynthesized molecules to be collected in the culture media for subsequent analyzes throughout the culture (hormones, growth factors, etc.) or even to reinject these media on another way to study the cellular effects (liposomes, growth factors, etc.).



*MIBA G2. Implementation of pumps and solenoid valves for fluid regulation - low flow flowmeters for the inputs of gas (CO2 5%) - integrated control electronics*

*Normoxia / hypoxia: Temperature and gas :* The partial oxygen pressure is managed by precision flowmeters by bubbling in the tank until saturation. Fluid management is essential for carrying out experiments in which the oxygen content varies (cellular hypoxia).



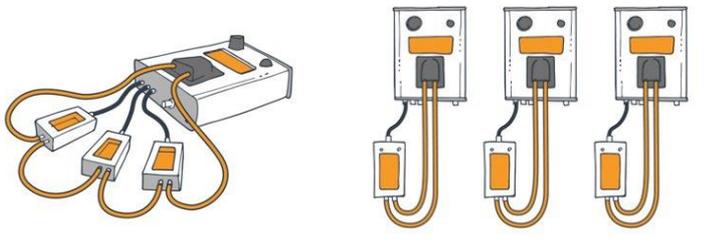
*Implementation of heat exchanger and internal temperature control (37,5°C)*

*Consumable - single use - cost of use :* MIBA uses a culture chamber (55mmx25mm) and for single use (of consumable type, and very widely available from many suppliers). The choice is also possible between one or more wells per chamber for multiply the tests. The cost of these culture chambers is therefore reduced.

## **Realization:**

The G2 prototype (2nd generation) has been classified TRL4 based on 3 MIBA micro-incubators and its monitoring (in serial and parallel configuration) is ensured by a controller interfaced to a workstation which manages each MIBA independently. Many parameters involved in cell culture (sterility, sealing, proliferation rate and cell viability, cleaning efficiency) have been successfully tested on several cell types (fibroblast, chondroblast, mesenchymal stem cells) under laboratory conditions (Biositech Lab, S Alilou). The results are very positive and concern the metrological validation of the G2

microincubator, and the development of a 3rd generation microincubator that integrates gas management.



Cell culture in parallel and serial configuration in 3 MIBA with external controller for independent monitoring.

### **Applications and valorization :**

The applications of MIBA modules are numerous and varied, but not limited to fluorescence microscopy and macroscopy (confocal, multiphoton), bright field or phase imaging (IPrasense as collaborative partner). When used in industrial test, MIBA modules allow for rapid testing of multiple culture conditions and remote data collection (imaging or Raman signal detection).



Test bench of several MIBA mounted in parallel configuration and observed in fluorescence mode and phase imaging.

The valorization of this work has been important with the constitution of an industrial and academic consortium around this innovative project under the impulse of the project with ESA, the publication of a scientific article on MIBA (AROIC, 2,1, 2021) and a patent that has been filed for the continuation of this development.

Innovations based on MIBA as a central module are being developed, in the field of automated cell culture, which will make it possible to maintain cultures over the very long term or applications of biological adaptability to extreme climate variations.