4.1 Final publishable summary report

4.1.1 Executive summary

The DIMID "Development of an Innovative Microfluidic Impedance-based Device for multi-parametric cell analysis" project is a highly interdisciplinary project where microfluidics, electronics, biology and informatics converge with the main aim of building a low cost impedance cytometer suitable for non-spherical cells and equipped with chips. To realize the device, the focus of the first project period was on the conceptual design of the device (a suited chip design in terms of geometry and electrode configuration, a proper stimulation and measuring scheme, a custom signal processing to extract features valuable for discrimination). During the last phase of the second project period, the DIMID consortium dedicated its efforts to the system integration and verification.

The device conceptual design phase of the DIMID project as well as the development phases, were strongly supported by numerical modeling and simulation. In order to assess the effectiveness of the conceptual designs and to guide their optimization, an in silico model of the DIMID device, referred to as the virtual DIMID device, has been developed, implemented and validated by UTV. Thanks to this versatile toolkit, with minor changes to the routine devoted to the creation of the geometry and to the routine implementing the stimulation and measurements scheme, any cytometer design can be simulated.

The design and production of the chip and fluidic system were pursued by the USOUTH and EPFL with two different approaches. A device with multiple sets of parallel facing electrodes was successfully fabricated by USOUTH, which enabled analysis of cell size (volume) and anisotropy (cell shape). Distinguishing red blood cells (bi-concave disks) from similarly sized spherical beads based on the anisotropy measurement was accomplished. The device is label free, requiring minimal sample preparation and has the potential for many shape-based measurement applications of cells such as identification of platelet activation.
The liquid electrodes device has been designed and fabricated in EPFL. This electrodes configuration presents some advantages compared to the standard micro-flow cytometry chip. Indeed, the simple manufacturing process allows the production of a simple low-cost device that could be disposable. This new device allows the discrimination of cells presenting different shaped, maintaining a simple manufacturing process and a simple data analyses procedure.

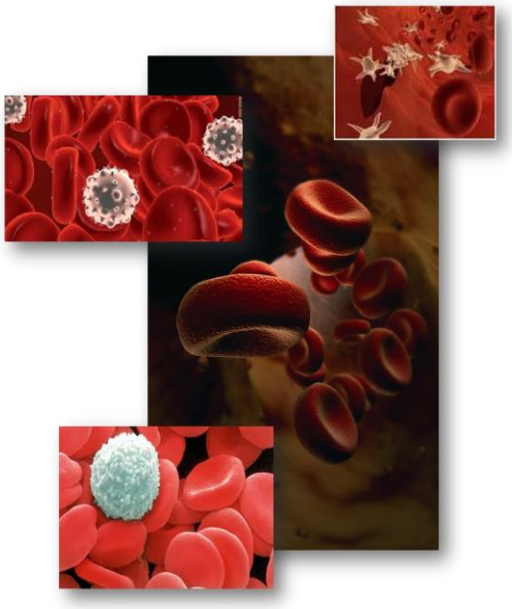
The design and implementation of the DIMID electronic unit was the main activity carried out by LABOR in close collaboration with ZURICH. The main output of the first phase of the Electronic Unit design is the functional block diagram. The second step was then to design and implement the DIMID electronic unit. The end result was the production of 10 boards for the DIMID electronic unit which were then assembled together to prototypes of the electronic measurement unit including a touch screen display and an embedded PC.

The Signal Processing Block was implemented by UTV and ZURICH and it is responsible for configuring and acquiring the impedance signal from the Impedance Spectroscope, isolating and segmenting the regions of interest that contain events/peaks (segmentation) and extracting suitable information from each event (feature extraction) that is used to classify the event as a certain cell type in the GUI and Analysis Block. The GUI, Analysis and Acquisition Modules were specifically designed by CYTOGNOS to run analysis in a easy to learn and friendly interface.

The system integration was mainly driven by CELLIX and ZURICH. A first complete DIMID system prototype was built at the premises of CELLIX with support of all consortium partners. The system was evaluated and several optimizations were done resulting in a second prototype. The second prototype was then used to do preliminary tests with red blood cells (RBC) and to do field tests on specific applications like dairy food quality testing. The bench marking showed that the DIMID device has clear advantages in terms of the electronic measurement unit, the analysis software, the portability and the cost structure.

4.1.2 Project context and objectives

Cell analysis has become an important technique and represents a fast growing market for a wealth of applications in the fields of life sciences, medicine and environmental analytics. Clinical (diagnostic) and research applications in the healthcare and drug discovery markets demand rather complex analyses at the single cell level, whereas routine and quality control applications in bioprocess monitoring must presently content themselves with simpler, quicker and cheaper analyses.



What these markets are calling for is a device comparable to the high-end solutions, allowing faster and less expensive analyses and therefore offering a valuable alternative for the more price-sensitive applications, or for real-time cell culture monitoring, **for which high-content analyses are presently not available**.

DIMID project has gathered a highly specialized Consortium of SMEs (ZURICH, CELLIX and CYTOGNOS) aiming to realize a simple, non-invasive method for counting, identifying and monitoring cellular functions. The system will enable a range of new analyses and diagnostic approaches that can be performed simply and quickly, without the use of a centralized resource. Specific examples include:

- **Real-time cell culture analysis;**
- **Haematology, in applications such as blood counting and analysis, measurement of leukocytes, erythrocytes and platelets;**
- **Identification of rare cells like tumour cells and stem cells;**
- **Measurement of endothelial microparticles, parasites in blood cells, etc…**

The **S&T objectives** of the DIMID project consist of:

- ✓ **Selection of the optimal design for the disposable chip**: different configurations will be devised and compared by simulation to achieve the highest discrimination sensitivity.
- ✓ **Definition of the optimal micro-fabrication technique for manufacturing the disposable chips**: the most important achievement will be the introduction of effective and reliable disposable chips, which will be mass produced. The challenge is to ensure the same performances of the glass chips, exploiting the cheaper production process of the chips.
- ✓ **Definition and implementation of the driving and measuring module**, which will the core endeavour of the electronic and signal processing work.
- ✓ **Implementation of a pilot prototype** and evaluation of its performances.
- ✓ **Evaluation of the technical and economic** impact of the technology.

The DIMID "Development of an Innovative Microfluidic Impedance-based Device for multi-parametric cell analysis" project is a highly interdisciplinary project where microfluidics, electronics, biology and informatics converge with the main aim of building a low cost impedance cytometer suitable for non-spherical cells and equipped with chips.

The two years DIMID project started in October 2011 funded under the FP7 "Research for SMES" programme. The DIMID consortium is formed by three SMES and four RTD performers from five different countries.

The DIMID device structure basically consists of the electronic unit developed by Zurich (Switzerland), the disposable chip designed by CELLIX (Ireland) and the system software created by CYTOGNOS (Spain). Those modules shape a unique integrated low cost impedance device allowing faster and less expensive cell analyses, obtaining results comparable to the currently available high-end solutions. An intensive research work and support has been carried out by the RTD performers on microfluidic chips (University of Southampton and École Polytechnique Fédérale de Lausanne), electronic control unit (LABOR) and DIMID signal processing algorithms (University of Tor Vergata and University of Southampton).

Therefore a closely collaboration between the SMEs aiming to obtain a new successful exploitable product, and the RTDs willing to obtain new knowledge has been a key factor of the project.

4.1.3 Main S&T results

The first period of the DIMID project (M1-M9) was aimed at achieving a major part of the results expected for the user and system requirements, with some preliminary results coming the device conceptual design and the chip design.

The first period of the project had its central goals in:

1. The definition of the DIMID application scenarios, user requirements and system requirements;
2. The conceptual planning and design of the DIMID system supported by in-silico experiments;
3. The design and production of the first demonstrator of the chip and fluidic system of the DIMID device;
4. The design and fabrication of the first demonstrator of a liquid electrode microfluidic chip for flow-cytometry;
5. The definition of the system architecture and its embedded core and the related design strategies for the DIMID Electronic Unit.
6. The design, development and publish of the DIMID website, design of the brochure and the poster as well as an initial strategy for the using and disseminating knowledge.

The first objective was pursued through a constant interaction with the beneficiary SMEs, fundamental for the identification of the European context and situation concerning the cytometry market field; in addition, the SMEs provided information on the end-users needs and expectations.

Laying on the output of the first objective, the second one was pursued by UTV. Thanks to an extensive campaign of in-silico experiments and discussion with the other performers, UTV identified a configuration, which potentially should be effective for the DIMID system.

The third objective was pursued by USOUTH. The current design of chip first demonstrator is able to detect biological cells, but different electrode designs and dimensions are being looked at to improve sensitivity.

Forth objective was pursued by EPFL. The first prototype of liquid electrode device developed in EPFL for impedance-based single cell viability measurement was design and fabricated. The fabrication process and preliminary results are reported in the deliverable.

LABOR pursued the fifth objective in collaboration with ZURICH. Based on the experience from previous designs, ZURICH ensured the application-fit of the design.

In the second period (months 10-24), the main efforts were on the implementation, integration and verification of the DIMID building blocks. Besides the design and the production of the disposable chips, the measurement subsystem design and prototype production was one of the key deliverables of that period. This included not only the development of the hardware, but as well the implementation of the entire signal processing and analysis software tool chain.

Finally, during the last phase of the second project period, the DIMID consortium dedicated its efforts to the system integration and verification. First, the two subsystems as depicted in Figure 1 were integrated and verified independently, before the entire DIMID system was tested in a systematic way according to a defined test plan.

During the last two months of the project, the DIMID device was successfully exposed to various field tests and a benchmarking against the actual state-of-the-art device was performed. As an outcome, the DIMID technology proved to have several competitive advantages, namely in terms of cost, throughput and sensitivity.

The DIMID device is an **innovative user-friendly low-cost microfluidic impedance-based device for multi-parametric cell analysis** with enhanced performance and discrimination capabilities with respect to state of the art devices. Efficient and reliable multi-frequency measurements allow cell discrimination based on geometric and dielectric properties. The **striking innovation** is the potentiality to perform a morphology-based discrimination. To achieve this goal, the key point is the possibility to probe flowing cells along different spatial orientations, thus revealing cell anisotropy.

To realize a device with the aforementioned features, the focus of the first project period (months 1-9) was on the conceptual design of the device,, in particular on the identification of:

- a suited chip design in terms of geometry and electrode configuration
- a proper stimulation and measuring scheme
- a custom signal processing to extract features valuable for discrimination

Accordingly, the basic blocks of the DIMID device are pictured in Figure 1 and described below.

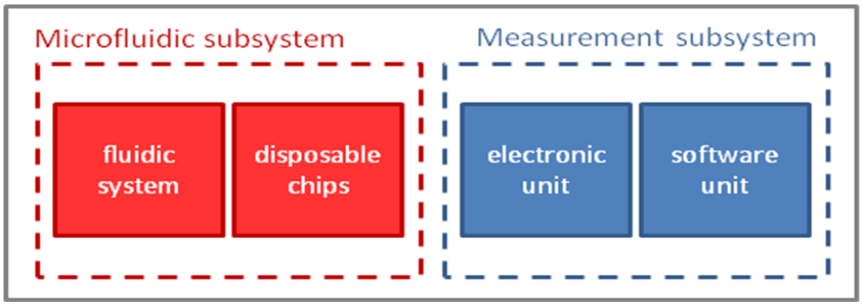


Figure 1 – DIMID device architecture

In the first phase of the second period (months 10-20), the main efforts were on the implementation, integration and verification of the DIMID building blocks. Besides the design and the production of the disposable chips, the measurement subsystem design and prototype production was one of the key deliverables of this period. This included not only the development of the hardware, but as well the implementation of the entire signal processing and analysis software tool chain.

During the last phase of the second project period (months 21-24), the DIMID consortium dedicated its efforts to the system integration and verification. First, the two subsystems as depicted in Figure 1 were integrated and verified independently, before the entire DIMID system was tested in a systematic way according to a defined test plan. Finally, the DIMID device was successfully exposed to field tests and a benchmarking against the state-of-the-art device of AMPHASYS (the leader in impedance flow cytometry) was performed. As an outcome, the DIMID device proved to have several competitive advantages, namely in terms of portably and cost structure, which will be an important enabler for the technology to establish outside of laboratories, for example milk quality testing directly at the farm or greenhouse applications. In the following sections, the work performed in the second period is described in detail.

Main building blocks of the DIMID device architecture can be organized as in the Figure 1, in particular:

☐ **Microfluidic subsystem**

- **fluidic system**: a microfluidic system delivering the sample to the cytometer chip.
- **disposable chip**: a chip scalable to mass production enabling single cell measurement along different spatial orientations.

☐ **Measurement subsystem**

- **electronic unit:** the hardware part of the measurement subsystem, whose core is a high performance impedance spectroscope.
- **software unit**: the software part of the measurement subsystem, an easy-to-use acquisition tool, processing raw data and yielding classification results. Several steps are involved in the signal processing chain, as pictured in Figure 2.

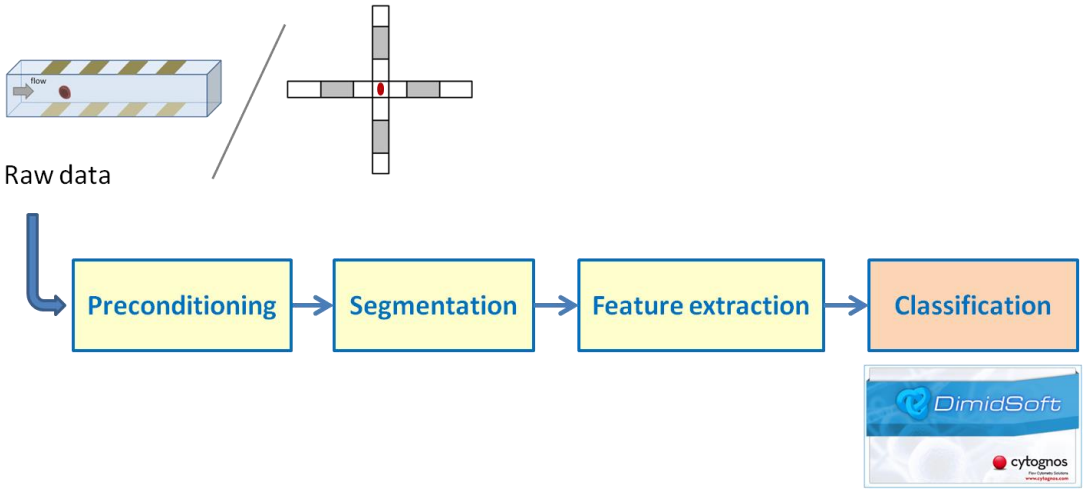


Figure 2 Software tool chain

In the following sections, the detailed activities performed under the RDT WPs are reported, together with the main S&T results.

WP1 - SYSTEM REQUIREMENTS

The very beginning of the project was dedicated to the activities for the identification of the suitable application scenarios for the DIMID device. Specific activities were performed to investigate the market, thanks to the presence and the experience of the SMEs.

The scenarios identified have been subjected to a further analysis with the purpose to understand the real needs of the SMEs involved and the most profitable markets for their business. This analysis permitted to have a prioritization of the scenarios identified and a most useful identification and definition of the requirements of the DIMID system.

The most important requirements were addressed by the RTDPs in order to focus the definition of the system requirements and specifications of the DIMID system.

All the activities performed under WP1 were carried on in collaboration with the SMEs and the RTDPs and the outcomes have been the input for the following technical work packages.

In summary, the main results obtained are:

- The definition of the interested application scenarios for the DIMID device;
- The definition of the end-users' technological needs and problems in order to address them in the development of the technology;
- The definition of the most important user requirements to be addressed into the system requirements;
- The definition of the standard procedure to be followed for the definition of the system requirements for the technology.

The **first task** of the DIMID project has been carried out with the support of the whole Consortium and great contribution came from the beneficiary SMEs. The report, produced at the end of this task, contains the definition of the scenarios in which the DIMID system should be applied and gives concrete scope for the project.

The result of the dedicated workshops set up for this WP, has been inserted into D1.1 and can be summed up in the Conclusion section of the document. The application scenarios identified for the DIMID are related with the fields of health, biotech industry and environment.

The choice of the scenarios has been conducted taking into account the innovation brought by DIMID system, the unmet needs of the final users and the interest of the SMEs involved in the exploitation of the system. For each scenario identified a deep analysis was conducted: a complete list of Pros and Cons has been of help for the decision about which scenario would have been suitable for the system.

In conclusion, the analysis performed in the DIMID project defined some specific markets, in which cell analysis is of primary importance. Moreover it has been shown that there is a strong need of novel devices able to perform cheap, reliable, high-contents single cell analysis.

In this framework, the most important requirements for the device have been:

- Need of a low-cost device
- Need of viability cell discrimination and tomography capability
- Enhanced sensibility of the device

The user requirements definition task was led applying the participatory design methodology. The Coordinator organized, managed and led the other partners in order to have interaction among them.

An analysis of the User requirements relative to each application scenario was developed. The availability of a questionnaire on hematology analyzer for researchers and labs technicians gave the consortium the possibility to highlight unmet clinical needs.

Once identified the problems and need, possible solutions have been addressed by describing the use cases. The consortium has identified three use cases as example for assessing short-term, mid-term and long-term goals. In order to translate the user requirements into system specifications, the consortium managed three different subtasks in respect to the main technological aspects of the DIMID system: the Electronic Unit, the Chip and the Software and HMI.

In each subtask, the corresponding workgroup has provided their requirements. To homologate the work of each group, the consortium adopted a subset of guidelines taken from the Volere engineering approach.

WP2 - DEVICE CONCEPTUAL DESIGN

The device conceptual design phase of the DIMID project as well as the development phases, were strongly supported by **numerical modeling and simulation.** In order to assess the effectiveness of the conceptual designs and to guide their optimizations, an in silico model of the DIMID device, referred to as the **virtual DIMID device** (Figure 3), has been developed, implemented and validated.

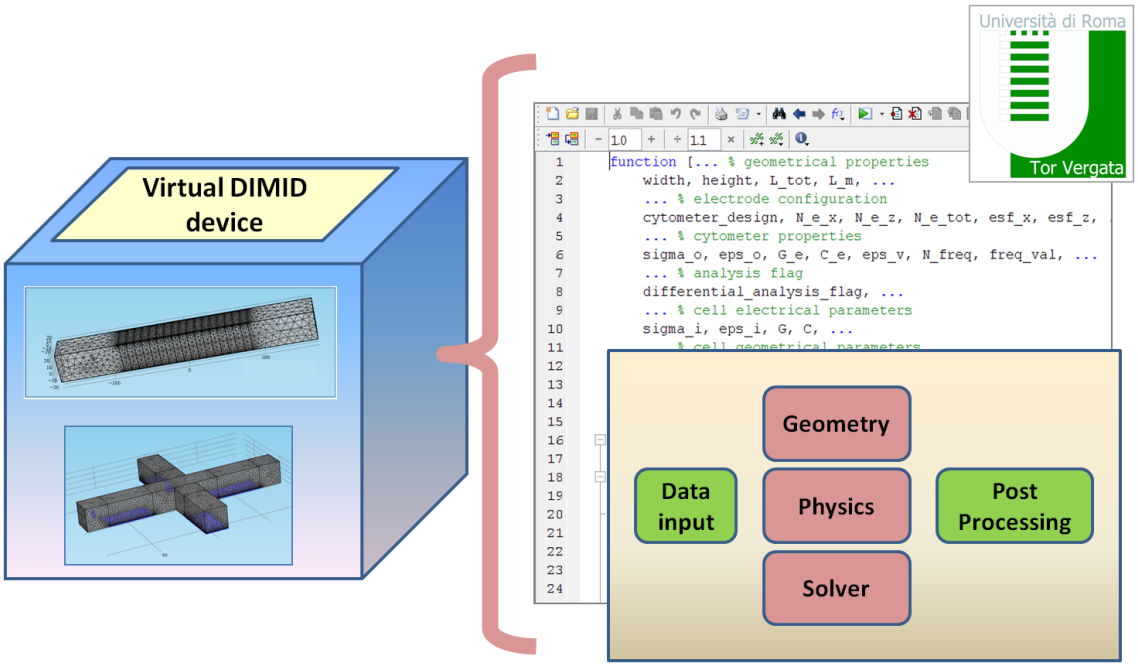


Figure 3 The virtual DIMID device

In particular, a complete **distributed 3D model** of the electric conduction in the microfluidic chip, comprising the cell, the microchannel, and the electrodes has been developed. It is characterized by the following **relevant features**:

- **both prototypes** (i.e., parallel-facing or liquid electrodes) are implemented;
- cells of **arbitrarily complex geometry** (e.g., spherocytes or erythrocytes) are allowed;
- the cell plasma membrane is treated as a **distributed conductive/capacitive interface**;
- the so called "**complete electrode model**", able to effectively match experimental measurements, is used for electrode modeling;

With a proper set of simulations, the model allows the determination, at each frequency, of the **admittance matrix** of the device. Such admittance matrix, in turn, allows the **simulation of the experimental measurements**, and hence it is exploited for the **analysis and comparison** of different **stimulation and measurement schemes**.

The model has been implemented into the commercial finite-element code COMSOL Multyphysics. In order to achieve the maximum **flexibility** and the most **efficient implementation**, the link between COMSOL and MATLAB has been exploited instead of the COMSOL graphical user interface. Accordingly, the virtual device consists of a **MATLAB toolkit**.

The **main blocks** of the toolkit are (Figure 3):

- the **data_input**, defining the geometric and dielectric properties of the device and of the flowing cell;
- the routines for the computation of the **admittance matrix** of the device (i.e., geometry creation & meshing, physics definition, problem solution);
- the **post_processing** routines, enabling the **simulation of any measurement scheme** starting from the admittance matrix.

The model has been validated by comparison with available numerical benchmarks and published data.

Thanks to this **versatile toolkit**, with minor changes to the routine devoted to the creation of the geometry and to the routine implementing the stimulation and measurements scheme, **any cytometer design can be simulated.**

The virtual DIMID device has been **exploited** to:

i) Test basic principles and **ideas** of impedance cytometry, such as the information content of the acquired signals according to the stimulation frequency and the resulting cell discrimination potentiality (Figure 4);

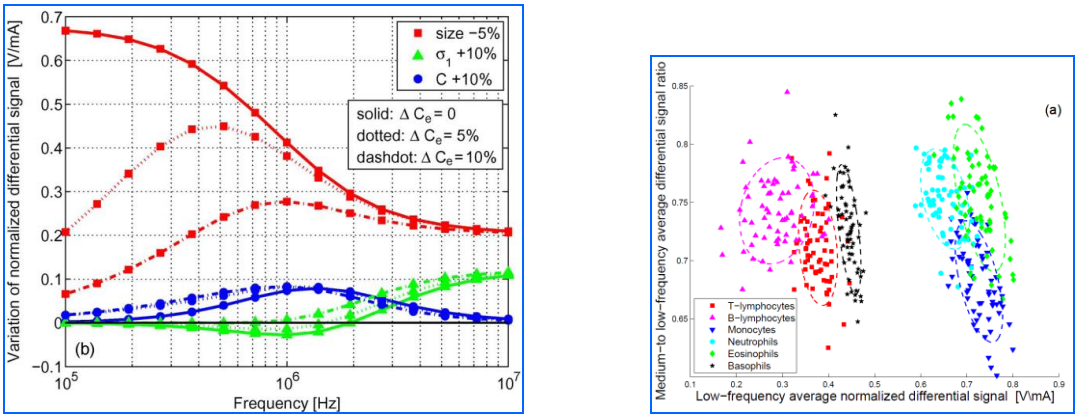


Figure 4 On the left, signal sensitivity to size, intracellular conductivity, and membrane capacitance; on the right, WBC discrimination

ii) Assist and guide the **device conceptual design**, exploring and testing ideas in simulation prior to implementation, with special emphasis on the morphology-based discrimination potentiality of the proposed designs. Several configurations resulted in principle effective for the DIMID objectives. Communication and collaboration among the consortium partners was instrumental to the **identification** of the **configurations most suited to the project**, as a compromise between device performance, microfabrication effort and electronic requirements;

iii) Perform in silico experiments tailored to the prototypes pursued in the project (Figure 5) in a synergic experimental-modellistic approach. The main objective of those numerical experiments was performance assessment and device optimization. In particular, the following features have been investigated:

- sensitivity to cell morphology;
- sensitivity to cell volume;
- sensitivity to cell dielectric properties;

with respect to their dependence on:

- channel and electrodes dimensions and geometric arrangement;

- measuring scheme (e.g. floating vs grounded electrodes, frequency);
- cell offcentering;
- cell orientation.

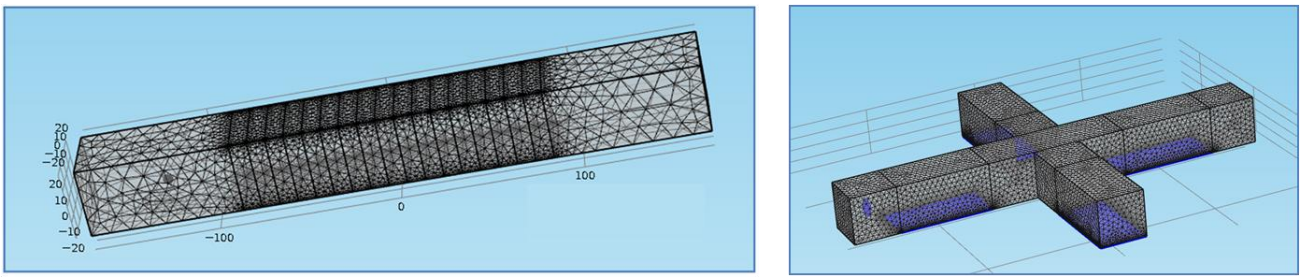


Figure 5 Virtual DIMID prototypes.

WP3 - CHIP MICROFABRICATION

Under the WP3, the design and production of the chip and fluidic system were pursued by the University of Southampton (USOUTH) and the École Polytechnique Fédérale de Lausanne (EPFL).

Two different approaches were followed by USOUTH and EPFL.

The design and material of the chip are being investigated by USOUTH with a view to improving the chips sensitivity and achieving a cost that can be considered disposable.

Before entering the chip samples often require some level of pre-processing such as dilution, lysis or labelling in order to distinguish types of cells and avoid double counting. Double counting occurs when more than one particle passes through the chip's region of interrogation and causes information on both to be lost. Protocols and fluidics aim to standardise these pre-processing steps and integrate them into the device as much as is possible.

In parallel with the development of the double-sided electrodes, a liquid electrode microfluidic chip for flow-cytometry have been designed and fabricated by EPFL.

Micro impedance cytometer with multiple sets of parallel facing electrodes

USOUTH developed a micro impedance cytometer with multiple sets of parallel facing electrodes to enable analysis of cell size (volume) and anisotropy (cell shape). The chip was tested with a range of different particles and could successfully distinguish red blood cells (bi-concave disks) from similarly sized spherical beads based on the anisotropy measurement.

Accurate dilution and delivery of sample is vital to achieve accurate results. A microfluidic system capable of diluting whole blood and delivering it to the cytometer chip was investigated. Mixing devices were first validated using coloured dyes. When replicated with blood and diluent (PBS), results were promising however repeatability issues were encountered due to the viscous nature of blood.

Serial dilution with pipettes was developed and a number of ways of introducing this diluted sample into the cytometer chip were examined. The working of the system relies on the exclusion of bubbles. The most reliable system was found to be pushing the sample through the cytometer chip with a syringe pump. The sample was loaded into a syringe. An initial fast flow was pushed through the cytometer chip to remove bubbles, followed by a steady state flow while the measurement was taken. A study of RBC count was taken

using this method and found to produce realistic absolute values with acceptable reproducibility between multiple repeats of the same sample.

A device with multiple sets of parallel facing electrodes was successfully fabricated which enabled analysis of cell size (volume) and anisotropy (cell shape). Distinguishing red blood cells (bi-concave disks) from similarly sized spherical beads based on the anisotropy measurement was accomplished. The device is label free, requiring minimal sample preparation and has the potential for many shape-based measurement applications of cells such as identification of platelet activation.

A specific 40µm channel version of this device in combination with a chip holder developed by USOUTH was then used for all DIMID integration and field tests.

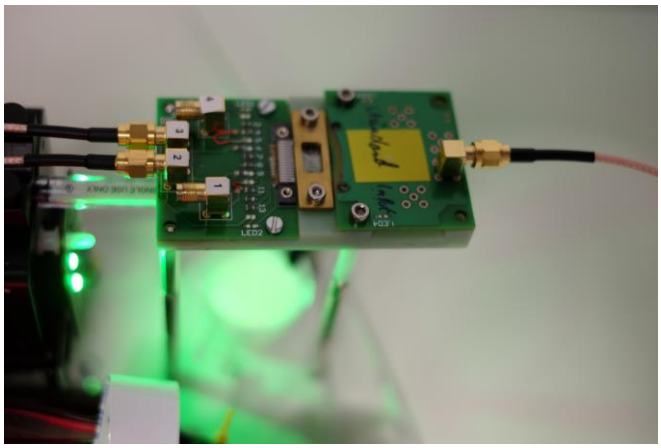


Figure 6 – USOUTH chip holder connecting the 40 µm device to the electronic measurement unit and the microfluidic subsystem

Liquid Electrodes Chip

As introduced in the previous section, EPFL worked for the development of a liquid electrodes device to satisfy the requirements identified in the project. The main fundamental achievement is the optimization of the manufacturing protocol and the definition of a simple two-masks microfabrication process. The patterning of the metal layer is performed by lift-off. This layer consists of 20nm of titanium (Ti) and 200nm of platinum (Pt). The Ti layer is used to enhance the adhesion of the Pt layer on the glass wafer. The fluidic network is produced on top of the electrodes by SU8 photolithography. The process is shown in Figure 7.

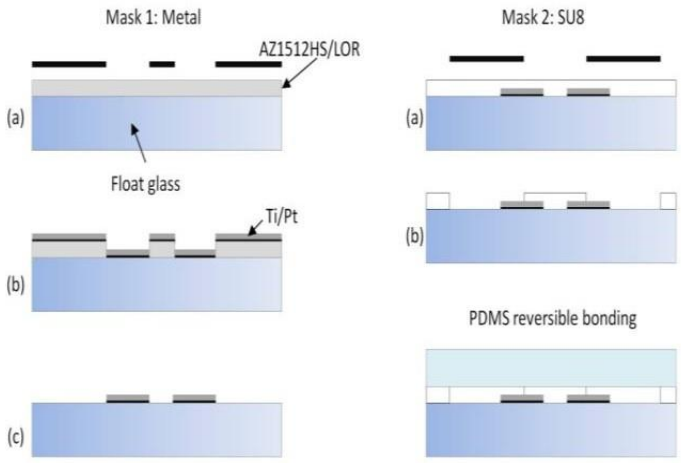


Figure 7 Process flow for the liquid electrodes chip fabrication

Different electrodes geometry have been produced and tested. The first liquid electrodes demonstrator presents four liquid electrodes coupled to create a sensing volume and a reference volume. The device is placed on a chip holder and a plastic block seals the fluidic network with the PDMS part by exerting a mechanical pressure. The handling of liquids is controlled using an external pneumatic pressure system. The electrical connection with the Zurich Instrument impedance analyser HF2SI is obtained by using a dedicated printed circuit board.

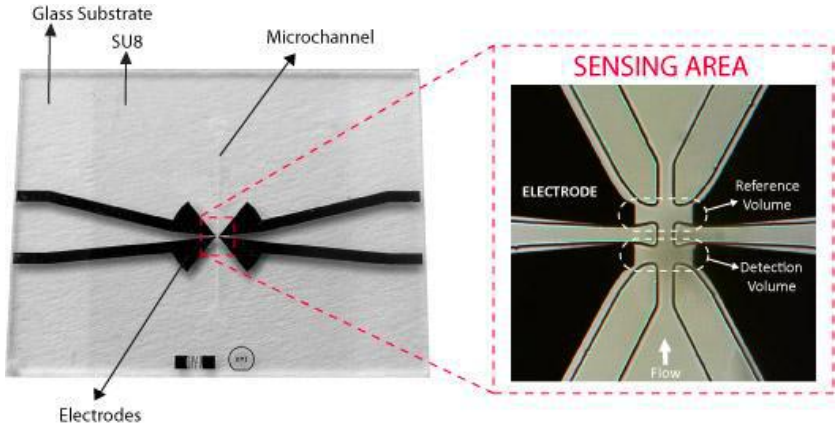


Figure 8 Picture of the first liquid electrodes demonstrator and zoom of the sensing part. Four liquid electrodes and the microchannels for fluids control are patterned on a glass substrate.

Using this device, multifrequency cells discrimination has been successfully performed on yeast cells. Mixed populations of dead yeasts and living yeasts can be discriminated by using an opacity threshold (opacity is defined as the ratio between the high frequency amplitude and the low frequency amplitude) previously determined experimentally. The results of the tests are reported in Figure 9, where the signals at 500kHz and 15MHz have been selected for viability discrimination.

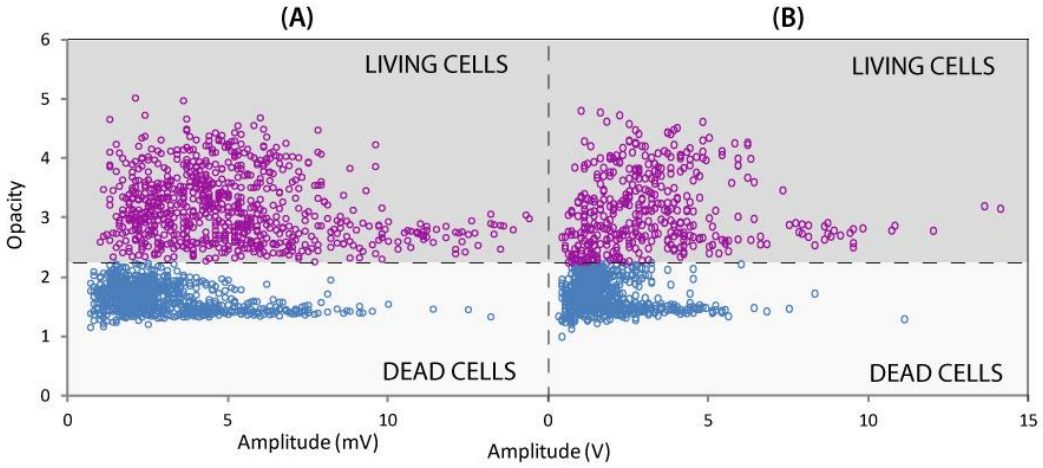


Figure 9 Dead/living yeasts cell discrimination obtained by the liquid electrodes first demonstrator. The scatter plot (a) is obtained from separated populations and it is used as calibration to define the threshold, which has been applied to distinguish between mixed population of dead and living yeasts (b).

A new electrodes design has been implemented during the second period of the project. The novel proposed device presents tomography capability, beside the standard cell analysis capability. To obtain shape information, the chip has been modified and the sensing electrodes have been fabricated in cross configuration. In this way, each single cell can be interrogated in two different directions. Moreover, focusing electrodes have been added on the main channel to align and orient the cells and, consequently to eliminate the dependency on the position within the channel. Orientation and focusing are obtained by dielectrophoresis. In specific condition of frequency and external medium conductivity, negative DEP acts on the cells that are repulsed in an equilibrium position in the midline of the channel. Moreover, ellipsoidal cells are oriented with the main axes parallel to the electric field lines (therefore, perpendicular to the flow).

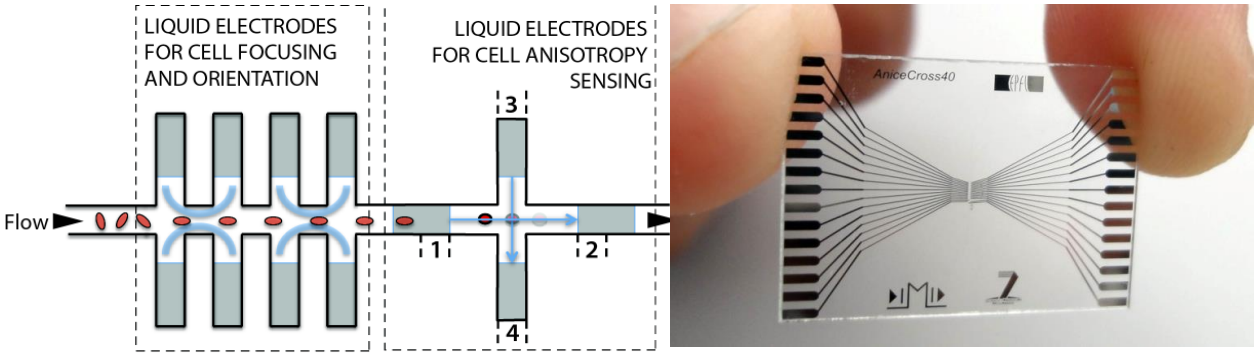


Figure 10 Working principle of the final liquid electrode demonstrator (left) and picture of the fabricated device (right)

Several experiments have been performed to study the performance of the final demonstrator. The fluidic and the electrical external setup have optimized for the new device.

In a first stage the focusing/orientation has been tested on different cell types. The results are shown in Figure 11. As expected, all the particles are focused in the midline of the channel. However it is interesting to notice that erythrocytes travel flat in the microchannel, with both electric field configuration (opposite and alternating electrodes). On the contrary, the orientation of the rods can be changed by selecting the electrodes configuration.

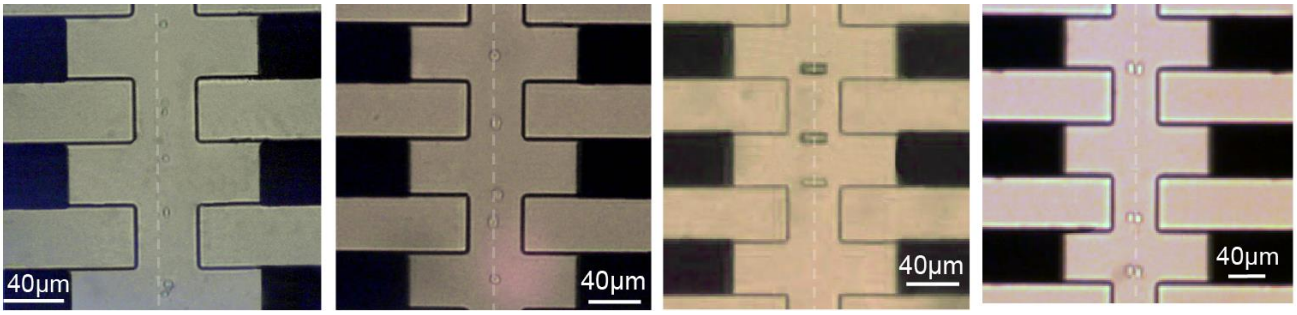


Figure 11 Focusing and orientation of spherical yeasts, erythrocyte, rods and budding yeasts (from left to right).

To obtain a proof of concept of the shape-based cell discrimination, yeasts cells and budding yeasts have been used. The typical obtained signals are reported in Figure 12.

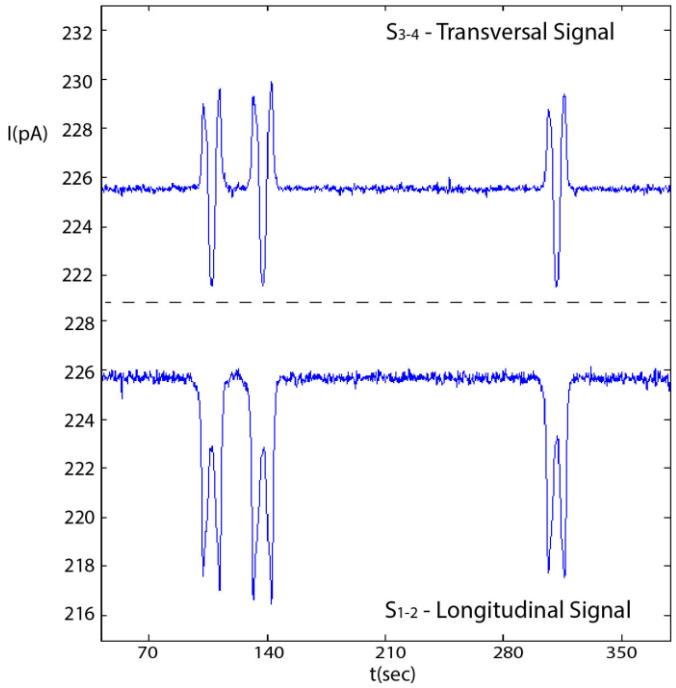


Figure 12 Transversal and longitudinal signals of yeasts passing within the sensing electrodes. To extract the anisotropy index the ratio of the peaks magnitude is calculated for each particles (AI=S1-2/S3-4). For spherical particle the peaks magnitude is expected to be equal in the two directions

The peaks of transversal and longitudinal signals are compared to obtain information about the cell shape and an anisotropy index is defined as the ration S1-2/S3-4. This simple data analysis allows the discrimination between spherical and ellipsoidal particles. Yeasts and beads are spherical particles and therefore, they present AI equal to 9.5-1. On the contrary, rods are cylindrical particles (5x20µm) and their anisotropy index is about 2. Budding yeasts present an AI between 1.2 and 1.4, according to the division stage and the dimension of the bud. This result is shown in Figure 13.

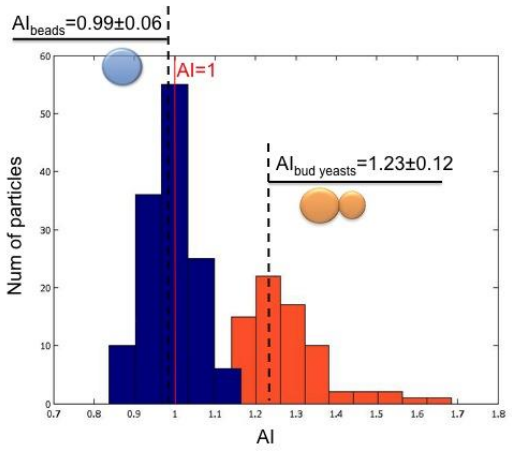


Figure 13 Histogram of the anistropy index of spherical yeasts and budding yeasts. Spherical cells have a AI=1, instead budding yeasts AI are greater than 1.

To summarize, the liquid electrodes chips developed in EPFL for the DIMID project enable vialibity discrimination and cell shape discrimination, maintaining a low-cost fabrication process and a simple data analysis.

WP4 - MEASUREMENT SYSTEM

Under the Measurement System work package, two main activities have been performed:

- **design and development of the electronic unit:** the hardware part of the measurement subsystem, whose core is a high performance impedance spectroscope.
- **Design and development of the software unit, signal processing block and GUI**: the software part of the measurement subsystem, an easy-to-use acquisition tool, processing raw data and yielding classification results. Several steps are involved in the signal processing chain.

The Electronic Unit prototype

The design and implementation of the DIMID electronic unit was the main activity carried out by Labor in the project. The first step was to define, together with ZURICH, an accurate and detailed design strategy to guide the DIMID electronic unit development. That is, a detailed functional block diagram, component selection, critical block simulation, schematic diagrams, PCB layout, board production, testing and enabling.

In fact, the development of the Electronic Unit prototype required a series of activities with a high level of mutual correlation and dependency. The Design Strategy was therefore a fundamental step to schedule such activities and define the path to follow in order to obtain the final result.

After a detailed and extensive analysis of the requirements, the Schematic Block Architecture of the Electronic Unit has been defined and used as the guiding document for the Electrical Schematics Drawing. Spice Circuit simulation also helped in some of the single component selection or specific schematic diagrams configuration selection.

Main result has been the Electrical Schematics Drawing for DIMID Electronic Unit, divided in:

- Power Module

- Digital Core: FPGA & Embedded CPU
- Digital I/O
- Low Frequency Analog Front End
- High Frequency Analog Front End
- Customized Components Library (schematic and PCB footprints)
- Documentation library
- PSpice Circuit Simulation to support diagram and/or components selection

Multiple technical review and critical adjustments took place during the schematic layout activity.

The main output of the first phase of the Electronic Unit design is reported on Figure 14, which is the functional block diagram.

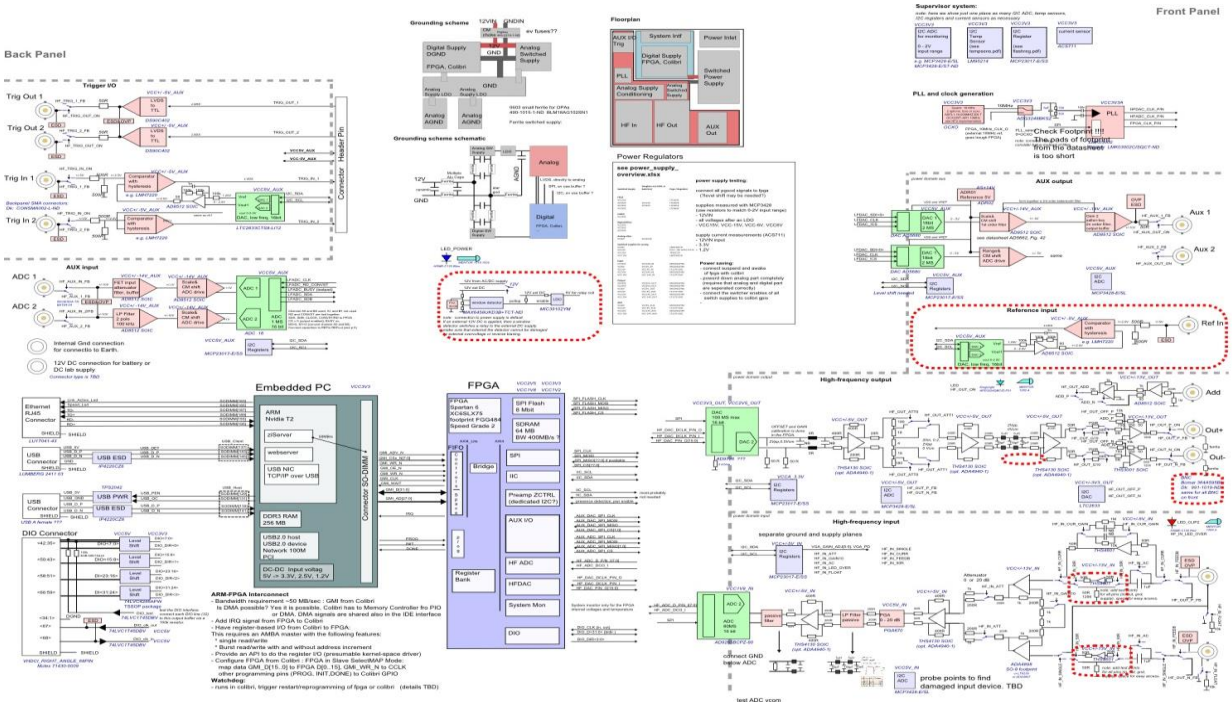


Figure 14 Functional Block Diagram

The second step was then to design and implement the DIMID electronic unit. The actual design work was executed by Labor in close collaboration with ZURICH. During weekly Skype calls, the progress was monitored and eventual deviations were discussed and mitigated by corrective actions. The design underwent a very thorough review process before it was released for prototype production.

In parallel, ZURICH defined a detailed testing specification consisting of 10 test phases. In the third and last step, those tests were successfully performed on the prototype unit. Apart from few minor issues found, all main functions of the electronic unit proved to be working.

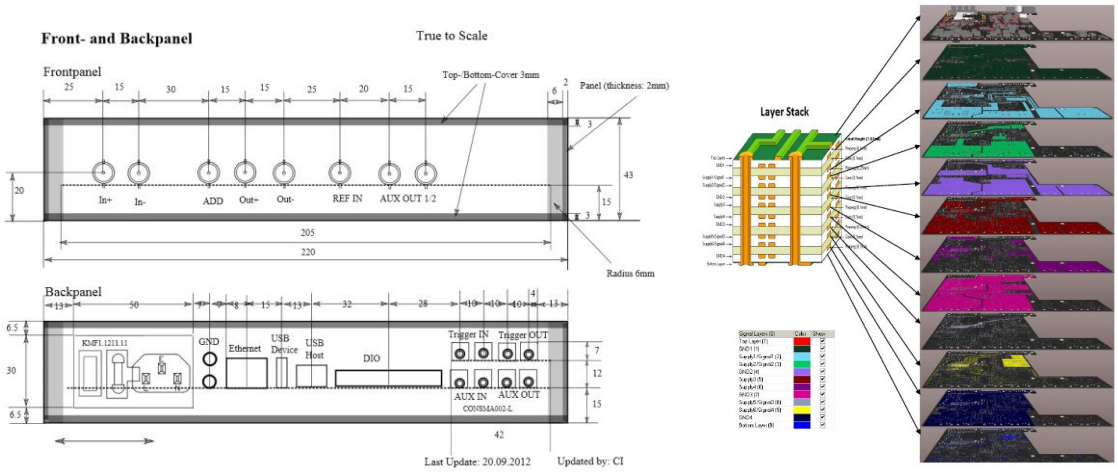


Figure 15 Left: Front and Back panel DIMID; Right: Layer Stack compact(left) and exploded(right) view

The end result was the production of 10 boards for the DIMID electronic unit which were then assembled together to prototypes of the electronic measurement unit including a touch screen display and an embedded PC. All the architectural components of the unit were integrated and tested according to a detailed test specification.

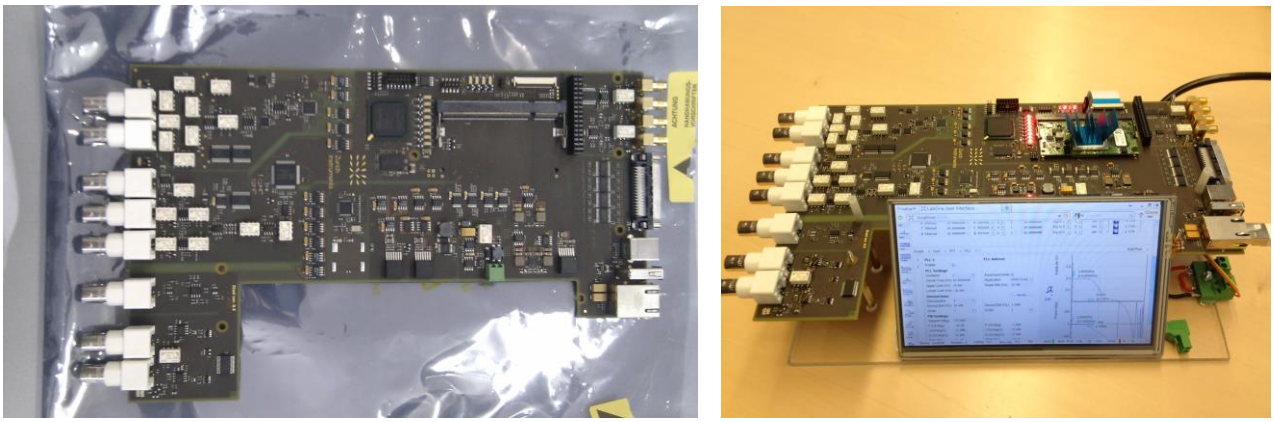


Figure 16 – (Left) DIMID board developed by LABOR and (Right) DIMID electronic unit prototype successfully tested and evaluated by ZURICH

With only minor design modifications, the prototype of the electronic measurement unit successfully passed all functional integration tests. This device was used to build the final prototype of the entire DIMID system as shown further below. In parallel, this prototype will be commercialized by ZURICH as a stand-alone product complementing its portfolio of lock-in amplifiers and impedance spectroscopes.

Software unit, signal processing block and GUI

The Signal Processing Block was implemented by UTV and ZURICH and it is responsible for configuring and acquiring the impedance signal from the HF2 Impedance Spectroscope, isolating and segmenting the regions of interest that contain events/peaks (segmentation) and extracting suitable information from each event (feature extraction) that is used to classify the event as a certain cell type in the GUI and Analysis Block.

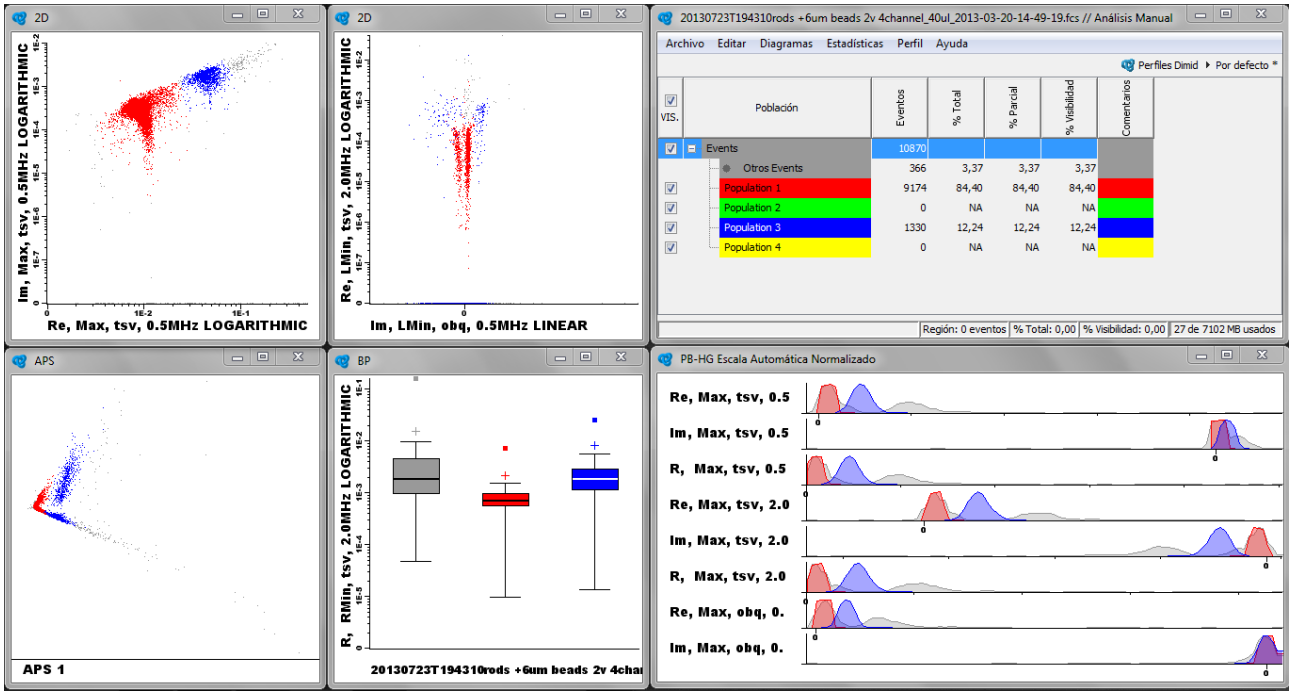
In summary the raw impedance data measured with the DIMID device is transformed into event parameter data readable by DimidSoft. Two different versions of the Signal Processing Block were developed. Each version consists of two code sub-blocks, one block was implemented within the framework of ZURICH LabOne GUI and the other was implemented by UTV in Matlab.

Finally, a flexible FCS (Flow Cytometry Standard) file writer has been implemented that writes the extracted features with clear labels describing each feature to an .FCS file that can be read by CYTOGNOS DimidSoft software.

The GUI, Analysis and Acquisition Modules were specifically designed by CYTOGNOS to run analysis in a easy to learn and friendly interface.
The modules include different components for accessing the provided functionality:

- Population Tree/Diagrams/Histogram
- Dotplot
- APS (Automatic Population Separator)
- Multidimensional
- Boxplot/ Report/ Strategies
- Export functions
- Profiles

The signal processing and analysis software developed by UTV, CYTOGNOS and ZURICH has been successfully tested during the DIMID system integration process and later during the field tests. ZURICH developed the required interfaces between the data acquisition unit and the analysis tool. Specifically, an FCS writer was developed, which allows to import the feature extracted impedance data provided by UTV signal processing algorithms into the DIMIDsoft analysis tool.



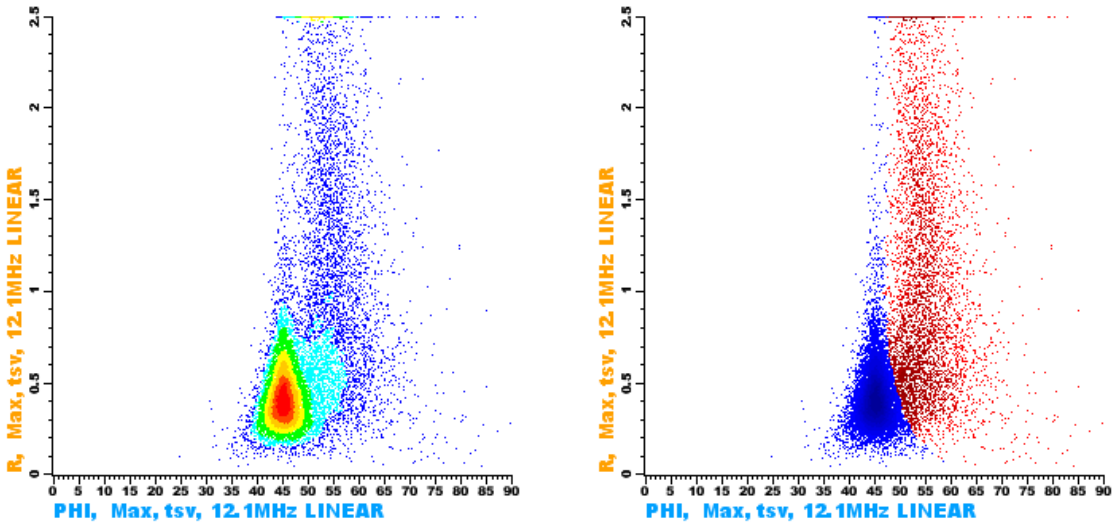


Figure 17 – Screenshots of DIMIDsoft analysis tool developed by CYTOGNOS and used during field tests

During the field tests, the DIMIDsoft analysis tool was intensively used and provided reliable results. The DIMIDsoft user interface proved to be intuitive, also for people without prior flow cytometry experience.

WP5 - PROTOTYPING AND TESTING

During the last six months of the project, the entire consortium worked closely together in order to prepare and execute the system integration steps and the field tests.

Figure 18: System integration of the DIMID device

This task required a lot of coordination among the partners. The system integration was mainly driven by CELLIX and ZURICH. In total, three DIMID system prototype milestones were defined. For each milestone, a set of tests were specified. One of the challenges during the system integration was to make sure that all the interfaces between the DIMID components were working as initially specified. This required close cooperation within the consortium. During regular Skype calls, issues were discussed and solved. The two DIMID subsystems, the microfluidic subsystem and the measurement subsystem were integrated and verified independently. Finally, a first complete DIMID system prototype was built at the premises of CELLIX with support of all consortium partners. The system was evaluated and several optimizations were done resulting in a second prototype. To keep changes to the system traceable, a configuration management with strict version control was introduced.

The second prototype was then used to do preliminary tests with red blood cells (RBC) and to do field tests on specific applications like dairy food quality testing.

WP6 - FIELD TESTS

The DIMID Prototype (ZURICH, CELLIX, CYTOGNOS)

The final and main outcome of the project is the DIMID prototype version 2.0, which has been further optimized in terms of portability and cost structure.

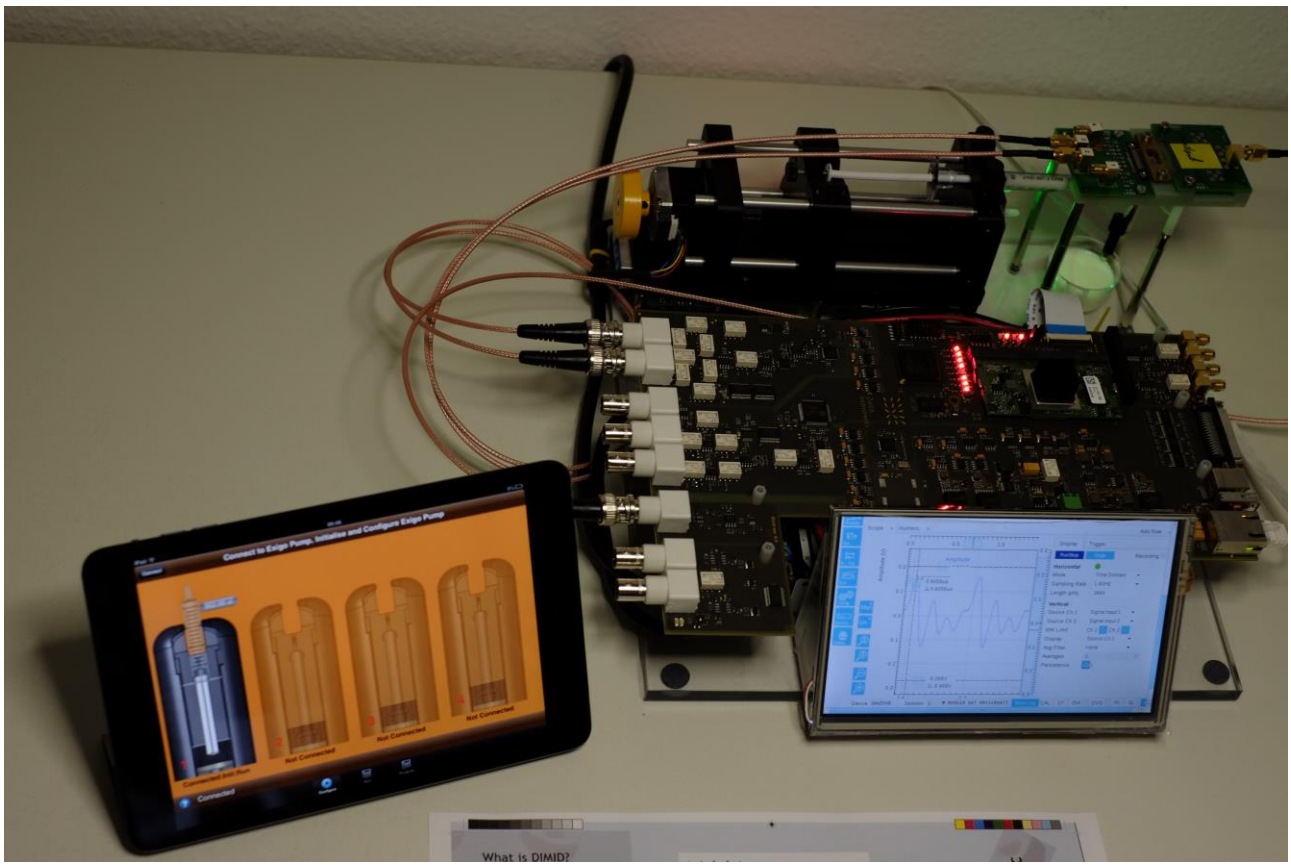


Figure 19 – DIMID prototype version 2.0 – A highly integrated impedance flow cytometer – The microfluidic pump as well as the measurement software itself can be controlled remotely from an iPad

Figure 20 – (Left) Embedded Linux PC module attached to the electronic measurement unit. (Centre) The user interface of the electronic measurement can run remote from an iPad. (Left) State-of-the-art device from ANPHASYS against which the DIMID device was bench marked.

During the field tests, the DIMID device has been successfully exposed to specific applications. In this context, the performance of the system together with other criterions like portability and cost where benchmarked with the state-of-the-art device of AMPHASYS. On the performance, a high level of correlation between the DIMID and the AMPHASYS device was obtained which speaks for the maturity of the technology.

The bench marking showed that the DIMID device has clear advantages in terms of the electronic measurement unit, the analysis software, the portability and the cost structure. Comparable results were achieved in sensitivity, throughput and microfluidic subsystem. The high score on the electronic unit origins from the fact, that it contains a network interface and an embedded PC, which makes the external PC obsolete. For many applications, portability is an important requirement, which was also confirmed by the outcome of several interviews with industry stakeholders conducted by CELLIX. In the mid-term, AMPHASYS has no technical solution at hand in order to avoid a separate computer.

4.1.4 Potential Impact

The aim of the European Union's Framework Programme call "Research for SMEs" is to develop new technology products and markets. In order to achieve this aim the DIMID project has developed a first prototype of an impedance-based microfluidic cytometer.

In order to assess the economic perspective of DIMID, a study has been performed which consists of three major parts. First, the results of the DIMID project and the maturity of the technology in general has been assessed and compared against the initial objectives as outlined in the DIMID project proposal.

Secondly, an updated study of the haematology market has been performed including latest market trends and requirements. This study was consolidated with feedback from interviews that CELLIX conducted with industry stakeholders.

In the third part, results and market requirements have been compared and were consolidated in a strategic evaluation matrix. This was done with a perspective to DIMIDplus, the DIMID follow-up demonstration project, which will be completed by AMPHASYS as consortium partner. As a result of this strategic analysis, an updated business scenario for DIMIDplus has been developed quantifying the economic success potential for all involved SME.

The study shows very realistic exploitation paths for all SME and recommends specific ways forward for each individual SME performer. While ZURICH and AMPHASYS will promote own products based on the DIMID technology directly to the market, CELLIX will focus on offering assays for haematology applications in the context of an OEM partnership.

Socio-economic impact

Socio-economic impacts are directly related with the future commercial exploitation and increase of employment in countries where the SMEs are placed. Societal implications are also expected improving the enforcement of EU legislations and having an impact in the health field.

Cell analysis has become an important technique and represents a fast growing market for a wealth of applications in the fields of medicine, life sciences, food and environmental analytics. The application scenarios that were analysed by the on-going DIMID project partners were framed in the three different areas of:

1. Medical and Veterinary Diagnostics,
2. Biotech Process Development,
3. Food and Environmental Analysis.

The DIMID prototype has been proved as an affordable, reduced in size and easy-to use impedance cytometer enable to perform fast and accurate analysis.

Each SME will be benefited of its participation on the DIMID project exploiting the DIMID components addressing wider markets and getting higher revenues.

- **CELLIX** enhances currently its microfluidic platform in order to expand its market. Haematology assay market is of high interest for CELLIX and can be estimated about 600 MEUR. This estimate is based on the total cytometry market size of 1,000 MEUR in 2009 and taking into account the different assays available. Three years after product launch (2015) revenue of 7 MEUR is planned. This includes revenue on DIMID devices and consumable. Six years after launch revenue of 21 MEUR is targeted.

- **ZURICH** Instruments will sell the DIMID electronics to CELLIX this will create revenue of 0.4 MEUR 3 years after market launch. In addition ZURICH is marketing the DIMID electronics in 2 other products (a lock-in amplifier and impedance analyser) directly to end users in physics and biological research markets. The total market size for DIMID-based lock-in amplifiers can be estimated around 50 MEUR. Three years after product launch this will generate 4 MEUR p.a. In the longer term ZURICH aims at a market share of 30% or about 12 MEUR with the lock-in amplifier. In addition the lock-in amplifier ZURICH exploits the DIMID electronics to commercialise an impedance analyser. The impedance analyser will enable researches world-wide to improve their impedance spectroscopy setups. The volume addressable by ZURICH can be estimate to be 10 MEUR p.a. today with the potential to grow to 80 MEUR in 10 years. Three years after product launch it will generate sales of 1 MEUR.

- **CYTOGNOS** will supply the DIMID analysis software to CELLIX, after six years of the launch turnover of 2.2 MEUR is targeted. In addition CYTOGNOS will sell IES analysis software to the unfilled biological research field meeting uncovered needs. Potential revenues are 0.2 MEUR after three years of launch and 0.8 MEUR after six years.

The results achieved during the project will bring to the SMEs **innovative advantages and competitive positioning** marketing.

- **CELLIX**, thanks to the technical developments achieved during the DIMID project, the DIMID device will allow obtaining results comparable to the currently available high-end solutions in terms of discrimination sensitivity, tomographic capability and measurements per second. The cost of the DMID device will be noticeably lower than the competence one allowing cost saving for many analysis cells applications.

- **ZURICH**, through its participation in the project will improve the quality of software and hardware electronic unit, potential scaling up the production will decrease cost enabling to address additional markets in physics and biology research and become technological leader in impedance spectroscopy.

- **CYTOGNOS** will increase business market as exclusive provider of the analysis DIMID software. CYTOGNOS will also increase its portfolio enabling to offer EIS analysis software to the unfilled biology research EIS field, future developments are expected to be performed in this new market niche.

A successful exploitation of the DIMID technology will generate employment throughout the consortium supply chain. Based on the estimation of the industrial experts we have consulted, CELLIX sales of DIMID devices per year will need about 4 manufacturing workers due to the labour-intensive assembly; for ZURICH and CYTOGNOS additional employment will be created in sales and extra production; so we can conservatively estimate the direct employment creation as follows:

Jobs/year	2015	2016	2017	2018	2019
	Implementation		Full Roll Out		
Device Manufacturing	4	6	8	10	12
Chip Manufacturing	4	8	12	14	16
Electronics Manufacturing	3	5	7	9	11
Software	3	2	1	1	2
Sales	4	6	10	12	18
Service	2	3	4	5	6
Total	**20**	**30**	**42**	**51**	**65**

The SMEs within the consortium clearly recognized a significant market potential of the DIMID device that would open a new market segment, generating new business opportunities. In order to launch a successful product costly marketing and demonstration activities were identified as key factors. Therefore, the DIMID project SMES decided to submit a proposal for a demonstration project under the FP7-SMES-2013 Demonstration Activities.

Societal implications

The DIMID device will bring considerable benefits to a wider research community being able to purchase the product at a lower price but still receiving a high end solution for carrying out high throughput screens of chemical libraries to identify new tools for biological research.

The DIMID device can be available for point-of-care use, applications in medicine and routine analysis. The low prize will facilitate the delivery of services in developing countries.

The DIMID device can also have an important impact on the **enforcement of EU legislations**, especially in the food safety domain. The main regulation that can be supported is the EC 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Besides, raw milk offered for sale within and into the European Union has to be produced according to the requirements of Commission Directive 89/362/EEC and to meet quality standards described in Council Directive 92/46/EEC.

Dissemination activities and exploitation results

A correct identification of the **target audiences** was important in order to make a better use of the resources committed to the dissemination issue. The target audience for the DIMID project was separated in two different main categories aiming for a successful future exploitation:

- Primary Audiences:
    - Hematology field, comprising biotech and pharmaceutical companies, research centers, public and private health bodies, universities and laboratories related with cell analysis for diagnostic, research or routine.
    - Greenhouse field, including companies for routine quality dairy and seeds analysis, research centers and labs related with the environmental field.
    - IES physics and biology research fields, including research centers, universities and laboratories aiming to research new applications related with the IES.
- Secondary Audiences: policy makers, national authorities, regulatory bodies and general public.

The primary audience required a technical description of the innovations related with each specific field, competitive advantages and cost saving, while the second audience required a general overview of the product enhancing its final advantages.

Dissemination activities related with the primary audience were carried out in specialized events, through direct communications or commercial contacts. Communication to the secondary audience has been performed through the website and press release publications.

In order to ensure a better dissemination and communication of the information, different **media** has been used during the project:

- Printed media: the oldest media form has been designed during the project, brochures and posters have been spread in conferences and events.
- Internet: internet is one of me more powerful and cheap tools for disseminating information, at this respect DIMID website has been designed and developed, and a press release in the Cordis website has been confirmed
- Audiovisual media: during the first term of the project a video presentation was performed by the EPFL presenting the liquid electrodes, this video can be found at DIMID website http://www.dimid-project.eu/ and youtube http://www.youtube.com/watch?v=IJg55_9yh6A. For the second term a video presenting a first prototype will be performed by CELLIX.

DIMID project information has been disseminated through brochures in fairs and exhibitions during 2012 and 2013 (list in 4.2 section A2).

What is DIMID?

DIMID is the first low-cost, portable impedance-based microfluidic cytometer equipped with disposable chips, which will enable a range of new analyses and diagnostic approaches that can be performed simply and quickly, without the use of a centralised resource.

cellix

Zurich Instruments

cytognos
Flow Cytometry Solutions

UNIVERSITY OF Southampton

LABOR

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

Tor Vergata

"The research leading to these results has received funding from the European Union's Seventh Framework Programme managed by REA - Research Executive Agency, FP7/2007-2013 under Grant Agreement no. 286692".

www.dimid-project.eu

DIMID - DEVELOPMENT OF AN INNOVATIVE MICROFLUIDIC IMPEDANCE BASED DEVICE FOR MULTI - PARAMETRIC CELL ANALYSIS

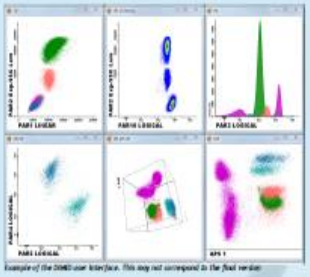
BENEFITS

Simplicity
An easy-to-use system, characterized by the absence of time consuming sample and reagent preparation, multi-step test procedures, tiresome cleanup.

Speed
Fastest testing on the market.

Accuracy
Quick, clean and accurate.

Cost-effectiveness
Time saving, money saving, lower cost per completed test.



DIMID:
the next-generation, integrated, point-of-care, and miniaturised system

DIMID PROJECT ID CARD

Full Title: *Development of an Innovative Microfluidic Impedance-based Device for multi-parametric cell analysis*

Funding Scheme: *FP7-SME-2011*

Grant Agreement n°: *286692*

Starting Date: *1st of October 2011*

Project Duration: 24 Months

Consortium: *7 Partners (3 SMEs and 4 RTDPs) coming from 5 Countries (Switzerland, Ireland, Spain, Italy, UK)*

INNOVATION

Extension of the frequency windows, both at the low and high ends.

Improvement of chip sensitivity.

Novel method that enable faster sampling and analysis.

A new graphical and intuitive software that enable the extraction of quantitative information on cell dielectric properties.

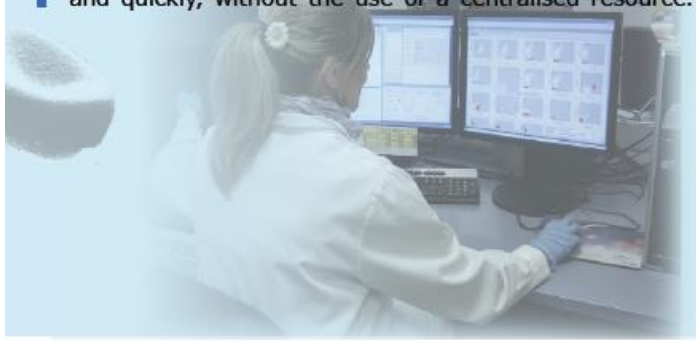
Project Coordinator: Dr. Sadik Hafizovic
ZURICH INSTRUMENT AG
e-mail: info@dimid-project.eu
Website: www.dimid-project.eu

DIMID Triptych brochure

DIMID - The next-generation, integrated, point-of-care, and miniaturised system

What is DIMID?

DIMID is the first low-cost, portable impedance-based microfluid cytometer equipped with disposble chips, which will enable a range of new analyses and diagnostic approaches that can be performed simply and quickly, without the use of a centralised resource.



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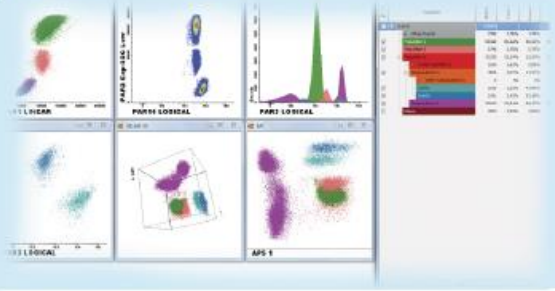

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Speed
Fastest testing on the market

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Quick, clean and accurate.

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Time saving, money saving, lower cost per completed test.

Partners

cellix · Zurich Instruments · EPFL École Polytechnique Fédérale de Lausanne · University of Southampton · cytognos Flow Cytometry Solutions · LABOR Industrial Research Lab

www.dimid-project.eu

Project Coordinator:
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DIMID - DEVELOPMENT OF AN INNOVATIVE MICROFLUIDIC IMPEDANCE BASED DEVICE FOR MULTI - PARAMETRIC CELL ANALYSIS

"The research leading to these results has received funding from the European Union's Seventh Framework Programme managed by REA- Research Executive Agency, FP7/2007-2013 under Grant Agreement no. 286692".

DIMID Poster landscape

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Community Research and Development Information Service

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Innovative microfluidic impedance-based device for multi-parametric cell analysis

Publication Date: 2013-07-01

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DIMID "Development of an Innovative Microfluidic Impedance-based Device for multi-parametric cell analysis" is a highly interdisciplinary project where microfluidics, electronics, biology and informatics converge with the aim of building a low cost impedance cytometer suitable for non-spherical cells and equipped with disposable chips.

DIMID "Development of an Innovative Microfluidic Impedance-based Device for multi-parametric cell analysis" is a highly interdisciplinary project where microfluidics, electronics, biology and informatics converge with the aim of building a low cost impedance cytometer suitable for non-spherical cells and equipped with disposable chips.
The two years DIMID project started in October 2011 funded under the FP7 "Research for SMES" programme. The DIMID consortium is formed by three SMES and four RTD performers from five different countries.
The DIMID device structure basically consists of the electronic unit developed by Zurich (Switzerland), the disposable chip designed by Cellix (Ireland) and the system software created by Cytognos (Spain). Those modules will shape a unique integrated low cost impedance device allowing faster and less expensive cell analyses, obtaining results comparable to the currently available high-end solutions. An intensive research work and support has been carried out by the RTD performers on microfluidic chips (University of Southampton and École Polytechnique Fédérale de Lausanne), electronic control unit (LABOR) and DIMID signal processing algorithms (LABOR, University of Tor Vergata and University of Southampton).
DIMID device will have broad potential applications including identification and counting of different blood cells populations, real-time cell culture analysis, identification of rare cells and green house applications (analysis and quality control of seeds).
At the end of its first year the SMEs within the consortium clearly recognized a significant market potential of the DIMID device. In order to launch a successful product costly marketing and demonstration activities were identified as key factors. Therefore, the original DIMID project SMES jointly with LAB64 (Germany) and AMPHASYS (Switzerland) decided to submit a proposal for the demonstration DIMIDplus project under the FP7-SMES-2013 Demonstration Activities.
In March 2013, the DIMIDplus proposal was successfully evaluated by the European Commission. First discussions with new consortium members took already place and a joined strategy on howto exploit the excellent results achieved in DIMID has been identified.
For more information on the DIMID project and its continuation DIMID-PLUS, please visit: http://www.dimid-project.eu

DIMID Cordiswire Press release

4.1.5 Project Public Website

The DIMID WEBSITE represents the windows of the DIMID project for the stakeholders, end users and everyone interested in such an innovative solution and it is one of the most important assets for the dissemination of the project.

During the design and test phase of the website one of the goals has been the definition of the key message to be communicated through the site and the way to communicate it. Particular attention was paid on the "receiving" (the end users), what they need to know about the project and how the message should be communicated.

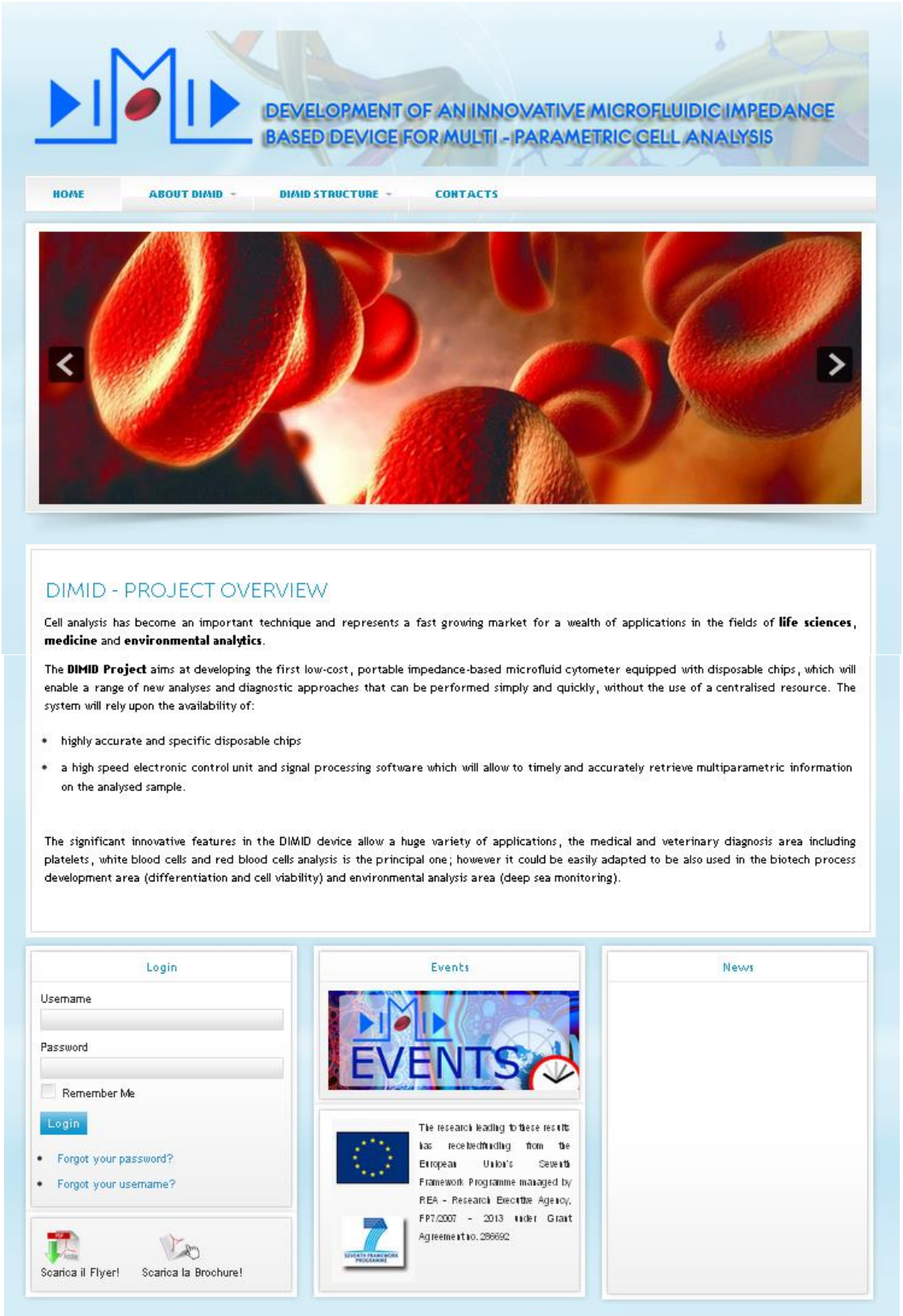
The project web site is the most versatile dissemination tool, by which the consortium informs stakeholders about the DIMID project, the findings and upcoming events/activities. The layout of the webpages has been studied with the purpose to create a screenshot that uniquely identifies the project.

The link of the website is the following: http://www.dimid-project.eu/.

The Home page welcomes you with the DIMID logo as the basis of the new product image, and its colours present the colour scheme of the webpage.

The web pages have been designed with a simple and clear structure. The structure aims at facilitating user access to the information.

In the public area the contact person is the Project Coordinator. It is possible to write a free text in a general purpose email info@dimid-project.eu or send a message directly through the form.



DIMID Homepage