Flexible monitoring system for automated detection of bacterial growth in a commercial specimen processing platform

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Abstract—Diagnosing the presence of bacteria in a biological sample is a task to accomplish as early as possible, since bacterial infections still represent a serious threat to human health. In the market, there are commercial systems carrying out pre-analytical tests on biological specimens. An example is provided by the WASPLab, by COPAN Italia S.p.A., which monitors bacterial growth on Petri dishes that contain the samples, by taking and processing images of one dish at a time in a completely automated way. In this paper, we propose a newly monitoring sensor system for all the Petri dishes at the same time for the detection of bacterial growth with the aim of integrating it directly in the WASPLab. It could permit to obtain quantitative information about bacterial activity in real time directly inside the incubator, offering a more rapid and complete diagnosis response and avoiding the movement of the samples. In addition, the user can set measurement parameters according to his/her needs. This allows the system reaching a great level of flexibility. We tested our solution in two ways. First, we analyzed the behavior of the proposed solution comparing the output signals with the data obtained using an impedance analyzer (HP4194A) as reference. We obtained an average deviation equal to 0.768 Ω in magnitude and 0.059 ° in phase angle. Second, we carried out a 24-hour test to monitor the activity of *Staphylococcus Aureus ATC 6538* in a climate chamber. We found that our system succeeded in observing bacterial growth, with an early detection time of 4 hours. Research is undergoing to integrate the proposed system in the WASPLab.

Keywords—bacterial growth detection; impedance measurement; instrumented Petri dish; WASPLab

I. INTRODUCTION

Bacterial infections represent a serious threat to human health, both in developed and developing countries. The fight against them is topical also nowadays, as some species are becoming more and more resistant to antibiotics, as reported by the World Health Organization [1]. Therefore, acting in a timely manner by delivering a suitable drug therapy when infection has not developed yet is critical for subject's health. For this reason, diagnosing the presence of bacteria in a biological sample is a task to accomplish as early as possible.

Systems able to perform a preliminary detection of bacterial growth in a biological specimen (pre-analytics) play a central role. In fact, they permit to recognize whether the sample is infected or not without waiting a detailed answer provided by expensive and time-consuming specific laboratory tests [2].

In the market, there are numerous commercial systems that carry out pre-analytical tests on biological specimens. Some works in the literature, such as [3], report different examples. They rely on various detection techniques, like surface plasmon resonance, amperometry, microcalorimetry, and bioluminescence [3]. This diversity of techniques has increasingly fostered the spread of different typologies of biosensors in the last decades [3]-[7].

However, in the Industry 4.0 era, a competitive system is supposed to reach very high levels of automation and connection with other devices and Internet. An advanced solution going in this direction is offered by the WASPLab (WASP means Walk-Away Specimen Processor), which is commercialized by company COPAN Italia S.p.A [8]. The WASPLab is a platform that monitors bacterial growth in a specimen, by taking and processing successive images of the Petri dish containing that sample. It handles the tasks of dish inoculation with a possibly infected specimen, its incubation, and image taking, processing, and storing, all in a completely automated way. However, the possibility to integrate in the WASPLab a system monitoring bacterial growth by carrying out a measurement through embedded sensors would make this platform even smarter. Indeed, quantitative data obtained from such measurements would complete the information acquired by processing specimen images. In addition, system monitoring signal could even anticipate image information, enhancing WASPLab capabilities to provide an early objective response about the presence of an infection.

For the previous reasons, in this paper we propose a monitoring system for the detection of bacterial growth. In its final version, such system could be integrated directly in the WASPLab for the monitoring of all the incubated Petri dishes at the same time. Its operation principle is based on the notions of impedance microbiology, which allow monitoring bacterial activity through a measurement of electrical impedance performed with a sensor [2]. The literature reports that main research efforts in the field of impedance

microbiology go towards the design, fabrication and test of technologically innovative sensors [9]-[14]. On the contrary, a very small number of works studies the realization of a complete ensemble including both sensor and an electronic readout unit. Therefore, the main innovation of the developed system is not the sensor itself but the whole system including the specific electronics. The system is designed to be easily integrated in the WASPLab in order to improve the accuracy of the bacterial growing detection. In the actual WASPLab a vision system is used to analyze the bacterial growth, the camera is placed outside from the incubator, so the Petri dishes need to be extracted by a robotic system. This process is quite time consuming and expose the dishes to a temperature not optimized for the growth. The aim of the paper is to solve the cited problems, the integration of our system in the WASPLab permits to estimate the growth leaving the dishes in the incubator avoiding any possible thermal shock. Furthermore, the test cycle of the all dishes in the incubator will be faster and can potentially reduce the growth detection time and allow the recognition of the typical growth phase (lag, exponential, stationary and death).

The second section of this paper will briefly describe the existing WASPLab platform. On the other hand, third section will treat our monitoring system, in all its parts, as it was developed so far. In this way, the benefits connected with the integration of our solution in the WASPLab will emerge. Then, last sections will illustrate the studies carried out to test the system and will present the obtained results.

II. THE WASPLab PROCESSING PLATFORM

Fig. 1 provides a picture of the WASPLab platform. Every single part of this system is labeled by a number.

1. WASP: it is the station where a sterile Petri dish containing a medium for proper bacterial growth is inoculated with a biological specimen possibly infected by a pathogen. All the operations of dish picking, opening, inoculation, closing, and transportation are performed by robotic manipulators in an automated way. A scanning module identifies a precise dish through a bar code. An image-based verification system supervises the entire procedure.

2. Image acquisition station: it scans the Petri dish by taking High Definition pictures of it. The first image is acquired just after inoculation step (i.e., at zero hour), in order to have at disposal reference information about the initial situation. Then, other pictures are taken at successive time instants, which are determined according to analysis protocol.

   Incubators: after having taken the image at zero hour, the Petri dish moves to an incubator, which recreates the appropriate environmental conditions needed to favorite a proper bacterial growth. The WASPLab is equipped with two incubators, which have different capacities. Internal temperature ranges from 4°C to 40°C. All dishes are stacked at a specific location inside each incubator, according to their own bar code. In particular, they are positioned upside down, in order to prevent the condensation caused by medium partial drying from falling onto the sample. According to analysis protocol,

the dish is removed from the incubator at different time intervals to be sent to the image acquisition station. Then, it comes back to allow bacterial culture to grow.

3. Digital imaging interface: it includes an image processing software and a web-based user interface. From this station, platform user can analyze all the acquired images and can monitor the time evolution of bacterial colony growth.

4. Dishes accumulation silos: they hold the Petri dishes that leave definitively the incubators, as bacterial growth inside them has been reached and analysis on their images has been finished. The user takes the silos and removes the dishes from the WASPLab. At this point, the dishes can be sent to another location for further analysis or can be disposed of.

5. WASPLab Central: it plays two roles in platform operation. On one side, it is a server storing all the images of the Petri dishes that have been taken and processed. On the other side, it provides a connection between the WASPLab and third party systems working in cooperation with it.

III. MONITORING SYSTEM

The proposed monitoring system can be separated in three different parts. The first part is a Petri dish that has been instrumented with an electrodes-based sensor. The second part is a portable electronic unit exciting the sensor with a known sinusoidal signal and evaluating its response. Finally, the third part is a computer program that manages electronic unit operation and stores the received data, providing information about bacterial growth in real time.

A. Instrumented Petri dish

Fig. 2 presents the geometric configuration of the sensor that is embedded in the Petri dish. Overall dish diameter is equal to 85 mm, like the ones used in the WASPLab. We considered the simplest sensor configuration as possible. In fact, we worked with a couple of steel electrodes having a rectangular section.

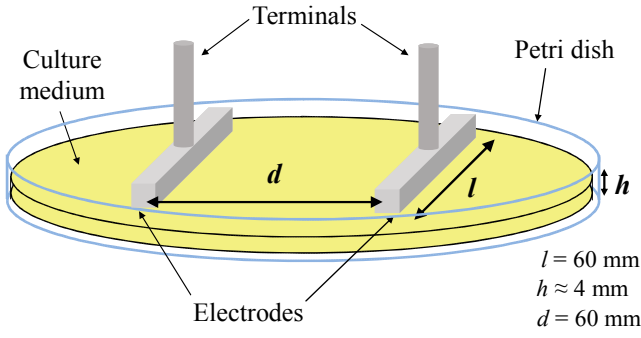Fig. 1. Picture of the WASPLab processing platform.

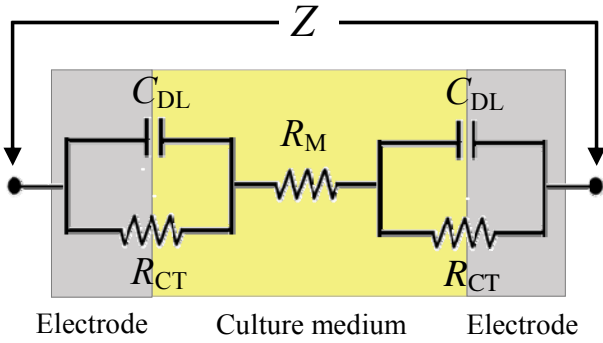Fig. 2. Schematic view of the instrumented Petri dish.

Fig. 3. Equivalent circuit model describing sensor impedance response when immersed in the culture medium.

With reference to Fig. 2, electrodes are 60 mm long and they were immersed in the culture medium for about 4 mm.

In addition, they were arranged in parallel to each other, at a distance of 60 mm. Thus, it is possible to evaluate the impedance Z between them. Furthermore, two contact terminals are screwed into the electrodes to provide an electrical interface to the portable measurement unit.

Sensor impedance response can be electrically described through numerous equivalent circuit models [2], [9], which are characterized by different levels of complexity and accuracy. The model chosen for this work is shown in Fig. 3. It exploits the parallel configuration between capacitance C_{DL} and resistance R_{CT} to represent sensor behavior at the interface electrode/medium. Parameter C_{DL} is the double layer capacitance at this interface, whereas R_{CT} models the charge transfer between electrode and medium. Since the two electrodes are nearly identical, it is assumed these parameters should have equal values for both of them. Last model element is resistance R_M, which is an estimation of medium conductivity. It is in the middle between the parallel configurations, in series with them. The estimation of the three parameters can be done by evaluating the overall impedance at least two frequencies f_1, f_2, and by applying the following mathematical formulas:

$$C_{DL} = \frac{f_1 / \mathrm{Im}(Z(f_1)) - f_2 / \mathrm{Im}(Z(f_2))}{\pi f_2^2 - \pi f_1^2} \quad (1)$$

$$R_{CT} = \sqrt{-\frac{\mathrm{Im}(Z(f_1))}{4\pi f_1 C_{DL} + 4\pi^2 f_1^2 C_{DL}^2 \, \mathrm{Im}(Z(f_1))}} \quad (2)$$

$$R_M = \mathrm{Re}(Z(f_2)) - \frac{2 R_{CT}}{1 + 4\pi^2 f_2^2 C_{DL}^2 R_{CT}^2} \quad (3)$$

$\mathrm{Im}(Z(f_1))$ and $\mathrm{Im}(Z(f_2))$ are impedance imaginary part at f_1 and f_2, respectively. $\mathrm{Re}(Z(f_2))$ is impedance real part in correspondence of f_2. Model parameters estimation allows having at disposal quantitative information about bacterial growth in the Petri dish.

B. Portable electronic u notions nit

The portable electronic unit permits to excite the sensor with sinusoidal waveforms at the two identified frequencies f_1, f_2, and to analyze its response. Since model parameters estimation needs to measure Z at only two frequencies, only two impedance points are required. Therefore, monitoring system can work with a simpler electronics. Unit core is AD5933 integrated circuit (IC), from Analog Devices [15]. This component is specifically designed to facilitate impedance measurements. The IC can be ideally divided into three main parts. Transmitter side is composed by a 27-bit direct digital synthesizer, a digital-to-analog converter and a programmable output amplifier that allows the generation of sinusoidal excitation waveforms with a frequency resolution of 0.1 Hz. Such waveforms present a 1.65-V dc-offset, whereas their amplitude can be chosen. On the other hand, receiver stage comprises an I-V converter, a programmable gain amplifier, an antialiasing filter, and a 12-bits analog-to-digital converter (ADC). The current signal corresponding to the unknown impedance response is converted to a proportional voltage value and presented to the ADC. The digitalized signal is then processed by the internal digital signal processor, which performs a Discrete Fourier Transform on the sampled data. Finally, results are written as impedance real part and imaginary part into specific registers, which are accessible through serial peripheral interface (SPI). To avoid any transient behavior, a settling time is established by setting a specific register of the AD5933 that determines the number of output excitation cycles allowed to pass through the unknown impedance before the ADC is triggered to perform a conversion of response signal [15].

Specific peripherals are added to control the IC by a computer through a USB connection and to comply with the measurement requirements. A microcontroller (MCU) works as an interpreter between the SPI interface of the AD5933 and computer's USB connection. The unit clock involved both in waveform generation and sampling process can be provided to AD5933 core through a dedicated pin. Clock frequency depends on the range which f_1 and f_2 belong to. In the proposed system, it is obtained starting from an oscillator through a phase-locked loop (PLL) used as a clock divider. Since in our study this range goes from some hertz to about 1 kHz, a 200-kHz clock is needed, according to the datasheet [15]. Therefore, we chose a 16 MHz oscillator (AEL-4303) and ADF4001 PLL with a division ratio of 80. Other clock frequencies can be obtained by reconfiguring PLL division ratio, giving a good level of flexibility to the electronic unit.
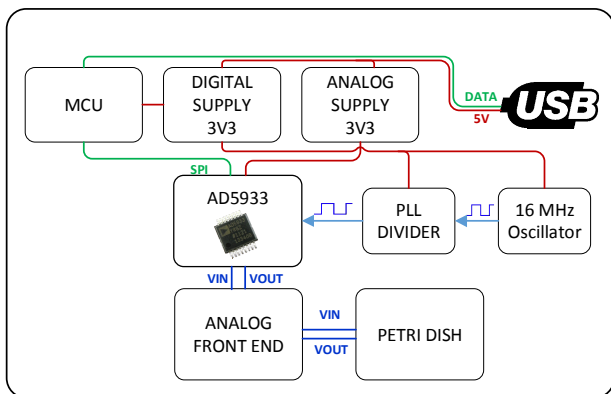
Fig. 4. Block diagram of the portable electronic unit.

The AD5933 output section can excite a sensor with an impedance greater than 500 Ω. On the contrary, below that limit, the current flowing through the unknown impedance increases, exceeding the output amplifier capacity. For this reason, an additional analog section was included, due to the low resistance characteristics of the Petri dishes (going between 50 and 150 Ω). Fig. 4 shows unit block diagram.

C. Computer program

The portable electronic unit is connected via USB to a personal computer (PC) on which a LabVIEW Virtual Instrument (VI) was implemented. Through such VI, aiming at testing the portable electronic unit, the software is programmed to acquire not only the two frequencies f_1 and f_2, but a specific number of impedance points by performing a linear frequency sweep in an interval permitted by unit clock. Furthermore, system operation can be controlled by the user through an intuitive interface. In particular, the VI has various functions. First, it allows setting measurement parameters, such as start frequency, the number of incremental steps, and incremental frequency, according to the desired number of impedance points to acquire. Also f_1 and f_2 can be set, for model elements estimation. Furthermore, the VI permits to choose to carry out single or continuous measurements. In the latter case, user has to decide measurement cycle duration and the number of cycles to repeat. However, then the system works automatically, without any actions from the user until measurement process ends. The possibility to choose such a number of parameters gives great flexibility to the proposed monitoring system. In this way, it is able to adapt to different operating conditions. Second, it acquires electronic unit outputs and calculates C_{DL}, R_{CT}, and R_M through the implementation of (1), (2), and (3), from the values of $Re(Z(f_2))$, $Im(Z(f_1))$, and $Im(Z(f_2))$. Third, the VI presents the data regarding measured impedance and calculated model elements, with either numerical indicators or graphs, in real time. In this way, system user has the possibility to monitor the time variation of the parameters associated to bacterial growth at any instant. Last, it saves all useful data in a .txt file. Therefore, they can be used for additional elaboration and visualization also after measurement session ends.

IV. COMPARISON WITH HP4194A ANALYZER

In the first kind of tests, we analyzed the behavior of the portable electronic unit with a gold standard, generally used for impedance measurement, at the starting conditions (sterile Petri dish). We analyzed system frequency response and we compared it with the one characterizing an impedance analyzer, which was exploited as reference instrument.

A. Performed test

We used an instrumented Petri dish that was filled with a culture medium based on Tryptone Soya Agar, which contains the nutrients required by a bacterial colony to grow properly. Anyway, such dish was kept sterile for the entire test.

We connected its terminals to a HP4194A impedance analyzer. Then, we performed a single frequency sweep, taking 401 impedance points from 100 Hz (which is the minimum frequency allowed by the instrument) to 100 kHz.
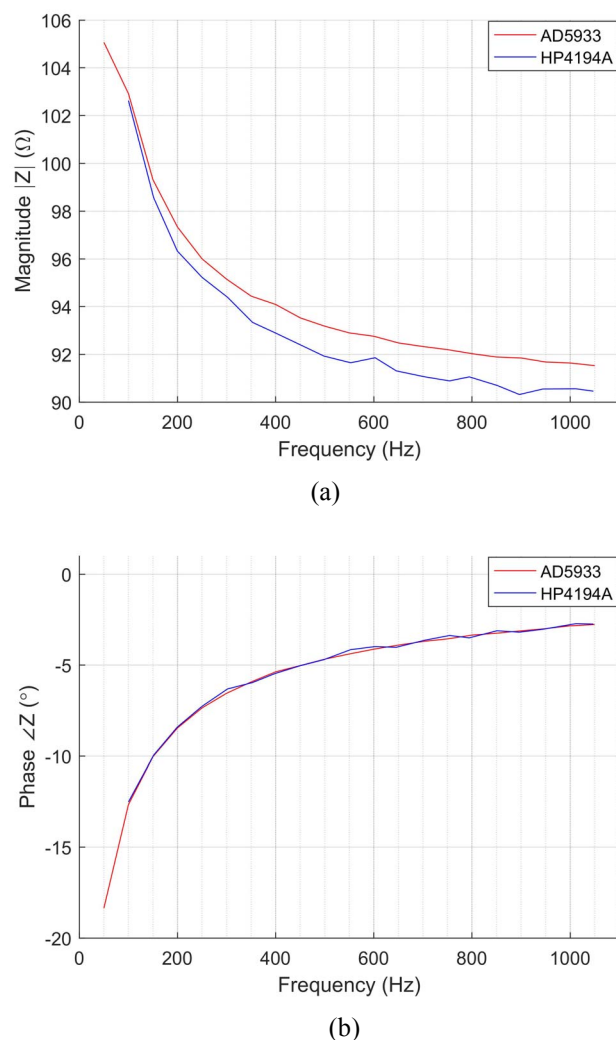

(a)

(b)

Fig. 5. Comparison between the measurements obtained from the AD5933 and the HP4194A of a sample sterile Petri dish divided in magnitude (a) and phase (b).

Afterwards, we worked with our system. We set the amplitude of the excitation sinusoid as 1 V_{pp}. In addition, the system needs always a onetime calibration before every measurement session can take place. This is necessary to calculate electronic unit's internal gain factor and compensate possible cable parasitic effects. For this purpose, we connected a resistor with a known value (i.e., about 100 Ω) to the measurement terminal and we carried out a single impedance acquisition. Then, the system was ready to operate. We connected the same Petri dish that had been used with the impedance analyzer, in order to measure the same component. We set all measurement parameters through the user program interface, in a way to obtain 22 impedance points, from 50 Hz to 1050 Hz, with a 50-Hz frequency interval between each point. This measurement was performed only once.

The entire test was conducted at ambient temperature.

B. Achieved results

The results obtained from the comparison between the designed portable electronic and the instruments used as reference (HP4194A) show, in the measurement range (100 – 1050 Hz), the average deviations are: 0.768 Ω for the magnitude and 0.059 ° for the phase. Fig. 5. reports the data of the test divided into module (Fig. 5a) and phase (Fig. 5b).

V. BACTERIAL GROWTH MONITORING

In the second session of tests, we used our system to monitor the growth of bacterial cultures in a favorable environment, in order to assess its detection capabilities. We show here the obtained preliminary results.

A. Followed protocol

We employed two instrumented Petri dishes in this test (Fig. 6), which were filled with Tryptone Soya Agar-based medium too. One of them was inoculated with a solution that contained an initial concentration of 1.5 McFarland of pathogen *Staphylococcus Aureus* ATC 6538. Inoculation step was carried out at ambient temperature. The other dish was maintained sterile, in order to have a reference for making a comparison with the behavior of the infected one.
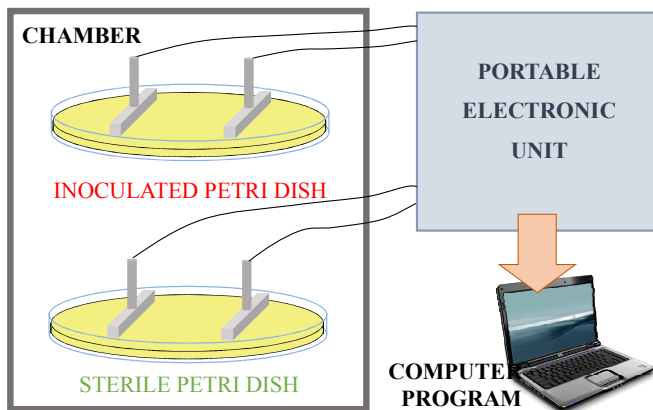
Just after the inoculation, we placed both Petri dishes in a climate chamber, which was exploited to recreate the ideal environmental conditions that are reached inside WASPLab incubators. We set chamber internal temperature to a constant value equal to 35 °C. Furthermore, we turned it on one hour before starting the measurement session, to assure uniform temperature conditions.

When the realized setup was ready, we could begin our analysis. Firstly, we calibrated the system through the procedure followed in the previous test. Then, we connected the Petri dishes to electronic unit terminals and we set the measurement parameters as described in section IV.A. In addition, we fixed f_1 = 150 Hz and f_2 = 250 Hz for model parameters estimation. Cycle duration was set to 2 minutes, whereas the number of cycles was defined in a way to allow the system working continuously for 24 hours from inoculation time.

A. Results and discussion

Fig. 7 presents the parameter of the equivalent electrical model of the sensor applied to the Petri dish for both the inoculated and sterile one. The curves present an initial transient that last about one hour that probably represents the medium temperature settling. The curves related to the sterile dish have only weak variations that may depends on the medium drying. Analyzing the inoculated dish parameters it is possible to observe the typical behavior associated to the bacterial growth curve [2], in particular a change in the curves slope can be appreciated after 4 hours for the parameter R_M (Fig. 7). Leaving out the transient time that occurs during the first hour a further variation in the curve slope can be observed. During the early hours, the parameter has a constant behavior like the one showed on the sterile dish, after 4 hours there is a clear change in the slope. The system recognized the pathogens activity after 4 hours considering the parameter R_M. During the growth phase the maximum parameters variation is about 5 Ω.

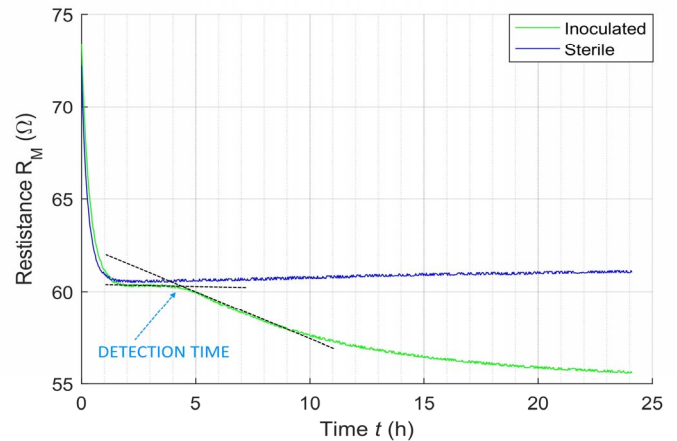Fig. 6. Block scheme of the experimental setup used during the test.

Fig. 7. Parameter R_M of the equivalent electrical model of the sensor collected from a 24 hours measurement session. Comparison between inoculated and sterile dishes.

Achieved results permit to observe that bacterial growth has a stronger influence on system behavior at the interface between electrodes and medium, with respect to medium itself. During the growth phase the maximum parameters variation is: 79.69 µF for C_{DL}, 2,81 Ω for R_{CT} and 4.67 Ω for R_M. This enhances system reliability.

Anyway, our solution allows carrying out a measurement at any phase of bacterial growth automatically. Thus, it is able to provide different quantitative data, which could integrate the information coming from WASPLab image processing action. In fact, a more complete information set could enhance WASPLab smartness and diagnosis capabilities. Indeed, WASPLab Central could store both image and quantitative data, and it can make them available whenever they are requested. For instance, it can send them through the cloud station to other platforms, which can use them directly or elaborate them through Big Data analytics software. Furthermore, obtaining an electrical signal that can anticipate image information could shorten the time needed to diagnose that a Petri dish contains a pathogen. In this way, not infected dishes can be removed from the WASPLab before taking additional images on them. In this way, new dishes can be placed in the incubators, augmenting pre-analytics process speed. Finally, our system goes towards the possibility to monitor bacterial growth directly inside the incubators. In this way, there would not be the need to bring the dishes out and to send them to the image acquisition station. Consequently, the introduction of possible perturbations on growth process could be avoided.

VI. CONCLUSIONS

In this paper, a monitoring system for the detection of bacterial growth in a Petri dish has been proposed, with the final aim to integrate it in WASPLab automated platform, commercialized by COPAN Italia S.p.A. This system provides real-time quantitative information about bacterial activity, starting from an impedance measurement performed with an electrode-based sensor embedded in the Petri dish. In addition, the user can set measurement parameters according to application requirements and his/her needs. This aspect gives the system a great level of flexibility. We have presented the parts that compose it. Furthermore, a study has been carried out to test it. We have described the related analyses and we have illustrated the obtained preliminary results.

Such preliminary results suggest there is the possibility to integrate the proposed system in the WASPLab. In fact, it permits to automatically monitor bacterial growth whenever it is desired. Furthermore, provided quantitative data could complete the information obtained from WASPLab image processing action.

Looking at the final aim, future activity is going towards the direction of optimizing the proposed system to integrate it in the WASPLab. Firstly, we are going to realize a more advanced configuration of electrodes (for instance, by employing interdigitated electrodes made of better materials),

to augment system sensitivity to bacterial activity. Secondly, we are going to improve the interaction between portable electronic unit and electrodes, in order to obtain simultaneous measurement information from all the Petri dishes in the WASPLab.

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