# A Web-based Software System for Behavior Analysis of Laboratory Animals

Simone Macrì, Luca Mainetti, Luigi Patrono, Stefano Pieretti, Andrea Secco, and Ilaria Sergi

Original scientific paper

*Abstract* – **The analysis of locomotion in laboratory animals plays a crucial role in many scientific research areas. In fact, important information on animals' behavior and their reaction to a particular stimulus is deduced from a careful analysis of their movements. The techniques commonly adopted to support such analysis have many limitations, which make the related systems particularly ineffective. On the one hand, the human observation and annotation process is strongly observer-dependent and expensive in terms of time and efforts. On the other hand, the use of more sophisticated systems based on video recordings and recognition algorithms is very expensive and complex. In order to face this challenge, this paper presents a tracking solution based on passive Radio Frequency Identification (RFID) technology in Ultra High Frequency (UHF) band, allowing the tracking of laboratory animals with a high accuracy. The overall solution consists of a hybrid system including hardware and software components. In particular, in this paper, the attention is focused on the software component as the hardware has already been described in previous works. The software component is a Web-oriented solution that offers a complete 2D and 3D information tool including reports, dashboards, and tracking graphs. The proposed solution was widely tested using twelve laboratory mice and compared with an automated video-tracking software (i.e., EthoVision) in order to demonstrate its effectiveness and reliability. The obtained results have demonstrated that the proposed solution is able to correctly detect and reconstruct the events occurring in the animals' cage, and to offer a complete and user-friendly tool to support researchers in behavioral analysis of small laboratory animals.**

*Index terms* – **animal behavior, Radio Frequency Identification, RFID tags, software architecture, UHF technology, Web application.**

## I. Introduction

Animal testing is a key step in research of new pharmaceutical products for human use. In fact, laboratory animals are often used as models for understanding particular biological characteristics of humans. More in detail, their use holds promise to aid the identification of disease etiology and the design of therapeutic approaches. The use of animals in biomedical experimentation is essential but also object of ethical disputes. These exhort to find alternative solutions to animal use and to improve experimental conditions in favor of a greater care of laboratory animals. These requirements have been described in the 3R principle [1], which summarizes three fundamental concepts: Replacement, Reduction and Refinement. Based on this principle, the researcher should initially try to replace its animal model with an alternative model. The second step is to try to minimize the number of animals used in experimental studies. Finally, the last R refers to the need to improve the experimental conditions of animals. In recent years, the new concept of rehabilitation [2] was added to the 3R principle. The scientific community and pharmaceutical companies agree on reducing the number of animals involved in the testing of new products, but nowadays there are still no valid alternatives able to completely replace animal testing. Some tests aim to detect emotional aspect of animals, extract qualitative and quantitative measures of locomotor activity or study animal attitude to explore an environment. In particular, the analysis of locomotor activity represents a key approach to extract important information and, for this reason, automated animal tracking provides a crucial and significant support for medical research and drug discovery. In preclinical behavioral analysis, the mostly used animals are mice and rats, which typically move in close and small environments. The techniques commonly adopted to investigate locomotor activity have many limitations, which make the corresponding systems particularly ineffective. In the manual registration of animal behavior performed by a skilled operator, the success of the analysis depends heavily on the researcher's capability to identify and monitor each animal involved in a test. This is a time and labor consuming process prone to experimenter bias and/or fatigue. Moreover, some movements may be too fast to be detected by human observation. Finally, in poor lighting conditions, animals similar to each other cannot be easily distinguished. In order to overcome these limitations, several automated systems exploiting video analysis have been developed [3]. An ad hoc software able to capture and process animal tracking data typically supports these systems but, usually, these software solutions are expensive.

In the present work, an effective software system for behavioral analysis of small laboratory animals based on passive Radio Frequency Identification (RFID) technology in Ultra High Frequency (UHF) band is presented.

The RFID technology is an emerging auto identification solution that exploits electromagnetic fields, generated by a reader system, to read an identifying code stored in

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a transponder. The RFID technology has been widely explored for the traceability of goods. Several studies have been performed, for example, on the use of this technology for the traceability of pharmaceutical products [4], [5] and on the possible effects of electromagnetic waves on such products [6], [7]. As the hardware component presented by Catarinucci et al. in [8] ensures the compliancy with EPC Class 1 Gen 2 standard [9] for multiple and simultaneous reading of several RFID tags, also the here proposed software solution guarantees the usability with small laboratory animals exploiting the Near Field (NF) technology that permits to realize very small tags.

Solutions based on RFID technology in Low Frequency (LF) or High Frequency (HF) bands already exist in the literature. Kritzler et al. [10] proposed a solution in which a LF RFID system is used in a semi-natural environment. The use of LF band, which provides a limited reading range, forces mice to follow predefined paths in order to detect their movements. Aguzzi et al. [11] exploited RFID technology in HF band to analyze marine animals' behavior. Both LF and HF bands do not support multiple readings of many tags because they are not compliant with the EPC Class 1 Gen 2 standard and are characterized by a low reading range. Then, these frequency bands do not permit the study of social interactions involving a large number of animals and are not able to ensure a natural environment that does not affect animal behavior.

Instead, the use of passive NF UHF RFID solutions is able to guarantee the observation of an animals' colony preserving a natural environment. In the proposed solution, the fundamental working assumption is that a passive NF UHF RFID tag is implanted in each mouse. More in detail, the implant technique of RFID tags in laboratory mice was planned in order to preserve the performance of implanted RFID tags and not to procure distress to the animals. Some results of this study were presented by Catarinucci et al. in [12] and detailed in [13]. In these works, the analysis performed on implanted mice confirmed the absence of pain, distress or changes in animal behavior as well as the long-term readability of the implanted tags.

In the current work, the performed tests aim to demonstrate the effectiveness and reliability of the developed software system. The tracking solution was validated against a visual system, using several laboratory mice in each of which an UHF RFID tag was implanted. The proposed solution allows obtaining a large set of information, in terms of 2D and 3D charts and statistical dashboards, reducing the number of involved animals, in accordance with the requirements of the international scientific community.

The rest of the paper is organized as follows. In Section II, the architecture of the proposed system is presented, with details regarding the software and hardware components. Test procedures are presented in Section III, whereas the results of the tests are presented and discussed in Section IV. Finally, the concluding remarks are given in Section V.

## II. Proposed Tracking System

### A. Hardware architecture

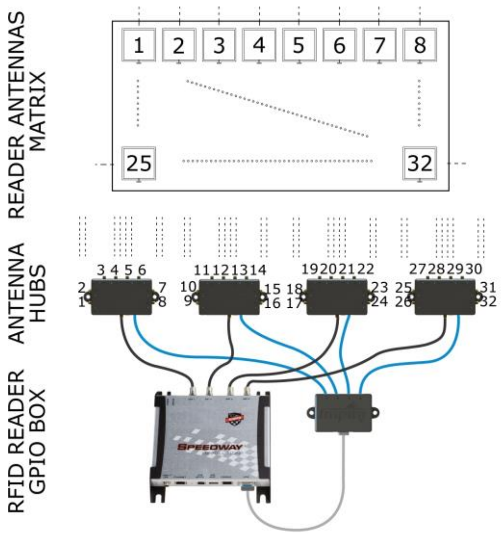


Fig. 1. Hardware architecture proposed in [8].

In order to better understand the functionality of the software component, a brief reminder on the hardware component of the system, introduced by Catarinucci et al. in [8] and detailed in [14], is required.

Fig. 1 shows the hardware architecture proposed in [8].

A prototypal reader antenna was designed in order to univocally localize the animal in an elementary cell and to meet specific requirements. More in detail, it is able to guarantee a uniform, homogeneous and confined magnetic field. The prototypal reader antenna size is about 12 cm x12 cm, which is comparable with the size of a mouse. The bottom of the mice cage is virtually split in cells. Each cell corresponds to one antenna. Therefore, the reader antennas system is installed under the mice cage and each cell represents a possible position (i.e., cell) occupied by the animal. In its maximum extension (i.e., using 32 reader antennas), the antennas system is very large (about 48 cm x 96 cm) and allows the observation of a mice colony.

The proposed system is able to work properly using also commercial NF UHF reader antennas. This makes the system very flexible and not constrained to customized solutions.

### B. Software architecture

In order to capture and process the positional information coming from the hardware component and to provide the user with correct behavioral information, a complete and efficient software infrastructure is needed.

Fig.2 shows the software architecture of the proposed

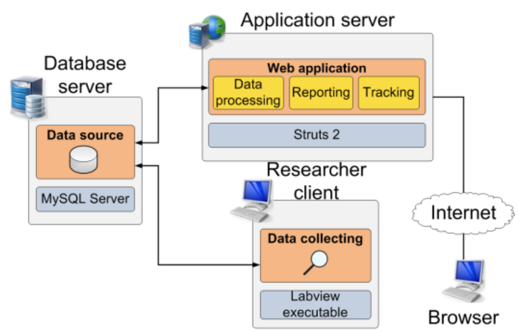


Fig. 2. Proposed software architecture.

tracking system. It is a 3-tier client-server architecture in which the functional process logic, computer data storage and user interface were decoupled as independent modules. A database server is used for the data management, whereas an application server is dedicated to the business logic. More in detail, by implementing Java Enterprise Edition (JEE) specifications [15], this last component provides specific services and allows the Web application execution.

The Web-oriented choice allows all authorized users to access locally or remotely animals' information, at the same time, by viewing different data according to the specific user profile and using a simple Internet access (i.e., a Web browser).

The software system consists mainly of two components: (i) the data-collecting tool described and validated by Catarinucci et al. in [16] that allows the management of the hardware component and the collection of the information coming from the reader antenna system, and (ii) the Web application that supports the researchers' activity.

### B.1 Data collecting

The data-collecting module, as widely discussed in [16], supports the hardware management using a middleware implemented by the graphical programming environment LabVIEW [17]. The middleware communicates with the RFID reader by sending appropriate Low Level Reader Protocol (LLRP) [18] messages containing the configuration parameters according to the user requirements, such as the power transmission of each reader antenna connected to the RFID reader. The reader replies by sending LLRP messages containing information about each connected reader antenna or the success or failure of a specific configuration. All this information is captured and processed by the middleware. An important task performed by the middleware consists in capturing and storing raw positional data in an ad hoc designed database, hosted in the database server. This database contains both the raw data captured by the middleware and the data processed by a specific module included in the Web application. MySQL [19], one of the most common open source database management systems (DBMS), was used for the creation and the management of the database, representing a secure and reliable solution able to guarantee flexibility, scalability, and high performance.

### B.2 Web application

The Web application supports researchers in the animal behavior analysis providing them with statistics, graphs and animal tracking information in a simple and effective way. In particular, the Web application processes raw data stored in the database and stores the processed data in the same database, in order to make them available to the end-user.

The main components of the Web application are: the data processing module, the reporting module, and the tracking module. Each module was designed independently in order to guarantee effectiveness and scalability. Moreover, all modules were developed using the JEE, adopting a client-server architecture. In particular, the Java language, able to guarantee multiplatform and object-oriented solutions, was used to develop the data processing module. Vice versa, Struts 2 [20] was chosen for the realization of the Web application. This framework, distributed by Apache, is completely based on Java.

A fundamental requirement was to design coherent and intuitive interfaces and, for this reason, different technological solutions were adopted. One of these is SiteMesh [21], a framework used for the creation and management of the Web pages and for the composition of the Web application template. SiteMesh offers an important support for the Web pages decoration and layout, by managing effectively the navigation, and allowing the generation of composed pages.

The Web solution provides interactive pages, which show test results in form of tables and graphs. This feature was obtained using JFreeChart [22], one of the most popular open source Java graphic libraries. This library can create charts dynamically at runtime exploiting data stored in the database.

With regard to the Object Relational Mapping (ORM) service, the Hibernate framework [23] was adopted.

In order to implement a 3D viewer within the Web application, the WebGL [24] technology was used. WebGL is able to generate and manage three-dimensional graphics directly on Web pages, allowing the interaction of the user with the 3D environment.

The main components of the Web application will be described in more detail in the follow.

*Data processing module.* The core of the data processing module, included in the Web application, consists in a complex algorithm able to filter the raw data collected during the data acquisition phase. The raw data are processed by the RSSI-Chebyshev-Ping-Pong (RCP) algorithm, which aims to discriminate the correct animal position. In fact, if several reader antennas read the same RFID tag in the same time, a positional ambiguity occurs, which can be solved by the RCP algorithm. It is structured in three phases:

(i) Identification of simultaneous tag readings and data discrimination based on the Received Signal Strength Indication (RSSI).

(ii) Evaluation of cells adjacency exploiting the Chebyshev distance [25] method.

(iii) Removal of the "ping pong" effect, which occurs when a tag is positioned between two cells, generating a continuous alternation of the position between the involved cells.

Fig. 3 shows in details the operational features of the RCP

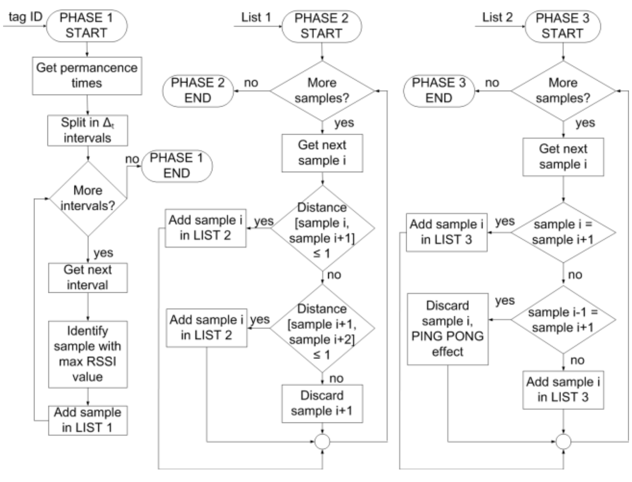


Fig. 3. Flow-charts of the RCP algorithm phases.

algorithm.

The processed tracking data are stored in an ad hoc table of the same relational database used to store the raw data.

RCP algorithm is characterized by high scalability. In fact, it is able to work with different hardware configurations (i.e., 3, 6, 12, 24 or 32 reader antennas).

*Reporting module.* The reporting module is able to summarize statistics and information about mice behavior and their locomotors activity using the processed data stored in the relational database. In detail, information about the visited cells, the time spent in each cell, and the occurrence of particular social events (i.e., aggregations and isolation phenomena) are reported. Each analysis is performed according to several parameters set by the user, such as:

(i) Isolation Time, it indicates how many seconds a mouse should stay alone in a cell cage in order to detect isolation phenomena;

(ii) Aggregation Indicator, it indicates the number of mice that have to be in the same cell in order to detect aggregation phenomena;

(iii) Aggregation Time, it indicates how many seconds N mice, where N is the Aggregation Indicator, should stay together in a cell in order to detect an aggregation phenomena.

(iv) Data, Start Time, End Time, they permit to select the desired observation interval for data analysis.

The statistics related to the visited cells show, in tabular form, the maximum, minimum, and average time spent by each mouse in each cell of the cage and a count of mice movements (i.e., cell changes).

The detection of isolation and aggregation phenomena is performed using an algorithm able to analyze individual positions in order to check if these are temporally continuous and meet the thresholds (i.e., Isolation Time and Aggregation Time) inserted by the user. Fig. 4 shows the operational features of this algorithm.

Finally, space-time graphs show the mice movements in the cage during the observation period. The user can select different mice in order to visualize animals' displacement in a single space-time graph. Along the abscissa axis, the selected observation period is reported whilst, along the ordinate axis, the cells cage are indicated (Fig. 5).

*3D tracking module.* The 3D tracking module consists of several three-dimensional interfaces able to provide the researcher with analysis about locomotor activity and behavior of small laboratory animals. The 3D tracking module offers a reconstruction in a three-dimensional scene of the movements performed by each mouse in the cage. The user can also insert several objects in the 3D scene (e.g., wheel, food or water container, etc.), placed into specific cells (Fig. 6.a). It is possible to visualize the path related to all the mice (one color for each mouse) or only some of them.

The 3D tracking module also allows the end-user to view a video that reconstructs the mice movements in the cage (Fig. 6.b). For the selected mice, it is possible to start, stop, pause or restart the video in order to observe how the animals approach to each other or to objects in the cage.

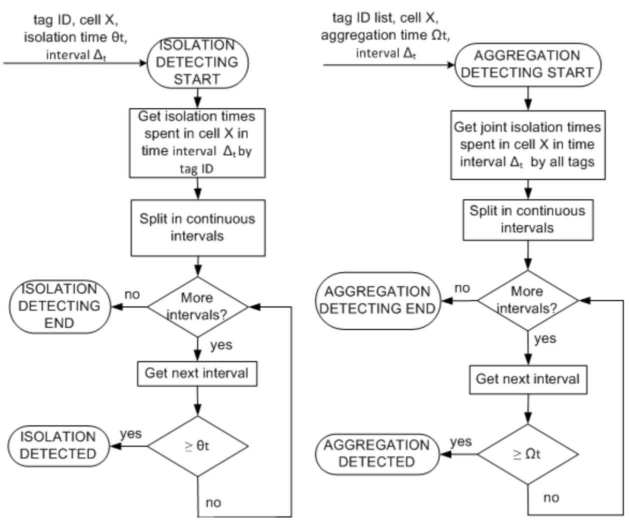


Fig. 4. Flow-chart of the algorithm for isolation and aggregation phenomena detecting.

## III. Methods and Procedures

### A. Animals

All the implanted passive RFID tags are constituted by an inlay composition characterized by aluminum on the top and polyester PET as substrate. In order to prevent the tag antenna corrosion due to the animal tissue composition, each tag was plasticized with adhesive PVC, with a thin plasticization of 80 μm per side. In tests involving laboratory animals, twelve male CD-1 mice (Harlan, Italy), which weight is about 50-60 g, approximately 300 days old, were used. Mice usually reside alone in their own Plexiglas home cage (33 cm long, 15 cm wide and 13 cm deep), with sawdust for bedding. The cage, equipped with overhead cover, due to its transparency allows a good view of the environment. Mice reside under standard light (light on from 7:00 a.m. to 7:00 p.m.), temperature (21±1°C), relative humidity (60±10%) with food pellets and water accessible ad libitum. The experimental protocol was approved by the Service for Biotechnology and Animal Welfare of the Italian National Institute of Health and authorized by the Italian Ministry of Health, according to Legislative Decree 116/92. This decree implemented the European Directive 86/609/EEC on laboratory animal protection in Italy. Veterinarians from the Service for Biotechnology and Animal Welfare routinely checked animal welfare. Tags implantation technique was carried out after choosing an appropriate tag position under the animal skin in

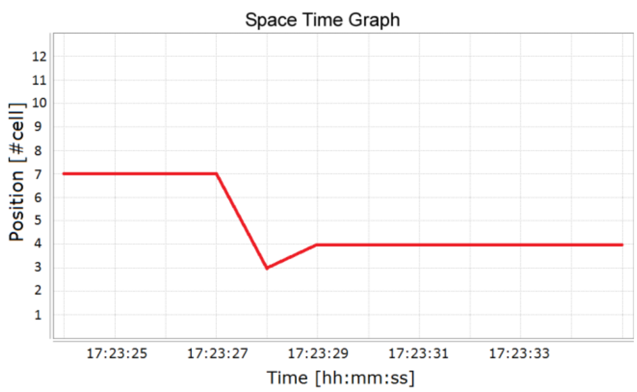


Fig. 5. Graph showing mice activity in the test cage.

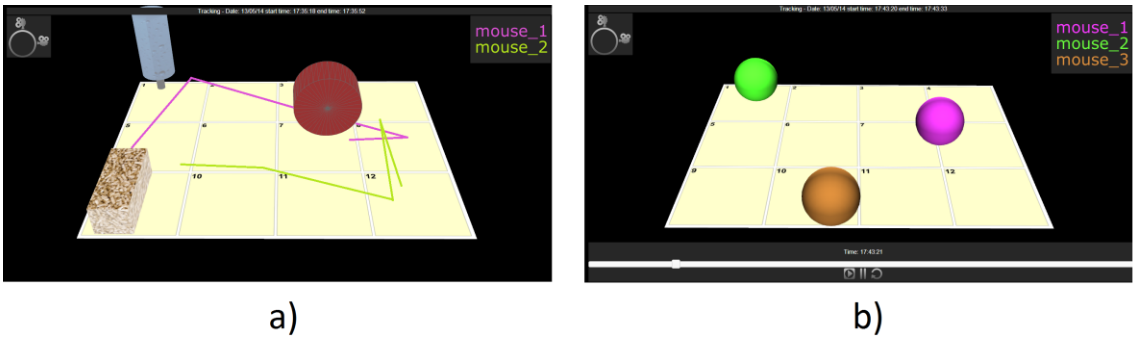


Fig. 6. A detail of the 3D tracking module.a) The 3D reconstruction of the mice movements in the cage; b) the Web interface showing the movie tracking.

order to ensure animal comfort and tolerance to mechanical stress. Furthermore, the implantation technique should guarantee a long-term readability of the implanted tags and should not affect their behavior.

The surgical tag implantation required multidisciplinary skills and this task was performed in cooperation with the Italian National Institute of Health, Rome. Mice were anesthetized with xylazine + ketamine (80+10 mg/kg, ip.). Ventral skin was shaved and washed with Betadine and a small (1 cm) horizontal inguinal incision was made. Then, a hemostat was inserted into the incision and, by opening and closing the jaws of the hemostat, a pocket for the RFID tag was created. The RFID tag was inserted into the pocket and the wound was closed with sutures. Sham-operated animals underwent the same procedures but they were not implanted with RFID tag. Mice were placed on a heated pad until they recovered from anesthesia. After the surgery, mice were placed in their home cage and observed, once a day, to verify the presence of pain or distress for at least 30 days. Pain and distress in laboratory animals can be detected by carefully observing thin changes in overall behavior. Every day, the activity level (inactivity or hyperactivity), attitude (arousal or awareness of surroundings), behavior (posture, vocalization, self-injury, hiding and aggression), food and water intake, fecal and urinary output, and wound closure were evaluated and recorded. Body weight was recorded four times per month. Tag functionality was verified, twice a month, by placing each mouse in proximity of a NF UHF RFID reader antenna, at different reader output power (from 20 dBm to 30 dBm).

## *B. Hardware and software environment*

The test environment used to validate the proposed tracking system is shown in Fig. 7. It consists of several components: a Speedway Revolution R420 RFID reader [26], an Impinj GPIO box, two Impinj Antenna Hubs [27] and an antenna platform based on a 3x4 matrix of prototypal reader antennas as described in [8] or commercial Impinj Mini-Guardrail antennas [28]. The 4-ports RFID reader is connected to the GPIO adapter via one HD15 cable. The GPIO adapter is able to connect up to four Antenna Hubs, each of which accepts up to eight reader antennas. The Antenna Hub operates as a multiplexer in order to extend the number of reader antennas that can be connected to the RFID reader. It is connected to the GPIO adapter via a straight Ethernet cable and to the RFID reader via a SMA-male to R-TNC-female coaxial cable, whereas each reader antenna is connected to its Antenna Hub using a SMA-male to SMA-male coaxial cable. The reader is connected to the computer via a cross Ethernet cable. The connected antennas are powered in time division through the four ports of the RFID reader. In detail, at a generic time, only a single antenna is powered, thus reducing energy wasting. The switching time between a reader antenna and the next connected to the same Antenna Hub is about 200 µs, whereas the switching time between two Antenna Hubs is about 25 ms. This high sampling rate ensures an accurate tracking, without loss of positional information. The acquisition module is installed on the PC connected to the RFID reader in order to store the raw tracking data coming from the hardware component. From the same PC, equipped with a Web browser, it is possible to access the Web application for the animals' behavioral analysis. In tests where a comparison between the proposed system and video analysis was provided, a video camera (Canon MVX 460, Canon Italia, Milano, Italy) was installed above the cage in order to record the animal behavior.

## *C. Test setting*

The tracking system was validated through three different validation tests. The first test was carried out in order to verify the effectiveness of the proposed system by using both prototypal reader antennas and a commercial solution. The second test, consisting in the so-called *sociability and social novelty preference test* [29], aimed to evaluate the capability of the proposed system to detect animals' behavior. In the last test, the system capability to provide summary information about the behavior of a mouse was verified through a

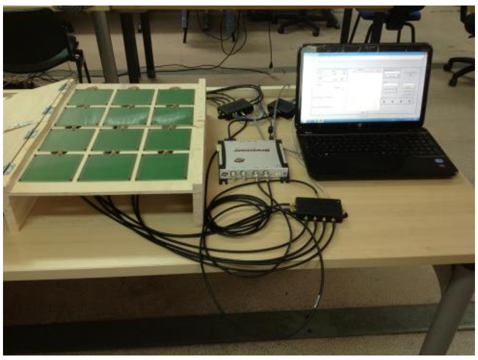

Fig. 7. Test environment with a particular focus on hardware architecture used for the system validation.

comparison with a visual system, commonly used in the research laboratories.

The first test, not involving animals, aimed to verify the correct detection of animals' movements in the cage, permanence times in each cell of the cage, and social phenomena (i.e., isolation and aggregation phenomena). This test was carried out using the proposed system with both prototypal and commercial reader antennas. RFID tags were applied to wood tabular supports and the power transmission of the RFID reader was set to 27 dBm. In order to verify the correct reconstruction of tags movements, nine random paths were fixed (Fig. 8, paths 1-9). Instead, to test the system capability to correctly detect the permanence time of one tag in different cells of the cage, two paths were fixed and the tag was stopped for 5 s in predetermined cells (Fig. 8, path 10-11). Finally, in order to test the system capability to detect particular behavioral phenomena, other three paths were defined involving up to three tags (Fig. 8, paths 12-14). In particular, the path 12was used to verify the system capability to detect the isolation phenomena whereas paths 13 and 14 were used to test the detection of the aggregation phenomena. Both Isolation and Aggregation Time parameters were set to a value of 5 s. Each test path was repeated 10 times for each system configuration (i.e., by using prototypal and commercial reader antennas).

The average error in the paths reconstruction (*err_path*) was calculated by using the following formula:

$$err_{path} = \frac{\sum_{path\,1}^{path\,9} \sum_{i=1}^{10} \frac{n^\circ \text{ incorrect cells in } i-\text{th replica}}{n^\circ \text{ involved cells } i-\text{th replica}}}{9} \quad (1)$$

The average error related to the permanence times (*err_p*) was calculatedas:

$$err_{p} = \frac{\sum_{path\,10}^{path\,11} \sum_{i=1}^{10} \frac{n^\circ \text{ incorrect perm. times in i - th replica}}{n^\circ \text{ occurred perm. events in i - th replica}}}{2} \quad (2)$$

Finally, the average error related to behavioral phenomena detection (*err_b*) was calculated as:

$$err_{b} = \frac{\sum_{path\,12}^{path\,14} \sum_{i=1}^{10} \frac{n^\circ \text{ detected social events in i - th replica}}{n^\circ \text{ simulated social events in i - th replica}}}{3} \quad (3)$$

The second test, involving animals, was performed by comparing the positional data acquired by the proposedRFID systemwith the data coming from video analysis performed by an operator inthe *sociability andsocial novelty preference test*. Usually, this test is usedto assess sociability and interest in social novelty in mice affected by disorders of the central nervous system (e.g., transgenic mice affected by autistic diseases). In particular, in the *sociability* session, the attitude of a mouse to interact with a fellow is verified. Instead, in the *social novelty* session, the attitude of a mouse to interact with an unfamiliar mouse rather than with a familiar mouse is verified. Based on these behavioral attitudes, this test allows

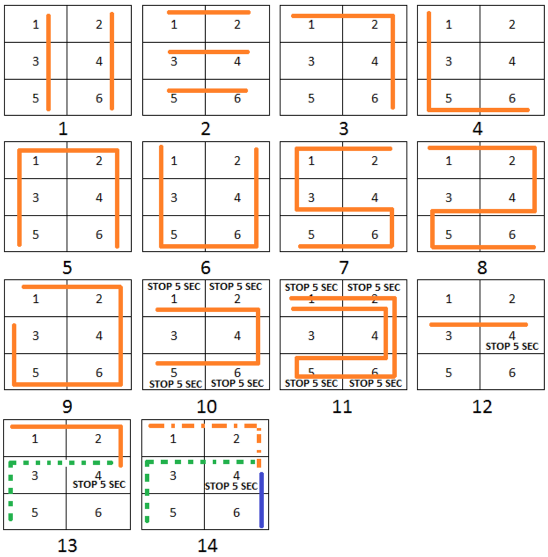


Fig. 8 Designed paths to test the correct detection of mice movements, permanence times in the cells cage and social phenomena.

the detection of mice with deficits in sociability and/or social novelty. Test was performed using a Plexiglas 40 cm x 35 cm x 33 cm three-chambered cage. Each chamber is 12 cm x 35 cm x 33 cm and is equipped with a removable door thatallows a mouse to access other chambers. In each replica, a mouse was initially placed into the empty middle chamber allowing the exploration of the environment for five minutes. In this phase, called habituation period, the other chambers were closed. After this period, an unfamiliar mouse was placed in one of the side chambers, in a small rounded box, and the separation door was removed for 10 minutes (Fig. 9.a), allowing the first mouse to approach the second one. Then, another unfamiliar mouse was placed in the last chamber in a small rounded box, the separation door was removed for other 10 minutes (Fig. 9.b), and the time spent by the first mouse in the exploration of the two mice was measured. The power transmission of the RFID reader was set to 30 dBm andthe cage was cleaned with alcohol after each replica.

In the last test, the positional data acquired by the RFID system were compared with the data obtained using a visual system in an open field test. The open field test is commonly used for evaluating animal behavior through the observation of the animal locomotion in a confined space. If a mouse is placed in a new empty environment without a shelter,

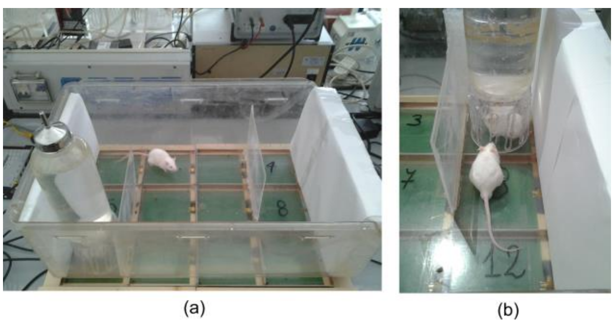


Fig. 9. Sociability and social novelty preference test: a) sociability session b) social novelty preference session with a particular of the interaction with an unfamiliar mouse.

itinitially moves along the arena perimeter, in an anxiety state, ignoring the central area of the arena. Only after the mouse is accustomed to the new environment, it moves in the center of the arena revealing attitude to the exploration. Furthermore, it is known that, in a new closed environment, a mouse explores the space moving near the walls, looking for tactile and visual landmarks [30]. In this last test, the results of the analysis carried out by the proposed system were compared with those provided by the EthoVision software [3]. A basic set-up of EthoVision consists of a camera connected to a PC equipped with EthoVision software. It analyzes each acquired frame in order to detect multiple animals, in both normal and infrared lighting conditions, and providing information about behavior, movements and activity of each observed animal. Moreover, EthoVision provides spatial measurements, such as distance and speed, and many parameters that are difficult to estimate through human observation. It works by virtually separating the space in which animal moves in different sections, each of which is called arena, in order to perform separate analysis for each arena. The test was carried out using a 40 cm x 35 cm x 33 cm animal cage with black walls. The video camera was connected to the same PC equipped with EthoVision. In particular, the EthoVision version used in this test (i.e., 3.0) is able to track only one animal in one arena. The RFID system was equipped with prototypal reader antennas and the power transmission of each antenna was set to 27 dBm. The animal cage was located above the prototypal reader antennas system. The arena was logically split into two areas: the perimeter area, called edge, and the central area (24 cm x 12 cm), called center. Four different mice, in four different time intervals, were placed in the cage and their behavior was monitored by using both systems(i.e., EthoVision and the RFID system) for 30 minutes, with ambient temperature very similar to the animal facility temperature (i.e., about 21° C) and during early afternoon. The arena was cleaned with water and soap after testing each mouse. In particular, the time spent by each mouse both in the edge and in the center of the arena was measured, as well as its locomotor activity. EthoVision was set in order to provide a report every three minutes for the analysis of the time spent in the different arenas and every five minutes for the analysis of mice locomotors activity. The same temporization was used for the data coming from the proposed system in order to obtain comparable data.

The correlation between the results obtained through the two systems was measured using the Pearson correlation coefficient (r). It measures the strength of a linear association between two variables, where the value r = 1 means a strong positive correlation and the value r = -1 means a strong negative correlation.

## IV. RESULTS

All tests reported in this paper were performed in order to ensure statistically relevant results, with a confidence interval of 95% and a maximum relative error of 5%.

The results related to the first validation test are reported in Table I. This table shows, for each system configuration, the average percentage error in path reconstruction (after RCP algorithm processing), the average percentage error in permanence time calculation, and the average percentage error in social phenomena detection. The comparison between commercial and prototypal reader antennas highlights the reliability of the adopted solution, able to reconstruct movements and to detect social phenomena. In particular, the tracking system is reliable and efficient using both commercial and prototypal reader antennas. The percentage error obtained using the commercial solution is less than the error obtained using prototypal solution but comparable. The reason is that the designed prototypal antenna has obvious limitations completely absent in the commercial solution, which adopts proper techniques (e.g., shielding techniques) in order to guarantee better performance. Obviously, the advantages of the prototypal solution reside in its low cost and in the possibility to customize the antenna shape.

Main results related to tests on*sociability and social novelty preference test*are reported in Table II. In particular, the values related to the time spent by each mouse in the second session of each replica (i.e., the social novelty session) are shown. The results obtained using the two methods are compatible and they demonstrate that, according to the literature, a mouseis more attracted by anunfamiliar fellow rather than a familiar one. Moreover, these values confirm that the RFID system is able to correctly detect the animal behavior.

In Table III, the results of the comparison between the proposed system and the EthoVision visual system in the open field test are reported. In details, the values represent, for each system, the percentage of permanence time in the arena center and in the edge and are very similar for the two systems. In fact, both systems detected a greater time spent by each mouse in the edge of the arena rather than in the center, according to the theory that a mouse, placed in a new environment, tends to explore the edge zones, in an anxiety state, ignoring the central area.

These considerations are also confirmed by the correlation graphs, reported in Fig. 10. In particular, Fig. 10.a shows, for each mouse,the correlation related to the permanence time in the arena center. In this case, the obtained Pearson correlation coefficients (r) for each mouse are: r = 0.94, r = 0.78, r = 0.90, and r = 0.96. These values indicate a strong positive correlation, which means that few time spent in the arena center detected by EthoVision system corresponds to few time spent in the same area detected by the proposed system. In particular, at each of these correlation values corresponds a p-value (i.e., an indicator of the correlation significance) inferior to 0.05, indicating a statistically significant correlation.

Vice versa, Fig. 10.b shows, for each mouse, the correlation related to the permanence time in the arena edge. In this case, the obtained Pearson correlation coefficients (r) are r = 0.76, r = 0.83, r = 0.84, and r = 0.75. These values indicate a strong

TABLE I
AVERAGE PERCENTAGE ERROR IN PATHS RECONSTRUCTION, PERMANENCE TIME CALCULATION AND SOCIAL PHENOMENA DETECTION USING BOTH PROTOTYPAL AND COMMERCIAL READER ANTENNAS

| | Prototypal solution | Commercial solution |
|---|---|---|
| Paths reconstruction | 8,6% | 0,0% |
| Permanence time | 9,0% | 3,0% |
| Social phenomena | 3,0% | 0,0% |

TABLE II
TIME SPENT BY A MOUSE INTERACTING WITH A FAMILIAR AND AN UNFAMILIAR MOUSE IN THE NOVELTY SOCIABILITY SESSION

| Mouse | RFID-based system | | Manual observer | |
|---|---|---|---|---|
| | Interaction with familiar mouse (sec) | Interaction with unfamiliar mouse (sec) | Interaction with familiar mouse (sec) | Interaction with unfamiliar mouse (sec) |
| 1 | 71 | 243 | 86 | 275 |
| 2 | 53 | 137 | 66 | 168 |
| 3 | 61 | 114 | 72 | 132 |
| 4 | 50 | 242 | 54 | 261 |
| 5 | 79 | 118 | 85 | 126 |
| 6 | 47 | 129 | 59 | 157 |
| 7 | 92 | 115 | 109 | 137 |
| 8 | 60 | 76 | 73 | 81 |
| 9 | 63 | 98 | 68 | 112 |
| 10 | 83 | 194 | 92 | 227 |

positive correlation, which means that a high time spent in the arena edge detected by EthoVisionvisual system corresponds to a high time spent in the same area detected by the proposed system. Moreover, also in this case, at each of these correlation values corresponds a p-value inferior to 0.05, indicating a statistically significant correlation.

Finally, Fig. 11 presents a comparison between the locomotor activity detected by both EthoVision visual system (continuous line) and the proposed system (dashed line), for each mouse. These results confirm the reliability of the proposed system to detect mice locomotors activity. Moreover, these graphs confirm an expected mouse behavior, i.e., a mouse placed in a new environment initially presents a very intense locomotors activity, performing many movements in the arena.Over the time, the mouse is accustomed to the new environment and then the movements decrease as well as the total covered distance in the cage.

## V. CONCLUSION

The proposed software system aims to provide an innovative solution able to support the tracking and the behavior analysis of small laboratory animals. The designed and developed solution preserves all the benefits derived from the systems commonly used in the research laboratories and overcomes all their limitations, offering a low-cost and user-friendly solution. The main challenge was to propose a complete and effective automatic system able to provide the end-user with a complete set of information. For this purpose, appropriatetechnological choices were made. Unlike some

TABLE III
PERCENTAGE PERMANENCE TIME IN THE CENTER AND IN THE EDGE OF THE ARENA USING BOTH THE RFID SYSTEM AND ETHOVISION SYSTEM

| Arena center | | Arena edge | |
|---|---|---|---|
| EthoVision system | RFID-based system | EthoVision system | RFID-based system |
| 7,34% | 9,77% | 92,66% | 90,23% |
| 9,54% | 8,29% | 90,46% | 91,71% |
| 5,40% | 4,11% | 94,60% | 95,89% |
| 5,49% | 4,16% | 94,51% | 95,84% |

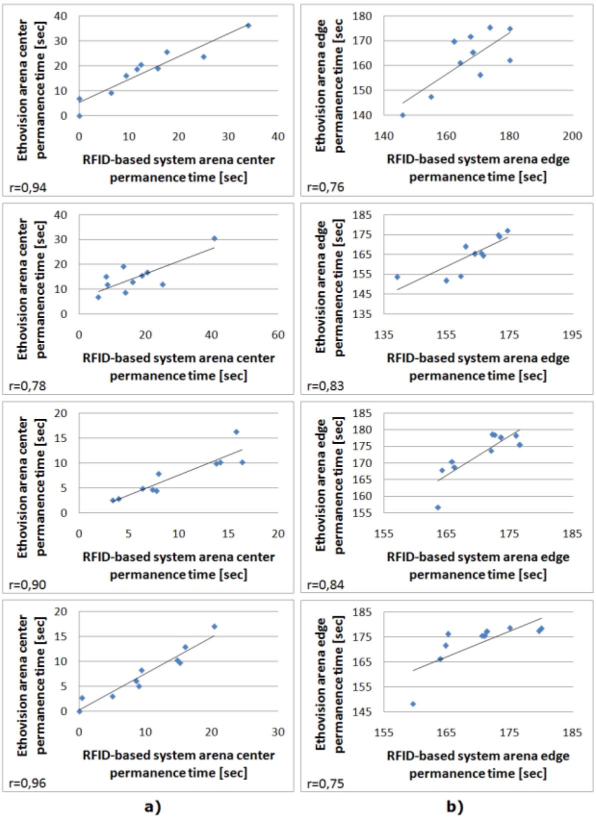


Fig. 10. Correlation graphs in the comparison between the RFID tracking system and the EthoVision system related to four mice: a) correlation related to the arena center b) correlation related to the arena edge.

solutions adopted in the literature, which use the RFID technology in LF and HF bands, the UHF technology allows multiple and simultaneous tags readings, offering a support for the monitoring of a colonyof animals in a small indoor environment. Furthermore, this technology is able to guarantee an accurate tracking in a natural environment, without forcing the animals to prefixed paths and without affecting their natural behavior. In addition, the characteristics of the passive NF tags and their dimensions favor its implantation in small laboratory animals. The proposed system provides the end-user with different types of information, including graphs and statistics on animals' behavior and information about specific social events, such as isolation and aggregation phenomena.

An innovative graphics solution, which is lacking in many other systems, offers interactive three-dimensional scenes, which allow a quick and easy identification of both social events and behavior analysis.

The validation tests, including the comparison with EthoVision visual system, demonstratethe effectiveness of the RFID system and its capability to provide accurate behavioral information.

As ongoing works, two features are currently under development. The first feature expects to apply a passive RFID tag on each object placed in the cage. In this way, as each object is uniquely identified, the system canautomatically reconstruct the virtual arena as exact copy of the real one. Then, the user will not need to interact with the Web application in order to manually configure the arena

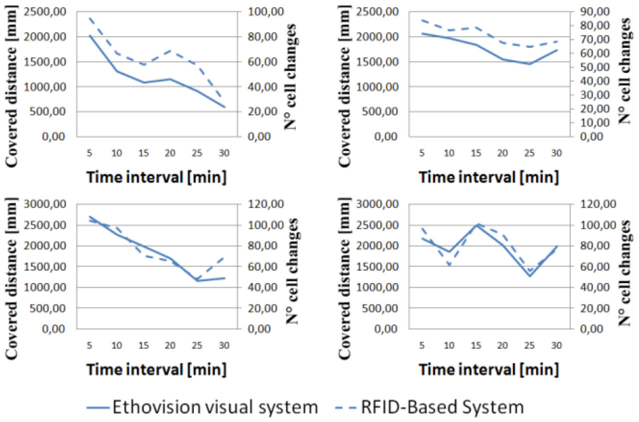


Fig. 11. Comparison between locomotors activity detected by EthoVision system and the proposed system for each observed mouse.

arrangement. The second feature is intended to detect the animal rearing by exploiting the RSSI value. Based on the electromagnetic characteristics of reader antennas, the RSSI value is high when the tag is close to the antenna surface, and decreases when the tag is distant from it. As during the animal rearing the area in which the tag is implanted moves away from the antenna, this behavior could be associated to a reduction of the RSSI value. A possible alternative to this solution consists of adding as a complement to the proposed system low cost devices (e.g., IR sensors) able to capture the animal rearing.

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