An RFID-based Smart Cage for Animal Behavior Analysis

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***Abstract*—An innovative tracking system based on passive Radio Frequency Identification technology in Ultra High Frequency band, able to perform behavior analysis of small laboratory animals, is presented in this work. The proposed smart system consists of both hardware and software components and it is able to extract main behavioral parameters exploiting raw animals tracking data captured by a Radio Frequency Identification reader system. The proposed solution allows overcoming some limits of typical analysis methods commonly used in research laboratories for the same purposes, such as systems based on video technology and human observations, while providing the same information content. It is cheaper and guarantees better performance even in case of strong similarity among animals and in poor lighting conditions. Different tests were carried out in order to demonstrate the feasibility and effectiveness of the proposed system using laboratory mice. The software component is able to provide, via Web, a user-friendly tool containing main animal behavioral information such as statistical analysis and graphs regarding animal displacements, indication about the locomotor activity and detection of specific conditions including isolation and aggregation phenomena.**

Keywords-animals behavior; animal tracking system; Radio Frequency Identification; UHF; Web application.

I. INTRODUCTION

The animal behavior analysis is a very important research area, which involves several scientific disciplines, such as biology, physiology, pharmacology, toxicology, and so on. It is used to analyze neuropsychiatric diseases or to test the effects of new drugs and vaccines before being placed on the market. The traditional method to collect this information exploits video recordings that can easily gather information about many aspects of the situation in which animals interact with each other or with the environment. In addition, the video recording makes offline research possible by a skilled operator that observes the video and annotates the animal behavior manually. This is a time and labor consuming process and the analysis results may vary among different observers that can introduce subjective interpretations.

For this reason, the study of animal behavior supported by automatic system based on video technology has become increasingly popular. The use of visual systems, equipped with proprietary software tools, enables automatic analysis of information acquired by video in order to obtain statistical data and behavioral information. However, these software solutions are often very expensive and the usability is generally restricted to basic functions.

In the literature, several works propose solutions able to automatically collect behavioral information using the Radio Frequency Identification (RFID) technology, which allows the identification of a tag when it is in the region covered by an electromagnetic field generated by an RFID reader antenna system. A significant example is presented by Kritzler et al. [1], in which Low Frequency (LF) RFID systems are used and a semi-natural environment is reproduced. Nevertheless, mice are forced to follow obligatory paths in order to detect their movements. In addition, LF band does not allow simultaneous reading of a lot of tags because it is not compatible with the EPC Class1 Gen2 standard [2] and it is only able to guarantee a very limited reading range, i.e., less than 1 cm distance between tag and reader antenna.

The RFID technology in High Frequency (HF) band is used by Aguzzi et al. [3] to capture the behavior of marine animals. Also in this context, HF band does not allow simultaneous reading of a lot of tags and, consequently, it cannot be used to track simultaneously a lot of animals in order to identify social behaviors.

In this paper, an innovative RFID-based tracking system able to overcome some limits previously described is presented. This smart system uses the passive Ultra High Frequency (UHF) RFID technology and it is able to track an entire animal colony. In fact, unlike the LF or HF bands, the RFID technology in the UHF band is compliant with the EPC Class1 Gen2 standard, allowing a reading of about 100-1500 tags/s. Furthermore, the proposed system is able to summarize, in terms of graphs and statistical dashboards, the behavior analysis of each laboratory animal. To make this possible, a passive Near Field (NF) UHF RFID tag must be implanted in every animal (e.g., mouse, rat, etc.). For such a reason, a strong effort has been dedicated to the implant technique in order to allow the RFID tags to keep on working in the time and not to procure distress to the animal. Some results of these experimental tests on laboratory mice are presented by Catarinucci et al. [4] and confirmed in this paper through an analysis of the implanted mice performed long time after the date of the tag implantation. Instead, the main results obtained in the tests performed on the hardware component (i.e., on the prototypal reader antenna system) and reported by Catarinucci et al. [5] allowed to establish the

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proper distance between two adjacent antennas and between the RFID tag and the reader antenna plane in order to guarantee a correct tracking of the animals in the cage.

In addition to long-term analysis on the implanted tags, the tests presented in this work had two major goals. The first goal was to demonstrate the scalability of the proposed system. To achieve this goal, the system should be able to effectively work regardless of the hardware configuration (i.e., the number of reader antennas) and of the number of RFID tags involved in tests (i.e., the number of mice in the smart cage). The second goal was to prove that the RFID system is able to extract correct behavioral information useful to support the operator work. In order to prove this capability, the results obtained by the proposed system were compared with a video analysis performed by a skilled operator.

The paper is structured as follows. In Section II, the architecture of the proposed smart system is presented whereas details regarding the test procedures are reported in Section III. The results of the performed tests are discussed in Section IV. Finally, the conclusions are drawn in Section V.

II. ANIMAL TRACKING SYSTEM ARCHITECTURE

The architecture of the proposed smart cage, shown in Figure 1, is a hybrid (i.e., hardware and software) system composed of two main components.

The hardware component is mainly based on a NF antenna system working in the UHF bandwidth (i.e., 860-960 MHz). Such a system is composed of a matrix of antennas each one able to univocally localize the animal in an elementary cell as large as 12 cm x 12 cm. In order to guarantee an accurate identification and localization of the animals moving within the cage, each single antenna satisfies specific requirements fulfilled through the design of optimized segmented loops: first, it irradiates a magnetic field as confined as possible in the related cell. Moreover, it guarantees a uniform magnetic field within the cell in order to minimize the localization uncertainty. Finally, it minimizes the far field radiation in order to avoid potential spurious readings of tags located in different cells. Such a system is thought to be positioned right below the cage in correspondence of each elementary cell and is connected to the RFID reader through a multiplexer allowing the management of up to 32 reader antennas. In this way, even large environments can be monitored and the behavior study of many animals at the same time can be performed. Let us observe that there are no obligatory paths for animal movements. The reader antenna system was widely tested and validated by the same authors [5].

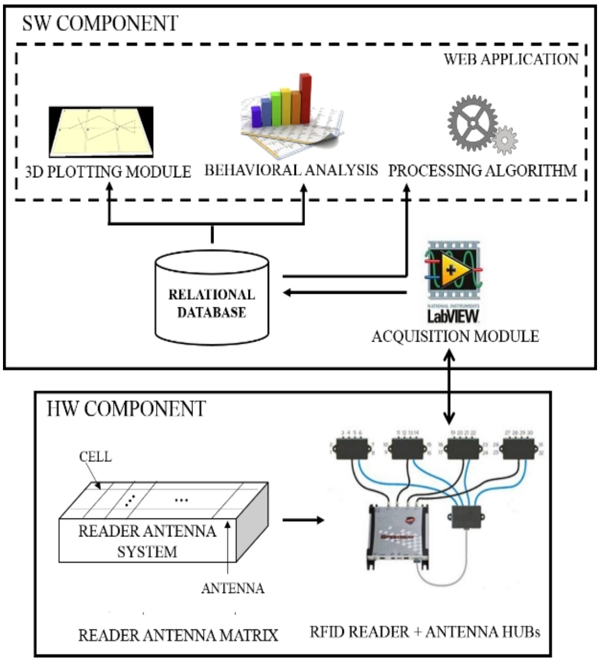


Figure 1. System architecture.

Instead, the software component consists mainly of two modules: (i) the acquisition module, described and validated by Catarinucci et al. [6], developed by using the graphical programming environment LabVIEW [7] and responsible for managing the hardware component and collecting the raw readings coming from the reader antenna system, and (ii) the Web application able to support the researchers in animal behavior analysis.

The Web application is able to extract raw data, stored in a MySQL [8] relational database, in order to provide the end-users with an effective analysis that summarizes main behavioral parameters. The choice to adopt a Web solution is motivated by the need to guarantee a behavioral data access from anywhere by using a simple Internet access. In addition, different operators can access the same data from different locations. In particular, the Web application firstly processes the raw data by using the three phases of the RCP (RSSI Chebyshev Ping-pong) algorithm able to discriminate the correct mouse position. In fact, when only one of the reader antennas reads an RFID tag, the animal position is uniquely individuated. Nevertheless, if several reader antennas read the same RFID tag, a position ambiguity occurs and the RCP algorithm works to solve it by using the following three processing phases:

1. Identification of simultaneous readings and data discrimination based on the Received Signal Strength Indication (RSSI) value.
2. Check of adjacent cells exploiting the Chebyshev distance [9] method.
3. Removal of the "ping-pong" effect, which occurs when a tag is positioned between two cells. This generates a continuous alternation of the position between the two involved cells.

The pseudo-code of the proposed algorithm is shown in Figure 2.

The Web application provides the end-user with several information about the behavior of each mouse in the smart cage in the form of tables or graphs. This feature was obtained using the JFreeChart [10] Java library. This open source library can dynamically create graphs starting from

```
for each tag ID{
    for each sampling interval{
        PHASE 1:
        store the sample with max RSSI value
      and all samples with RSSI value in the
      interval(RSSImax – RSSI) in List1.
    }
    PHASE2:
        for each sample in List1{
            if distance [i , i+1] ≤ 2
                add i+1 sample in List2;
            else if distance [i + 1, i + 2]>2
                discarded i+1 sample;
                    else add i+1 sample in
List2;
        }
        PHASE3:
        for each sample in List2{
            if i ≠ i+1{
                if i-1=1+1
                    discarded i sample: ping-
pong effect!;
                else add i sample in List3;
            else add i sample in List3;
            }
        store all sample in List3 in the
database
        }
```

Figure 2. The pseudo-code of the proposed RCP algorithm.

the source data stored in the database. The Web application adopts a client-server architecture and is developed by using Struts2 [11]. This framework, distributed by Apache, is completely based on Java. In order to manage the Web pages, SiteMesh framework [12] was used. It offers an important support for the Web pages decoration and layout, by effectively managing the navigation and allowing the generation of composed pages. Finally, a tracking module allows the user to show a screen in which the smart cage surface is replicated in order to provide information about the displacement of each mouse in the cage (Figure 3.a). In this way, it is possible to track and evaluate animal behavior and reactions to particular solicitations (e.g., drugs or vaccines administration). The Web application also provides a 3D video that reproduces the mice movements in the cage. The user can manage the video reproduction by using the Play, Stop and Restart buttons. Furthermore, the user can insert several objects in the experimental scene (i.e., cage) reproducing accurately the real scene. In this scene, a sphere of a different color that moves in the cage represents each mouse (Figure 3.b). In order to develop the 3D component, the WebGL [13] technology was chosen because it is able to generate and manage three-dimensional graphics directly on Web pages, allowing the interaction with the 3D environment.

From the computational point of view, it is worth highlighting that the acquisition module was designed and implemented separately from the others components in order to guarantee effectiveness and scalability. In fact, in this way, the acquisition module and the processing module may evolve independently allowing, for instance, different processing systems to be associated with the same acquisition system. Furthermore, this choice relieves the computational load of the acquisition module.

It is worth noting that the implantation of an RFID tag in each mouse represents a decisive procedure. For this reason, much time was spent in this direction. First, a careful technological scouting was performed in order to select, among all the commercial tags, those most suitable to be implanted in laboratory mice, characterized by small size and high performance. Then, the best tag implantation technique in laboratory animals was evaluated through some tests carried out by using mice.

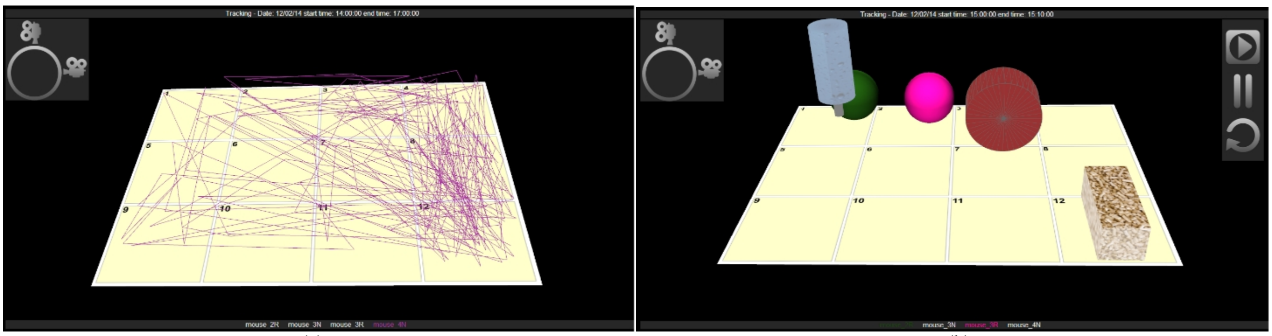
In particular, the choice of the better tag to implant in the mouse took into account the main requirements reported below: (i) to guarantee a long-term readability of implanted tags, and (ii) not cause discomfort to the animals or changes in their behavior.

III. MATERIALS AND METHODS

In this section, details regarding the test procedures and the proposed goals for each test are reported.

A. *Tags implantation procedure*

The first phase of this work focused on the choice of the best passive UHF RFID tag able to satisfy the stringent requirements such as high tolerance to mechanical stress, high reading performance in presence of liquids, and low cost. Preliminary tests, presented by Catarinucci et al. [4], were carried out in order to select the best candidate tag able to ensure high performance for a long time after its implantation in laboratory mice. Furthermore, a feasibility



(a) (b)

Figure 3. Screenshots of the tracking module: (a) displacement of a mouse in the smart cage; (b) 3D reconstruction of the smart cage.

study on the tag implantation methodology in the animal was performed. As result of these preliminary tests, the tag with higher performance has a layout characterized by an antenna size of 15 mm x10 mm, die-cut size of 19 mm x14 mm, an Impinj Monza4D chip, and a PVC plasticization with thickness of 80 μm per side. The plasticization preserves the tag conservation, protects it from corrosion due to animal tissue composition, and ensures the reliability of the implanted tags in the time. The first tests, performed to select the best tag, lasted for about a month. Instead, the tests presented in this study are long-term analysis and covered a period of about one year. In this regard, the long-term analysis concerned several mice in each of which the selected tag was implanted. In order to evaluate the readability of all implanted tags, each implanted mouse was placed in proximity of the reader antenna for 30 seconds. Then, the ratio between the number of readings performed by the system and the total number of expected readings was calculated in different time instants starting from the implantation date (i.e., 1, 3, 6, and 12 months after the implantation date). Because the system, in a configuration with a single reader antenna, is able to intercept about twelve readings per second, and the considered time interval was 30 seconds long, the total number of expected readings is 360. Finally, for each time instant, the performance of the tags was evaluated as the percentage of the average readings.

The surgical tag implantation required multidisciplinary skills and this task was executed in cooperation with the Istituto Superiore di Sanità (Italian National Institute of Health) in Rome, which made possible the testing of passive UHF RFID solutions in laboratory mice. The surgical implantation details are reported by Catarinucci et al. [4]. All procedures involving animals were 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, which implemented the European Directive 86/609/EEC on laboratory animal protection in Italy.

B. RFID devices

The proposed tracking system consists of a 4-port Impinj Speedway Revolution R420 reader connected to an Impinj GPIO adapter via one HD15 cable. The GPIO adapter allows for the connection up to four Impinj Antenna Hub, acting as multiplexers, each of which accepts up to eight reader antennas. Each Antenna Hub is connected to the GPIO adapter via a straight Ethernet cable and to the reader with a SMA-male to R-TNC-female coaxial cable. Each reader antenna is connected to its Antenna Hub via a SMA-male to SMA-male coaxial cable. The reader antennas are powered in time division through the four ports of the RFID reader. More specifically, at a generic time, only a single antenna is powered, thus reducing potential array effects and energy wasting. In such a context, the multiplexing system allows a switching time between reader antennas inferior to 200 μs and a switching time between two Antenna Hubs amounted to 25 ms. This means that, even in presence of the worst computational case (i.e., with 32 reader antennas), a time distance between two consecutive interrogation of the same antenna is inferior to 6.4 ms, which is about eight times less of the minimum time required for a mouse to cross a cell. This ensures an accurate tracking, without loss of positional information. If all 32 reader antennas are connected based on a 4x8 matrix, the size of the arena can reach about 48 cm x 96 cm, enabling the testing of a high number of animals.

C. Tests setting for system scalability and animal behavior

In the test aimed at evaluating the scalability of the proposed system, different system configurations were used, as reported in Table I. In each cell, in correspondence of each reader antenna, two RFID tags were placed: one was placed in the center of the cell and the other tag was placed randomly on the cell. The power transmission of each reader antenna was set to 27 dBm and, for each system configuration, the test was 30 minutes long and was repeated five times. Then, the percentage of incorrect tag localizations was calculated before and after the data processing carried out by the RCP algorithm. The percentage of incorrect localizations was calculated as the ratio between the number of localization errors and the total number of readings detected by the RFID system.

A final test was performed in order to evaluate the effectiveness of the proposed system to correctly detect animal behavior. In this three hours long test, a system configuration, consisting of twelve reader antennas according to a 4x3 matrix and four implanted mice, was used. The test environment is shown in Figure 4. A cage of dimension 36 cm x 48 cm was used and on the bottom of the test cage, the sawdust was placed in order to absorb mice feces and urine. The transmission power of the reader

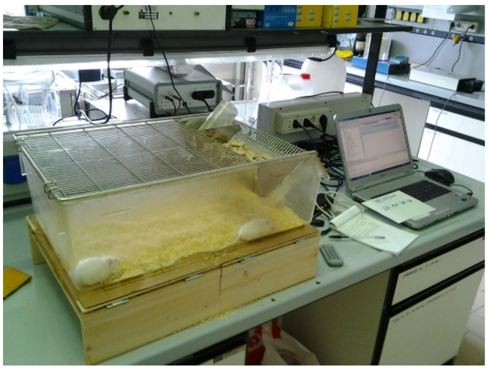


Figure 4. The proposed system during a test performed by using laboratory mice.

TABLE I. SYSTEM CONFIGURATIONS USED IN THE SCALABILITY TEST

Number of cells	*Number of tags*
3	6
6	12
12	24
24	48
32	64

antennas was set to 30 dBm. The behavior of the mice in the cage was monitored by using both the proposed RFID system and a video camera (i.e., a Canon MVK 460). In this way, it was possible to compare the results obtained through the proposed system with those obtained by a video analysis performed by a skilled operator. The operator, by using a stopwatch, measured and recorded how many seconds the mice spent alone, manifesting an isolation attitude, or in group of at least two mice, manifesting an aggregation attitude. In particular, an event was considered an isolation phenomena if a mouse remained alone in a cell cage for at least 100 seconds. Vice versa, an event was considered an aggregation phenomena if at least two mice remained in the same cell for at least 25 seconds. The correlation between the results obtained through the two methods was measured by using the Pearson correlation coefficient (r). It measures the strength of a linear association between two variables, where the value r = 1 means a perfect positive correlation and the value r = -1 means a perfect 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%.

Before analyzing the results obtained in the tests aimed to evaluate the scalability of the proposed system and its capability to detect behavioral information, it is worth highlighting the high performance of the selected RFID tag. In fact, one year after the surgical implantation, all implanted tags were still readable. Table II shows that the average percentage of readings is 100% one month after the implantation date (i.e., all tags are still readable) and it is not below 98% even one year after the implantation date. This is a very interesting result that demonstrates not only the robustness of the RFID technology in the UHF band for animal tracking purposes, but also that the tag implantation does not cause pain or discomfort to the mice. In fact, after one year, the mice had not removed the tag and did not suffer from health effects. Regular checks on the implanted animals carried out by the researchers of the Italian National Institute of Health in Rome, Italy, demonstrated that this surgical practice does not affect the animals' behavior. In fact, implanted mice behavior is not different from that of sham operated mice as well as their health conditions.

The results regarding the system scalability are reported in Figure 5 where, for each system configuration, the average percentage of incorrect localization, before and after data processing performed by the RCP algorithm, are reported. It is possible to notice that, before the data processing, the percentage of incorrect locations varies between 33.74% (in the system configuration with three cells) to 58.73% (in the system configuration with six cells). These localization errors were caused by tags positioned between two cells, which were read simultaneously from more than one antenna, leading to an ambiguity of their position. After the data processing, the percentage of incorrect localization was less than 0.4% in all system configurations. These results prove the effectiveness of the RCP algorithm and that the system is scalable because it works well regardless the number of considered cells and involved tags.

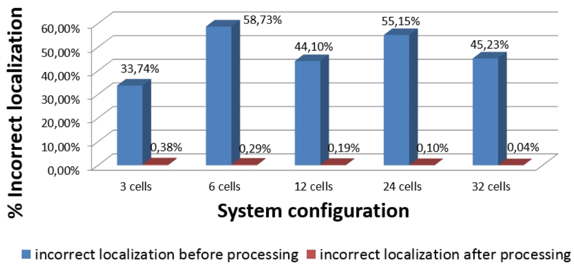


Figure 5. Percentage of incorrect localizations before and after the RCP algorithm processing for different RFID system configurations.

In Table III, the results of the comparison between the proposed system and the operator analysis in the test aimed to evaluate the ability of the RFID system to detect animal behavior are reported. In particular, the values represent the average isolation time and the average aggregation time detected during the test by both the RFID system and the operator analysis. The Table III shows as the results obtained using the two methods are very similar to each other and prove that the proposed system is able to correctly detect the animal behavior. These considerations are also proved by the correlation graphs, reported in Figure 6. In fact, the correlation values are 0.97 and 0.92 in the detecting of isolation phenomena (Figure 6.a) and aggregation phenomena (Figure 6.b), respectively. This reveals a strong positive correlation between the two compared methods and indicates that high values of the isolation (aggregation) time detected by the RFID-based system corresponds to high values of the isolation (aggregation) time detected by the operator video analysis (and vice versa). The RFID system extracts higher values compared those recorded by the operator because the proposed system is able to provide measurements that are more accurate.

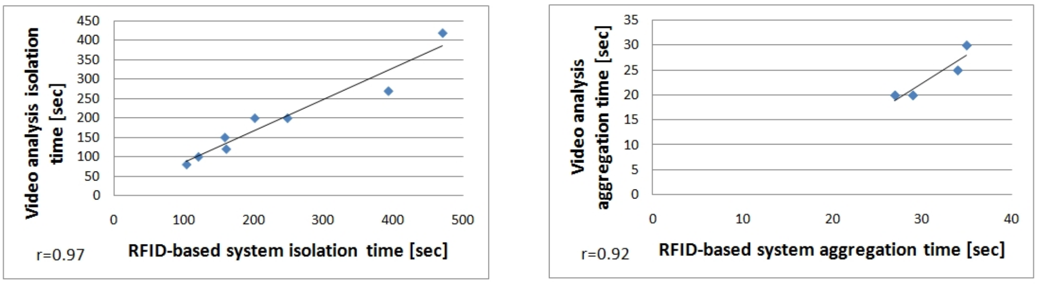
TABLE II. AVERAGE PERCENTAGE OF READINGS FOR ALL IMPLANTED TAGS 1, 3, 6, AND 12 MONTHS AFTER THE IMPLANTATION DATE

Observation time	% reading
1 month	100%
3 months	99%
6 months	98%
12 months	98%

TABLE III. SOCIAL INTERACTIONS TESTS, DETECTION OF THE ISOLATION AND AGGREGATION EVENTS

	RFID-based system	Skilled operator
Isolation time [s]	231.80[a]	192.5[a]
Aggregation time [s]	31.3[a]	28.75[a]

a. Data are reported as mean of the values obtained using four mice in 3 hours test.



(a) (b)

Figure 6. Graphs of the correlation between the proposed RFID system and the operator video analysis: (a) detecting of the isolation time; (b) detecting of the aggregation time.

V. Conclusion and Future Work

In this work, an innovative and complete UHF RFID-based system able to extract behavioral analysis of small laboratory mice is presented.

Although RFID technology in LF or HF (Near Field Communication – NFC) bands allows the simultaneous reading of tags, the number of tags that can be read at the same time is limited. RFID tags in LF or HF band are generally used for proximity applications or when the distance and the population of the tags are not excessive. Instead, the UHF RFID technology allows reading many tags simultaneously, making it the ideal candidate for the study of colonial animals' behavior. Although some materials (e.g, liquids or metals) may absorb or reflect the waves, altering the conditions of writing and reading of UHF tags, the results obtained in tests on the proposed system shown that the use of this technology is effective for the declared purposes.

The proposed smart cage allows overcoming some of the inherent limitations of methods commonly used in research laboratories for the same purposes, such as systems based on video technology and human observations by using a hybrid system consisting of hardware and software components.

Different tests were carried out in order to demonstrate the feasibility of the proposed system, its scalability and its efficiency in terms of detection of social events involving the observed animals. The performed tests demonstrated the feasibility of implantation of a small RFID tag in a mouse and the effectiveness of the RFID technology for the behavior analysis of laboratory mice. Moreover, the proposed system offers a low-cost, user-friendly and time-saving solution to support the researches in the animal behavior analysis.

Currently, the RFID system is under evaluation at the Italian National Institute of Health, Rome, Italy, where skilled operators are testing it in real-world scenarios.

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