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VETBIONET

Veterinary Biocontained facility Network for excellence in animal infectiology research and experimentation

Deliverable D9.1

Prototype telemetry sensors and behavioral analysis software

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Organization name of lead contractor: Noldus Information Technology

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This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement N°731014

1. Summary

This report is a deliverable of Work Package 9 (Development and validation of advanced approaches to the monitoring and evaluation of clinical and pathological outcomes of experimental infections), in particular Task 9.2, which focuses on automated behavioral and physiological monitoring of animals.

As a first step, we performed a survey among VetBioNet partners to create an inventory of use cases and requirements from BSL3 facilities. Data were obtained from 13 BSL3 labs. The animal species used by most partners is cattle, followed by pigs, sheep and chickens. The most important behavioral and physiological output parameters to be measured are body temperature, general activity, eating behavior, drinking behavior and respiration.

In this report, we present the solutions for telemetric monitoring of animal behavior, both in terms of data acquisition and data analysis, which we have completed so far and that are ready for testing in partner facilities. Solutions for monitoring body temperature and respiration are still under investigation and development.

Deliverable 9.1 is the result of the effort of the following VetBioNet partners: Noldus Information Technology BV, Erasmus Medical Center Rotterdam and Wageningen BioVeterinary Research.

2. Introduction

Veterinary biocontainment laboratories accommodate research on contagious diseases of animals, some of which may also affect humans (zoonoses). Animals on study in Biosafety Level 3 (BSL3) biocontainment facilities should be closely observed and monitored for any clinical signs of disease, both as a scientific outcome and in order to manage animal welfare. Conventional practices involve laborious manual data collection by animal caretakers, with frequent transitions in and out the biocontainment facilities, which are time-consuming and occasionally risky procedures. By performing behavioral observations and physiological measurements remotely through sensors that refine and (partially) replace the direct observation by humans, time can be saved, risks can be reduced and data quality and quantity can be increased. Such innovations will help us to achieve a more objective definition and scoring of Humane Endpoint (HEP) criteria, and to reducing transitions of humans in and out of BSL3 facilities.

One of the aims of VetBioNet WP9, entitled “Development and validation of advanced approaches to the monitoring and evaluation of clinical and pathological outcomes of experimental infections” is to develop and validate tools for automated behavioral and physiological monitoring of animals (Task 9.2). The clinical evaluation of the animals is key to the scientific outcomes of studies and also to the management of animal welfare. Specific attention is paid to the monitoring of socially housed experimental groups with cameras and other telemetric sensors transmitting images, location data and physiological parameters. Here we report on the results achieved in the first 24 months of the project and tools developed so far.

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In 2018, a survey was conducted among VetBioNet partners, in order to create an inventory of the use cases and requirements from BSL3 facilities and their users and to make an inventory of gaps in our knowledge of methods used by BLS3 facilities. The questionnaire was designed by WP9 partners Norbert Stockhofe (WBVR), Martje Fentener van Vlissingen (EMC), Sandra Thoomes-Wildschut and Lucas Noldus (NOLDUS). Preliminary results of the survey were presented at the VetBioNet annual meeting in Weybridge, May 2018. Additional questionnaires were collected after the meeting, after which the report was completed and distributed to the consortium partners in September 2018.

The partner survey forms an important and valuable source of information and the findings are used to prioritize the use cases. Subsequently, in order to obtain a deeper understanding of the requirements of each partner with respect to each use case, we started (in Q4 2018) to conduct in-depth interviews with the individual partners, either on-site or remotely. The process of gathering requirements from the partners is in progress and will be finished in Q2 2019.

Noldus Information Technology has developed a range of software tools and integrated systems that are suitable for behavioral and physiological monitoring of animals in BSL3 facilities. Some of these tools have been used and under further development for many years and have been upgraded since the start of the VetBioNet project, based on partner requirements, others are novel developments. The selected systems are: Viso, The Observer XT, EthoVision XT and TrackLab. These systems are described in more detail below. Not all software screenshots originate from veterinary research applications and details may be hard to read; they are meant for illustrative purposes only.

3. Results

3.1 Results of the partner survey

From the VetBioNet consortium, 13 partners participated in the survey and submitted 26 completed questionnaires. A large variety of animal species is investigated in the BSL3 facilities of the VetBioNet consortium, including large mammals, small mammals, birds and fish. The results of the partner survey are summarized as follows:

- The animal species used by most partners is cattle, followed by pig, sheep and chicken (see table below).
- The most important behavioral and physiological output parameters to be measured are body temperature, general activity, eating behavior, drinking behavior and respiration.
- Current data collection methods used by the respondents have multiple drawbacks:
    - Rectal temperature measurement can be stressful for the animals since it requires restraining/handling of the animal. Therefore, body temperature measurement is usually carried out only once a day or even less frequently.
    - Most temperature implants presently used are loggers, which do not offer real-time temperature information. As a result, data is only available after the experiment. Real-time temperature monitoring is important for indication of the animal's health status and objective application of predefined Humane End Point criteria.

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- Manual scoring of activity is subjective, suffering from inter-observer variability and other sources of non-experimental variables.
    - Eating and drinking behavior cannot be measured individually in group-housed animals, so only qualitative individual data is available.
    - There is no tool for minimally invasive automated measurement of respiratory rate in freely moving animals.
- The requirements for better tools derived from the survey are the following:
    - Generic design, suitable for multiple species
    - Continuous measurement
    - Real-time information & feedback
    - Scalable solution (from one to many animals, from one to many rooms)

	Number of facilities			Number of facilities
Large mammals			**Birds**	
Cow	8		Chicken	6
Pig	7		Turkey	2
Sheep	6		Duck	2
Goat	5		Partridge	1
Horse	2		Magpie	1
Alpaca	1			
Badger	1		**Fish**	
			Trout	3
Small mammals			Sea bass	1
Mouse	5		Sea bream	1
Rabbit	2		Salmon	1
Ferret	2		Carp	1
Guinea pig	1		Zebrafish	1
Rat	1			

3.2 Telemetry sensors and behavioral analysis software

In this deliverable, we focus on two sensor technologies for the monitoring, automated measurement and quantitative analysis of animal behavior: video imaging and ultra wideband positioning. These have reached the stage of practical use in BSL3 facilities, meeting the requirements listed above.

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- Video imaging can be used for several purposes:
    - Remote monitoring of the animals from outside the BSL3 facility. This is provided by the Viso system (section 3.3).
    - Recording of animal behavior for post-hoc playback, inspection, and – if desired – manual annotation and detailed analysis by a human expert. This is provided by the combination of Viso and The Observer XT (section 3.3).
    - Recording of animal behavior for post-processing with an automated video tracking system, such as EthoVision XT (section 3.4).
    - Real-time video capture and automated processing with an automated video tracking system, such as EthoVision XT (section 3.4).
- Ultra wideband positioning. This radiographic real-time location technique is ideally suited for tracking the movement and behavior of multiple animals in large enclosures. The tool developed for this purpose is TrackLab (section 3.5).

3.3 Viso + The Observer XT

Brief description of the system

Viso® is a versatile software tool designed for recording video and audio streams in multiple independent locations. Video/audio recording can be controlled for any computer in the Viso network, allowing intrusion-free observations. Video can be reviewed and annotated for further analysis. Viso is a network-based system (see Figure 1), which makes data collection, transfer and analysis extremely fast and flexible.

The Observer® XT is a software package for coding, storing and analyzing observational data. Video material can be imported directly from Viso. In a typical study carried out with The Observer XT, the researcher defines a *coding scheme* that describes the relevant ethogram, that is, the various behavioral events and states that are important in the study. Compared with Viso, The Observer XT can support larger and more complex coding schemes. When watching video, the researcher manually codes the events and behaviors in an event log. Physiological signals (e.g. body temperature, respiration rate) can also be imported for an integrated analysis. The Observer XT can also be used as an analysis tool of event data scored in Viso. It provides descriptive statistics such as average rates and duration of events, and offers additional functions like reliability analysis and lag-sequential analysis of behavioral data.

Special features of Viso for veterinary research in high-containment facilities

- The system works with video cameras in robust housing (dome cameras) that can be decontaminated.
- Scalable, networked architecture, connecting multiple enclosures, recording rooms and users.
- Easy control of camera orientation (pan, tilt, zoom) from within the software.
- Remote viewing on Windows PC or web browser on PC, notebook, tablet or smartphone.

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Diagram of the system

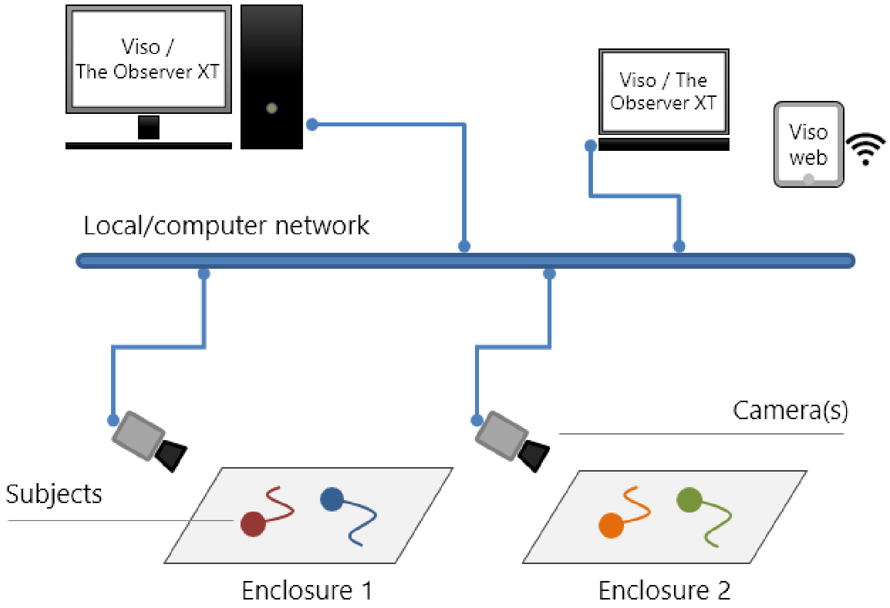

Figure 1. General scheme of the Viso system for controlling video acquisition from multiple locations and devices. Each location can be monitored with up to four cameras. The Observer XT can be used as an analysis extension of Viso or for coding and analyzing complex events.

Screenshots of the software

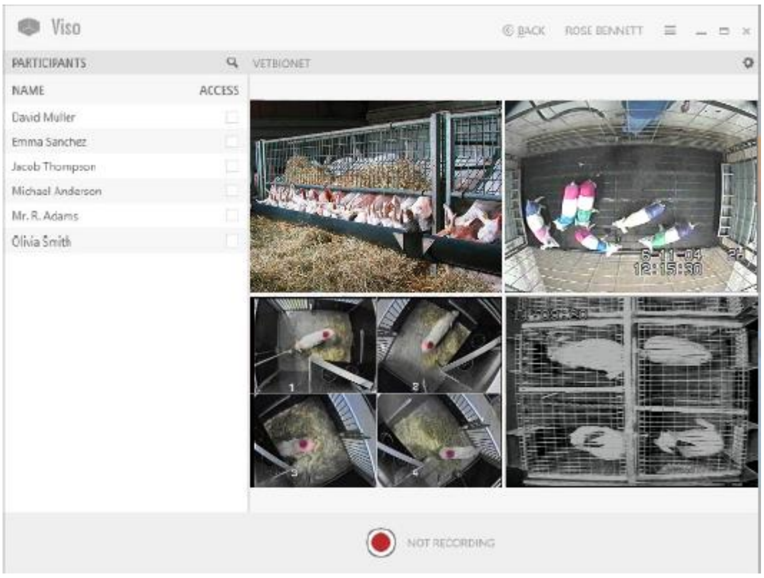

Figure 2. Screenshot of the Viso recording console. From the console you can control start/stop recording, review video, control camera orientation. Different users may have access to specific locations.

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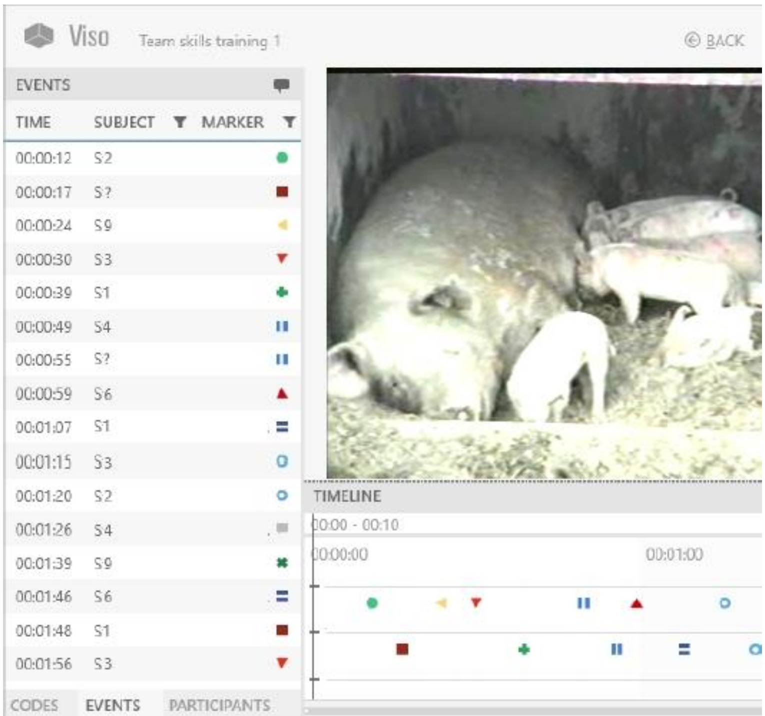

Figure 3. Screenshot of the annotation module in Viso. Code significant behaviors and events with mouse clicks or key presses when reviewing the video material. A more extended function for complex ethograms is available in The Observer XT.

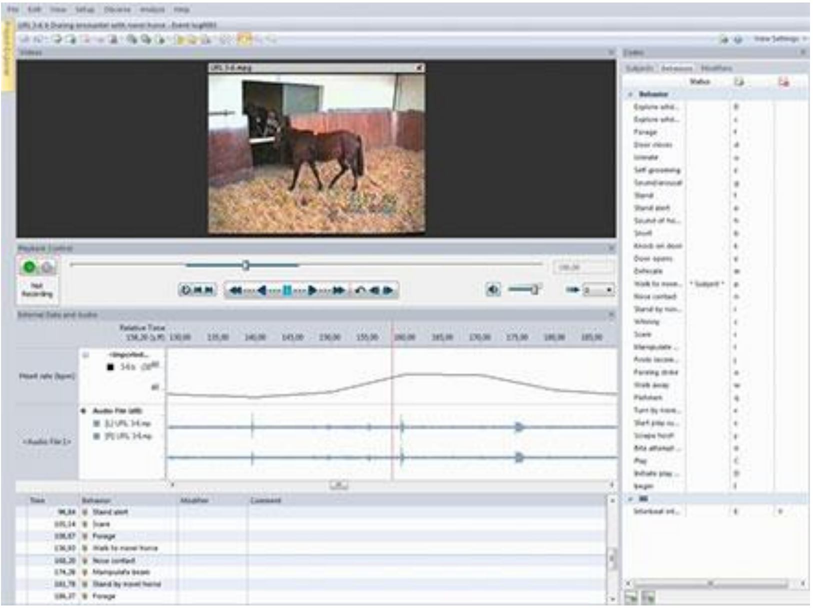

Figure 4. Screenshot of The Observer XT during a coding session. From top to bottom: Video window, Playback control widow, Physiological data window, Event log window. On the right-hand side, the coding scheme listing the behavioral elements defined by the investigator.

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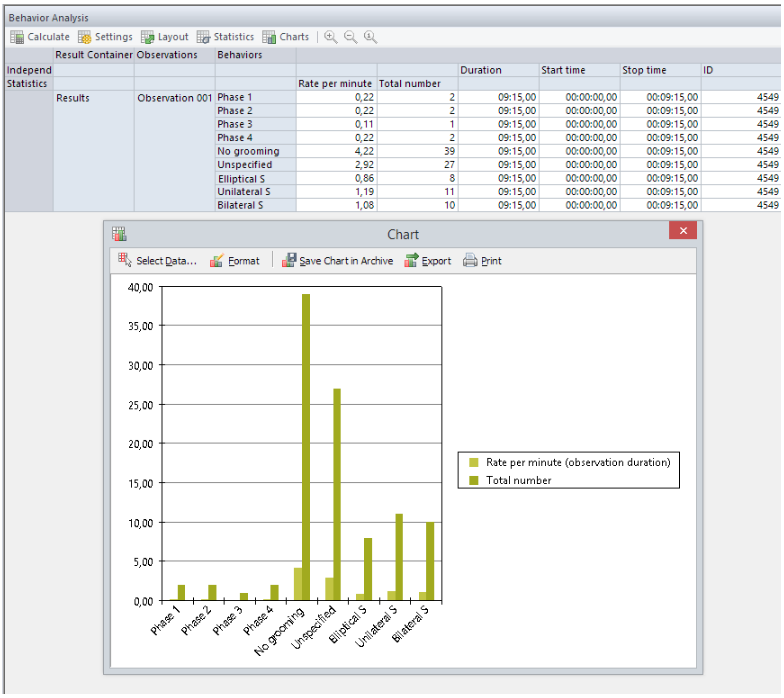

Figure 5. Example of behavior analysis in The Observer XT.

<u>Applications and limitations for different animal species</u>

- The tools are very flexible: Viso and The Observer XT work with any animal species, and any behavior that can be observed by a human expert can be recorded with the software.
- The behavioral annotation requires the time and effort of a human observer, which limits the throughput of this type of data collection. They are excellently suited for detailed behavioral analysis but not for continuous automated monitoring of animal activity and behavior.

3.4 EthoVision XT

<u>Brief description of the system</u>

EthoVision® XT is a software package for fully automated video tracking and analysis of locomotion and spatial behavior. For some species (rats and mice) it also offers automated recognition of specific body postures and behaviors. For any other species, behavioral readouts can be derived from track analysis parameters. EthoVision acquires and processes images from an analog or digital video camera overseeing one or more animals in an enclosure. A digital camera allows recording video at higher resolution and frame rate than an analog camera. When EthoVision XT analyzes the video, it detects the animal(s) against the background and extracts features such as the location of the center of gravity, other body points and the shape of the animal. From these data, a wide range of analysis parameters are computed (see screenshots below), and data can be visualized in various ways.

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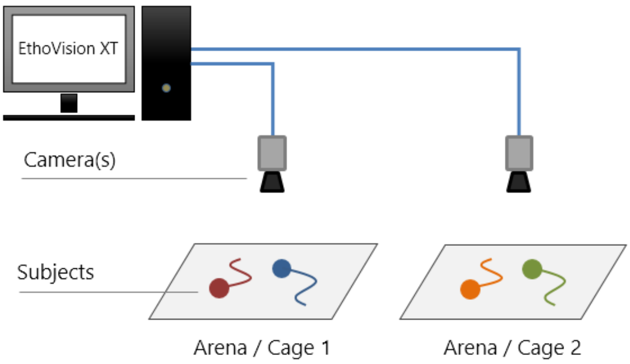
Special features for veterinary research in high-containment facilities

- High spatial and temporal accuracy.
- Automated behavior recognition: readily available for rat and mouse; can be developed for other species.
- The software supports studies with one or more animals per enclosure. In the case of multiple animals, these can either be tracked individually (if they are visually distinct, e.g. due to body size or color differences, or through color marking), or movement and/or activity levels can be quantified without identification.
- Flexibility. The system can be used in a variety of cages and enclosures. It can monitor multiple cages simultaneously.
- Fully automated, long term (i.e. multi-day) monitoring, no human operator required.
- The software allows integration of feeding and drinking monitors.
- Real-time analysis, with the option to generate alerts to the operator if a specific condition occurs (e.g. low mobility or absence of movement for a certain amount of time).
- Management of experiments, transfer of data, backup function.
- Remote viewing possible via Windows Remote Desktop.
- Quality Assurance module to help users create GLP-compliant experiments (e.g. logging of events and changes occurred to the system and data).

Diagram of the system



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Screenshots of the software

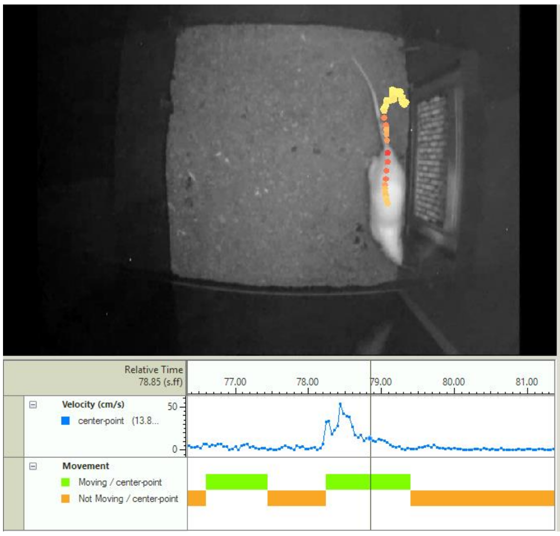

Figure 6. Extracting position data of one animal in a cage. The video shows the collection of spatial coordinates (track). The plots show examples of behavior endpoints, in this case (1) velocity and (2) a category variable that quantifies the duration of activity bouts.

Movement
- Distance moved
- Velocity
- Movement
- Acceleration
- Acceleration state

Location
- In zone
- Distance to zone
- Distance to point

Path
- Meander
- Target visits and errors
- Zone alternation
- Zone transition

Direction
- Heading
- Heading to point
- Head direction
- Head directed to zone
- Turn angle
- Angular velocity

Body
- Body elongation
- Body elongation state
- Body angle
- Body angle state
- Mobility
- Mobility state
- Rotation

Trial Control
- Trial Control event
- Trial Control state

External data
- Mouse call.Chevron
- Mouse call.Chevron state
- Mouse call.Ripple
- Mouse call.Ripple state

Figure 7. Overview of analysis parameters in EthoVision. Each of the endpoints in the list can be customized by the user. Besides parameters derived from image features, the software can also include trial control events (e.g. interaction with stimulus or response devices) and external data (e.g. vocalizations) in the analysis.

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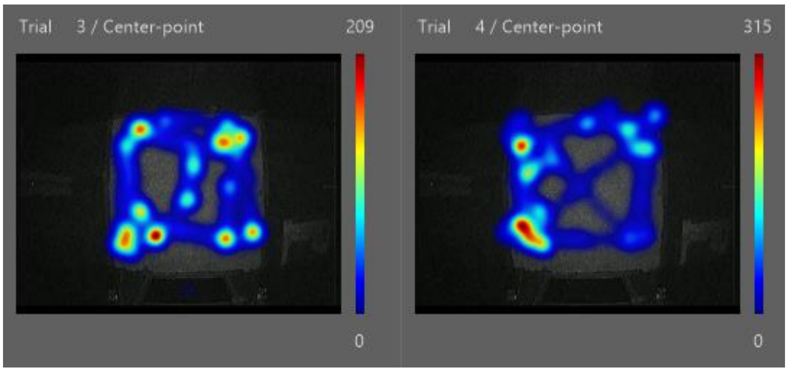

Figure 8. Example of data visualization with heatmaps. Heatmaps allow qualitative analysis of spatial behaviors and help detecting hotspots.

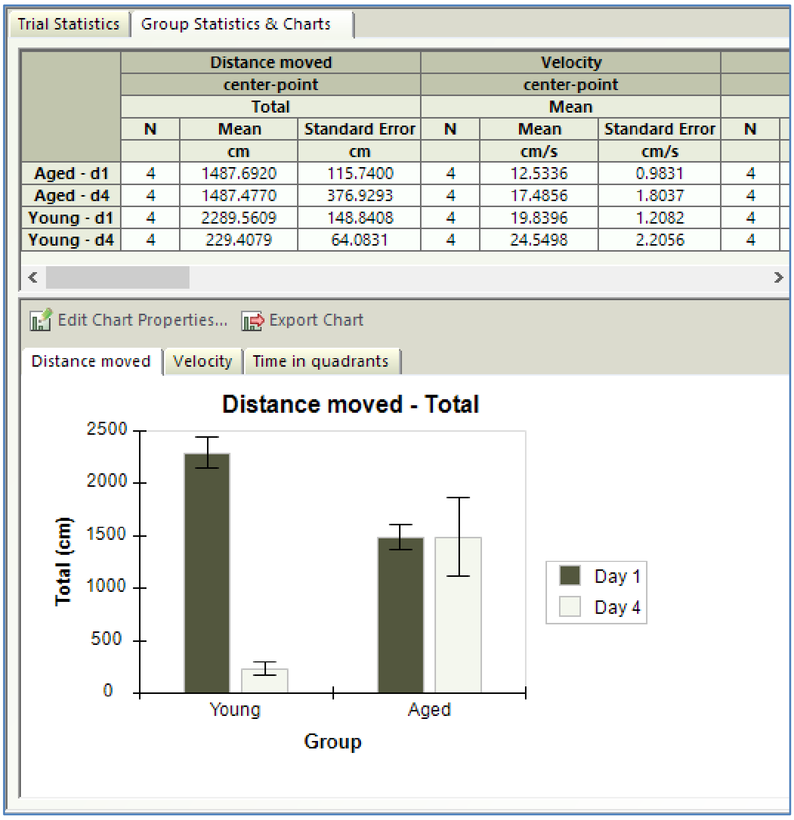

Figure 9. EthoVision XT provides a wide range of descriptive statistics and charts visualizations for quick comparisons of results. Data, tables and charts can be easily exported.

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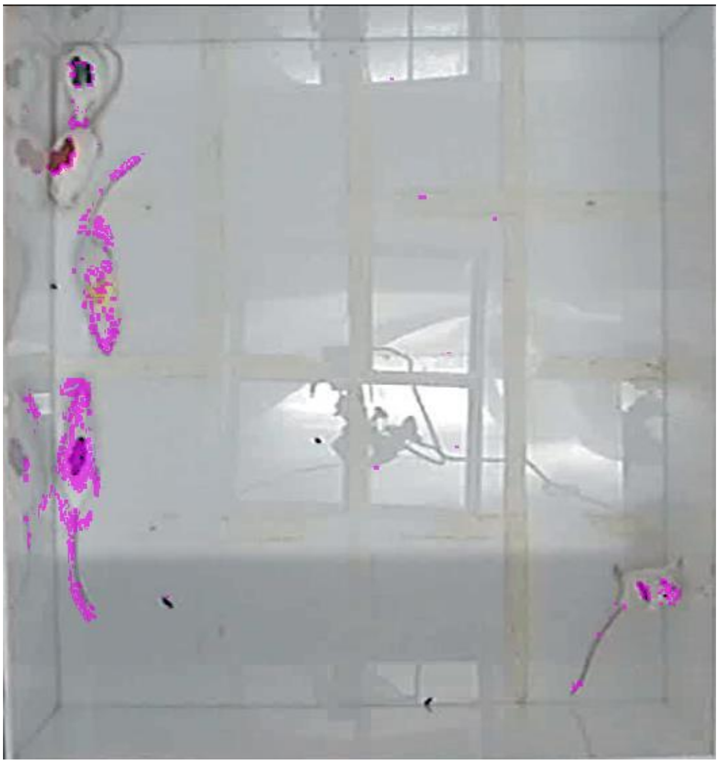

Figure 10. Measuring the activity of five socially housed rats (without tracking).

<u>Applications and limitations for different animal species</u>

- Can be used with any animal species observed via a video camera pointed at the enclosure (e.g. cage).
- Not suitable for enclosures that are too large to be overseen by a single video camera (e.g. cow barn).
- Rat, mouse: automatic behavior recognition available; other animal species: tracking of body center only (other functions, when needed, require additional development).
- Group-housed animals: if individual tracking is needed, marking method required; if not needed, group-based tracking and analysis.
- For locomotion tracking: vertical camera needed.
- For 24/7 operation: IR camera and IR illumination needed during dark periods.

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3.5 TrackLab

Brief description of the system

TrackLab™ is an integrated solution for recognition and analysis of spatial behavior of large animals. Using ultra-wideband (UWB) tracking technology, TrackLab records accurate and reliable position data in real-time. It is possible to monitor user-defined zones for activity and design algorithms to detect and classify key behavioral indicators. The collected data can be visualized, filtered, and analyzed.

Special features for veterinary research in high-containment facilities

- Tags (mounted on animal) and sensors (mounted on walls or ceiling) can be decontaminated using a wide range of disinfectants. The sensors are IP69K specified.
- Scalable, networked architecture, connecting multiple rooms and users.
- Remote viewing via TrackLab on Windows PC or notebook.

Diagram of the system

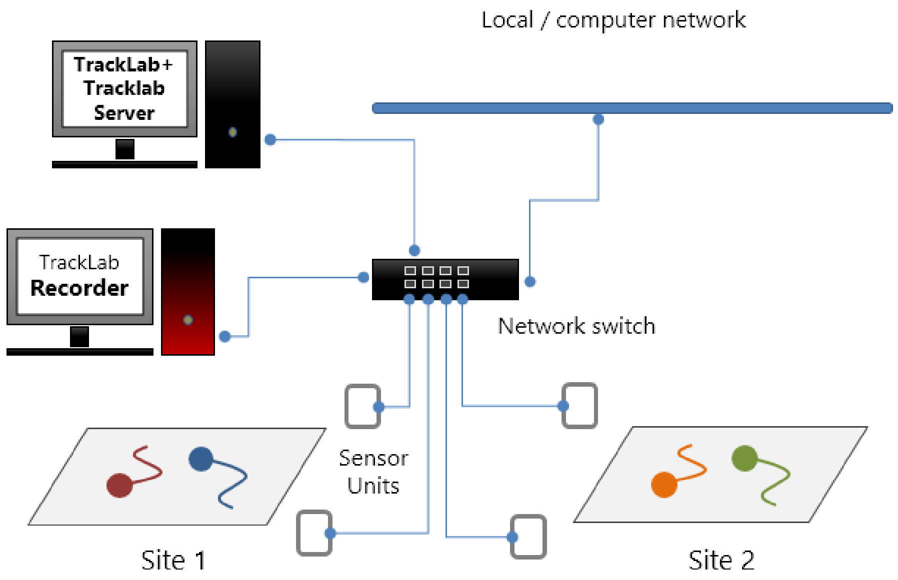

Figure 11. General scheme of a TrackLab setup for data recording in two enclosures (named Sites). The three software components TrackLab, TrackLab Server and TrackLab Recorder, can in principle be installed on one computer. From TrackLab, one can access one or more sites. The TrackLab Recorder collects the actual coordinate data for each subject. TrackLab Server stores the data and makes them available for analysis.

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Screenshots of the software

Figure 12. Screenshot of TrackLab when defining a map for a site. In this phase the user calibrates the map, so that TrackLab can provide accurate spatial data.

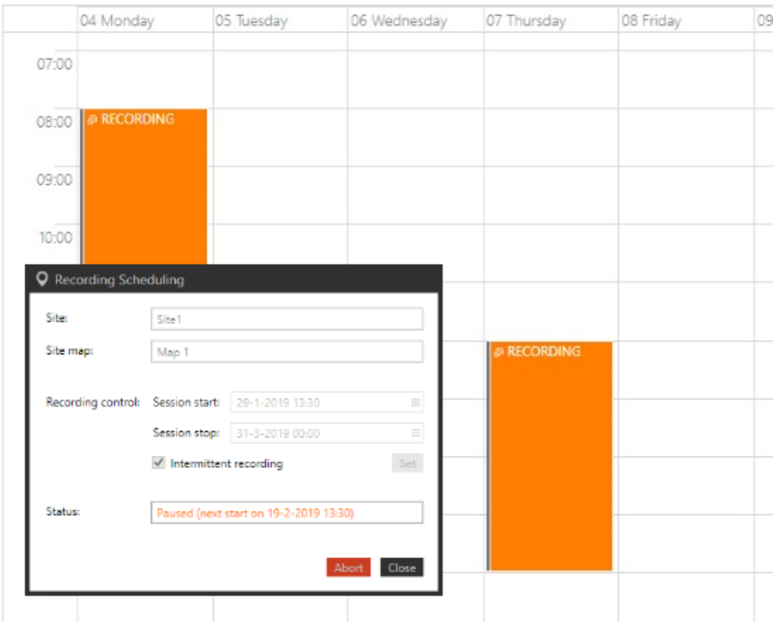

Figure 13. With the *Scheduling* function the user can plan recordings in a calendar-like fashion. For example, every Monday and every Thursday. Data acquisition starts and stops automatically in the selected intervals.

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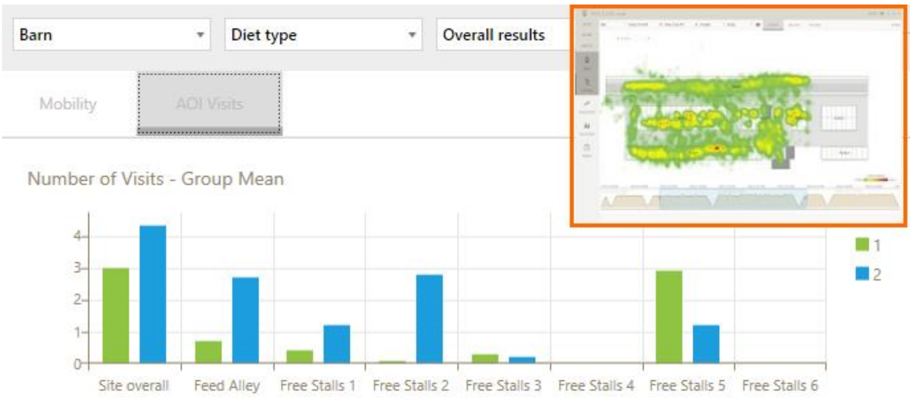

Figure 14. Statistics and charts aim at quantifying detected behaviors, like zone entries, activity levels etc. In the inset, a heatmap representing density of utilization of space by one or more animals.

<u>Applications and limitations for different animal species</u>

- Suitable for large numbers of animals housed in groups, no visual marking needed
- Specially suitable for animals in large spaces (e.g. cattle, pigs, chickens)
- Electronic tag must be attached to animal, e.g. on neck collar (cattle, goat), ear tag (pig), backpack (chicken, sheep), so not suitable for small animals (such as rodents) or for fish

3.6 Other telemetry sensors

Using video imaging and ultra-wideband positioning technology, we have developed three telemetric monitoring tools for recording of animal behavior (described in the sections above). These also offer extensive behavioral analysis functionality.

A third sensor technology, 3D accelerometry, can be used for automatic recognition of body posture, orientation and behavioral patterns. The body-worn sensor is not telemetric in itself, but can be combined with radio telemetry to provide a remote monitoring solution. This technique is currently under development for use with cows. As soon as a prototype solution is available it will be offered to VetBioNet partners for testing.

Technologies for telemetric monitoring of body temperature and respiration are currently under investigation. For body temperature, the selection and/or development of the most suitable sensor is in progress. Once the requirements analysis has been completed, we will proceed with development of an end-to-end solution for remote monitoring with real-time data transmission for the most relevant animal species. As soon as a prototype solution is available it will be offered to VetBioNet partners for testing. Technology for telemetric measurement of respiration will be next on the agenda.

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4. Conclusions

In the first 24 months of the VetBioNet project, we have developed and improved three systems for remote monitoring of animal behavior in BSL3 facilities. These systems provide significant added value compared to conventional manual data collection, both in terms of data acquisition and analysis. This applies in particular to EthoVision and TrackLab, which are fully automated systems for small and large animals, respectively. Specific benefits provided by these tools include:

- The researcher obtains better insight in what happens during experiments.
- Timely detection of critical events and Humane End Point criteria.
- Fewer human transitions in and out the BSL3 facility for extra observations.
- Real-time access to the continuous data allows quick intervention in case of abnormal events.
- Time is saved, because less time needs to be spent on inspection inside the biocontainment facility. Automated registration provides data that are immediately available for analysis, without the need for transcription.
- Higher operational efficiency.

For the measurement of body temperature and respiration, selection and/or development of the best sensor for various species still needs to be completed. This will be the main focus of the next project period, with body temperature measurement having the highest priority.

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