# Tracking Location and Orientation of Multiple Group Housed Rodents

## Master Thesis

by

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# Preface

Plenty of advancements in the medical field and technical field go hand in hand. I wanted my thesis subject to touch both fields, due to my interest spanning the mechanical, technical, and the medical field. Thankfully prof. dr. ir. W.A. Serdijn got me involved in the Neuromate project January 2017. It is a collaboration project between the TU Delft and the Erasmus MC. Lead by prof. dr. ir. W.A. Serdijn and Prof. dr. F.E. Hoebeek respectively. The Neuromate project aims to create a test setup using new technological advancements allow all kind of new neuroscientific studies.

August 2017 I got a laryngitis. Ever since I struggle with Chronic fatigue syndrome (CFS), also called myalgic encephalomyelitis (ME). CFS severely limited what I can do on any given day due to a lack of energy. This was very noticeable both physically and cognitively, but the cognitive aspect affected my studies the most. Over the course of my master graduation my workload has averaged between 10 to 20 %.

After a little over 5 years this thesis will finally be at an end. It has been a long way, but thankfully I have not been doing it by myself. I am very lucky that everyone in my circle is very understanding and supportive of my situation. Though I would like to mention the following people by name for their contribution to my graduation.

The first I am in debt of gratitude to are my parents Astrid Kingma and Jos Röling, who have always been an advocate for education. They guided and supported me every step of the way. I would like to thank both my professors Wouter Serdijn and Freek Hoebeek for getting me involved in the Neuromate and for keeping the focus on my graduation. When time got difficult due to CFS my sister Marloes Röling gave me regular support through coaching, which really helped and I am very thankful for. I am thankful that de Chaumont, F. et al. [1] were kind enough to let me use and adjust their setup. Additionally I am very thankful to my brother, Mark Röling, for helping me make the required adjustment to the Live Mouse Tracker and coding. Thanks to Thyco Engelshoven for his thorough review of my report. Last but not least I am thankful for all her support and help with documentation, Tessa Mol.

I would like to especially thank Wouter Serdijn for his compassion, encouragement, and dedication. And I would like to show special thanks to my father Jos Röling, because he took over the coaching from my sister and for all the extra time and effort he put in the second half of the graduation.

It has been quite the ride with plenty of rough patches. I learned a lot, but I am thankful it is at an end. I could not have done this without the guidance and support of all those mentioned. Except for the CFS. I could have done this without you buddy.

*J. Röling*
*Delft, March 2022*

# Abstract

Recent technological advancements have made it possible to study complex social behaviors in rodents using automated observations. The Neuromate project of the Erasmus Medical Center and Delft University of Technology aims to expand the research possibilities with this technology by enabling closed-loop neurostimulation and observation in group-housed mice. This thesis tackles a crucial aspect of the Neuromate project: the tracking of the mice.

This tracking is done using a 3D infrared (IR) camera in combination with 2D IR markers, located on the head of the mice. The tracking system is tested using dummy mice in two sets of test conditions. Each set consists of a baseline test and a test with a complication. The first set consists of a single mouse run as a baseline and a single mouse run with an obstruction as the complication. The second set consists of a run with two mice as a baseline and a run with two mice with a close encounter as the complication.

Results showed that the test setup was capable of successfully tracking the mice during all test conditions. The test with the close encounter showed the lowest rate of positive identifications (PI) per second of the 2D IR markers ($12.86 \pm 0.89$ PI/s). The single mouse baseline test showed the worst percentage of false positives (1.23% of all identifications). The test setup proves that by changing the camera setup to increase the spatial resolution, for example by using a higher resolution camera, the tracking method would be capable of fulfilling the requirements of the Neuromate.

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# 1 Introduction

## 1.1. The Neuromate project

The Neuromate project aims to enable the Neuroscience department of Erasmus Medical Center (Erasmus MC) to perform their desired studies on mice, that is taken as the main functional [mf] [1] criteria. The study goals tend to change over time[dg], having a flexible application of the solution [dg-fl] is therefore essential. The group leader at the Neuroscience department Erasmus MC & UMC Utrecht, F. E. Hoebeek, has stated that the current research goal is to study: "The long-term effects of early life events and their treatments".To meet this goal the Neuromate will have to meet a large set of criteria.

The Neuromate project originates from collaboration between the section Bioelectronics of the Delft University of Technology (TU-Delft) and the Neuroscience department of the Erasmus MC. This project was initiated by the Erasmus MC to fulfill the ambition to increase the possibilities in neuroscientific studies on mice by applying the latest technology [nt] to the mice test bed / observatorium. The Erasmus MC and TU-Delft have a client-developer relationship, where the Erasmus MC defines the desired functions and criteria of the Neuromate Project.

## 1.2. Neuroscientific research with the Neuromate

The Neuromate will be a test bed that can record data [da] to be used as control input to influencing brain activity [cb] of multiple rodents [mr] in a group housing [gh] for long periods of time [ls]. The method should be applicable to a variety of different types of neuro-scientific studies with minimal human intervention [fl][mi]. Such a novel method would enable Erasmus MC to conduct ground breaking studies.

To enable group housing of mice [gh] , remote monitoring and control of the rodents is required [gh-wl]. A setup with multiple wired rodents in a single cage would result in tangled and damaged wires. Additionally, wires will limit mobility, making it hard for mice to hide behind or under placed obstacles. This is important for the well-being of the mice, as they mingle as social animals and hide for security. Some new studies use wireless implants [5–9], most of the research that uses neurostimulation is done on solitary rodents that are physically constrained due to an electrical wire or optic fiber attached to the head [10–19]. Additional benefits of a wireless solution are the possibility for complex free roaming [mi-fr] and ethological relevant model studies [fl] with minimal human intervention [mi] [20]. These studies include navigating obstacles, small spaces, and mazes [fl].

One of the goals of the Neuromate is to enable longitudinal studies [ls] on group-housed rodents [mr][gh]. Neuroscientific studies are currently done by either human observers or automated systems. Two advantages associated with minimizing human observers for longitudinal studies are the reduction of man hours and the reduction or elimination of an observer bias. Automated observation is commonly used, with some studies

[1] In this report numerical references [1..9] refer to external sources listed in the bibliography, alphanumerical references [a..z] refer to criteria which are collected in chapter 4.1. An alphabetical list of the criteria used can be found in Table 4.1

also using automated control [1, 21–30]. Readily available systems that can perform automated observations either use force plate location tracking (Laboras and BASi FPA) or camera-based observation(Ethovision, ANY-maze, Homecagescan or Phenotyper).

These systems however, require a considerable amount of data storage for longitudinal studies, especially camera based systems. Furthermore, these systems are designed to observe a single animal and currently don't support the observation of multiple rodents. The quantity of data [da-l] the Neuromate will have to process will be higher than that processed by other systems, because it records multiple modalities of data [da] of multiple rodents [mr].

The Neuromate requires two functions to manage the information and to eliminate the need for human observers [er]. Firstly, it will require a system that can observe multiple rodents in a single enclosure. Secondly, these observations must be used in a closed control loop as illustrated in Figure 1.1 to influence the brain activity for each individual rodent [cb]. This requires the Neuromate to analyze different types of data in real time [cb-an]; rodent location [da-tr] and ElectroCorticoGraphy (ECoG) [da-ec]. The algorithm in Neuromate analyzing the data can score the relevance based on the data characteristics [da-l]. With this approach only relevant data is stored, reducing the size of storage required. Additionally, researchers could automatically flag data patterns, deemed relevant or important for their research. This would greatly reduce the time spent processing vast amounts of data for their research.

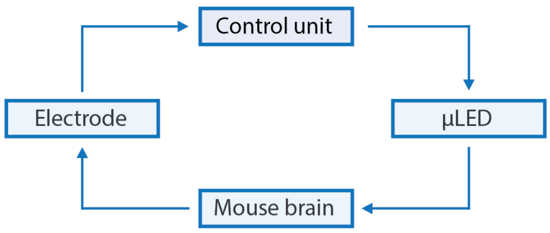


Figure 1.1: Depiction of the signal flow of the closed loop control. The control unit sends a signal to the µLEDs, which in then activate and influence the rodent brain activity. The brain activity is measured with electrodes and sent to the control unit. The control unit uses this input to determine the correct µLEDs activation.

A small [mi-s] and light [mi-w] wireless device linked to the rodent brain [cb][da-ec] would make more research topics possible. As such a minimally invasive [mi-in] device would allow long-term [ls] neurological studies on freely-moving [mi-fr] group housed [gh] rodents [mr]. The device would also allow the automated observations [ao] of complex and sensitive social behaviors [ao-sb], while simultaneously performing individual neuro-stimulation [cb] [20]. In conclusion, the Neuromate will increase the possibilities within neuroscientific research, the well-being of the rodents, the reliability of study results, and reduce the number of human hours to perform studies.

## 1.3. Desired functionality of the Neuromate

The data should not support the conclusion, but the conclusion should be supported by the data. For a successful functioning of Neuromate, behavioral and free roaming neuroscientific studies should be possible. From these studies the relevant data must be collected. Meaning you need the relevant data to be able to make the conclusion. The data is only relevant if it is directly related to the effects of the phenomena of interest. The Neuromate would obtain this evidence with automated observations.

Measuring brain activity [da] is an important tool in neuroscientific studies. The Neuromate will accomplish this with ECoG [da-ec]. The ECoG data gathered in studies on social interactions can provide novel insights in the underlying neural networks.

To study social interactions they need to be measured. Rodents have multiple ways of communicating, such as vocalizations, whisker contact, and sniffing [31]. Vocalizations could be recorded, but the vocalization of the individual mice would need to be distinguished to make the data valuable [32, 33]. In larger cages with multiple rodents whisker contact and sniffing are hard to monitor, because multiple animals need to be recorded and the movements are subtle.

Social interactions based on whisker contact and sniffing can be estimated using the proximity and orientation of the rodents. For whisker contact and sniffing they need to be in close proximity to a companion with their nose or whiskers. Furthermore, location and orientation data can be used to evaluate other kinds of experiments, which would grant Neuromate a wider range of applications [dg-fl].

## 1.4. Project goal

The goal of this thesis is to design and evaluate a technical approach which could track the locations and orientations of group housed rodents for the Neuromate project.

### 1.4.1. Research questions

In order to achieve this goal the following question need to be answered.

- What is the problem to be solved?
- What information and methods are available, relevant for the defined problem?
- What are the criteria defining the design space for a proposed solution?
- Which method has the most potential as a solution for the defined problem?
- What are the safety concerns with the proposed solution?
- What is required to determine the success of the solution for the defined problem?
- Does the proposed solution fit the criteria adequately?

## 1.5. Thesis outline

The 10 chapters of the report are grouped in 4 major sections; Context, Problem, Experiments and Conclusions. The first section sets the context and defines the research area. The second section articulates the problem definition and conducts a literature study as a base for the research question. In the third section the design and execution of the experiments are described including the validated results. Finally, the last section describes the findings and lists the recommendations. The structure is depicted in Figure 1.2.

**Section 1 - Context**

This section gives the context of the research.

Chapter 1 - Introduction

Introduction of the goal of this study, the Neuromate project, and it's desired functions.

Chapter 2 - Problem analysis

This chapter dives deeper into the Neuromate by discussing earlier work done on the project. The focus is defined as well as the problem definition of this thesis.

**Section 2 - Problem**

This section describes the problem definition.

Chapter 3 - Literature study

The literature study starts with a list of the search methods used to select papers. Thereafter, it describes the basic functionality of the 6 different tracking methods identified in the selected literature. The chapter ends with a comparison between methods.

Chapter 4 - Design criteria

Throughout the thesis criteria are labeled when they come up. This starts in the introduction. The criteria are gathered here and are further elaborated on when needed.

Chapter 5 - Valid tracking solution alternatives
This chapter compares the identified tracking methods in the context of Neuromate. The tracking methods are rated against the design criteria in a Harris profile. From this profile, a tracking method is selected.

**Section 3 - Experiments**

This section details the experiments performed.

Chapter 6 - Safety
This chapter is dedicated to the safety of the camera infrared marker tracking method. This includes the safety of the operators, animals, environment, and ethics.

Chapter 7 - Test method
This chapter starts with the research topic, main research question, and sub-questions. Thereafter, the experiment setup is laid out. This description is divided into the test subjects, the 2D IR marker design, and a list of used materials. Then follow the test conditions and a general description of each test run on the setup.

Chapter 8 - Results
This chapter reports the raw and processed test data. Additionally, it includes a section about the accuracy of the location and orientation. These are not directly measured during the test, but are estimated based on findings, informed assumptions, and parameters.

**Section 4 - Conclusions**

This section describes the conclusions and recommendations.

Chapter 9 - Discussion
In this chapter the performance of the test setup is discussed based on the data from the previous chapter. The performance is evaluated for each test condition and a subset of the important metrics, which includes false positives and accuracy. This is followed by a discussion of the limitations of the test setup and of the extent to which the criteria have been met.

Chapter 10 - Conclusion and recommendations
In this chapter the research questions are answered. It summarizes the extent to which criteria have been met and answers the project goal. Thereafter, it outlines the contributions of this thesis and it ends with future recommendations for further study of this topic and the Neuromate.

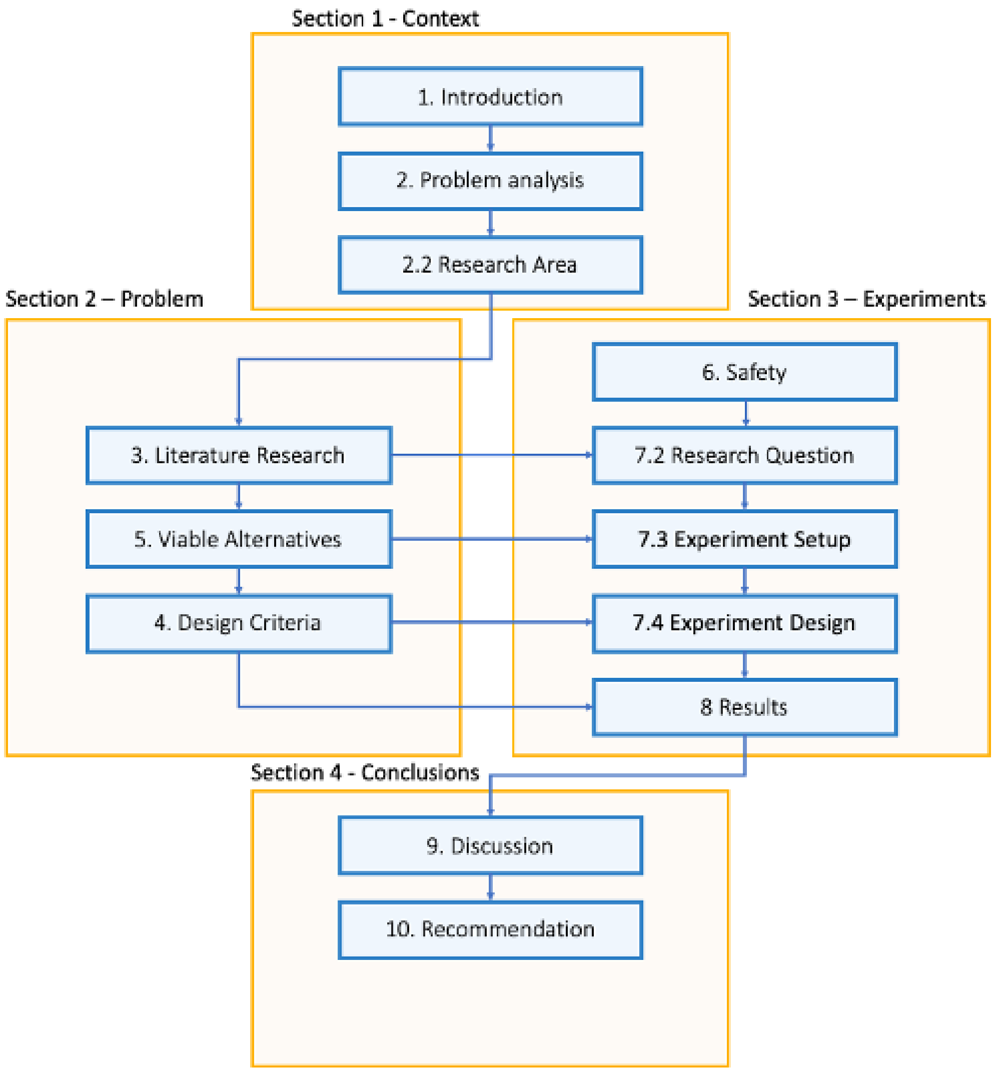


Figure 1.2: Structure of this research is divided in four major sections: context; problem definition; experiments; findings and recommendation

# 2

# Problem analysis

## 2.1. Previous work on the Neuromate

The Neuromate project is divided into several sub-projects. Several of these sub-projects were already completed or ongoing at the time this study was performed. The aspects of the Neuromate which have been or are being studied are illustrated in Figure 2.1. The gray cube represents the enclosure with the mice inside with the dimensions of 40x40x20 cm (lxwxh). The mice, labeled 1 to 4, have a box on their head. This box shows the location of the wearable device on the mice with implanted electrodes and μLEDs. The small lightning symbol indicates the wireless power transfer via inductive coupling. Directly above the wearable devices are illustrations of wireless connections. These represent the back scattering up-link. There is also a wireless illustration at the top of the cage, which represents the wireless Terahertz down-link. The bottom right Figure shows the frequency spectrum with indications on the frequencies used by the wireless signals. The monitor drawn on the top right shows the signals of ECoG originating from the neural interfaces of each mice. Illustration by Jinne Geelen [34]

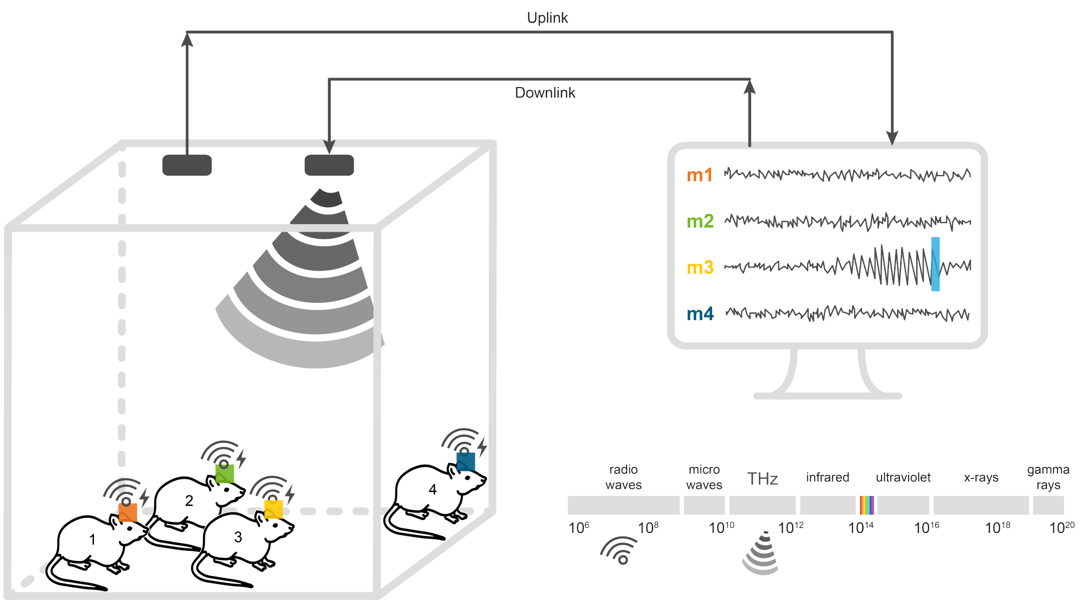


Figure 2.1: Illustration of the Neuromate project setup, including all the different components that have been or are being studied.

### 2.1.1. Neural interface

The neural interface is the implanted part of the wearable device. The wearable device is illustrated in Figure 2.1 and 2.3 with the (colored) squares on the heads of the rodents. In order to perform neuroscientific research, the Neuromate needs to be able to monitor and influence the brain activity [da][cb] of the mouse. The links to the mouse brain will consist of implanted electrodes [da-ec] and μLEDs [cb-op] [5, 6, 8, 9, 35], surgically implanted in the desired locations. The electrodes are used for brain monitoring by measuring electrical activity. The μLEDs are used for brain stimulation with optogenetics. In optogenetics, specific neurons are genetically modified to express light sensitive ion channels, allowing the μLEDs to influence the electrical excitability of these neurons [5, 6, 8–19, 35].

Electrodes and optogenetics can both be used for measuring and influencing brain activity. However a combination of the two would result in the optimal performance, as they function in different spectra (light vs. electromagnetic). If only electrical or optics is used for stimulation and observation, the accuracy of the measurements would degrade during the simulations. A combination of both methods leads to only a minimal amount of interference, which allows for simultaneous measurement and stimulation. The combination reduces crosstalk, but interference created by the light is still experienced due to the photoelectric effect [36].

Optogenetic stimulation offers a high selectivity, which allows research into very specific brain areas and neuron types. Electric stimulation would also be possible, but it is not a specific method. It stimulates all cells in the surrounding area. Opto-genetics could also be used to measure brain activity in vivo. This is called bio-luminescence imaging (BLI), but this is harder to implement than electrode readout and offers little advantages [37, 38]. Therefore, using opto-genetics for stimulation and electrodes for measurement of brain activity makes an effective and practical neural interface with very limited crosstalk as shown in Figure 2.2.

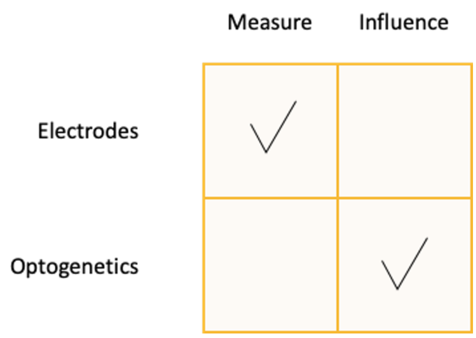


Figure 2.2: The application of different technologies for the neural interface as used by the Neuromate project.

### 2.1.2. Wearable wireless device

The Neuromate design criteria are based on the application on mice. The Neuromate should not prevent a mouse from free roaming [mi-fr] and should be suitable for group housing [gh]. Mice are taken as the target group for two reasons. Firstly, they are commonly used in labs and are Erasmus MC's rodent of choice. Secondly, if the Neuromate is of little hindrance [mi] for small rodents, other larger lab animals, e.g. rats, it is expected that they should also experience little hindrance as well. This makes the device more versatile. To be suitable for mice, the device needs to be small lightweight, and wireless.

### 2.1.3. Power supply

The Neuromate requires a connection to the implanted neural interface to operate it. This is achieved by a wearable device on the head [wd]. Not all components of this device have been determined yet, but the provisioning of power to the wearable device [wd-ps] is. Although small, the implanted μLEDs and electrodes require electrical power to operate. The electrical power supply needs to sufficient to create a waveform for the μLEDs and to record, buffer, and analyze the signals from the electrode. Since the goals of the Neuromate exceed the bare minimum requirements of the device, the power supply will also require some additional capacity for future applications. The power source must last for at least a period of 4 months to allow for experiments of the long-term effects. A battery is not suitable as power supply due to the weight restrictions of the device [mi-w].

Farnaz Nassirinia graduated on the wireless power solution [wd-wp] [39]. Power was transferred with inductive coupling between a coil around the cage and small coils in the wearable head fixated devices, as illustrated in Figure 2.3. The wearable devices have power if the coil around the cage is powered and sufficiently aligned with the small coils in the devices. The wireless power link is illustrated in Figure 2.1 with the small lightning symbols.

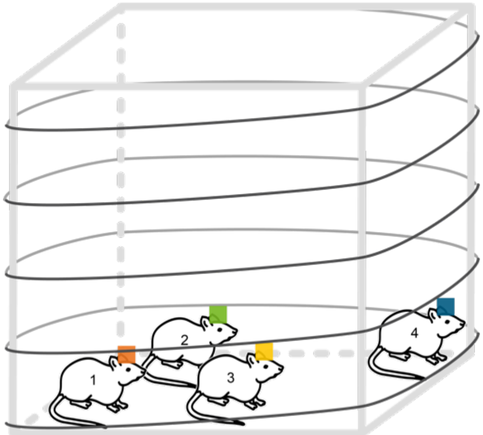


Figure 2.3: Illustration of the Neuromate cage with the coil around it, that transfers wireless power from outside to inside the cage. Illustration by Jinne Geelen.

### 2.1.4. Data storage

Relevant data must be available [da] and stored [da-st] for each individual device. This data is the basis of the evidence to deduce the correct outcome from an experiment. A wearable storage solution or wireless data transfer method to external storage is required. The storage solution will store sensor data for the duration of the experiments, to explore longitudinal effects. This would require a considerable amount of local storage capacity. Furthermore, researchers would be unable to tell if the equipment is malfunctioning. This would risk the introduction of measurement errors, without interfering with the mice and therefor the experiment [ri]. Such measurement errors could be caused by a loose wire or improperly placed implant. A wireless up-link [wd-ul] would allow for real-time evaluation [ri-re] which can lead to earlier detection of measurement error.

Ide Swager graduated on the concept development and testing of an up-link which uses back scattering technique [40]. Minghui Liu graduated on further development of this method, focusing on the receiver [41]. The up-link is visualized with the small wireless symbol above the squares in the cage in Figure 2.1.

### 2.1.5. Control of stimuli

The timing and intensity of the µLEDs need to be controlled to affect the brain as desired [cb-c]. The control input can be regulated internally in the wearable device or externally and transmitted with a wireless down-link [wd-dl]. An internal control loop would require processing capabilities in the wearable device. The input could be the output of the electrodes and the latter could be provided by a microprocessor. A solely internal control loop cannot be altered by the researchers without a physical connection, which would influence the experiment. Therefore, a down-link would be desirable [wd-dl]. This would result in the control loop accessing additional data from measurements outside the cage or other devices [da]. Additionally, outside the cage more computational power is available which enables the use of more complex control loops. The latency in the transmission would be a factor to consider in the control loop. A hybrid control system as depicted in Figure 2.4 could run a fast control loop to activate the µLEDs and the wireless down-link could be used to switch control states or adjust parameters of the loop. This way, the effects of the transmission delay can be minimized in the control system, but the wireless down-link can still supply the additional data.

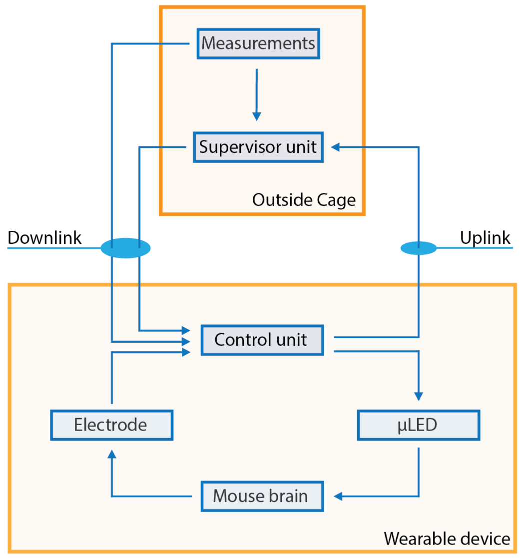


Figure 2.4: The control loop depicted in Figure 1.1 is situated inside wearable device. This system is expanded with an up- and down-link to the system components outside the cage. The outside system consists of external measurement equipment and a supervisor unit located outside the cage. The external measurement equipment is used to obtain new data otherwise unavailable to both the supervisor and control units. The up-link is used to send any information required by the supervisor unit. This could consist of current control values, control states, µLEDs activity, and electrode measurements. The supervisor unit determines new control values and states, based on uplink data and the external measurements. The down-link sends these new values, states, and the external measurement data down to the wearable device control unit.

Jinne Geelen was developing the down-link in her graduation project [34]. Her project is drawn in Figure 2.1 with the large wireless symbol originating from the top of the cage. She researched the possibility of THz torching, but found there are still some major issues with this method, such as a slow data transfer, obstructions, interference, and form factor. However, there are other methods, which may prove more viable to achieve a desirable down-link.

## 2.2. The focus

The Neuromate project has still several aspects that are to be resolved, in addition to early implementations of subsystems that needs to be improved. In the next sections the problem area for this thesis will be described.

### 2.2.1. Unsolved aspects

Both the control system [cb-c] and signal processing [cb-an] of the Neuromate are aspects which are not yet addressed. For either of these it is essential to, identify the system variables, especially inputs and outputs. During an initial mapping of the desired inputs for the system, the input criteria were not met. One of these criteria is the wireless tracking of location and orientation of the mice in the enclosure [da-tr]. This is necessary to support complex free roaming [mi-fr], and ethological studies with minimal intervention [mi] [20]. The location and orientation of mice can be used for research on navigating constricting obstacles, small spaces and mazes [mi-fr] [da-tr], sensitive social interactions [si] [ao-sb], simultaneous stimulation of multiple rodents [cb] and home cage behaviors [mi]. Social interaction can be estimated by proximity and orientation between rodents [da-tr] since they can only perform sniffing or facial interaction when they are near and facing each other. Mental states like fear and paranoia can be evaluated by measuring the percentage of time spent exploring large light spaces or hiding in small dark spaces [da-tr]. The location and orientation of the rodents is crucial to take advantage of the opportunities presented by a wireless neural interfacing close loop device.

### 2.2.2. Problem evaluation

Before defining and evaluating problem definition additional criteria are explored to help reduce the scope of the problem space. An important aspect to know is the proximity of a given mouse to the other mice [da-tr-pr]. To determine this, the simultaneous tracking of location and orientation of multiple mice is needed [da-tr]. An specific accuracy, defined further in section 4.1, is required to make a useful estimation of the position of mice confined in a small space. The tracking solution should not reduce, nor negatively influence the functionality of the other components of the Neuromate [or], such as the neural interface [da-ec][cb-op], up-link [wd-ul], down-link [wd-dl] and power-link [wd-wp]. If the solution is not compliant with the existing parts of the Neuromate the solution is considered to be insufficient.

### 2.2.3. Problem definition

How can the location and orientation of multiple group-housed mice be acquired with a minimal accuracy of 8 mm and 10 deg, without interfering with other aspects of the Neuromate?

# 3
# Literature study

## 3.1. Search methods

The initial sources for this research were supplied by F. Hoebeek and J. Geelen. These sources described the research field of the Erasmus MC & UMC Utrecht and studies with a goal like to that of the Neuromate. Additional papers were found using Mendeley and in the references of papers, but the vast majority were found using a variety of search engines. Google Scholar was used primarily. It has the largest selection, including the papers of the other search engines and it provided the Bibtex references. The used search entries included: rodents, mice, optogenetics, multiple, low power, locomotor, behavior, sleep, automated observation, automation, surveillance, accelerometer, force-plate, inertial sensor, localization, indoor, ultra-wide-band, positioning, and ultrasound.

## 3.2. Tracking methods

A variety of methods is currently used in the research field to track the movements and related activities of rodents. Several of these solutions are still in research phase, while others are developed and available. The next sections describe the different technological principles behind the tracking methods and gives an indication of their accuracy where possible.

### 3.2.1. Force plates

Force plates are commonly used to analyze walking gaits but can be an effective tracking method of mice as well [42]. Force plates used for research on rodents are most often rectangular with tri-axial force transducers on the corners [29, 43–45]. The ground reaction forces and torques measured by the tri-axial force transducers are used to determine the combined Center of Mass (CoM) of the set-up and the rodent. The CoM of the rodent can be derived from the combined CoM and the CoM of the set-up. This method can only determine a single unknown CoM and cannot track multiple rodents. Literature [29, 42–47] states these forces can also be used to analyze a variety of different movements including: climbing, grooming, eating, drinking, scratching, seizures, purposeless chewing, hind limb licking, wet dog shakes, head shakes, head twitches, distance traveled, wall rears, wall rear duration, number of low mobility bouts, in-place movements, number of high velocity runs, and gait parameters (stride rate, stride length, and velocity) .

A couple of force plate systems can be purchased for research purposes. The BASi force plate acti-meter uses a square plate and can perform gait parameter measurements and location tracking [29, 46]. Another system is the LABORAS by Metris B.V., which uses a triangular plate [47–50] and can perform tracking and a variety of behavior analysis. However, no performance tests on these systems performed by an independent party were found.

The papers of Fowler et al. and Parkison et al. [29, 42, 44] do give an accurate indication, by stating that the spatial resolution is lower than 1 mm, near 1 mm, and 2 mm respectively. Other authors do not quantify the spatial resolution. They do qualitatively compare their methods to exceed the capabilities of other tracking systems.

### 3.2.2. Camera

Rodent tracking is most performed with a camera system. This method is also often used in addition to other systems to enhance or evaluate their accuracy [1, 24, 25, 29, 42, 43, 51]. Others compare automated observation systems with two or more human observers [1, 25, 42, 51, 52]. The performance of camera tracking systems is deemed sufficient if the system compares to human observers or better.

Camera tracking for rodents uses infrared (IR) camera with IR lighting. Mice and rats are nocturnal animals which rely mainly on their olfactory system. Their vision is rod based, and only about 3% of the vision consists of cones. They have two types of cones, one for short wavelengths (360nm ultraviolet) and one for medium wavelengths (511nm green) [53]. The rodents are therefore unable to see IR light. Observations requiring constant IR lighting can be performed around the clock without interfering with the animals.

The spatial resolution refers to the effective data points per square cm. The spatial resolution of a camera system is determined by the amount of pixels per cm and the image processing of the system. The paper by Shis y. et al. [25] stated that traditional photocell matrices get a planar resolution of approximately 8 mm and that their combined system of camera and accelero-meter got a resolution of 1.2 mm. Ou-Yang et al. 2011 [27] reported an average root mean square error (RMSE) of about 11 pixels when comparing their tracking results to human observers.

### 3.2.3. Radio frequency identification (RFID)

Radio frequency identification is a technique used to identify rodents marked with a small unique tag chip within a setup. Detection coils strategically located near narrow corridors, or points of interest such as feeders, bridges, or scales, are used to determine the rodent's location [24]. For example, Schaefer A. T. et al. [26] uses RFID in tubes with a gate on either end to isolate single rodents in a test chamber to perform measurements on them within a social construct. Such experiments experience issues when rodents pass each other near the coils. For this literature study the RFID implementation of Weissbrod A. et al. 2013 [51] is more suitable. They were able to track and identify multiple rodents in a single cage using a camera system and a grid of antennas with RFID decoders located underneath the cage. The camera tracked rodents and the RFID information was used to identify them.

The RFID systems are mainly a tool of identifying different rodents by a unique tag. The system requires an additional tracking system, such as a camera, to perform a locating task. Where the accuracy of the tracking system is determined by the tracking method. An RFID system would allow for the detection of a rodent's general presence when the animal is outside the tracking range. For example, when the vision of a camera system is obstructed because the rodent is covered by another rodent or hidden under an object.

### 3.2.4. Radio and ultrasound positioning

**Self and remote positioning**

Most methods for wireless positioning can be implemented as either a self-positioning or remote positioning solution. For self-positioning a receiver determines its own location using the signals from transmitters with a known location. Whereas for remote-positioning receivers at known locations locate a transmitter with an unknown location. Which tracking method is more suitable depends on the application of the system. [3, 54]

**Different signal types**

Wireless positioning can be achieved with different types of signals: electromagnetic radiation waves such as WiFi (2.4 GHz or 5 GHz) and ultrawideband (UWB) (3.1 to 10.6 GHz), or pressure waves such as ultrasound (higher than audible >20kHz). Different signal types and frequencies have different properties which will influence the performance of the system such as propagation velocity, attenuation, penetration, and deflection. A UWB signal utilizes the different properties of signals, by transmitting a selection of different carrier frequencies and analyzing the discrepancies between them. The differences are used to solve multi-path components, caused by penetration and deflection as illustrated in Figure 3.1 [55]. The following paragraphs elaborate on seven different methods to achieve wireless positioning. These methods can be implemented as either self or remote-positioning and can use all discussed signal types, unless stated otherwise. Only the self-positioning methods are explained for the sake of simplicity.

**Proximity and received signal strength positioning**

Two relatively simple methods to implement are proximity-positioning and received signal strength (RSS). The proximity-positioning method is usually self positioning and thus requires multiple transmitters with a known location. The receiver scans for all transmitter signals which are in range. As depicted in Figure 3.2, the location is set to be the center of the area covered by all the transmitters detected. Increasing the number of transmitters increases the number of areas and will therefore increase accuracy, provided there are no interference issues. The RSS method uses the signal strength drop over distance to estimate the distance to the transmitter from the received signal strength. However, reflections from surfaces can reinforce or dampen the signal, which makes it hard to implement this method indoors. This method can be used on its own or in addition to the proximity-positioning method to increase the performance of the system. [2, 56, 57]

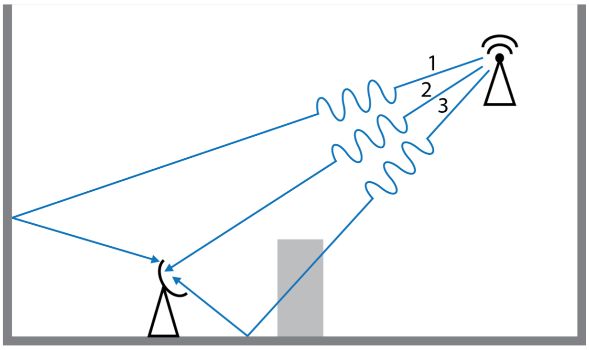


Figure 3.1: Illustration of a signal traveling multiple pathways in a cage from the transmitter (from the top right) to the receiver (at the bottom left). Signal 1 reflects off the wall increasing travel distance and therefore, increasing time compared to signal 2, which travels in a direct path. Signal 3 penetrates an obstruction and reflects off the floor, possibly creating an even greater travel delay. Illustration by Marloes Röling.

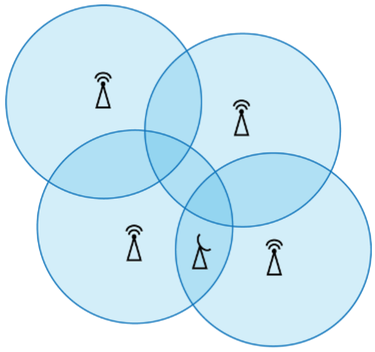

Figure 3.2: This figure shows the signal ranges of multiple transmitters, creating areas with coverage of specific transmitters only. The receiver can determine in which area it is located by detecting which transmitters are in range. Illustration by Marloes Röling.

**Time of flight and time of arrival positioning**

The time of arrival method (TOA) uses the signal time of flight (TOF). The TOF can be multiplied by the signal travel velocity to obtain the traveled distance. TOA registers the times at which a signal feature is sent by a transmitter and detected by the receiver. The time difference is the TOF as illustrated in Figure 3.3 with the bar-graph labeled *signal travel time*. This method requires the receiver and transmitters to share a clock. The clock can be predetermined or modulated into the signal, but in practice they will never be completely synchronized, which will result in an error. Figure 3.3 shows an self-positioning setup of this method with the minimum required 3 transmitters. [2, 3, 54, 56, 58–61]

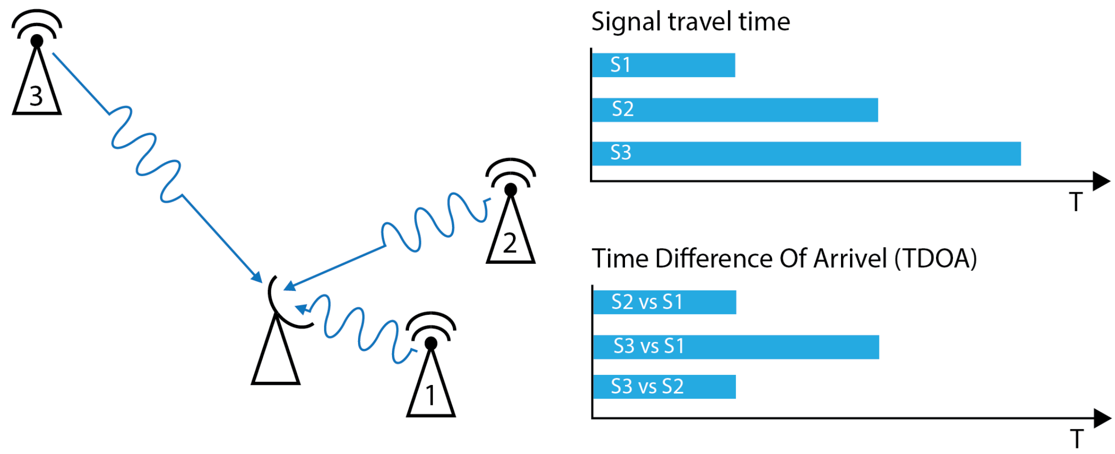


Figure 3.3: This setup depicts 3 transmitters located at different distances to the receiver. The signal travel times from each transmitter to the receiver will therefore vary. The upper bar graph shows an indication of the relative signal travel times from each transmitter. These values are used directly for Time of Arrival (TOA) triangulation. The lower bar graph shows the time differences between the received signals. The time differences are directly related to the difference in distance to the transmitter towers assuming Line Of Sight (LOS). These values are used for the Time Difference of Arrival (TDOA) method.

**Time difference in arrival positioning**

The time difference of arrival (TDOA) method resembles the TOA as in Figure 3.3 but reduces error due to clock offset. Only the transmitters must share a clock if the method is implemented as self-positioning. As illustrated in Figure 3.3 with the bar-graph labeled *time difference of arrival*, the TDOA detects the difference in the time of arrival between signal features sent at the same time from different transmitters. This difference can be translated into the difference in the distance to the transmitters. This method does not use the absolute TOF and does therefore not require the receiver to share a clock with the transmitters. Any offset between the clocks of the transmitters can be eliminated using a reference receiver at a known location. This receiver detects the transmitter signals and transfers the TDOA to the mobile receiver. The mobile receiver can use this extra measurement to increase accuracy. Although the clock, and therefore the clock offset of the transmitters is no longer part of the equation the clock offset between the reference receiver and receiver will cause a positioning error. [2, 3, 54, 56–62]

#### Round trip time positioning

The last method making use of TOF is the two-way ranging method. This method requires stations which both transmit and receive signals. The time a signal takes to be transmitted and returned to the same station is called the round-trip time (RTT). The signal is returned by a second station after detection and a set time interval. The average range is half the RTT minus the interval, multiplied by signal travel velocity. No clock synchronization is required, as the return signal is triggered by the incoming signal. The range error consists mainly of the clock drift of the original source and the response time differences of the bouncing station, which in most cases is a noise-like error. When moving, the measured range between stations is the average range of the start and end position. [2, 3, 54, 56–59, 61, 62]

#### Angle of arrival positioning

Angular positioning uses the angle of approach (AOA) from multiple sources to get the location. Either three AOA are required or two and the orientation of the receiver as illustrated in Figure 3.4. Assuming the orientation is known the location can be determined by drawing lines from the origin at the AOA. The relative position to the transmitter's location is the point at which the AOAs intersect. This method does not require a shared clock, but the receiver needs to be more complex. To obtain the AOA a rotating directional sensor or sensor array is required. The directional sensor has a specific directional sensitivity pattern. By rotating the sensor and recording the signal strength an accurate AOA can be determined. A sensor array has multiple sensor points in set positions. By comparing the time of arrivals, the TDOA is obtained, which used to calculate the AOA. [3, 54]

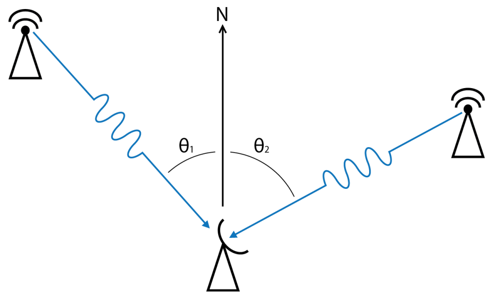


Figure 3.4: Angulation: two transmitter towers send signals, and the receiver detects their angle of arrival. The receiver can calculate its location using these angles and the direction of true north.

#### Ultrawideband pattern matching

The pattern matching method makes uses a UWB signal to cover an area. This area is divided into a grid and on each node a sensor maps the local signal pattern. Each grid point is presumably unique due to the complex reflection from the complex UWB signal. The receiver can determine the location by comparing its received signal to the mapped signal patterns on the grid. [3, 54, 58]

#### Performances

Wireless positioning has large variety of methods and performances, some of which have a considerably high accuracy and a large range. Davide Dardari et al. 2015 [54] published a table of wireless positioning system accuracies with similar results to those illustrated in the graphs 3.7. These findings align with those in the other literature used for this study, except for ultrasound. Ultrasound performed better in the other literature, with an error mean below 10 cm [57, 61, 62]. The best accuracy was achieved using an UWB signal, which was reported in the dissertation by Giovanni Bellusci [59] to achieve a standard deviation range error of less than 2 cm with Line Of Sight (LOS).

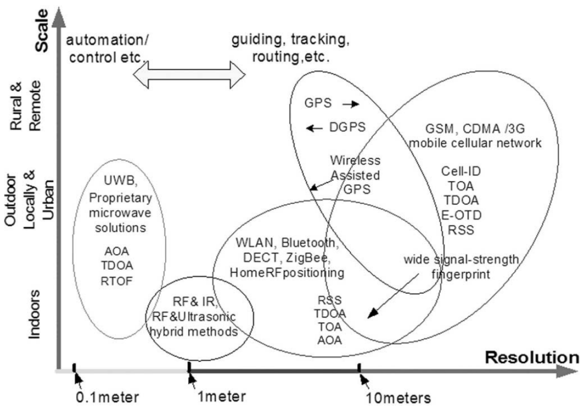


Figure 3.5: By Liu, Hui et al. 2007 Survey of wireless indoor positioning techniques and systems [2]

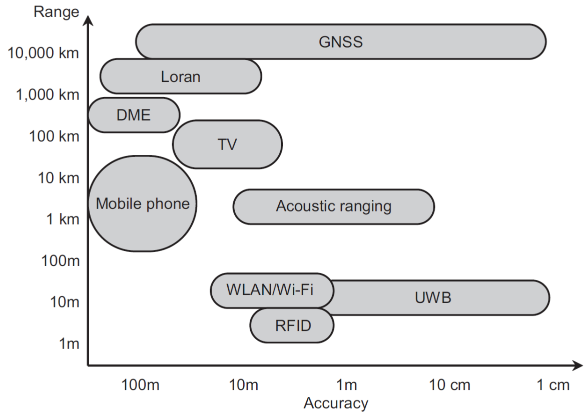


Figure 3.6: By Groves, Paul D. 2013 Principles of GNSS, Inertial, and Multi-sensor Integrated Navigation Systems [3]

Figure 3.7: These graphs indicate the system performance of different methods and signals. The horizontal axis represents the system resolution and the vertical axis the operating range. [2, 3].

### 3.2.5. Inertial navigation

An inertial navigation system uses accelerometers and gyroscope sensors to track the location of an object [63–65]. These sensors measure linear and angular accelerations. There are different sensor layouts possible, such as the mass spring sensor. The sensor uses capacitance to measure the displacement of an internal mass suspended by a spring. The acceleration of the sensor exerts forces on this mass and causes the spring to extend or compress which changes the position of the mass, leading to a capacitance change. Therefore, the capacitance is directly linked to accelerations. Damping and excitation should be considered when designing a mass spring system.

The linear and angular accelerations are used to determine the object's new location by integrating once over time to obtain velocity and twice to obtain the new position [3, 54]. This kind of positioning is a type of dead reckoning. The drawback of a dead reckoning is the location error drift caused by error propagation over time. Measured errors in acceleration will cause an error in the estimated velocity and thus the estimated position, but without measuring the real velocity or position these errors are not corrected and new errors will only compound. This can be reset by using a frame of reference. A commonly used framework is formed by a magnetic compass to determine north and the detection of the gravitational field to determine down. This way the sensor orientation can be determined without the angular acceleration and angular dead reckoning errors are reset.

The data from an inertial sensor could yield additional information depending on the situation, which could increase positioning accuracy. Such an example is the Pedestrian Dead Reckoning method (PDR) [66]. A person walking has a clear pattern on the vertical axis, allowing to estimate the number of steps and the step time. With reference points the step length can be estimated. This way propagation of the person can be estimated from the acceleration values by counting steps. This is still considered a dead reckoning method. As it requires the previous location the error can still compound over time.

Inertial sensors are used already in the medical research field, but are mainly used to measure body position, movement, accelerations, or forces [67–69]. Other fields use inertial sensors as a tracking method, but only if high frequency position updates are required and if a point of reference is hard to obtain. For example quadcopters or planes; movement ranges make obtaining reference points difficult and for which the orientation update speed is crucial for control stability.

Inertia navigation systems are applicable because they have a high accuracy, require little power, and are self-sufficient. However, they suffer greatly from error drift. Without a frame of reference, the error can increase significantly. Cenk Acar [63] evaluated the drift for an average system and has concluded the system will have an error of 22 meters per horizontal axis after 300 seconds from the accelerometer noise power spectrum density (PSD) alone. Some papers report significantly better performance characteristics with their design [63–65] and better sensors are on the market for use in fields such as aviation. Although these sensors experience significantly reduced drift, the problem is still present. If an inertia sensor would get regular location and orientation updates to reinitialize, the error increase would be low and the tracking method accurate.

### 3.2.6. Touch sensitive floor

This method uses a type of Touch surface on the floor to detect the paws of the mice. A simple way to construct such a setup is with an acrylic cage on a touchpad, as done in the paper by O.S. Mabrouk [70]. Such a setup allows to use the touch screen lighting to aid in the research. However, any type of touch sensitive kind of surface would suffice. Although a lot of different touch surfaces are available for purchase, they use either resistive of capacitive touch.

A resistive touch surface consists of two layers with a small separation. In each layer are parallel conducting lines. The layers are setup such that the conductive lines are orthogonal to the other layer. When a force is applied to the screen the layers are pushed together closing the gap. This creates an electric circuit loop between the conducting lines of the different layers. By measuring the resistance between each individual line, which lines are touching can be determined. Knowing the location where the wires cross is the point at which the force is applied to the resistive touch surface.

A capacitive touch surface uses either a single conducting film with four voltage sources or a grid of small areas each holding a small charge. When the screen with a single conducting film is touched the volt gap result in a small current into the user. The longer the distance to the voltage source the larger the resistance by the film, due to the increased length. Therefor relative current drawn from each source directly corresponds to the relative distance to the touch location. When a screen using a grid of areas is touched the charge will flow into the user, thus dropping the charge in the area. The location of the charge drop directly relates to the touch location.

O.S. Mabrouk [70] reports one instance where a camera system is used in tandem with a touch system, but never mentions the accuracy of either the separate or combined tracking system. However, they use an available touch screen device. Accuracy of such devices seems rarely mentioned in a specification. However, anyone who has hold a old and modern touch screen can tell that the accuracy has been improving and that they are quite accurate nowadays.

It should also be mentioned that not all touch-screens can handle multiple touch, but in principle the resistive touch and the capacitive touch with multiple areas could with the correct measuring equipment.

### 3.2.7. Tracking methods comparison

All methods found have their own strengths and weaknesses. Surveys like Liu, Hui et al. 2007 [2] and Groves, Paul D. 2013 [3] show a general idea of performance to tracking distance as depicted in Figure 3.5, 3.6. However, these leave a lot of information out which is important to the Neuromate. Table 3.1 shows all the methods with the additional required information on the necessary criteria from the next conclusion section. When viewing the table, it should be considered that a combination of methods often increases the performance. As the paper by Shis y. et al. [25] did by adding accelerometer to the camera system and increasing the accuracy from 8 mm to 1.2 mm. An common example of combined tracking methods is the smartphone, which uses WIFI, cell towers, GPS and inertia sensors for accurate location tracking.

Table 3.1 has several fields stated to be *undetermined*. This label can mean several things. The camera systems have an undetermined value for the orientation accuracy. This is because none of the systems have a reported orientational accuracy, except for the inertial sensor systems. However, the camera systems are likely to be able to meet the orientation accuracy criteria. For the time being, the ultrasonic solutions do not meet the criteria for portable devices due to the possible dimensions of an omnidirectional ultrasonic sensor. The UWB and AOA methods have an unclear satisfaction of the limitations wearable device, because the UWB sensor and the required rotating directional or sensor array for AOA require more power. Tracking with an inertial sensor has indeterminate positioning and orientation accuracy because it depends on the frequency of the reference points and the type and quality of the sensor. Inertial sensors often use a magnetic field sensor to improve orientation. It is used to sense the earth's magnetic field. However, in this case, the cage has a large oscillating magnetic field for the power link, so the magnetic north can probably not be determined.

Whether it is still possible to use the sensor to find north or to use the oscillating magnetic field to determine orientation can only be determined with tests. This is the second reason for the orientation accuracy to be undetermined for inertial sensors in the table. The last indeterminate field is for the localization accuracy for the touch surface method. Although this is likely to be greater than the required 8 mm, it has not been reported.

Table 3.1: The fit of the discussed methods to the criteria from the problem definition. The listed locating accuracies are the lowest reported in the papers, except for the radio methods which are an estimation acquired from the table and figures from [2, 3]. For the other criteria in the table a *no* indicates an unmet criterion and a *yes*, a fulfillment. All radio methods and frequencies other than UWB and AOA are grouped due similarities with concern to the criteria. The table contains several *undetermined* fields. The fit of the method for that criterion is unknown.

| **Method** | **location <8 mm** [da-tr-la] | **orientation <10 deg** [da-tr-oa] | **multiple rodents** [mr] | **limitations wearable device** [wd-ps][mi-w][mi-s] |
|---|---|---|---|---|
| Force plate | 1 mm | no | no | yes |
| Camera | 8 mm | undetermined | yes | yes |
| RFID | room size | no | no | yes |
| Ultrasound | 100 mm | no | yes | undetermined |
| UWB | 20 mm | no | yes | undetermined |
| Radio AOA | 2 m | undetermined | yes | undetermined |
| Radio other | 2 m | no | yes | yes |
| Inertial | undetermined | undetermined | yes | yes |
| Touch | undetermined | no | yes | yes |

## 3.3. Conclusion

*The goal of this literature study is to find any technical approach which could track the locations and orientations of group housed rodents.* Many tracking methods have been found, each with different strengths and weaknesses. To determine if any of these tracking methods is viable for the Neuromate and in extension if Neuromate tracking would make for a viable graduation topic, the following question needs to be answered: *How can the location and orientation of multiple group-housed mice be acquired with a minimal accuracy of 8 mm and 10 deg, without interfering with the other aspects of the Neuromate?* This question contains criteria which the chosen methods need to fulfill to be feasible for the Neuromate. It is not necessary for a single method to fit both the location and orientation criteria [da-tr] since a combination of methods could be used. However, the other criteria need to be met by any implemented method. These are the discriminatory criteria and are *simultaneous single cage multiple rodents tracking* [gh][mr] and *no interference with the other aspects of the Neuromate* [or]. The latter is a combination of criteria [1] . Among those the important criteria to consider are the power [wd-ps] and physical dimensions limitations [mi-w][mi-s] of the wearable device. Table 3.1 shows the different methods and their fit to the criteria. Camera tracking with the lowest tracking resolution of 8mm, capability to track multiple rodents, and its lack of wearable device could meet all the important tracking requirements of Neuromate. Although the tracking resolution needs to be determined, since it is effected by implementation and sensor performance. The fit of camera tracking to the main tracking criteria means there are viable tracking methods found in the literature study and thus the topic is viable for graduation.

[1] [a..z] refer to criteria, which are collected in chapter 4.1. An alphabetical list of the criteria used can be found in Table 4.1

# 4
# Design criteria

Criteria appear at various parts in this report, each uniquely indicated using a reference with [a..z]. They are labeled and fitted in the tractability matrix along with a description. This chapter collects all criteria including their properties. In the next section we will take a criteria and quantify its properties.

## 4.1. Criteria quantification

To validate if the proposed solution fits the criteria, some identified criteria are lacking proper quantification. In this paragraph we specify the quantification with the citations to the sources used.

Multiple [mu]

To observe social interaction and to allow the establishment of a social hierarchy multiple mice are required for an experiment. Although two mice will suffice to get social interactions, additional mice are needed for more complex social constructs. The mean litter size of a mouse is between 5-7.5 offspring. The criteria used by previous works on the Neuromate and mentioned during the meeting is a desire to support a group of at least four mice. This will be considered the minimum for the multiple mice criteria [71].

Long term [lt]

F. E. Hoebeek gave an estimation for the duration of studies dealing with the effects and treatment of early life events of about 3 months. Such studies can draw a conclusion with data from the early life events, this may be an event before birth, until reaching metal maturity. The time it takes for a rodent to be mentally mature is some debate among the scientist in the field. However there seems to be consensus that it takes between 6 to 8 weeks, or 3 months [72]. Therefore, a minimum study duration of 3 months is required to be able to satisfy everyone's definition of maturity in the field.

Sensitive behaviors - Track - Location accuracy [sb-tr-la]

To determine the desired tracking accuracy the size of the rodent and offset from the measured location to the point of interest should be considered. For instance the center of mass is measured and the head of the rodent is of interest to the study. This would results in an unknown offset distance, which needs to be compensated and would create an tracking error. It is stated in [42] that a spatial resolution lower than 5 mm is not needed for home cage behavioral research. A Center of Mass (CoM) estimation lower than 5 mm does seem sufficient. A mouse head plus body length is between 77 and 97 mm [71]. Rounding the size of a small mouse to 80 mm the accuracy of 5 mm is relatively 6.25 % of the body length. However, the main function of the location tracking is the estimation of the social interaction, which is mostly related to the location of the head. To quantify most the criteria the value is set to be about 10% of the relevant mouse characteristics. Considering the length of a mouse head and body is roughly 80 mm the location tracking criteria requires an accuracy of 8 mm.

Sensitive behaviors - Track - Orientation accuracy [sb-tr-oa]

To determine the accuracy criteria for the orientation tracking, the location error created by the orientation error should be considered. This is not easy to determine as it depends on the tracking method. For example accelerometers rely heavily on the orientation to determine the next position. However, a camera system does not require the orientation for location tracking unless some Euler estimation is done using velocity. To get around this the absolute error created by the angle relative to body length is considered. If the nose is what is tracked and the location of the start of the tail is known, an error of 10% body length would occur at an angle of $arcsin(8/80) \approx 10$ deg.

Social interactions - Wearable device - Weight [si-wd-we]
To deem the wearable device minimally invasive it should not be too heavy. By previous works of the Neuromate the weight criteria of the wearable device have been set at 1g. 1g would be below the 10% compared to the complete body weight, since a mouse weighs between 15 and 26 g [71]. However, the weight should be compared to that of the head, since that is the specific part of the body it will be mounted to. Then it is relatively heavy compared to the head, but light considering the functions it will perform. It is unlikely to create a lighter wearable device.

Social interactions - Wearable device - Volume [si-wd-vo]
The volume of the wearable device should be limited for the same reasons. Previous works have set the volume limit to be 10x10x10 mm. Mice are known to be able to cope with a wearable device of this size even though it is large compared to the size of the head. The $\leq 10mm^3$ is a small volume for all the desired functions and therefor cannot be set any smaller without becoming a serious limitation.

## 4.2. Traceability matrix

In order to keep track of the different criteria that appear in this document they get a letter label [a..z] and are placed in the traceability matrix 4.2. This overview reduces the chance of missing a criterion in the design stage and it will make it easier to track their origin and meaning. Each criterion is sorted in one of the following abstract levels: customer, system, and component. This way we can construct and analyze the criteria hierarchy.

Each criterion is uniquely labeled using the abbreviation codes listed in Table 4.1

Table 4.1: Criteria abbreviations used in this paper.

| | |
|---|---|
| ar = analysis real-time | nt = newest technology |
| cn = correlated neurolink | oa = automated observations |
| co = control input | oa = orientation accuracy |
| dg = different goals | oc = off-the-shelf |
| di = data importance | op = optogenetics |
| dl = down-link | pc = power consumption |
| ec = ECoG | pr = proximity |
| fl = flexible | ps = power supply |
| fs = functional state | rf = roaming freely |
| gh = group housing | ri = researcher interface |
| ib = influence the brain | sb = sensitive behaviors |
| in = interfere | sd = store data |
| in = invasive | se = separation |
| la = location | si = social interactions |
| ld = large data | tr = tracks |
| lt = long term | ul = up-link |
| mf = main function | vo = volume |
| mi = minimal intervention | wd = wearable device |
| mu = multiple | we = weight |
| mv = measures a variety | wi = wireless |

Table 4.2: Traceability matrix. Lists all the criteria grouped by (sub)system component. Each criterion has two underlined letters which form the label for that criterion. The criteria are sorted in three different abstraction layers: costumer, system, and component.

| Tracking | Customer | System | Component | Description |
|---|---|---|---|---|
| 1 | mf | | | The device's main function is to enable the Erasmus MC to perform their desired studies on mice |
| 2 | dg | | | The device is applicable for different study goals |
| | dg | ib | | The device can influence the brain activity of individual mice |
| | dg | ib | op | The device µLEDs and optogenetics to influence brain activity |
| | dg | co | | The device can determine and set the timing and intensity of control inputs |
| 3 | dg | fl | | The device is multi-purpose to accommodate a flexible study goal |
| 4 | dg | mv | | The device measures a variety of types of data |
| | dg | ec | | The device records ECoG data |
| 5 | nt | | | The device is developed with the newest technology |
| 6 | nt | oc | | The device is developed mainly using off-the-shelf components |
| 7 | mu | | | The device allows the study of multiple, ≥ 4 mice, at the same time |
| 8 | gh | | | The device allows the group housing of mice in a cage of 40x40x20 cm (lxwxh) |
| 9 | lt | | | The device is capable of long-term studies, ≥ 3 months |
| 10 | lt | ld | | The device can handle a large amount of data |
| 11 | lt | ld | ar | The device analyzes data in real-time |
| 12 | lt | ld | di | The device can determine data importance |
| 13 | lt | ld | sd | The device is required to store specific data of interest |
| | lt | ps | | The wearable device requires a power supply |
| 14 | lt | ps | pc | The wearable device power consumption should be $\leq 50mW$ |
| 15 | mi | | | The device performs with minimal intervention |
| 16 | mi | in | | The device is minimally invasive |
| 17 | mi | rf | | The device allows the mice to roam freely |
| 18 | ri | | | The device eliminates the need for researcher interference |
| 19 | ri | ao | | The device is capable of automated observations |
| 20 | ri | ao | fs | The device's functional state can be monitored during an experiment |
| | ri | ul | | The wearable device has an up-link |
| | ri | dl | | The wearable device has an down-link |
| 21 | si | | | The device allows the mice to engage in social interactions |
| | si | wd | | The Neuromate has a wearable device component with implants and a box fixated to the head |
| 22 | si | wd | wi | The wearable device is wireless |
| 23 | si | wd | we | The wearable device weight is $\leq 1g$ |
| 24 | si | wd | vo | The wearable device volume is $\leq 1cm^3$ |
| 25 | sb | | | The device can determine sensitive social behaviors |
| 26 | sb | tr | | The device tracks the location and orientation of each mouse in the cage |
| 27 | sb | tr | la | The device needs to track location with an accuracy of $\leq 8mm$ |
| 28 | sb | tr | oa | The device needs to track orientation with an accuracy of $\leq 10deg$ |
| 29 | sb | tr | pr | The device needs to determine mouse proximity and relative orientation |
| 30 | sb | tr | cn | The tracking data needs to be correlated to the neurolink data |
| 31 | | | se | Interference between test setups should be eliminated with separation of $\geq 100cm$ |
| 32 | | | in | additional components of the neuromate must not interfere with the others |

## 4.3. Tracking design criteria

The criteria labeled in Table 4.2 with a numerical value in the track column is considered an design criteria applicable for the tracking component of the Neuromate project. To determine the importance of these criteria they are sorted into three different groups.

If a criterion is considered crucial and the device won't function if the criteria is not met, it is an must have. Should haves are important for the functionality, but not meeting these criteria will not break the device. Could haves improve the performance of the criteria but are not needed or applicable to the direct functionality. This criteria classification originated from software development in 1994 and is known as the MosCoW method [73].

The total list of criteria is ranked and the results of "Must have" are listed in Table 4.3, the criteria results of "Should have" are listed in Table 4.4, the criteria results of "Could have" are listed in Table 4.5.

Table 4.3: This table collects the "Must have" criteria. These criteria prioritization is used in the Harris method.

| Must have | |
|---|---|
| nr | Description |
| 1 | The device's main function is to enable the Erasmus MC to perform their desired studies on mice |
| 4 | The device measures a variety of types of data |
| 7 | The device allows the study of multiple, ≤ 4 mice, at the same time |
| 8 | The device allows the group housing of mice in a cage of 40x40x20 cm ($l$x$w$x$h$) |
| 10 | The device can handle a large amount of data |
| 11 | The device analyzes data in real-time |
| 13 | The device is required to store specific data of interest |
| 14 | The wearable device power consumption should be $\leq 50mW$ |
| 21 | The device's functional state can be monitored during an experiment |
| 22 | The wearable device is wireless |
| 23 | The wearable device weight is $\leq 1g$ |
| 24 | The wearable device volume is $\leq 1cm^3$ |
| 9.1 | The device can determine sensitive social behaviors |
| 26 | The device tracks the location and orientation of each mouse in the cage |
| 29 | The device needs to determine mouse proximity and relative orientation |
| 32 | additional components of the Neuromate must not interfere with the others |

Table 4.4: This table collects the "Should have" criteria. These criteria prioritization is used in the Harris method.

| Should have | |
|---|---|
| nr | Description |
| 2 | The device is applicable to different studie goals |
| 3 | The device is multipurpose to accommodate a flexible study goal |
| 6 | The device is developed mainly using off-the-shelf components |
| 9 | The device is capable of long-term studies, ≥ 3 months |
| 12 | The device can determine data importance |
| 15 | The device performs with minimal intervention |
| 16 | The device is minimally invasive |
| 17 | The device allows the mice to roam freely |
| 19 | The device is capable of automated observations |
| 27 | The device needs to track location with an accuracy of $\leq 8mm$ |
| 28 | The device needs to track orientation with an accuracy of ≤ 10 deg |
| 30 | The tracking data needs to be correlated to the neurolink data |

Table 4.5: This table collects the "Could have" criteria. These criteria prioritization is used in the Harris method.

| Could have | |
|---|---|
| nr | Description |
| 5 | The device is developed with the newest technology |
| 18 | The device eliminates the need for researcher interference |
| 20 | The device's functional state can be monitored during an experiment |
| 31 | Interference between test setups should be eliminated with separation of $\geq 100cm$ |

For the Harris Profile we use the customer criteria prioritized using MoSCoW [73] ranking.

# 5
# Valid tracking solution alternatives

## 5.1. Tracking methods and their fit for application in the Neuromate project

All methods with a potential capability to fulfill all *must have* criteria of the Neuromate project are elaborated. The elaborations contain estimation for when the method would be applied in the Neuromate project. This overview acts as the basis for a decision in what technology to apply. This is the base for an informed decision, based on the methods available found in chapter 3. The five methods considered are:

1. Ultrawideband
2. Ultrasound
3. Inertial navigation
4. Touch sensitive surface
5. Camera

The analyzed methods are compared using a Harris profile to find an optimal combination, suitable for application in the Neuromate project.

### 5.1.1. Ultrawideband (UWB)

One of the methods of detecting object is the application of reflection of signals by objects, well known examples are RADAR for aviation and SONAR in marine application.

**Central sensor**

Ultra wide-band is a method that emits signals in a large range of frequencies and has an array of sensors to capture the reflections. As each surface type has specific reflection and absorption characteristics, the object can be detected based on the signals received. An advantage of using UWB for indoor tracking is the use of pattern matching; to take advantage of the unique frequency-profiles created by signals bouncing and penetrating objects and surfaces. This requires a mapping of the patterns on each point of the grid.

In order to detect an object by reflection line-of-sight (LOS) makes the detection easier/less error-prone. In the Neuromate project, mice can hide behind or under objects. When the test bed contains objects, a base map needs to be created before the mice are introduced.Creating these base maps are required as part of the test calibration at the start of each test run. Something to consider is that movable objects can distort the results when displaced by the mice.

When the mapping is done, the situation for our setup is similar to other research projects. Reaching a location accuracy of about 20 mm [55]. This method does not reach the desired accuracy of 8mm [27].

**Wearable sensor**

An alternative implementation of UWB would be having the wearable device receive UWB signals to be able to track its location. With the UWB available on the wearable device it is easier match the time-stamp of the location data, Neurolink data, and the closed loop control. Additionally the controller has quicker access to this information. This means extra electronics will need to be fitted in the wearable device, including an antenna. To track the proximity between mice, continuous comparisons of the locations in the need to be made. The location on the wearable device needs to be shared with the supervisory control to be able to calculate the proximity of the mice. If the control signal to the mice is dependent on the proximity of other mice, the supervisor needs to transfer back the proximity. This round trip communication introduces delay as it requires extra steps.

Another aspect to consider is the interference that is caused by different neighbouring cages with other experiments, where multiple tests are running in parallel. This can be accounted for if the cages are not positioned relative close to each other.

**Preliminary assessment**

In case the UWB and WiFi use (parts of) the same frequency spectrum, this UWB solution would suffer reliability as its signals are significantly weaker compared to WiFi signals.

As the UWB described above only detects the location, evidence is needed to prove that this method can determine the orientation by applying multiple antennas on the wearable device. It is also uncertain if it would fit within the wearable device constraints, as all the space is occupied by the other required components of the Neuromate project. The orientation criteria could be fulfilled by an additional implemented tracking method. However despite the orientation it does fit the other *must have* criteria. The method also fulfills most of the other *should and could have* criteria, except for the location accuracy criterion [27].

### 5.1.2. Ultrasound

For the methods that determine the location by the time of flight (TOF) the velocity of the signal is an important factor. In order to accurately determine the object location the arrival times need to be determined with an accuracy relative to the travel velocity. The most feasible method using the travel time is Travel Distance Over Air (TDOA). In the 40 by 40 cm cage a distance from the sender inside the cage to a receiver outside the cage could be 20 cm for example.

In free space a electromagnetic (WiFi) signal has a velocity of 299,700 km/s. The time of flight to from the sender to receiver is $\frac{0.2}{299700000} = 667$ ps. Now, lets take 8 mm as the minimum required spatial resolution.
In an ideal situation, the rodent moves the 8 mm closer or away in the direction of one of the other receivers. In this case the measured time of flight difference of 8 mm to be detected is $\frac{8^{e-3}}{299700000} = 26.7$ ps. With an ideal accuracy of 10 %, a minimal measurement frequency is needed of $1/2.67e-12 = 375$ GHz.

In order to make the detection of location and orientation based on ultrasound work, a lower velocity could be used like when applying ultrasound. Sound travels at 343 m/s though air at 20C °.
The travel time to from the sender to receiver is than $\frac{0.2}{343} = 0.583$ ms. This would result in a required measurement time of $\frac{0.2}{343} - \frac{0.192}{343} = 23.3$ us. With an accuracy of 10 % this would require a minimum measurement frequency of $1/2.33^{e-6} = 429$ kHz. For implementation within the project the measurement frequency can be chosen to be considerably higher.

Using ultrasound the signal strength experiences a gradual loss that due to its propagation through air, which needs to be considered. To elaborate on this attenuation for ultrasound waves traveling trough air the following simplified equation is used:

$$\text{Attenuation} = \alpha \times \ell \times f \tag{5.1}$$

Here $\alpha$ is a constant value that depends on the medium, [dB/(MHz × cm], $\ell$ is the travel distance [cm] and $f$ is the signal frequency[MHz]. This equation has a linear attenuation relative to distance[dB]. In situations where the echoes also arrive at the location, or when signals are blocked by a object with a different value for $\alpha$ the measurement using this method will be distorted.

To optimize the received signal strength the absorption and echo effects could be reduced by calibrating the attenuation, therefore accounting for reflections from stationary object, for example the cage itself.

To determine if the reflected echoes can be distinguished from the direct signals the signal strength of the echo should be compared to the direct signal. A high attenuation would be preferred as the additional distance from the echo travels would result in a larger signal strength loss. According to A. Vladišauskas et al. 2004 [74] higher frequencies have a larger attenuation. The attenuation for a 1 MHz signal at 20 C°is about 160 dB/m.

To get an idea for the expected performance the a worst-case scenario is considered. For this scenario the signal will be traveling perpendicular to cage wall and bounce back to the receiver. We assume a required spacial resolution of 1 cm. Thus the least amount of additional travel occurs in the situation where the wearable device is 1 cm from the wall as this add the least amount of additional travel distance for the echo. Echo strength is reduced compared to the direct signal by the loss during reflection and the additional travel distance.

The signal strength difference due to travel distance is $160 \times 0.02 = 3.2$ dB. The amount of reflection can be determined by the acoustic impedance of the different materials [75]. Assuming the cage is made of Plexiglas, since it is easy to use and allows for visuals observations by the scientist, the reflection is obtained with the following equation.

$$\alpha_R = (\frac{Z_1 - Z_2}{Z_1 + Z_2})^2 = (\frac{0.0004e-4-3.2e-4}{0.0004e-4+3.2e-4})^2 = 0.9995 \tag{5.2}$$

This is a power decrease of about 0.0022 dB. The echo will be about 3.2 dB weaker than the mean signal. Considering this would be on the other side of the cage the mean signal would have lost $160 \times 0.29 = 46.4dB$ already. The 3 dB difference would be challenging to filter.

Other methods that use ultrasound for tracking are dealing with the unwanted echoes by ensuring that the reflections arrive in 'another' time window, such that the echos arrive outside the time window of the desired signals and can be removed [60–62].

Since the rodents will get close to the cage wall this is not possible unless there is no cage wall or the wall does not reflect sound. Both of which would require a significant challenge to implement with other undesired side effects.

**Preliminary assessment**

In this scenario the ultrasound receiver is installed in the wearable device. The receiver would require at least three sensors. This would cause the wearable device on the mice to exceed the maximum size.

In the second ultrasound scenario the emitter is installed in the wearable device. The emitted signal would need to be powerful enough to not attenuate too much after one reflection. This would stress the power budget of the wearable device.

### 5.1.3. Inertial measurement unit (IMU)

Inertial sensors, also known as internal measurement units in the field of aviation, come in a large variety of specifications. As our power and size limits the performance of these systems below their minimum threshold. An example of what kind of accuracy you can expect from these inertial navigation sensors is given by Bhattacharyya et al. [4] and listed in Table 5.1. A lower accuracy will result in faster runaway errors. These errors can be mitigated by more frequent calibrations.

The calibration frequency is determined by the acceptable error. Assuming the worst-case scenario and the accuracy numbers listed in Table 5.1 we get a relative error of $20\% \times 40cm = 0.8cm = 8mm$ after 5.5 minutes. The heading error of 10 degrees is harder to determine, but due to the increasing error gain over time with dead reckoning and the time to heading error of 3 degrees at 7.2 minutes and 25 degrees instability after an hour calibration should occur every 30 minutes.

In order to stay within our location criteria a calibration needs to be sent every 5.5 minutes. A calibration at every 5.5 minutes is too frequent to run any kind of test, but it is an acceptable window in order to send a calibration point if a decent additional tracking system is used in tandem.

Comparison of gyro grade with respect to the effect of uncompensated bias to the PDR error build-up

| | Navigation | Tactical | Consumer |
|---|---|---|---|
| Bias instability (°/h) | 0.0035 | 1 | 25 |
| Time to 2% relative position error | 27 days | 2.3 h | 5.5 min |
| Time to 3% relative position error | 41 days | 3.4 h | 8.3 min |
| Time to 3° heading error | 35 days | 3.0 h | 7.2 min |

Figure 5.1: This table indicates the what kind of accuracy to expect from different kinds of inertial sensors. The table originates from page 74 of the 3rd edition Handbook of Signal Processing Systems Bhattacharyya et al. [4].

**Additional arguments for using an IMU**

As a second tracking solution is required in order to correct the position error of an inertial sensors to function well enough for Neuromate, what benefits does the inertial sensor contribute?

The power and weight of the inertial sensor are within our criteria, but nonetheless it will take valuable space and power, which could be used for other functions. Secondly, it will take bandwidth from the up-link at least, and from the down-link as well.

The advantage from using an inertial sensor is that the acceleration data from the mouse movements inside the cage can be obtained. Each signal available inside the wearable device can be used as input for a closed loop control system. The acceleration data can be used by itself to determine certain behaviors such as; sleep patterns, breathing, and eating.

Higher location accuracy and more frequent location updates could be critical for experiments. Finally the data could be used to identify the mice even when close to other mice, which could be a compelling argument for inertial sensor implementation.

The benefits of the availability of acceleration data to determine some simple behaviors can be done through vibrations. The update frequency of location data is directly related to the frequency by which the data of the inertial sensor can be saved, which is relatively high compared to other solutions.

The increased accuracy realized by combining multiple tracking systems is realized by comparing the outputs of the systems. Available selection strategies are, averaging the values of the different systems, or taking the best value if the error margin is known. If the error margin of both tracking solutions are independent, taking the average will cancel out a portion of the errors. If the error margins are dependent, taking the value with the smallest error margin is preferred.

If the inertial tracking is under performing an additional sensor can be used for this missing time period to determine the position. The deduced reckoning (known as dead reckoning) becomes a problem when the time span between calibration points gets longer.

**Compensating dead reckoning**

There are two ways to correct the dead-reckoning calculations. One approach is to upload the acceleration data as it is read from the sensors. With this data, an estimation on the reliability of the data can be made, and the best one can be chosen to calibrate the inertial sensor data. This method is easier to implement than a wearable device, but due to latency would not allow the dead reckoning positioning to be used in a closed loop system within the wearable device on the mice. The direct acceleration data could still be used for the closed-loop system to detect vibrations related to their behaviors.

The second method is by sending reliable position and orientation data to the wearable device in order for the microprocessor to re-calibrate the dead-reckoning positioning. In this case the wearable device would be able to determine its own location with a high frequency for the closed-loop system. However this takes additional computer power at the central system for calculating the location for each mouse as well as additional bandwidth in order to send the correct re-calibration data to the correct mice.

One last important note, an inertial sensor should also allow the system to identify individual mice. The identification accuracy is hard to estimate without testing it with live mice. As each mouse will move independently allowing its unique acceleration pattern to be determined given enough observation time. The amount of samples needed to identify a mouse depends on the activity of the mice. The IMU recorded acceleration pattern would be uploaded and for comparison by the central system to correlate this data with a second tracking solution to determine the identification of each mouse.

**Preliminary assessment**

Initially the inertial measurement unit is a viable solution. However, because it has a dead reckoning problem it requires location updates. This means that a secondary tracking solution is required for the inertial sensor to work. With another tracking solution the inertial tracking is obsolete unless the system requires a higher accuracy or an identification method. When considering to add inertial sensors to the tracking solution other drawbacks are to be considered. The drawbacks are:

1. additional space required by the IMU chip;
2. the power-budget for the IMU;
3. the bandwidth criteria for the communication;
4. the need to solve the dead reckoning.

### 5.1.4. Touch-sensitive surface

When the floor of the cage is divided in segments equipped with a sensor, the presence of a mouse in that segment can be determined. In order to be relevant for multi-rodent tracking a multi-touch capability is needed of which two types are available: resistive touch and capacitive touch.

For our test setup high-end touch surfaces may be needed in order to be able to detect 4 mice each with 4 legs in a single enclosure, requiring at least $4 \times 4 = 16$ points without problems.

Multi-touch sensor are very common for detecting human fingers input, however detecting 16 concurrent touch-points are less common.

The resistive touch surface requires a force above the threshold on a single point to detect an impression. Assuming the weights range of a mouse is 15 - 26 g [71]. A paw would support $\frac{15}{4} = 3.75$ g on average. Although the required touch force of a screen is a hard to find in the specifications, detecting a touch force of only 3.75 g would be close the the minimum threshold.

The capacitive touch surface requires that there are no conductive materials in the cage and that the paws directly touch the sensor. This would mean that the rodents could not have sawdust on the floor for comfort in the enclosure, as the sawdust would interfere with the required direct touch of the sensor by the paws.

**Preliminary assessment**

Although the location tracking using a touch sensitive surface may be a potential fit for the Neuromate project, the real interest is in the location of the snout. There would be some error introduced by guessing the location of the snout from the location of the paws. This method is likely to have issues determining the heading of the mice, as it would not be able to distinguish between front and rear paws. Although impossible to say without testing, a force touch pad might be able estimate the heading of the mouse by detecting the weight distribution.

An advantage of this method is the absence of additional components required in the wearable device and thereby no issues with power constraints, volume or weight. The location information is available outside the cage. This means that it is easy to determine proximity between mice. The proximity needs to be sent to the wearable device in order to be available for the closed-loop control. However the paws can not be distinguished between different mice. When mice are close to each other it can be difficult to determine which paw belongs to which mouse. Lastly the capacitance version of the touch surface is very likely to interfere with the magnetic field oscillating inside the cage to power the wearable device and vice versa. The touch surface would consist of conducting materials, which would change or even block the magnetic field. Conversely the electric charges for the capacitive touch screen would be influenced and the resistive field conducting lines would act like antennas. They would receive and conduct power from the field into the measuring electronics. This would render the touchscreen unable to function properly.

In summary a touch surface is an easy to implement and a high-accuracy tracking method for multiple group housed mice. It also provides additional information on the walking gait. However in case of the Neuromate the interference with the essential magnetic field deems these type of sensors inapplicable.

### 5.1.5. Camera

Most mice tracking methods apply infrared cameras for observation and tracking, as mice are unaffected by it. Generally there are two methods for detecting mice. The first method uses the difference in hue compared to the background. The second method works by recognizing rodent features by means of pattern matching, such as an eye or the base of an ear.

Both methods are self-calibrating and auto detecting. This gives the camera based methods a big advantage in mice tracking applications as its supports swapping of mice without the need for reconfiguration of the setup. Camera based methods are also animal friendly as these methods do not rely on markers that rodents are likely to remove. However, in the case of the Neuromate, the mice are wearing a small computing and communication device on the top of their head. They are unlikely to try to remove the device because of its strong attachment.

For camera tracking of rodents, the availability of these small devices offers an unique opportunity to associate a marker to each rodent, which could increase the tracking accuracy. The marker would be attached to the device on the head of the rodent, enabling a more accurate determination of the location of the snout. The marker would also allow us to label each mice to distinguish them with a camera.

Two types of markers could be used. An active marker sending infrared (IR) signals using IR light emitting diodes (LEDs) or a passive marker reflecting the IR light with a pattern.

**Active markers**

Using an IR LEDs marker to determine the location of a mouse can be done with a single LED on each mouse and a central IR camera. The IR camera picks up the signal emitted by the LED and determines its location based on the location in the image. To also obtain the orientation of the mouse the marker requires additional LEDs that can be distinguished by the camera from the first LED. On the IR image the two LEDs can be used as two points of a line, a single LED gives a known start location and the slope of the line indicates the orientation. In order to distinguish the two LEDs each LED should have a unique property. This unique property can be realized using a unique coloring or the LED have a unique blinking sequence. Since the testing is done with IR as to not disturb the rodents, a unique blinking sequence would be preferable.

One way of organizing this would be by having one LED on at all times and have the other blink at a specific rate. If a LED marker is used to distinguish between different mice a unique property needs to be added. Since the blinking pattern or frequency of one LED is still free this pattern could be used for this purpose. Each mouse therefor can get a unique blinking pattern that indicates which mouse it is. The blinking pattern needs to be in a specific frequency band. The low end is needed to make sure the orientation tracking is not lost when the LED is off for too long. The high end is set to to the limitation of camera frame-rate. The sampling rate, in units of samples per second, is at least twice the highest frequency (bandwidth) in Hz of a function or signal to be sampled. A standard camera has a frame-rate of about 30 frames per second. In that case the maximum frequency would be ≈ 29 Hz.

Assuming the LED is not blocked and its bright light can only be detected by a single pixel of the IR camera, the worst-case scenario would be that the LED is positioned in the corner of a pixel. The error would be

$$\text{Error} = \left(\frac{d_\Delta}{2}\right) \times \sqrt{2} \tag{5.3}$$

Where $d_\Delta$ is the distance between pixels in mm. To meet the criterion, this value should be below 8 mm [27], the distance between pixels need to be smaller than

$$\frac{8}{\sqrt{2}} \times 2 \approx 11.31 \text{ mm} \tag{5.4}$$

So, for the enclosure of 40 by 40 cm, a camera with a minimum resolution of

$$\frac{400}{11.31} \approx 35.36 \text{ pixels} \tag{5.5}$$

Therefor 36 by 36 pixels is at least required to meet the 8 mm location tracking criterion.

The orientation accuracy criterion is likely to increase the specifications for the camera. Assuming a LED is attached to the front and back-side of the box on the head of the mouse, the maximum distance between the LEDs would be 1 cm. The maximum orientation error would happen if the maximum error for each LED location would be in opposite directions. For 10 degrees the maximum error for each side would be:

$$\frac{tan(10)}{2} \approx 0.88 \text{ mm} \tag{5.6}$$

For the same tracking scenario a location error of 0.88 mm would mean a pixel distance of:

$$\frac{0.88}{\sqrt{2}} \times 2 \approx 1.2 \text{ mm} \tag{5.7}$$

To cover the total cage, the minimum camera resolution would be:

$$\frac{400}{1.2} \approx 320.8 \text{ pixels} \tag{5.8}$$

This means a minimum amount of pixels for the camera to accurately track the mice would be 321 by 321 pixels.

**Passive marker**

A passive marker is a IR reflecting pattern that be applied on the box attached to the head of the mice. The maximum size of the marker is the surface area of the wearable device. An observation position from the top of the cage is preferable as in this position it is most likely that the mice are visible for the camera with a small chance for other mice to block the visibility.

A custom made 2D IR marker design is shown in Figure 5.2. The corners of the 2D IR marker have to contrast with the fur of the the mouse. These squares are used to locate the 2D IR marker. The subsequent two squares between the corners on the front-side are black and the two squares between the corners on the back-side are white. With this pattern the front-end and back-end can be determined and help with the 2D IR marker validation. In the remaining area there are two sets of squares, one set on each side. Set A has 4 squares and is exclusively used for the ID, set B has 2 squares. Each set has to consist of one white and one black square. The two sets A and B combined encode part the ID and help with 2D IR marker validation. In total the 2D IR marker has 6 ID squares, one on each side and 4 in the middle. This allows for $2^6$ =64 unique IDs. As the typical sample groups are smaller, some of the squares may be allocated for ID error checking. Even a single square allocation would greatly reduce the number for false IDs. With one square for error detection the remaining squares would allow for $2^5$ = 32 unique IDs and with a two-square error correction the remaining squares would allow for $2^4$ = 16 unique IDs.

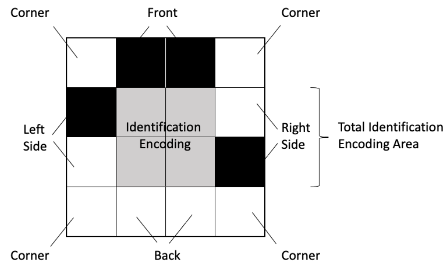


Figure 5.2: This is an example of the passive IR marker based on a 4 by 4 grid. The corner squares are used to find the marker and are in strong contrast with the mouse color. The bottom and to squares are orientation and marker validity squares. The right and left side are marker validity squares and ID squares. The center 4 squares are for ID only.

**Prelimenary assessment**

In summary, although some of the worst-case scenarios are not considered when calculating the required resolution for the active marker, such as dust, motion blur and the imperfect pixel spread across the area are not taken into account. Additional steps in the software could be used to increase accuracy as well, such as averaging of several IR camera images or other types of filtering. Thus the estimation is that the passive marker could be track with a relative low resolution (640×480) IR camera, but the 2D IR marker would require a higher resolution. Additional calculation are required to get a good estimate of the required camera resolution for the passive marker, but its likely that a higher resolution (1920x1080) IR camera's would be sufficient. Different marker types have different criteria. Although the active LED marker requires a lower camera resolution, but it does require some power and input for the blinking LED. These will be small compared to other parts of the wearable device and are within the criteria. Compared to the active marker, the passive 2D IR marker requires an higher camera resolution, but would be able to identify the mice at a higher frequency. The camera tracking method also fulfills almost all other *must-, should- and could-have* criteria. The difficulties for this method are:

1. The height of the test setup may be increased to get the camera at the correct distance.
2. Real-time image processing.
3. Large data handling and compression.

Although these challenges with the method should be considered, they are manageable.

## 5.2. Most suitable concept

For the Neuromate project, a method to provide tracking and identification of multiple group-housed rodents to support the Neuromate program is desired.

Table 5.1: Harris criteria matrix. Lists all the major user criteria.

| Requirement | MoSCoW | Description |
|---|---|---|
| 1 | Must have | The device's main function should allow ErasmusMC, LUMC and UMCU to perform their desired neuroscientific studies on mice |
| 7 | Must have | The device allows the study of multiple (≥ 4), mice, at the same time |
| 21 | Must have | The device allows the mice to engage in social interactions |
| 9.1 | Must have | The device can determine sensitive social behaviors |
| 32 | Must have | Additional components of the neuromate must not interfere with the others |
| 2 | Should have | The device is applicable for different study goals |
| 9 | Should have | The device is capable of long term studies, ≥ 3 months |
| 15 | Should have | The device performs with minimal intervention |
| 31 | Cloud have | No interference between test setups, when cages are separated ≥ $100cm$ |
| 5 | Could have | The device is developed with the newest technology |
| 18 | Cloud have | Eliminate of the need for researcher interference |

### 5.2.1. Harris profile

Based on the criteria elicitation from Section 5.1, the prioritized customer criteria, listed in Table 5.1 using the Harris method. For each described method in Section 5.1 the rating of major criteria of Table 5.1 are scored. The result of this scoring is reported in Table 5.2. In the Harris profile tables, the method that uses the camera has the best fit to the major criteria for the Neuromate project.

Table 5.2: Harris profile of the major criteria for each method described in 5.1 for the Neurmate project

| Requirements | | Ultrawideband | | | | | Ultrasound | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | - - | - | + | ++ | | - - | - | + | ++ |
| criteria 1 | | | | | ✓ | | | | ✓ | |
| criteria 7 | | ✓ | | | | | | | ✓ | |
| criteria 21 | | | ✓ | | | | | ✓ | | |
| criteria 9.1 | | ✓ | | | | | ✓ | | | |
| criteria 32 | | | ✓ | | | | | ✓ | | |
| criteria 2 | | | ✓ | | | | | | ✓ | |
| criteria 9 | | | ✓ | | | | | | ✓ | |
| criteria 15 | | | | ✓ | | | | | ✓ | |
| criteria 31 | | | | | ✓ | | | | | ✓ |
| criteria 5 | | | | ✓ | | | | | ✓ | |
| criteria 18 | | | ✓ | | | | | | ✓ | |

| Requirements | | Passive camera | | | | | Active camera | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | - - | - | + | ++ | | - - | - | + | ++ |
| criteria 1 | | | | | ✓ | | | | | ✓ |
| criteria 7 | | | | | ✓ | | | | | ✓ |
| criteria 21 | | | | ✓ | | | | | ✓ | |
| criteria 9.1 | | | | | ✓ | | | | | ✓ |
| criteria 32 | | | | | ✓ | | | | ✓ | |
| criteria 2 | | | | | ✓ | | | | | ✓ |
| criteria 9 | | | | ✓ | | | | | ✓ | |
| criteria 15 | | | | | ✓ | | | | | ✓ |
| criteria 31 | | | | | ✓ | | | | | ✓ |
| criteria 5 | | | | | ✓ | | | | | ✓ |
| criteria 18 | | | | | ✓ | | | | | ✓ |

| Requirements | | Touch-sensitive surface | | | | | Inertial navigation | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | - - | - | + | ++ | | - - | - | + | ++ |
| criteria 1 | | | ✓ | | | | | ✓ | | |
| criteria 7 | | ✓ | | | | | | ✓ | | |
| criteria 21 | | ✓ | | | | | | | | ✓ |
| criteria 9.1 | | ✓ | | | | | | | ✓ | |
| criteria 32 | | | ✓ | | | | | | | ✓ |
| criteria 2 | | ✓ | | | | | | | ✓ | |
| criteria 9 | | | ✓ | | | | | | ✓ | |
| criteria 15 | | ✓ | | | | | ✓ | | | |
| criteria 31 | | | | | ✓ | | | | | ✓ |
| criteria 5 | | | | ✓ | | | | | | ✓ |
| criteria 18 | | | ✓ | | | | | ✓ | | |

### 5.2.2. Winning combination

Methods can be combined, it may not always result in a better solution however. Adding more methods may result in little improvement at, a relatively, high cost. An example of a good combination of subsystems is a camera-based tracking system with the addition of inertial navigation.

The paper by Shih [25] states that traditional photocell matrices get a planar resolution of approximately 8 mm and that their combined system of a camera and an inertial navigation got a resolution of 1.2 mm.

From the Harris profile it is clear that a solution involving a camera component delivers the best performance. However the camera solution alone has problems with the identification of individual mice. This means that an additional method is required for the identification. This can be an auxiliary system like an accelerometer, or a system that relies on the camera. An additional camera based method has been described in 5.1.5. The described passive marker seemed to perform the best if the size was large enough. It does not require any power of from the wearable device, and can be tracked fast and often.

## 5.3. Elaboration of solution

In [1] the application of a camera system in a the live mouse tracker is described. It is a system that is capable to perform a lot of the desired functions of the neuro-e-mit. The plan is to build a replica setup with two modifications, first removing the RFID, which can not be used with the wireless power of the wearable devices, and secondly change their code so mice can be identified using 2D IR markers.

The test setup selected for the remainder of the work applies a IR camera mounted on a dedicated bar. The location of the camera is calibrated to cover the full area of the test-bed that has the same dimensions found in cages to study and observe rodents in the Neuromate program.

The camera applied in this research is the "Microsoft Xbox One Kinect V2 Sensor", Model: 1520, Serial: 006654763674.
The mounting bar is custom built and gives the camera an elevation of 60 cm above the test surface.
The coverage of the camera is an area of 40 cm x 40 cm.
The standard sensor can also cover long distances, with its high power IR transmitter. To prevent interference by this high power IR transmitter one minor modification is applied to the sensor, by simply blocking the high power IR transmitter by covering power IR transmitter with a piece of scotch paper tape.

There is a image crop effect, caused by the angle not being perpendicular to the camera, as depicted in Figure 5.3.A. This effect is most prevalent at the minimal camera angle in the corners of the set bed, when the rodent is facing outwards.

The tracking software that analyzes the data coming from the IR camera needs to be able to determine the location of two 2.5 mm x 2.5 mm tracking squares in the 2D IR marker. The maximum error would be the distance from the center of the square to the corner of the square. The minimum accuracy will be:

$$\frac{2.5}{2} \times \sqrt{2} \approx 1.8 \text{ mm} \tag{5.9}$$

This accuracy would fulfill the location accuracy criterion [27].

To detect a pattern, the Nyquist equation states that a sampling frequency of at least twice the desired detection frequency is required. The marker has a tracking square frequency of 4 per cm. Therefore, the required pixels per cm in each direction is:

$$f_s > 4 \times 2 = 8 \text{ pixels} \tag{5.10}$$

However, the system needs to be able to track the mice even when the marker is not situated in the perfect orientation relative to the camera. The worst position of a marker is at a 45 degree incline, this would reduce the visible surface area by $\frac{1}{\sqrt{2}}$. The incline would reduce the square surface, and therefor increase the pattern frequency by:

$$\frac{4}{\frac{1}{\sqrt{2}}} \approx 5.66 \text{ cm} \tag{5.11}$$

for a minimum required pixel density of:

$$5.66 \times 2 = 11.3 \tag{5.12}$$

To make sure the mice can be tracked everywhere in the enclosure the minimum pixel criteria of 11.3 per cm should be true for every cm. The size of the enclosure is 40 by 40 cm criterion [8], so the total required pixel resolution of the camera is $11.3 \times 40 \approx 454$. The minimum camera resolution to track the tracking squares in this scenario would be 454 by 454 pixels.

The maximum location tracking error with a positive ID occurs when the squares are detected on the corners. This would result in an error of:

$$\frac{2.5}{2} \times \sqrt{2} \approx 1.8 \text{ mm} \tag{5.13}$$

The maximum orientation tracking error with a positive ID occurs when the maximum tracking error occurs at either end of the 2D IR marker.This would mean that the orientation error is:

$$arctan(\frac{\frac{2.5}{2}}{\frac{10}{2}}) = 14 \text{ degrees} \tag{5.14}$$

This would not fulfill the orientation accuracy of 10 degrees criterion [28]. However the maximum error is very unlikely, if not impossible, to occur and the pixel density might be higher than the minimal increasing accuracy. Thus the the orientation accuracy of 10 degrees criterion [28] might still be met in the final solution.

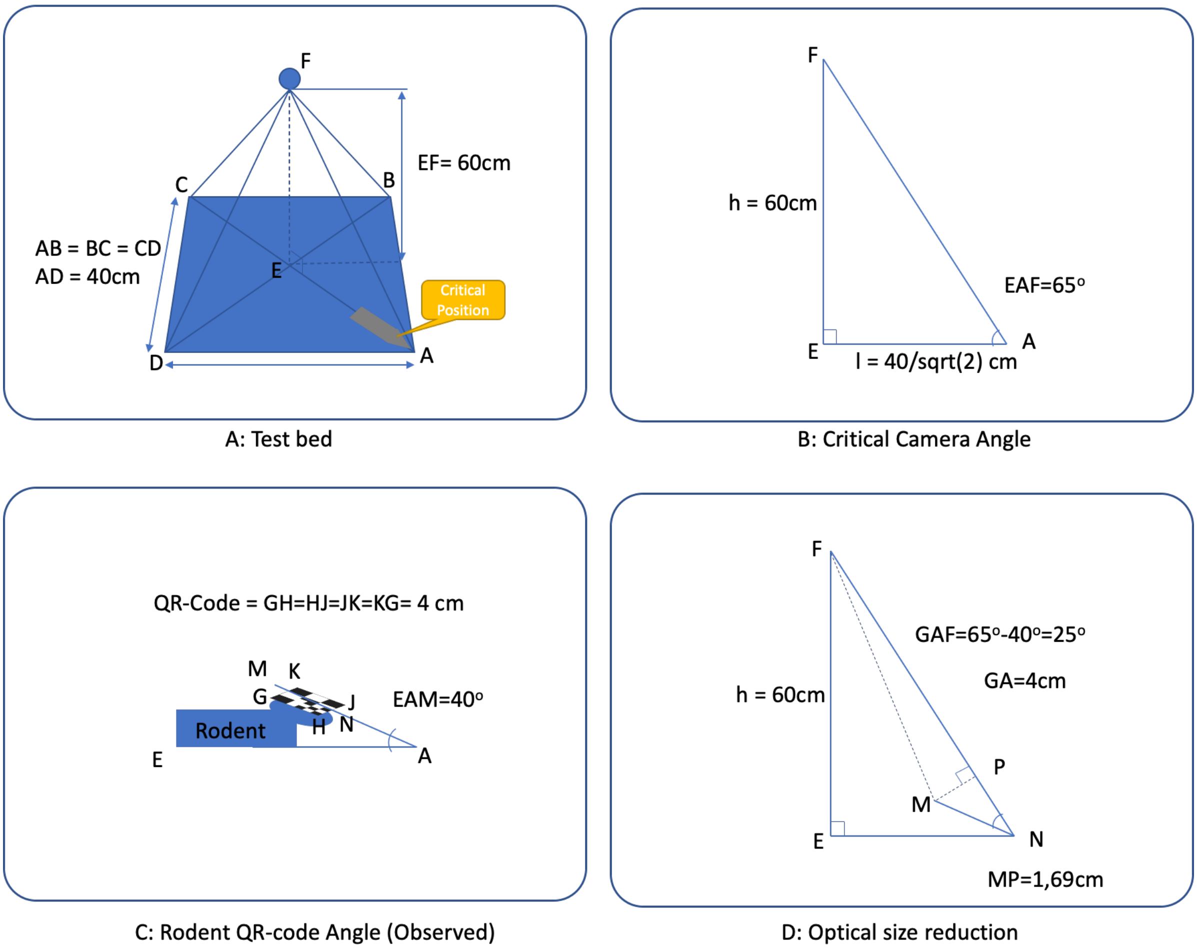


Figure 5.3: In this figure the optical size reduction is visualized. A: The test setup with its dimensions is depicted. B: The minimal camera angle at a corner location is depicted. C: The observed angle of the 2D IR marker on a rodent is depicted. D: The image size reduction is depicted for the situation a rodent is in the corner position (worst case).

Besides the height of the camera, and the lens used, a third determinant factor is the resolution of the IR camera used. In the diagram of Figure 5.4 the relationship of the image-sensor resolution and the coverage resolution at the observation surface in the cage is shown.

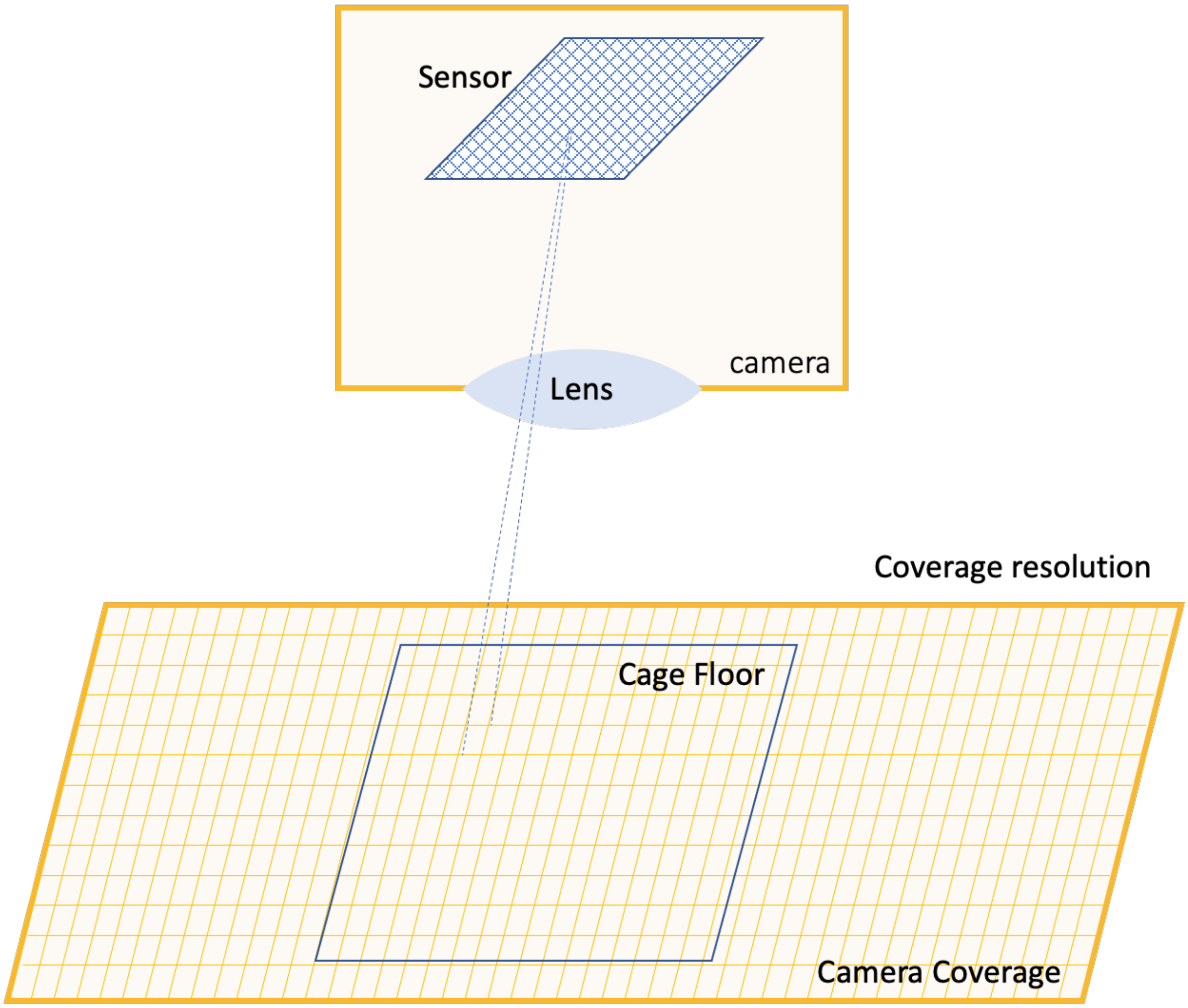


Figure 5.4: This diagram depicts the relation between the resolution of the sensor in the camera and the coverage on the observation surface.

# 6
# Safety and ethics

There are safety aspects unique for the test setup used for this research, which differ from the target application of the proposed solution in the Neuromate program involving animals. For the experiments performed for this paper the safety can be divided into two aspects. First there is safety for the human operator, second the safety regarding the environment and materials used.
For the target application the safety aspects are extended to include the animals undergoing the test. In the next sections each aspect is considered in the context of the winning concept, using an infrared camera in combination with passive markers. The finals sections will address the ethical aspects of the application of observation techniques.

## 6.1. Human operator safety

One theoretical safety risk for the human operator is introduced by the IR-transmitters, when exposed to the eye or skin. For our application we can use IR-transmitters with a very low power and therefore will likely not cause harm to the human skin, even if exposed for a longer period. The light spectrum is outside the frequency range of the human eye, and combined with the very low power will likely will not cause harm to the human eye. The method will not introduce changes in the operating procedures that could impose additional safety risks.

## 6.2. Test setup specific safety

For the test setup, a full scale replica of the Neuromate observatory is built however, without the cage walls for easy access. This setup is placed on a table surface with free access just underneath the table surface, where the table surface is approximately 25mm thick. An custom construction is created to hold the camera and its IR transmitters at the correct location above the observation surface. In our tests we use a equivalent to the existing IR-camera technology of the Neuromate program The weight of the camera and IR-transmitter is 450grams, the camera mounting construction is built using 12mm copper piping, normally used for drinking water. In order to make the dummy mouse move in the test bed, a set of two magnets is used. One magnet is mounted in the dummy mouse, the other is manipulated by the test operator under the table surface.

One risk for human safety is the collapse of the construction hitting the human on the hand when modifying the specimen position on the test field. A second risk for the human operator is introduced by the IR-transmitters, in our experiment we use an Microsoft XBOX One Kinect that is approved to be used in consumer application with children.

There are no animals involved in the test experiment. However the test should be performed in an environment that prevents any animals passing by being impacted by the IR-light.

One safety risk for the environment is also introduced by the IR-transmitters, there might be other objects or living organisms near the observation setup, that are sensitive to IR. Having a environment eliminated from living organisms will eliminate this risk.

## 6.3. Animal safety

One safety risk for the animals is introduced by the IR-transmitters, the power of the IR-transmitters is very low and will most likely not cause harm to mice fur even if the animals are exposed to IR-light for a longer period. The IR-light spectrum is outside the frequency range of the rodent eye, and combined with the low power, most likely will not cause harm to the rodent eye.
The IR-camera mounting construction should be able to withstand human interference, such to prevent the camera dropping in the observation cage and possibly hurting a rodent.

## 6.4. Environment and materials

In the proposed location and orientation method there are no consumables required for this experiment. Each wearable device will have a unique QR-code before installation on a Rodent. The ink used to print the IR QR-code on the wearable device, is considered to be environmentally friendly. Even when disposed properly with the wearable device .

The consumables involved in the test method used for this paper are limited to paper based QR-codes with hook-and-loop fastener and are also considered not to be environmentally harmful, when disposed separately. The materials used to build the test setup, copper pipes, magnets, are considered not to be environmentally harmful, when disposed separately. As such the safety risks of the materials aspect can be neglected.

## 6.5. Ethics

The ethical questions that observations have on social behavior and specifically the neuro-feedback based observations using the method described in this report are considered a topic to be discussed at the Neuromate program level, and beyond the scope of this work.

The test setup is performed with only a human test operator and without living subjects. The ethical implications of this research will be considered in the following domains:

- the Neuromate program,
- testing scenarios involving animals,
- human.

The use of an IR camera and transmitter as well as the passive markers are points of discussion. The direct and indirect influences these two aspects have will be discussed. The ethics of the Neuromate project itself, as stated previously, will not be directly handled as that is outside the scope of this research.

### 6.5.1. Passive markers

In the context where each rodent is equipped with a cube fixed on its head, additional aspects originate from the color of the cube. It is unlikely that a rodent, in this situation, will experience a negative impact when the cube has a unique pattern to reflect IR-light. Also it is very unlikely that group housed rodents identify each other based on the cube patterns rather than their natural capability of recognition and social interaction. Is this an ethical or experimental dilemma? Seems more like a consideration an experimenter might have to exclude this as an influence on the experiment.

- **Neuromate Domain:** Passive markers will not have influence on other aspects of the neuromate project
- **Other Animal Experimentation Domain:** The proposed tracking method would have similar ethical implications no matter the situation. This method does not address any ethical problems which may arise from applying the method in experiments involving any animals or humans. In this case the ethics of those experiments should also be considered separately,
- **Human Domain:** A very extreme case would be that humans are receiving an IR-tattoo without their consent. Based on this IR-tattoo and IR-capable surveillance cameras, accurate identification and tracking of people becomes possible. Based on this application of the method a social suppression system could be constructed. Such applications would are not the intention of this work nor will be supported by the parties involved in this research.

### 6.5.2. IR transmitter and camera

The context of the rodents in a traditional confined observation setting is extended by an observation camera placed well above the living plane of the rodents. The rodents will not be able to reach the camera. It is expected that the rodents do not have interest in the stationary camera and IR-transmitter. Also it can be expected that the IR-transmitter and camera will not have an impact on the well being of the rodent and its social interaction.

The IR-transmitter and camera are not likely to influence other aspects of the Neuromate program, as the cage subsystems are using electromagnetic technologies.

Impact on other observatory settings can be large. As camera imaging techniques are improving, this method can be applied in other settings as well, for observations involving other types of animals or even in human studies.

In the situation where high resolution IR-cameras are added to the existing surveillance cameras, another identification layer is added, on top of the snout recognition. This would increase the accuracy of positive identification with all the use cases this supports.

# 7
# Test method

This chapter redefine the goal of this study into research questions that are specific for the chosen tracking method. The chapter concludes by motivating and describing the test method.

## 7.1. Research topic

The Neuromate Project investigates the social behavior of a limited number of group-housed mice. The social behavior can be determined by analyzing the free movement of the mice. The Neuromate project requires an accurate constant tracking of each individual mouse, for its social behavioral studies. This research investigates the influence of a camera-based solution for which a 2D IR marker is added to each mouse and the performance of individual mouse tracking.

By using the existing live mouse tracker setup [1], of which I exchanged the standard RFID identification with the camera based identification using 2D IR markers, the first research question becomes:

*Can the proposed camera based system track individual mice?*

Then the followup question becomes:

*Does the 2D IR markers improve the system compared to a system that uses RFID tracking?*

This followup question can be broken down in two parts:

1. Does the new system provide additional tracking data to increase tracking accuracy, such as the facing and location orientation data?
2. Does the new identification method improve the system by adding new types of information, such as tracking information during encounters and head orientation for social interactions?

To fully specify our experiment we need the following assumptions:

**Assumption 1:** The accuracy of the identification needs to be at least 75 % [A1]

**Assumption 2:** In order to be relevant for live mice, we need to be able to detect the a ID of the mice within 1 s [A2]

**Assumption 3:** If we lose the ID of a mouse when encountered, we want to be able to positively find its ID when the mice is seperated one mice bodylength, in our case 5 cm [A3]

**Assumption 4:** The available surface to hold an passive identification code is 1 side of the 1 $cm^3$ cube shaped wearable device [A4]

## 7.2. Research question

Based on the questions and assumptions mentioned in section 7.1, the main research question becomes:

*Can a IR camera identify a single mouse[26], wearing a 1 by 1 cm [A4] unique 2D IR marker, within 1 second [A2] with a true/false ratio of at least 3 to 1 [A1], which is positioned 5cm away [A3] from any other mice in a 40 by 40 cm cage [8] with 4 group housed mice [7]?*

To validate the research question, a proof of concept of the tracking method is needed in order to execute some tests. The proof of concept would preferably be designed for the specific task derived from the research question. The proof of concept should be close enough to the final application, such that a good argument can be made for a system relying on this tracking method that can meet the criteria from the research question.

There are two types of tests required to answer the main research question.

1. A test to prove that a marker can be identified. This can be a simple test with a single mouse.
2. A test involving at least two mice, moving from close proximity (<5cm) to a position in which their distance is more than 5 cm.

Important measurements are

1. true/false ID detection rate,
2. ID detection frequency,
3. time between the engage and separation phase

all with an proximity (<5cm) and first positive ID detection. Finally, in a third the 2D IR marker is completely lost in the tracking system, and after a limited time the 2D IR marker reappears again. Such a test would show what would happen in the scenario in which a mice ID is totally lost.

### 7.2.1. Sub-question 1

Can the location and orientation of a 1 by 1 cm [24] unique 2D IR marker on a single mouse [26] group housed with 4 [7] others in a 40 by 40 cage [8] be determined with an accuracy below 8 mm[27] and 10 deg [28] in the horizontal plane using a IR camera?

The proof of concepts for this sub-question is identical to the one used to answer the main research question. These consist of a test to validate the ability to identify a marker and a test validating the ability to track with multiple mice in the cage. Both of these can be validated test conditions including two mice. Which is either a social or encounter trajectory. However, to make a statement about the tracking performance a method is required to get a tracking accuracy estimation from the ability to identify the 2D IR marker successfully.

### 7.2.2. Sub-question 2

Can the identification of a 1 by 1 cm [24] unique 2D IR marker on each mouse [26] with a IR camera continue tracking mice during close encounters?

The proof of concept for answering this question can be identical of the one used to answer the main research question. The close encounter tests, the second test for the main research question, could be used to answer this sub-question by collecting data during the close encounter period of the test.

## 7.3. Experimental setup

The test setup was constructed using the materials below as pictured in Figure 7.1. This setup and its components are replicated from the Neuromate program for easy of comparison with exsting opertions. However this is also an constraint to our setup and experiments. The Microsoft Xbox Kinect camera was mounted on a bracket of copper pipes. The outer marker on the surface indicates the tracking area, with a size identical to a Neuromate cage. As the Neuromate software takes some time to start tracking the mice, we have created an

smaller inner square to be used for measurements. The area between the measurement field and the tracking area is used in order for by the Neuromate code to detect the mice, as these movements are outside the measurement field they will not impact the test results.
A block-diagram depicting the sensors and connection with the laptop is shown in Figure 7.2

The experiment setup consists of:

1. Test surface with marking for calibration and trajectory planning.
2. Kinect 2.0 with appropriate cables.
3. Kinect fixture above test surface at appropriate height.
4. Computer compatible with the Kinect.
5. Neuromate software, consisting of Eclipse based Icy , with additional code which locates and identifies the 2D IR markers.

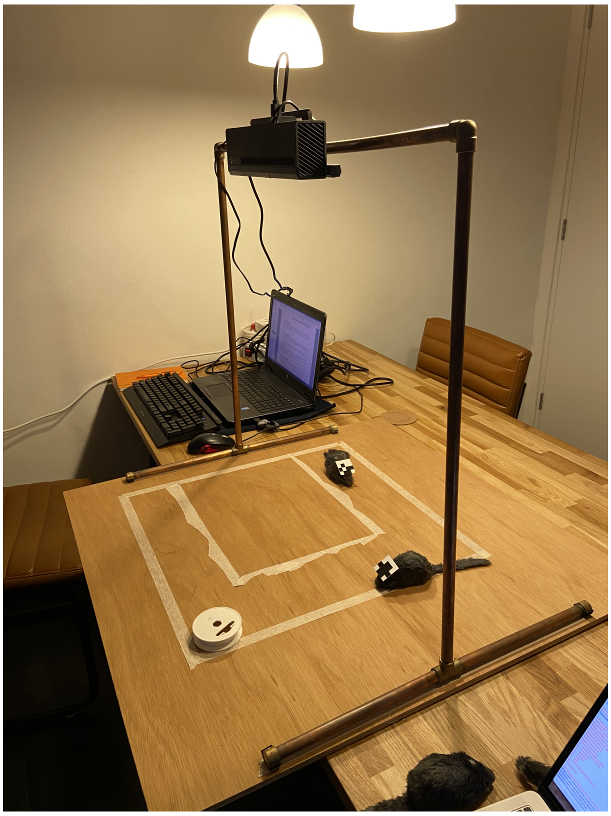

Figure 7.1: Photo of the test setup in action, with the laptop, the bracket with Microsft Xbox Kinect sensor above the test surface, the test surface with size indicators and two dummy mice. One mice being connected and operated by a nylon string on a spool. The other mouse is stuffed with a magnet and is operated by means of a second magnet, by the operator, holding the second magnet close the mouse but under the test surface table.

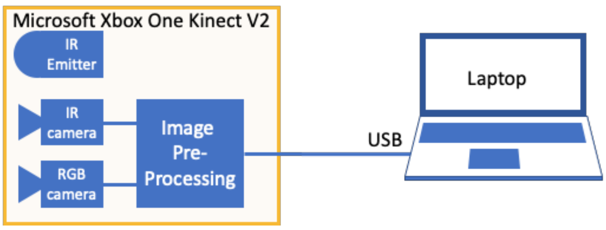


Figure 7.2: This block diagram shows the sensors that are within the Microsoft Xbox Kinect and the connection to the laptop running the Eclipse based Icy software.

### 7.3.1. Dummy mice

The specimens are made with the materials below. The dummy mice I obtained where gray, so they were colored black using spray paint to increase the contrast. This better simulates the actual fur color of the mice used for these kind of experiments. Small bits of Velcro where hot-glued to the head of each dummy mice for the application of a 2D IR marker. Magnets are glued to the bottom of the head for 3 of the 4 dummy mice. The printed 2D IR markers were attached to cardboard for rigidity and glued to the hooks of a hook and loop mounting system. The head and tail the fourth dummy mouse were hot-glued to two lines of nylon fishing wire. The two wires were wrapped around the testing surface and tied together. This allowed the mouse to be moved forward and backward along a single line. The 2D IR markers are stuck to the dummy mice using the hooks from the hook and loop system.

### 7.3.2. 2D IR marker design

The 2D IR marker design is depicted in Figure 5.2. At first the markers were 1 by 1 cm as per criteria 24, but during initial testing these proved too small for reasons discussed in Subsection 9.2.3. Therefor, the size was increased to 4 by 4 cm for testing. The corners of the 2D IR marker have to contrast the mouse, hence a white color is used.

### 7.3.3. Materials

The following materials are required:

1. four dummy white or black colored mice of appropriate size
2. four strong magnets,
3. four unique printed 4 by 4 cm 2D IR markers
4. fishing wire
5. glue
6. hook and loop attachment system

### 7.3.4. Code

The test setup uses a combination of programming languages for different aspects of the setup: Icy, Eclipse, SQL, and Matlab. The functionality of each language will be described in the following Paragraphs. The handling of the data during and after the test by the software is described in the next section.

#### Icy

Icy is an open source platform for bioimage informatics. Icy is mainly used for analyzing images of dyed cells. The great advantage of the program is that it is open source, so anyone can build plugins. These plugins are functional components written in Java. The plugins greatly increases the capabilities for the user by adding functionality to the core Icy platform.
The test setup used for this thesis is based on the Live Mouse Tracker design [1]. The Live Mouse Tracker uses a multitude of plugins in order for Icy to work with the Microsoft XBox One kinect camera and to be able to:

1. record
2. track
3. analyze

living mouse behavior. The Live Mouse Tracker team provided a working version of Icy including all the required plugins to be used for this thesis.
For the test setup needed for this thesis the Icy plugins were altered and new plugins added to allow the setup to locate and identify 2D IR makers. The Icy software was used in the tests to capture the images and identify mice as structured data. The data acquired from the test was stored in a SQL database to further analyzed after the tests were executed.

**Eclipse**

Eclipse is a program used to code in java. For this thesis it was mainly used to alter and add plugins. There was a considerable amount of code altered and added. The main modifications were changes to the main Live Mouse Tracker plugin, to redirect the existing identification process to a newly created plugin. This new plugin uses the image of the detected mouse and processes this image to get the 2D IR marker ID, location, and orientation and store these parameters as structured data in a SQL database.

**SQL**

A SQL database was used to store the data from the tests, such as the time stamp, frame number, and mouse ID. The database is later opened in Matlab to be further analyzed.

**Matlab**

Matlab was used to analyze the data in the SQL database and make plots of the data. This included the error analysis and the box plots.

### 7.3.5. Data processing

The handling of the test data is done in a series of sequential steps, in a linear data pipeline. For the test data the following data processing steps in a single pipeline is used by the Icy code:

1. Capture the visual image
2. Capture the IR image
3. Determine the area of interest (AoI)
4. Filter image to get a clearer 2D IR marker
5. Locate corners of the 2D IR marker in the AoI
6. Determine the location and orientation
7. Determine 2D IR marker identity
8. Write data to sqlite file and save video

Image filtering consists of 3 steps. First the image is turned black and white with a simple threshold. Next the image is filtered by first eroding and then diluting the image.
After Icy has saved the data, Matlab is used for further analysis and plotting of the data using the following steps:

9. Filter location data
10. Calculate the speed
11. Separate run in different tests
12. Delete invalid tests (the criteria for an invalid test are listed in Table 7.4)
13. Calculate other data such as True Positives/s (TP/s)
14. Save to .mat file

The filtering of the location data is done using a simple low-pass filter, that removes abnormal (Table 7.4) movements, which are sometimes created by stick-slip, a phonomena that creates a spontaneous jerking motion that can occur while two objects are sliding over each other. By applying a filter the average speed will smoothen as well.

The data processing pipeline is depicted in Figure 7.3

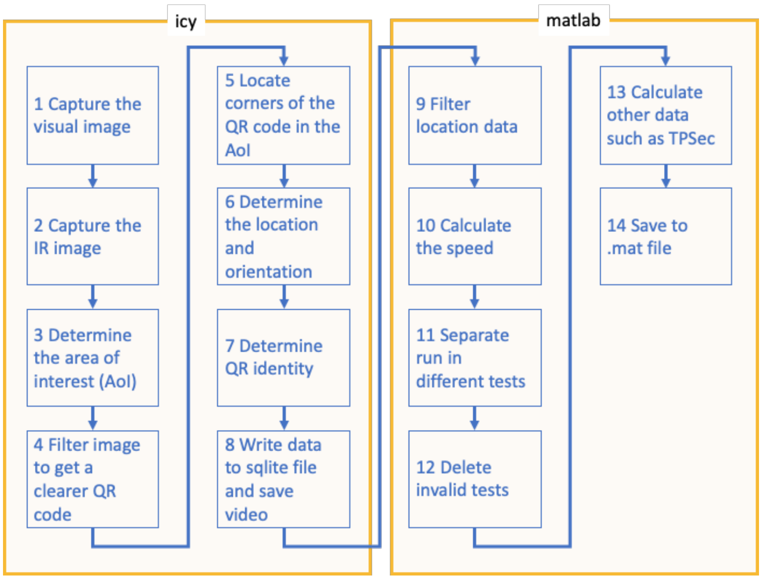


Figure 7.3: Block diagram of the data processing pipeline split in two sub-system. The icy subsystem, capturing the camera images and process these into a SQL database. The MatLab processing for analysis and calculating the measurements.

## 7.4. Experiment design

### 7.4.1. Test conditions

There are 2 main test conditions to consider. The 2D IR marker combinations used and the trajectory of the mice over the test area. For the 2D IR marker combination, each 2D IR marker is used in an equal amount of tests, in order to reduce random variables on the experiment results.

**Trajectory**

In order to represent a real life situations and simultaneously be repeatable, a series of abstract trajectories are designed. Each trajectory is a distinct representation of a fundamental real life trajectory, they are mutual exclusive and collectively exhaustive. The trajectory of the dummy mice during a test is a straight line, performed by a human test operator. The desired trajectory does not have to be perfect, since it simulates a real mouse.

The four basic trajectories used in the experiments are:

1. The single mouse test will use a trajectory, which will run through the test area from the bottom and exit at the top. This depicted in Figure 7.4.
2. The social test will use 2 mice that each have a similar independent trajectory, similar to the single mouse test. This depicted in Figure 7.5
3. The encounter test will use 2 mice, M1 is attached to a string instead of a magnet. The mouse M1 has a straight path up. The mouse M2 on a magnet starts at a encounter course towards mouse M1. Then M2 meets M2, after which they move together for a little while. Finally they separate clearly before both leaving the test area at the top.This is depicted in Figure 7.6.
4. As mice cages frequently have objects to enhance the well being of the mice, a trajectory with an object blocking the camera from observation is added. The obstruction test will use a single mouse with a similar trajectory as for the single mouse, but there is an object covering the mouse right before it enters the test area, as depicted in Figure 7.7.

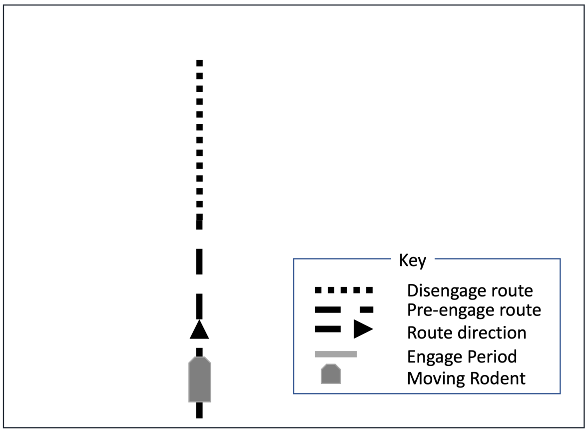


Figure 7.4: Graphical representation of the mouse in the test area in a single straight line.

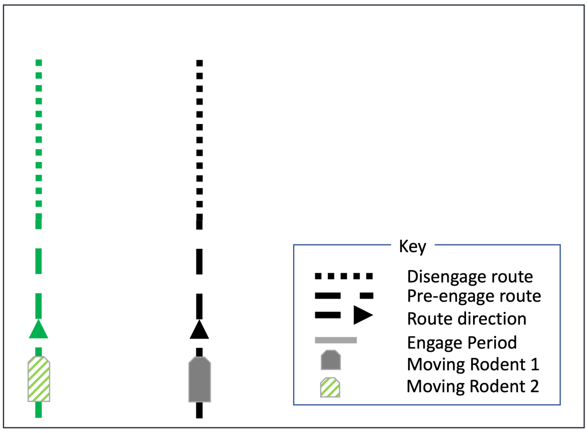


Figure 7.5: Graphical representation of two mice in the test area in an independent single straight line.

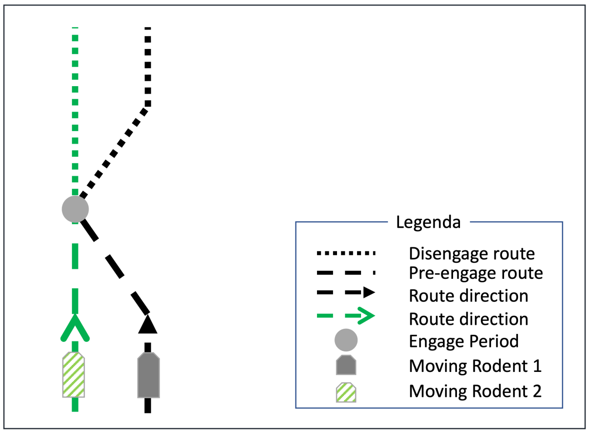


Figure 7.6: Graphical representation of two mice in the test area. On parallel tracks with a momentary engagement.

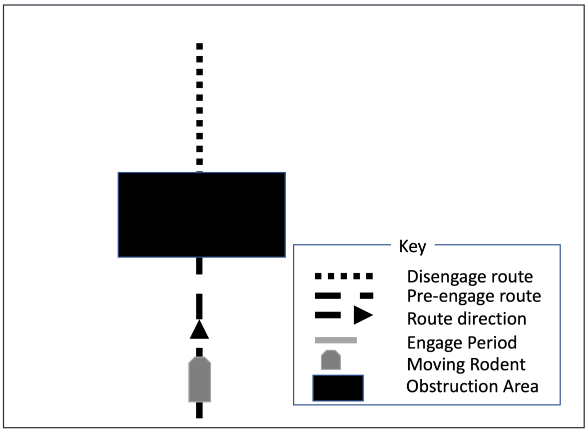


Figure 7.7: Graphical representation of a single mouse is moving across the test field and is shielded from the camera as it crosses an obstruction.

Figure 7.8: Test trajectories used in this research.

**Condition matrix**

The combination of the trajectory and the mouse 2D IR marker combination are fitted and listed in Table 7.1. In the table the test conditions are listed, for each item in the table a code is indicating the Experimental Condition *hyphen* and the mice involved. (eg. EC2-m2m3 represents Experimental Condition 2 with Mouse 2 and Mouse 3)

Table 7.1: Condition Matrix

| Mouse & 2D IR marker / Trajectory | 1 | 2 | 3 |
|---|---|---|---|
| **1 Single** | EC1-m1 | EC1-m2 | EC1-m3 |
| **2 Social** | EC2-m1m2 | EC2-m2m3 | EC2-m1m3 |
| **3 Encounter** | EC3-m1m4 | EC3-m2m4 | EC3-m3m4 |
| **4 Obstruction** | EC4-m1 | EC4-m2 | EC4-m3 |

### 7.4.2. Pilot test

The performance of the system is mainly evaluated by the number of true positive detections per second of the 2D IR markers. Its value is estimated by taking the mean of the measurements. To be sure that the estimated mean does not differ too much from the real mean value the estimated mean error is calculated using Equation (7.1) [76] [77].

$$E = Z\frac{\sigma}{\sqrt{n}} \tag{7.1}$$

- Z is the value from the table of probabilities of the standard normal distribution for the desired confidence level (e.g., Z = 1.96 for 95% confidence)
- $\sigma$ is the standard deviation of the outcome of interest
- n is the sample size

By refactoring Equation (7.1) the number of tests required to get a error within set margins can be obtained from Equation (7.2).

$$n \geq \left(\frac{Z\sigma}{E}\right)^2 \tag{7.2}$$

$\sigma$ is calculated using:

$$\sigma = \sqrt{\frac{1}{N}\sum_{i-1}^{N}(x_i - \mu)^2} \tag{7.3}$$

- N is number of measurements
- $x_i$ is an individual measurement
- $\mu$ is the mean of the measurements

A small pilot test was performed. In Table 7.2 the number of runs in the pilot test are listed in the first column, the standard deviation for the True positives per second (TP/s) in the second, and the calculated number for runs needed in the last.

To calculate the required amount of tests per trajectory, Equation (7.2) was used. In order to fill in the equation the Z value, standard deviation, $\sigma$, and estimated error, E are required. The Z value can be looked up, and depends on data distribution and desired confidence interval. In this case Z=1.96 for a 95% confidence interval. $\sigma$ is calculated from the pilot test using Equation (7.3). The values for $\sigma$ are shown in Table 7.2. E for this thesis is set to be related to the mean true positives per second (TP/s). E is determined by what would be an acceptable error. In this case E is set to be about 1. This means that with a 95% confidence the real mean TP/s does not deviate from the estimated TP/s by more than 1. A E value of 1 is acceptable, because such an error would have no effect on the assessment of the system performance. With this information the required amount of test is calculated per trajectory and listed in Table 7.2.

Table 7.2: The results of the pilot tests for each of the tested trajectories. The number of runs in the pilot test set are listed in the first column, the standard deviation for the true positives per second (TP/s) in the second, and the calculated number for runs needed in the last.

| Trajectory | Number of runs | $\sigma$ Standard Deviation | n Sample size |
|---|---|---|---|
| Single | 9 | 0.82 | 3 |
| Obstruction | 12 | 2.04 | 16 |
| Social | 8 | 2.45 | 23 |
| Encounter | 8 | 1.75 | 12 |

### 7.4.3. Runtable

For the experiment a number of test phases, covering each base type social interaction are scheduled. Each test phase consists of a number of test runs, and a test run consists of a number of tests. A pilot test was conducted to determine the total number of test required and the number of tests in a test run. The duration of tests in a test run is depending on the performance of the test equipment, experienced as a reduced frame-rate, as the setup was not able to keep up with the data stream received over a longer period of time. The number of tests per test run is dependent on the number of concurrent used mice, single mouse test would allow 6 to 8 tests per test run, in two mice settings this reduces to two tests per test run.

In constructing the run table the the number of times a mouse, with its unique 2D IR marker, is used in a test phase must be equal to mitigate the mouse ID as a nuisance variable. There are four mice used in the test, each uniquely tagged with a 2D IR marker encoding the IDs: 30, 36, 43, and 53.

The numbered items below identify the combination of test phase, number of test runs and number of mice used as follows:

**1 =** The single trajectory using one test-run per mouse - 2D IR marker combination.

**2 =** The social trajectory using 5 test-runs per mice - 2D IR marker combination.

**3 =** The encounter trajectory using 5 test-runs per mice - 2D IR marker combination.

**4 =** The obstruction trajectory using one test-run per mouse - 2D IR marker combination.

The order in which test tests are performed are randomized, with Matlab, to reduce the effect of nuisance variables. The resulting run-table can be found in the appendix, Table A.1 and an abbreviated of the run-table in Table 7.3.

The run-table 7.3 consists of a three columns the first is the test sequence number, the second columns specifies the test phase from the list above and the third columns specifies the mouse id(s) used.

The number representing the ID of the mouse for the test phases. Phase one and four use a single mouse with a magnet. The a single mouse is represented as follows:

**1 =** ID 53

**2 =** ID 30

**3 =** ID 43

For test phase two, which uses the social trajectory, the number represents a mice combination consisting of two mice with magnets. The mice combinations are represented in the run table as follows:

**4 =** ID 53 & ID 30

**5 =** ID 30 & ID 43

**6 =** ID 43 & ID 50

For test phase three, which uses the encounter trajectory, the number represents a mice combination with one wired mouse and one mouse with a magnet. There is only one wired mouse which has ID number 36. The mice combinations are represented in the run table as follows:

**7 =** ID 53 & ID 36

**8 =** ID 30 & ID 36

**9 =** ID 43 & ID 36

The lists above with the ID numbers are the result of a random generator in order to make the 2D IR marker patterns an independent variable.

Table 7.3: First section of the Run Table, the full Run Table is listed in Table A.1

| Run-sequence number | Test phase | Mouse/mice used |
|---|---|---|
| 1 | 3 | 8 |
| 2 | 3 | 9 |
| 3 | 3 | 7 |
| 4 | 3 | 7 |
| 5 | 2 | 4 |
| 6 | 3 | 7 |
| 7 | 3 | 7 |
| 8 | 4 | 3 |
| 9 | 2 | 6 |
| 10 | 2 | 5 |
| ... | ... | ... |

### 7.4.4. Experiment initialization

Before testing starts, the setup has to be initialized. This is done according to the following steps:

1. Setup the test configuration, as depicted and described.
2. Ensure the camera is positioned correctly. This is done in the following way:
   (a) Start Eclipse
   (b) run Icy.
   (c) run the calibration plugin found under plugins->Other plugins->fab->live-mouse-tracker->calibration This is done to make sure the camera position is correct.
   (d) Make sure there are no objects nearby which could interfere with the depth tracking of the camera.
3. Check the 2D IR markers: The mice each have a different unique 2D IR marker. Those need to stay the same.
4. Perform a couple of trial runs to get a feel for the right movement speeds, patterns and check values to ensure the setup is operating according to expectation.

### 7.4.5. Experiment protocol

Runs are performed according to the following steps:

1. Place the correct mouse 2D IR marker combinations for the next test in the observable area outside the test area.
2. Run Icy code from Eclipse.
3. Move mouse/mice until they are detected by Icy.
4. Execute the trajectory according with the correct run from the run table.
5. Close Icy code.
6. Run output through Matlab to see if it is a valid test. The criteria for an invalid test are listed in Table 7.4..
7. If the test is valid, save results and move on. Otherwise delete results and repeat the run.

The tests with two mice have two tests per run. If one of the tests is considered invalid, the whole run is considered invalid and deleted. Single-mouse experiments run longer and can include up to 8 runs per test. A single wrong run per set is acceptable in such a group. If more abnormal runs are present, the set is deleted. Abnormalities identified during the tests are listed in Table 7.4.

Table 7.4: Abnormal test results

| | |
|---|---|
| 1 | Frame rate of test is too low (below 25 fps). Caused by low computation power when the testrun is to long. |
| 2 | Large deviations from the mean desired run speed (values between 3.5 and 5.5 cm/sec). These can be caused by human error. Large uneven distributed deviations of the speed may influence outcome. The speed range was chosen as it seemed realistic and did not result in manageable test times, which could effect frame rate. |
| 3 | Unexpected differences between the number of measurements in a single test run between mice. For example a social trajectory one of the mice has 5 the other two detected measurements even though there should be only two. This is caused by human error. Code has been upgraded with a run matching algorithm, which is capable of discarding bad measurements. |
| 4 | Encounter test runs that fail to have a valid encounter (either never get close enough or fail to separate before the run ends). |

## 7.5. Summary

In the first part of this chapter the test setup and test sequence is described and motivates that this setup provides the information required to evaluate the tracking method of the research question. In the second part the layout of the execution of the experiment is described. The design is an adapted setup from the Live Mouse Tracker design[1]. The most important changes are the use of dummy mice and the change from the RFID technology to to the 2D IR markers technology for determining the location and orientation. Based on the research questions the test conditions are set and with the use of pilot tests the run table is constructed. This chapter finishes by describing the test protocol.

# 8
# Results

## 8.1. Raw data

The data collected is in the infrared domain, shown in gray scale, in Figure 8.1. In the images an overlay an be seen of some of the processing done in Icy. The inner yellow square are the bottom edges of the assumed cage., the outer yellow square indicate height cage. White lines indicates the detected outlines of the mice. The red line is the transverse center line and the yellow line is the longitudinal center line. The white dot on the red lines indicates the front of the mouse. A couple of snapshots of an encounter trajectory are shown in Figures 8.5. These images were chosen to be highlighted, because they show some abnormalities even though they are typical for any encounter trajectory test. The images are analyzed according to the procedure described in Chapter 7.3.5.

In Figure 8.1 the tracking is initialized. The white dot representing the facing direction, is wrong for the mouse on the right. For a mouse to be detected it need to be moved. The mouse facing is mainly determined by its with and length ratio and recent movement direction. The right mouse is attached to a operating wire. During initialization the mouse is moved backwards, to position the mouse into the start location for the test hence the detected facing direction is incorrectly. The facing is corrected during all the test performed, as shown in Figures 8.3 and 8.4, in these cases the mouse will be moving forwards.

Figure 8.2 shows the start of a close encounter. The outlines appear gray and the other marker for the mice are both gone. This is due to the close encounter. When the mice are touching, the software is not able to tell them apart, even as the code attempts to separate the mice. The software can only create a squiggly separating gray line between the mice. The lack of information about each individual mouse due to the close proximity the code is unable to determine the other markers, therefor they are lacking in the image.

Figure 8.3 shows the continuation of the close encounter. The markers have reappeared. Although this is still a close encounter there is sufficient separation between the mice for the software to make a clear distinction, and display the markers in the image.

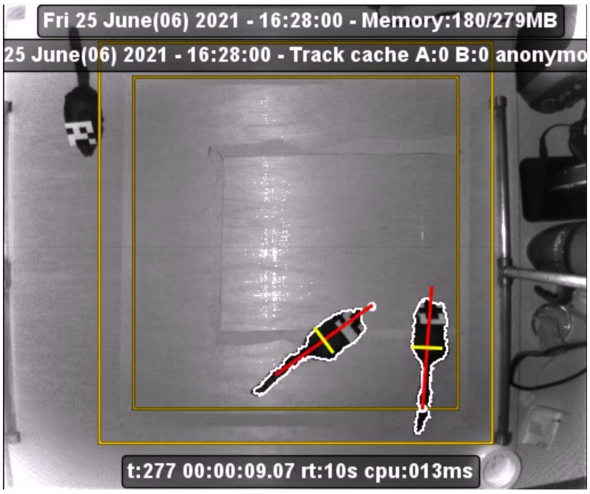


Figure 8.1: First position, the test starts when the mice enter the testing square.

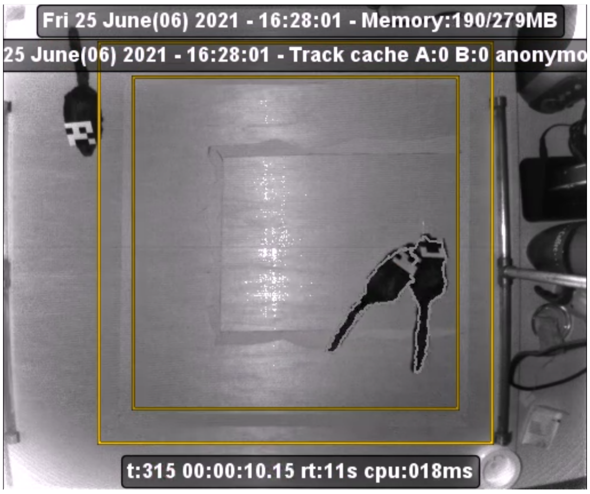


Figure 8.2: Second position, the mice just started the encounter.

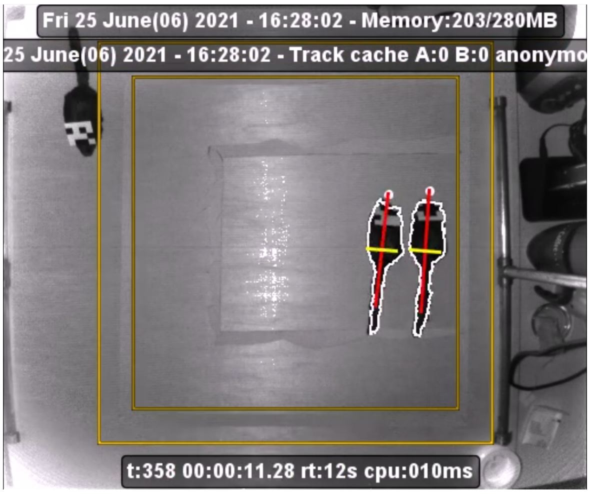


Figure 8.3: Third position, the mice stay close for some time before separation.

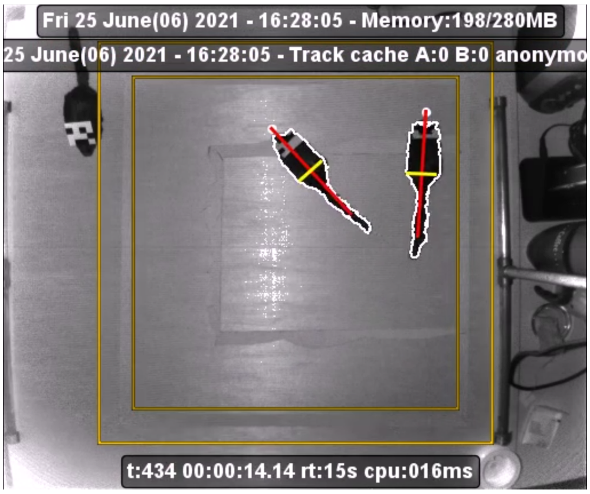


Figure 8.4: Fourth position, the test ends when the mice leave the test square.

Figure 8.5: Image analysis

## 8.2. Data

As described in Chapter 7.4, a total of 100 experiments were performed. The results from these test can be found in Figures 8.6, and 8.7. For all the test the true-positives per second are measured, as indicator of the performance of the system.

For each test phase the true positive results are depicted in the boxplot of Figure 8.6, the red line indicates the mean, the lower box limit the 25 percentile, the upper box limit the 75 percentile, the whiskers the +/–2.7*deviation or approximately 99.3 percentile and lastly the red crosses indicate the location of outliers. Besides the true-positives per second values there a two other metrics specifically relevant for the encounter trajectory. The rediscover time after loosing the mice during the encounter and true-positives per seconds during the encounter. These data are shown in the boxplots of Figure 8.7.

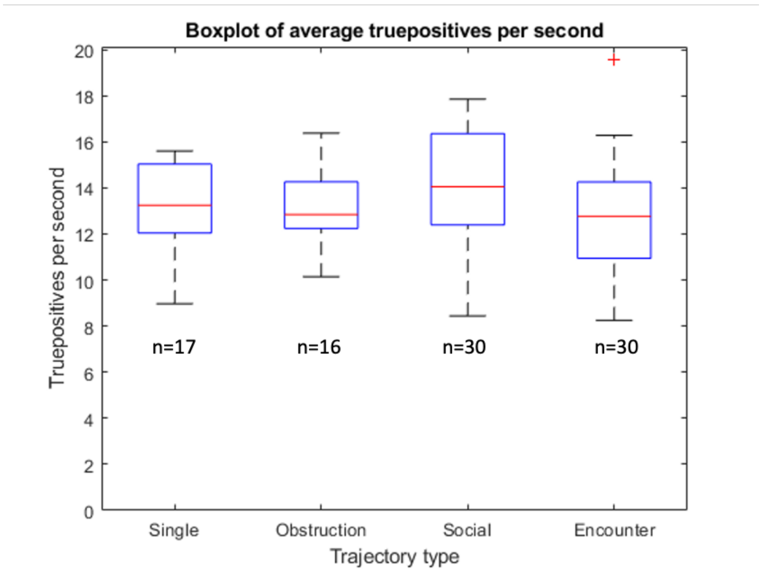


Figure 8.6: Boxplot of the true-positive results from each experiment, with sample size n.

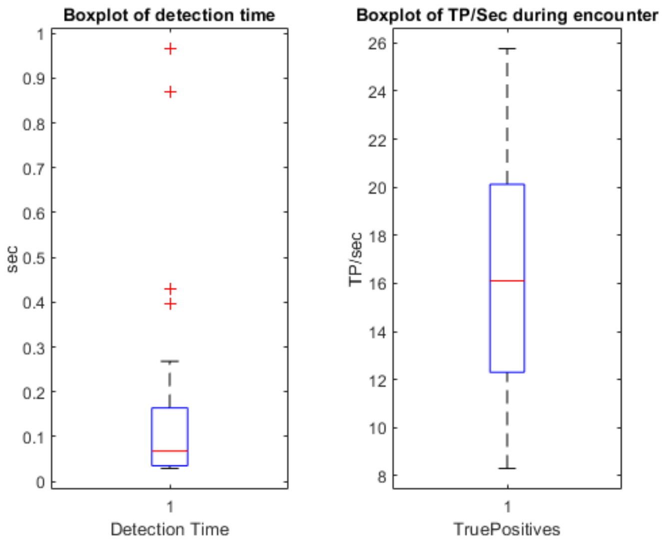


Figure 8.7: Box-plot of the test performance during and after an encounter. Sample size n=30

## 8.3. Statistical power

Statistical power represent the chance that conclusion drawn from data are false. This graduation topic requires the development of a system which meets the defined criteria. In this thesis the statistical power represents the chance of false conclusions based on the measured system performance. The statistical power is therefor the chance that the real performance of the system deviates from the measured performance enough that criteria are falsely presumed to be met. This chance is calculated with the estimated mean error. This value indicates the deviation of the real error deviation from the observed error for a set confidence interval. The confidence interval is represented by $Z$, and the allowed error is set to be 1 TP/s. Such deviation should have no effect on the conclusion drawn about the system performance. Therefor the measurements can be used to draw conclusion with confidence.

To obtain the estimated mean error ($E$) we use Equation (7.1). Where:

1. $Z = 1.96$ for a 95% confidence interval.
2. $\sigma$ is the standard deviation of the test for a single trajectory
3. $n$ is the sample size of the trajectory.

The results are shown in Table 8.1 and Table 8.2 for the performance during the encounter.

## 8.4. Processed data

Table 8.1 lists the average successful identification rate during different trajectory types. Where Table 8.2 shows the ratio between true-positive and false-positive identification. This ratio gives the reliability of any positive detection. The system does not require a very high reliability detecting the 2D IR makers, because the mice are tracked over time. For the true-positive ID to be dominant, the reliability must be sufficient during any tracked time span. During encounters an important performance indicator is the time it takes for the rediscover after the mice separate. The detection rate during the encounter and the true-positive ratio during the encounter.

The average detection time after separation in seconds $0.17 \pm 0.08$
The average true-positives per second during the encounter $16.78 \pm 1.75$

Table 8.1: The results for the various trajectories that are performed in the tests. Where true positives are abbreviated as TP.

| Trajectory | n Sample size | $\sigma$ Standard Deviation | Mean TP/s | TP/s mean error |
|---|---|---|---|---|
| Single | 17 | 2.02 | 13.15 | ± 0.93 |
| Obstruction | 16 | 1.72 | 13.17 | ± 0.82 |
| Social | 30 | 2.39 | 14.32 | ± 0.84 |
| Encounter | 30 | 2.54 | 12.86 | ± 0.89 |

Table 8.2: The critical success values, True-positive rate and False-positive rate for the various trajectories that are performed in the tests.

| Trajectory | True-positives | $\sigma$ False-positives | Mean TP/FP ratio | Percentage False-Positive of detections |
|---|---|---|---|---|
| Single | 1527 | 19 | 80 | 1.23% |
| Obstruction | 1813 | 1 | 1813 | 0.06% |
| Social | 5268 | 46 | 115 | 0.87% |
| Encounter | 5454 | 6 | 909 | 0.11% |
| Total | 14062 | 72 | 195 | 0.51% |

## 8.5. Location and orientation accuracy

Previously, the maximum error in the location and orientation was determined when the system detects a positive ID, assuming a single measurement point was within each square of the 2D IR marker. However, the image is now filtered to deal with anomalies. This changes some of the assumptions. The filter works with two steps; erosion and dilution step.

For dark mice, white 2D IR markers, would have white corner squares to make them stand out. The first step of the filtering process was removing white pixel abnormalities in the detected mouse area where there are no white squares. This was done by turning white pixels black when there was less than four adjacent white pixels, including diagonals. This is called eroding the image. Dilution is the process of filtering any black pixel with 3 or more white neighbors and turning them white. With these two processes single or small groups of white pixels are eroded away completely. The clusters that remain are grown with the dilution step to closer resemble what they were before eroding. The anomalies where removed and won't come back with dilution. This image filtering process allowed the test setup to detect 2D IR markers.

This image filtering has a large effect on the position accuracy. A minimum cluster of 5 pixels is required to maintain at least one white pixel after erosion. Conversely, a cluster of at least 7 pixels is necessary to be restored through dilution. Assuming the full resolution of the camera, 1920 x 1080 pixels, spans the complete cage area, 50 x 50 cm, a maximum pixel density of 2.16 pixels/mm can be achieved. This means that in ideal circumstances, the theoretical minimum required area to maintain a white pixel after erosion is $1.93mm^2$, assuming these 5 pixels fit in a 3 x 3 pixel square.

Assuming a square 2D IR marker of 100 mm$^2$, the individual squares in the marker would be 2.5 x 2.5 mm. The maximum displacement error occurs when the 2D IR marker is rotated 45 degrees compared to the pixel grouping. To find the maximum displacement error, half the difference between the size of the pixel grouping and the length of a marker square is taken, as shown in Equation 8.1:

$$\sqrt{2 * d^2} - t/2 = E_{dMax} \tag{8.1}$$

Where d is the length of a single marker square (mm), t is minimum cluster size (mm), and $E_{dMax}$ is the maximum displacement error (mm). When applying this in our situation, a maximum error of 1.07mm is found.

The maximum orientation error occurs when the 2D IR marker is rotated around the center. The maximum rotation occurs for the maximum possible displacement error is present on the corner squares. The maximum orientation error in degrees can be calculated with Equation 8.2:

$$arctan(\frac{E_{dMax}}{r_c}) = E_{oMax} \tag{8.2}$$

Where $r_c$ is the length from 2D IR marker center to the center of a corner square (mm), $E_{dMax}$ is the maximum displacement error (mm), and $E_{oMax}$ is the maximum orientation error (deg). $r_c$ is calculated using Equation 8.3. When applying this in our situation, a maximum orientation error of 11.41 degrees is found.

$$\sqrt{2 * (2 * d)^2} - \frac{\sqrt{2 * d^2}}{2} = r_c \tag{8.3}$$

In the test setup, the size of the 2D IR marker used is 4 times larger than just described. During the pilot test the system was unable to detect a square marker 2D IR marker of 100 mm$^2$. The size of the 2D IR marker was increased to make it possible to test the validity of the tracking method using the available test setup.

Although the tested solution is unable to meet the criteria for the marker size, the data gathered with the larger makers gives valuable insight in the potential application of the tracking method. The increase of the marker size does effect the calculated maximum positional error. It becomes 4 times larger, 4.28 mm. The maximum orientation error is unaffected, since it is related to the maximum positional error and the distance from the center, which scale proportionally.

# 9

# Discussion

## 9.1. Performance of the test setup

In this chapter we are discussing the performance of the test setup based on the results from the experiments.

**True-positives per second**

The performance of different trajectories measured in true-positives per second (TP/s) is depicted in Figure 8.6. True-positives are correct identifications. The difference between the TP/s mean does not exceed 1.5. This indicates that the system performance is robust and able to support multiple trajectories.

The identification rate should distinguish mice the majority of the time during an observation period. This is desired in order to track and classify the complex social interactions . Since the proposed solution is built for the Live Mouse Tracker [1], the 2D IR markers are a replacement for the RFID solution of the original Live Mouse Tracker. The original RFID solution detects the ID of a lone single mouse in close proximity of a RFID sensor. This is only possible when the RFID chips in the mice do not interfere with one another. These periodic identifications are sufficient, because the tracking is done with the images from the camera. The identification of the marker is only required as verification and correction in case the tracked mice IDs got swapped at some point.

For the 2D IR marker solution to be successful it would need to function at least as well as the current RFID solution. The RFID system is only able to verify mouse ID when the mouse is separated from the others. So the 2D IR system would be considered a capable replacement if it is at least capable of getting a positive detection within 1 second after the mouse is positioned 5 cm away from any other mouse. Thus this criteria is met when the amount of true positives per second (TP/s) never drops below 1 or the detection time after separation is lower than 1 second.

Figure 8.6 shows the 2D IR marker based detection rate is on average ~13 TP/s for all trajectories tested. Only in some situations this will drop to 8 TP/s during any run. During the encounter trajectory the detection time after separation in seconds is measured at $0.17 \pm 0.08$. Both of these metrics are significantly better than the minimum required 1 TP/s and 1 second respectively. Based on this performance we can conclude that the performance of the 2D IR marker tracker can be used in the Neuromate program to improve some of the capabilities and the reliability of the system as a whole.

In section 9.1.1 to 9.1.4 the performance of the individual tests will be further discussed.

**Estimated mean error**

The estimated mean error indicates the statistical power of the study. In Table 8.1 the estimated TP/s mean error is listed. This shows the maximum deviation of the real mean from the estimated mean TP/s with a 95% certainty. For example for the single mouse trajectory the mean TP/s is 13.15 with an error of ± 0.93. Therefor the real mean TP/s is between 12.22 and 14.08 with 95% certainty.

In Section 7.4.2 the data from the pilot tests was used to calculate, using formula (7.1), an estimate for the number of tests required to get a TP/s mean error of below 1. The allowed TP/s mean error of 1 was set as acceptable, because such an error would have no effect on the system performance.

After running the tests the TP/s mean error is calculated for each trajectory. All values related to the TP/s mean error (the number of test run, mean TP/s during those tests and the TP/s mean error) are reported for each trajectory in 8.1.

### 9.1.1. Single trajectory

The single trajectory test run was used to test the performance of the system in a simple scenario and to set a baseline for comparison with other trajectories. The performance of the system during the single trajectory test measured is > 10 TP/s and is considered acceptable the system performed about the same for the other trajectories .

### 9.1.2. Obstruction trajectory

The obstruction test was designed to evaluate the speed at which the test setup is capable of finding the 2D IR marker after having lost sight of its current location. The obstruction trajectory has the mice go through an opaque tunnel just before entering the testing area. This way the time spent in view of the camera in the test area is the same as that of the single mouse trajectory. Thus, when comparing the two test trajectories, a dip in the detection rate of the obstruction test results would indicate that it has taken some time to re-find the mouse. If the detection rate of both tests are the same this would indicate that there is no problem at all with the obstruction.

The difference in mean TP/s between these test was 0.02 TP/s. This is a negligible variance and the performance of the trajectories can be considered equal. Therefor the test setup is successful in finding the mice after the QR-location is lost.

### 9.1.3. Social trajectory

The social trajectory was chosen to simulate the interaction test between mice. The social trajectory was used to test the performance of the system using two mice with a simple trajectory and as a counterpart to the encounter trajectory. The difference between the two is the encounter of the mice during the trajectory. This gives a good base for comparison for the effect that an encounter between mice may have on the performance of the system. Since this is the first test which uses two mice the effects on the percentage of false-positives is important, because in this scenario a high percentage could lead to ID swaps.
The test show a very low percentage of false positives to true positives (0.87%) and the total amount of TP/s (14.32 ± 0.84) is even higher than for the single mouse tests. Overall the system seems very capable of multi object tracking.

### 9.1.4. Encounter trajectory

The encounter test was the second test to simulate mice interactions. The test was created to determine if close proximity of mice and therefor 2D IR markers would be a problem with the identification. This is important, as social encounters between mice is often in close proximity. The main concern would be that the close proximity of different 2D IR markers might result in a situations where the individual 2D IR markers can not be distinguished from one-another, resulting in identification issues. Figure 8.7 shows that there is no problem with identification even during the encounters.
During the encounter test the TP/s (16.78 ± 1.75) is high compared to other tests. The true-positive results of the encounter overall is a little lower (12.86 ± 0.89), but not significantly so.
There are some variables that might have influenced the results, namely:

1. **Speed:** In order to reduce the effect of the speed of movement of the mice, the average speed of the mice must be within a certain margin for the test to be valid. However, the speed just before and after the encounter was measured higher than average. This speed bump could increase the detection rate during the encounter while having the expected average detection rate.
2. **Visibility:** The location of the mice relative to the camera could result in the 2D IR marker being more visible than on average of the entire test.
3. **Area:** I suspect that the proximity of two mice with their 2D IR markers made it easier for the algorithm to find the 2D IR markers, due to the fact that combined the two 2D IR markers covered a larger area.

In Figure 8.1 at the start of the test run, one of the mice headings is wrongly detected as can be noticed by the white dot at the end of the red center line of a mice. This is expected, as this is the mouse connected to a wire which caused an unnatural movement. In order to get the Icy code to track a mouse it must move. Because it is on a wire the mouse can only move forward and backwards. Moving backwards results in the erroneous detection of the facing direction. The second reason is that the code has trouble with the mice when they are too close together. The these situations the distinction between the mice is lost. Therefore, the outline changes and the red and yellow lines disappear. Both of these problems are easily solved with the 2D IR marker, but cannot be solved with the traditional RFID setup.

With the 2D IR marker fixated on the head it will give the location and orientation of the mouse head when the 2D IR marker is identified, even during close contact. Hence the criteria set by the Neuromate program are met.

### 9.1.5. False positives

The ratio of false positives to true positives is listed in Table 8.2. The values are low and on average about 0.5% of the detections are a false positive. False positives are only a problem when they have an effect on the test results. Even with more of false positives there are simple solutions to reduce the chances for the increased rate of false positives to have an effect on the test results.

One solution involves camera tracking. When a mouse is tracked over time, an forecast can be made of possible locations it may be detected in the next image. This would depend on the current velocity and time between detection's. If a mouse would be detected outside the forecast area the software ignores the detection. In the case of a single ID swap incident for example the mouse would seem to teleport to the location of the other mouse for a single detection period and then teleport back. This would be considered as an unlikely movement and be rejected as valid data point.

A second solution is done with a database of ID's currently in use. The system would check detected ID against the list of ID currently in the cage. If a false positive results in a ID not listed as an ID that is present in the cage the detection can be safely be rejected.

Lastly one or more of ID squares in the 2D IR marker could be used as error detection or error correction bits as described in Section 5.1.5. This would reduce the amount of possible distinguishable ID's, but would result in flags in case of a false positives or in case for the error correction would auto correct the ID.

### 9.1.6. Accuracy

The maximum location and orientation error is calculated in section 8.5. However, the practical maximum location error will be lower than the maximum possible error calculated. The error calculations assumes worst case scenarios, to an impractical extent. Secondly, even in the worst case scenario the chance of a positive ID is very low. In the case of no positive ID there is no location and orientation data of the 2D IR marker. Therefor there would be no location and orientation data with a high error. Thus reducing the experienced maximum error measured.

The assumptions were required to make prediction of the accuracy of the system. The assumptions were made such that the accuracy of the system would not be overestimated. An example would be the assumption of perfect camera coverage and the assumption of equal pixel distribution in the image. The area covered by the camera is not 50 cm by 50 cm, but closer to 73 cm by 73 cm and the pixel density is not equal everywhere due to image deformation from the lens. Thus the pixel density is actually lower and unequal.

A lower pixel density results in a higher location and orientation accuracy, although the TP/s would drop. The lower pixel density requires a lower error in order to make a positive identification. There is no location and orientation error without a positive 2D IR marker detection. Thus the remaining detection have a higher location and orientation accuracy.

There is no location and orientation error without a positive 2D IR marker detection. So a lower pixel density results in a higher accuracy. This is because the minimum pixel grouping size to detect a square would be relatively larger. Therefor to get a successful marker detection there is less room for error. Thus a lower pixel density would reduce TP/s value, but would reduce the maximum error.

Although the maximum location and orientation error calculated (1.07 mm and 11.41°) are higher than the allowed error set in the criteria chapter (1 mm [sb-tr-la] and 10 degrees [sb-tr-oa]). The error of the setup is expected to be within the limits when measured due to the factors just explained.

## 9.2. Limitations of experiments

### 9.2.1. Dummy mice

The dummy mice were good and representative placeholders for real mice for the tests performed. The dummy mice helped to prove the concept but avoided the stringent testing requirements according to the METC if real mice were used. However, this does create a discrepancy between my tests results and those of the other tracking solution reported test results.

The majority of reported tracking solutions specify their performance based on experiments that are using real mice. The tested performance of the automated tracking solution is usually evaluated by comparing it with a human marking the images. To get a fair view of the performance of the automated system we compare the tracking deviations in two scenarios: a) the difference between the automated system and a human observer and b) the difference between human observers.

A true performance validation of an automated tracking solution can only be done with real mice and an extra observer. However, tests with this proof of concept indicates that changes, as described in the recommendations section 10.3, are needed to make it a full solution. Only after maturing the proof of concept into a product tests using real mice is valuable and worthwhile.

### 9.2.2. Number of mice

The tests are only performed using two mice. This setup should be capable of tracking four mice. There were two main reasons to test with only two mice. First, the performance of the used laptop was the limiting factor when testing with two mice for a couple of minutes. With more mice this would have been exacerbated, resulting in losing data at the data capture stage. The other reason was that most of the dummy mice used magnets in order to move around. This meant that they had to maintain some distance in order to not be influence each other in undesired or unexpected ways.

By showing the capability of the setup with two mice it is demonstrated that the system could handle multiple mice if scaled up. Scaling this system to a larger implementation is limited more by the hardware and budget than the inherent choices made in the design of the system. Therefore the two mice test is a representative proof of concept.

### 9.2.3. Larger 2D IR markers

As described in subsection 5.1.5, it is estimated that a HD-camera would be able to detect a 1 by 1 cm sized 2D IR marker. This assumed ideal conditions. However there were two reasons that caused the 1 by 1 cm markers to be too small for our setup.

**Camera coverage**

The camera used is a Microsoft Xbox One Kinect, which is also used as a depth camera. For the depth camera to operate, a minimum distance to the floor of the cage is required. From the required minimal height, the camera covers a larger area than just the cage size. This resulted in half the pixel-density that could have been achieved if this was not the case.

**Picture imperfections**

When working on the 2D IR marker detection it became clear that some filtering of the image was required to compensate for image noise. I implemented a conservative image filter, which combined with error correcting information in the 2D IR marker as described in section 5.1.5, resulted in limited loss of information. The filtering operation increased the required pixel density from the Nyquist calculated value in section 5.1.5 of 9 pixels/$cm^2$ to a minimum of $5 \times 4 = 20$ pixels/$cm^2$.

These two factors combined increased the required pixel density significantly. However the camera could not be changed for this setup due to software dependencies, see section on experiment setup 7.3. Therefore the size for the 2D IR markers was increased instead. This does not completely invalidate the calculations of subsection 5.1.5. However, they did serve their purpose, which was to give an estimate figure in order to determine feasibility of the solution. This means that the system has become a proof of concept instead of a direct application. It can still validate whether this tracking method, with some changes, would be a good solution.

## 9.3. Satisfaction of the criteria

A series of criteria for the Neuromate project, was introduced in Chapter 4. In this chapter, we document to which degree these criteria have been met by introducing a 2D IR marker based method for observation to the project.

Out of all *must have* qualified criteria, 9 criteria are met, and the other 7 are qualified as non applicable for the 2D IR marker observation sub-system.
Out of all *should have* qualified criteria, 9 criteria are met, one criteria is partially met, one is qualified as not applicable, and one criteria is outside the scope of this project and left "to be determined".
Out of all *could have* qualified criteria, 2 criteria are met, one criteria is partially met, and one criteria is outside the scope of this project and left to be determined.
The tables below represent the individual scores of each of the criteria introduced in 4.

Table 9.1: Satisfaction of the Moscow grouped criteria for the Neuromate project by the 2D IR marker observation module designed for the Neuromate.

| Must have | | |
|---|---|---|
| nr | Requirement | Score |
| 1 | The device's main function is to enable the Erasmus MC to perform their desired studies on mice | Met |
| 4 | The device measures a variety of types of data | N/A |
| 7 | The device allows the study of multiple, ≥ 4 mice, at the same time | Met |
| 8 | The device allows the group housing of mice in a cage of 40x40x20 cm ($l$x$w$x$h$) | Met |
| 10 | The device can handle a large amount of data | N/A |
| 11 | The device analyzes data in real-time | Met |
| 13 | The device is required to store specific data of interest | Met |
| 14 | The wearable device power consumption should be $\leq 50mW$ | N/A |
| 21 | The device's functional state can be monitored during an experiment | N/A |
| 22 | The wearable device is wireless | N/A |
| 23 | The wearable device weight is $\leq 1g$ | N/A |
| 24 | The wearable device volume is $\leq 1cm^3$ | N/A |
| 9.1 | The device can determine sensitive social behaviors | Met |
| 26 | The device tracks the location and orientation of each mouse in the cage | Met |
| 29 | The device needs to determine mouse proximity and relative orientation | Met |
| 32 | additional components of the Neuromate must not interfere with the others | Met |

| Should have | | |
|---|---|---|
| nr | Requirement | Score |
| 2 | The device is applicable to different study goals | Met |
| 3 | The device is multipurpose to accommodate a flexible study goal | Met |
| 6 | The device is developed mainly using off-the-shelf components | Met |
| 9 | The device is capable of long term studies, ≥ 3 months | Met |
| 12 | The device can determine data importance | N/A |
| 15 | The device performs with minimal intervention | Met |
| 16 | The device is minimally invasive | Met |
| 17 | The device allows the mice to roam freely | Met |
| 19 | The device is capable of automated observations | Partial |
| 27 | The device needs to track location with a accuracy of $\leq 8mm$ | Met |
| 28 | The device needs to track orientation with a accuracy of ≤ 10 deg | Met |
| 30 | The tracking data needs to be correlated to the neurolink data. | TBD |

| Could have | | |
|---|---|---|
| nr | Requirement | Score |
| 5 | The device is developed with the newest technology | Partial |
| 18 | The device eliminate the need for researcher interference | Met |
| 20 | The device's functional state can be monitored during an experiment | Met |
| 31 | Interference between test setups should be eliminated with separation of $\geq 100cm$ | TBD |

The major criteria for a location and orientation solution in the Neuromate program are:

1. Track multiple group housed rodents
2. 15 observations/sec
3. Track location
4. Track orientation
5. Observe sensitive social behaviors

Table 9.2 the life mouse tracker systems RFID and the proposed 2D IR marker solution are compared. All the aspects which will be influenced with the change to a 2D IR marker are listed and compared. As visible in Table 9.2 the change from the RFID setup to a 2D IR marker setup results in an increase in the reliability and frequency of the identification at the cost of requiring a wearable device. The increased reliability and frequency of identifications means frequent additional information of the ID, location, and orientation.

Table 9.2: summary of differences in the capabilities of the tracking solution using RFID vs 2D IR marker

| **Capability** | **Neuromate** (RFID) | **Life Mouse tracker** (marker) |
|---|---|---|
| ID frequency | single lone mouse | ≈ 13 per second |
| ID performance | mota score >0.97 | unmeasured, but expected to be improved with higher ID frequency |
| Location accuracy | N.A. | additional location data from marker ≈ 13 per second |
| Orientation | N.A. | additional orientation data from marker ≈ 13 per second |
| Interference with other aspects of Neuromate | yes | no |
| Observe sensitive social behaviors | yes | improved with more ID, location, and orientation data available during close encounters |
| Evasiveness | none | fixated wearable marker |

In Table 9.3 all the considered different methods are listed and compared against the major capabilities.

Table 9.3: The tracking method proposed in this paper (Neuromate) and alternative tracking methods compared with the major capabilities

| **Capability** | **Neuromate** (IR+marker) | **Life Mouse tracker** (IR+RFID) | **Force plate** [46] | **Gated** (RFID) [26] | **Touch Sensitive Floor** [70] |
|---|---|---|---|---|---|
| Multiple rodents | yes | yes | no | partial | partial |
| 15 observations/sec | yes | yes | yes | no | yes |
| Location | yes | yes | yes | no | yes |
| Orientation | yes | yes | no | no | no |
| Social behaviors | yes | partial | no | no | partial |

# 10

# Conclusions and Recommendations

The overarching goal of the Neuromate is to enable the Neuroscience department of Erasmus Medical Center (Erasmus MC) to perform their desired studies on multiple group housed mice. Tracking the location and orientation of multiple group housed rodents is essential for there studies. A sound tracking solution would allow to automated observation of mice behavior. To accomplish this task this thesis proposed and validated an IR-camera tracked 2D IR marker system as a replacement to the current RFID based solution. This chapter concludes the findings and discussion of this thesis, contains recommendations for further research, and discusses the contribution of this work.

## 10.1. Conclusions

### 10.1.1. Research question

**Main research question:**
The main research question is:

*Can a IR camera identify a 1 cm by 1 cm unique 2D IR marker on a single mouse, within 1 second with a true/false ratio of at least 3/1, which is positioned 5 cm away from any other mouse in a 40 cm by 40 cm cage with 4 group housed mice?*

We will break down this questions into its components and discuss these separately:

- **Can an IR camera identify a 1 cm by 1 cm unique 2D IR marker on a single mouse?**
  The current state of the system has not directly proven that this is possible. Due to limitations in the IR camera hardware, the camera resolution was not ideally spread over the test area. This reduced the usable resolution for experiments in this investigation, therefor requiring larger 2D IR markers. However, these larger 2D IR markers were scaled proportionally to the available resolution of the camera, indicating that proper hardware could identify markers with a 1cm$^2$ area. This would need to be validated but this investigation does not reject the possibility of such capabilities.

- **Can the method identify a mouse within 1 second when it is positioned 5 cm away from any other mouse?**
  The encounter data shows that the identification after separation happens within 0.17 s ± 0.08 s, successfully proving this is possible. The system is also able to detect the markers during the close encounter with a TP/s of 16.78 ± 1.75.

- **Can the method perform with a false/positive ratio of at least 33.3%?**
  The maximum false/positive ratio observed in all tests performed is 1.29% for any trajectory. This is significantly better than a false/positive ratio of 33.3%.

- **Can the method be used with 4 mice in a 40 by 40 cm cage?**
  All tests are done with a 40 cm by 40 cm cage, but only up to 2 mice. Tests with 4 mice have not been performed for 2 main reasons.

  - The dummy mice could not be in sufficient close proximity to each other, because magnets were used to move mice during tests by the operator. Were two mice to get in close proximity the magnets would result in the mice attracting each other and become inseparable for the remainder of the test. This would make the test uncontrollable.
  - The laptop used could not process more than 2 minutes with 2 mice. Adding mice would significantly reduce the maximum run-time of the tests to the point that it would provide no useful results.

  However the successful tests with 2 mice prove that multiple mice can be tracked. By using a more powerful computer it can be expected that this setup could scale to such an extent that tracking at least four mice is possible.

To summarize the answer of these subquestions to answer the main question:
Through the use of the designed proof of concept, an IR camera can identify unique 2D IR marker on a single mouse within 1 second with a true/false ratio of at least 3/1, which is positioned 5 cm away from any other mouse in a 40 by 40 cm cage. From the lessons learned from this proof of concept, with further development it can be expected that an IR Camera system can identify a 1 by 1cm unique 2D IR marker with 4 group housed mice.

**Sub-question 1**

*Can the location and orientation of a 1 cm by 1 cm unique 2D IR marker on a single mouse housed with 4 others in a 40 cm by 40 cm cage be determined with an accuracy below 8 mm and 10 degrees in the horizontal plane using an IR camera?*

If the ID of an 2D IR marker is detected the location of the corner-squares of the 2D IR marker are known to a certain degree. For the test setup, the maximum error of the location is 4.8 mm and the maximum error for the orientation 12.7 degrees.
For a 1 cm by 1 cm marker the location error would go down to 1.2 mm. So the location accuracy is well within the expected error margin.
The real orientation error is expected to be lower than 12.7 degrees. This reduction in error is predicted to be below 10 degrees with this test setup relying only on the 2D IR marker. The accuracy can be improved by improving the pixel density and/or using tracking of the head and tail location. But, it is not proven that this test setup performs well enough for the orientation angle to be determined with the required accuracy.

**Subquestion 2**

*Can the identification of a 1 cm by 1 cm unique 2D IR marker with an IR camera help track mice during close encounters?*

The average true-positives per second recorded during the encounter test runs is $16.78 \pm 1.75$ TP/s.
The identification of a 1 cm by 1 cm unique 2D IR marker with an IR camera can help track mice during close encounters. Even with the proof of concept setup, the performance during the encounters is very good.

### 10.1.2. Criteria

The elicitation of the criteria, with its prioritization using MOSCOW described in section 4.3, for the test-setup are applicable for the Neuromate program as a whole. Therefore, many of the criterion are not-applicable to the tracking method. However, all tracking method relevant criterion are either met, or not applicable, in which case they were not violated, with the following exceptions:

- *should-have*: one criteria was partially met, and one criteria could not been determined with this setup.
- *could-have*: one criteria was partially met, and one criteria could not been determined with this setup.

### 10.1.3. Project goal

The goal of this thesis is to design and evaluate a technical approach for tracking both the locations and orientations of group-housed rodents to be applied in the Neuromate project.

Many tracking solutions have been considered, and an IR camera based system with a 2D IR marker has been selected to be explored further. The effectiveness of the tracking solution was proven by the test results from a proof of concept implementation, which showed that the solution would be a good fit for the goals of the Neuromate project.

## 10.2. Contribution

This thesis furthers the development of the Neuromate, which aims to extend the scope of what is possible in the neuroscience research field. It will allow new types of neurological studies on rodents and ease the labor requirements for this type of research.

The principal contributions of this thesis are:

1. An analysis of the current state of the Neuromate and its design criteria. (Analysis: Chapters 1 and 2, Criteria: Chapter 4)
2. An overview of tracking methods including their suitability for tracking rodents to the criteria of the Neuromate. An camera only tracking or a combination of tracking methods including camera tracking was deemed the most suitable. (General overview: Chapter 3, Application Neuromate: Chapter 5)
3. A new tracking solution for the Neuromate, adapted from the Live Mouse Tracker [1]. The Live Mouse Tracker is a state of the art tracking system which combines camera tracking with RFID to observe free roaming group housed rodents. In this thesis the system's identification technique is changed from RFID to an IR camera based system. It uses an IR camera to track individual rodents equipped with a 2D IR marker small enough to be of little hindrance to rodents. This system improves the tracking performance, especially during close interactions of multiple rodents, which is a focus for the Neuromate. The elimination of the RFID-technology frees up the RFID-frequency band. This allows the use of other applications which require similar frequencies, such as the wireless power transfer for the wearable device used by the Neuromate. Testing showed the combination of tracking methods (camera, 2D IR marker) to be valid. However, the current setup needs a redesign to meet the Neuromate's criteria. (Design: Chapter 7, Results: Chapter 8, Discussion: Chapter 9)
4. A new test setup using dummy mice for ethically evaluating tracking solutions during early stages of development. This setup revealed the limitation in the current version of the new tracking solution. (Design: Chapter 7, Results: Chapter 8, Discussion: Chapter 9)

## 10.3. Recommendations

This Section will discuss the recommendations for the IR camera tracking of a passive visual 2D IR marker, now that the effectiveness has been determined with the proof-of-concept. This will be done in two steps. First, changes that are crucial for the proof-of-concept to be fit as a tracking solution for the Neuromate will be discussed. Secondly, other recommendations are considered related to the limitation of the proof-of-concept as discussed in Chapter 9.2. These might prove useful to consider when further developing the proof-of-concept.

### 10.3.1. Crucial changes

There is a large change required to create a suitable tracking solution that meets the criterion of the Neuromate Program. This change is needed to make sure that the tracking solution can track 2D IR markers as small as 1 cm by 1 cm. To achieve this the pixel density has to be increased. This can be done in three ways.

#### Camera

The first method to increase pixel density is by improving the camera coverage of the cage. The Microsoft Xbox One Kinect has a significant area of the camera outside the cage. This means that a lot of the pixels are not used for tracking mice. However, the height of the Microsoft Xbox One Kinect is fixed, because the depth sensing does not work when the camera gets close to the cage floor. In order to implement better coverage a different camera is required, and even then a HD-IR camera with sufficient coverage will not be enough to increase the pixel density to detect the small markers reliably. Modifying a consumer device by replacing the camera lens is not a viable option, as lens corrections is expected to embedded in the camera ASIC or firmware.

The second method to increase pixel density is by increasing the number of required cameras. With 2 or 4 cameras the view area of the cameras can be reduced to half or a quarter of the cage size, thus effectively increasing the pixel density. This would mean multiple camera inputs, which would complicate image processing. Imposing a substantial software change and challenge.

The last method to increase the pixel density is by using a camera with a higher-quality image. Infrared cameras with 2k or 4k pixels are on the consumer market. Some of these do have a frame-rate below the 30 frames per second, but these would be able to increase the pixel density enough so that a 1 cm by 1 cm 2D IR marker will be reliably detected. At which point a lower frame rate is not very limiting.

In conclusion, the tracking solution requires a different camera system, which means it will be a completely different solution form the Live Mouse Tracker [1], or effort has to be put in to let the Live Mouse Tracker support an other camera brand and type. Either way this will be a large change for the Live Mouse Tracker system. This change would however bring the proof of concept to a much more complete state.

**Verification**

After the camera upgrade, the solution needs to be verified. The first tests should be performed with dummy mice, similar to the tests of the proof-of-concept. These tests need to be done with 4 dummy mice with 2D IR markers. These tests are needed to make sure the system can keep up with 4 mice at once using a higher resolution camera. After these tests, a real mice test is required.

The tests with 4 real mice are needed to be able to determine the true performance of the tracking solution and to be able to compare it to other tracking systems on the market. The tracking solution should be implemented with a test that uses real mice. Based on the video recordings the tracking also needs to be done by at least two humans, who will mark the images from the same camera feed. The differences between humans as well as the differences between the tracking solution and a human tracker can be determined, preferably using a standardized metric such as the MOTA and the MOTP metrics from the paper by Bernardin, K. et al. (2006) [78].

### 10.3.2. Other recommendations

Besides the larger changes in the setup and testing as described above, there are some other changes that may improve the tracking system.

The proof-of-concept searches for the the 2D IR markers in the snippets from the detected mice by the Live Mouse Tracker code using the Microsoft Xbox One Kinect depth sensor. To improve the system, the marker could be looked for in the entire image. This could result in marker detection when a mouse is not detected. This helps the tracking system in situations where the mice would otherwise be lost by the tracking system.

If, during testing, it turns out that 2D IR markers are often found, but not always correctly identified some of the bits could be re-allocated to become error-bits, or even error-correction bits. For example, using one bit indicating whether the number of white square is odd, a single flip in the ID bits could be detected, but not corrected. This would lead to a lower rate of false ID's, but would reduce the available number of unique ID codes from $2^6$ to $2^5$.

Error correction is harder using a total of 6 bits. Optimal error correction for 1 bit flip uses the Hamming codes. Where r>=2 is the number of correction bits the number of message bits (k) is $k = 2^r - r - 1$. With 3 error correction bits ($r = 3$) there could be 4 message bits $k = 4$, which is a total of 7 bits. This is a larger number than the 6 available, but for r=2 and k=1 for a total of 3 bits, which is lower. For the 6 bits the most efficient way to perform bit correction is by repeating the message. Thus a 2 bit message can be sent 3 times. This would result in a low amount of possible IDs, namely 4. This is just enough for a unique ID for each of the 4 mice in a cage.

If, during testing with real mice, the angle of the 2D IR marker to the camera exceeds 45 degrees with some frequency, additional markers could be added to each of the faces of the wireless device. As the top face of the device turns above the 45 degree angle another face will be turned towards the camera. A problem does arise as the 2D IR marker would no longer give a direct indication of the mouse heading. This could be corrected for by making the markers on each face unique. This would require 2 bits. This reduces the number of ID bits to 4 bits.

It is hard to predict the exact required amount of computational power required for a the final tracking solution as it is dependent on multiple aspects. However it is likely to be a demanding task to run the tracking solution. Therefore, it is recommended to use a computer with good computational power, a large amount of memory storage and fast long term storage. At the very least a good computer will compensate for code optimizations during early development of the tracking solution.

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# A
# Runtable

The numbered items below identify the combination of test phase, number of test runs and number of mice used as follows:

**1 =** The single trajectory using one test-run per mouse - 2D IR marker combination.

**2 =** The social trajectory using 5 test-runs per mice - 2D IR marker combination.

**3 =** The encounter trajectory using 5 test-runs per mice - 2D IR marker combination.

**4 =** The obstruction trajectory using one test-run per mouse - 2D IR marker combination.

The order in which test tests are performed are randomized, with Matlab, to reduce the effect of nuisance variables. The resulting run-table can be found in the appendix, Table A.1 and an abbreviated of the run-table in Table 7.3.

The run-table 7.3 consists of a three columns the first is the test sequence number, the second columns specifies the test phase from the list above and the third columns specifies the mouse id(s) used.

The number representing the ID of the mouse for the test phases, applicable to phase one and four, with a single mouse is as follows:

**1 =** ID 53

**2 =** ID 30

**3 =** ID 43

For test phase two using the social trajectory the number representing both random selected ID's, one for each of the mouse, is as follows:

**4 =** ID 53 & ID 30

**5 =** ID 30 & ID 43

**6 =** ID 43 & ID 50

For test phase three using the encounter trajectory the number representing both random selected ID's, one for each of the mouse, is as follows:

**7 =** ID 53 & ID 36

**8 =** ID 30 & ID 36

**9 =** ID 43 & ID 36

The lists above with the ID numbers are the result of a random generator in order to make the 2D IR marker patterns an independent variable.

Table A.1: run table

| Run-sequence number | Test phase | Mouse/mice used |
|---|---|---|
| 1 | 3 | 8 |
| 2 | 3 | 9 |
| 3 | 3 | 7 |
| 4 | 3 | 7 |
| 5 | 2 | 4 |
| 6 | 3 | 7 |
| 7 | 3 | 7 |
| 8 | 4 | 3 |
| 9 | 2 | 6 |
| 10 | 2 | 5 |
| 11 | 2 | 5 |
| 12 | 1 | 2 |
| 13 | 2 | 6 |
| 14 | 2 | 4 |
| 15 | 2 | 5 |
| 16 | 2 | 4 |
| 17 | 4 | 2 |
| 18 | 2 | 3 |
| 19 | 1 | 3 |
| 20 | 2 | 6 |
| 21 | 3 | 8 |
| 22 | 3 | 7 |
| 23 | 1 | 1 |
| 24 | 2 | 6 |
| 25 | 2 | 4 |
| 26 | 3 | 8 |
| 27 | 3 | 8 |
| 28 | 4 | 1 |
| 29 | 2 | 5 |
| 30 | 3 | 9 |
| 31 | 3 | 9 |
| 32 | 2 | 5 |
| 33 | 3 | 8 |
| 34 | 3 | 9 |
| 35 | 2 | 6 |
| 36 | 3 | 9 |

# B

# Test Variables

## B.1. Dependent variable

The following dependent variables are delivering the results of the experiments:

1. 2D IR marker identification.
2. Location
3. Orientation

The dependent variables are influenced by the following parameters:

1. Deviations from the desired trajectory
2. Variations in the size of the 2D IR marker
3. Orientation shift of the mean periodic system code during the experiment
4. Ambient light conditions
5. Variation in type of the Kinect
6. Temperature
7. Humidity
8. Speed during the trajectory
9. Performance of the machine learning
10. Laptop performance
11. Mouse friction to surface
12. Movement stutters
13. Lose mouse magnet
14. Camera placement
15. 2D IR marker infrared effectiveness
16. Color threshold
17. Program auto-calibrations

18. Trajectory
19. Location 2D IR marker
20. Orientation 2D IR marker

Some of above parameter are independent others nuisance, as determined by the research question.

## B.2. Independent variables

From the long list of the influencing parameters in the previous chapter the research questions gives us the following independent variables.

1. Trajectory
2. 2D IR marker
3. Mouse size
4. Mouse color

## B.3. Nuisance variables

With the given test setup and environmental conditions it is expected that the major nuisance variable is:

1. Ambient light conditions, as this might influence the camera recording.

Table B.1 collects all nuisance variables and their motivated treatment, using the following coding :

F = fixed value (specify value)

R = depend on randomization

O = observe or measure (explain how)

T = Taken out of the experiment

Table B.1: Identified nuisance variables and how they are treated.

| Variable | Treatment | Motivation |
|---|---|---|
| 1. Deviations from the desired trajectory | T. | If the mouse is outside the desired test area the measurement will be discarded. |
| 2. Variations in the size of the 2D IR marker | F. | Uniform size |
| 3. Orientation shift of the 2D IR marker during the experiment | F. | |
| 4. Ambient light conditions | R. | |
| 5. Variation in type of the Kinect | F. | |
| 6. Temperature | R. | |
| 7. Humidity | R. | |
| 8. Speed during the trajectory | O. R. T. | Will be calculated using the location data from the live-mouse-tracker. Only than desired speeds are kept, or multiple measurements can be done to plot different speeds. |
| 9. Performance of the machine learning | T. | |
| 10. Laptop performance | O. | Measured by dropped frames. |
| 11. Mouse friction to surface | F. | |
| 12. Movement stutters | R. | Should be about the same for all experiments and should on average be present the same amount in all experiments. |
| 13. Lose mouse magnet | T. | If magnet disconnect the results are discarded. |
| 14. Camera placement | F. | |
| 15. 2D IR marker infrared reflectiveness | F. | |
| 16. Color threshold | F. | |
| 17. Program backdrop auto-calibrations | R. | |
| 18. Trajectory | F. | |
| 19. Location 2D IR marker | O. | |
| 20. Orientation 2D IR marker | O. | |
| 21. Mouse size | F. | The 2D IR marker mice combinations are set for ease of the experiment. Different mice and 2D IR markers make sure that are no anomalies with certain mice or 2D IR markers. |
| 22. Mouse color | F. | Same as previous. |