



Mila Maria Pamplona Barbosa

**Ritmos diários de forrageamento e
corte de folhas em uma colônia de *Atta
sexdens***

Daily rhythms of foraging and leaf-cutting on
an *Atta sexdens* colony

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Dedicatória

Aos meus pais, Janinha e
Carlinhos, pelo apoio
incondicional na busca
pela realização dos meus
sonhos.



Epígrafe

Stonehenge

One of my goals in these closing sections has been to illustrate - and enjoy! - what I take to be the real adventure in the scientific enterprise, which is, among other things, a search for pattern and meaning in what one observes. It is the necessarily conjectural nature of that search, at least at the outset, which entails hazard and makes the enterprise adventurous, not only for the observer himself, but for his observations and ideas. "Escape from light" is just such an adventure, which one enjoys for its own sake, and possibly will prove useful, perhaps even correct.

Stonehenge

Um de meus objetivos nestas seções finais tem sido ilustrar - e aproveitar! - o que considero ser a verdadeira aventura no empreendimento científico, que é, entre outras coisas, uma busca por padrão e significado no que se observa. É a natureza necessariamente conjectural dessa busca, pelo menos no início, que acarreta perigos e torna o empreendimento aventureiro, não apenas para o próprio observador, mas para suas observações e idéias. "Fugir da luz" é exatamente uma tal aventura, que se desfruta por si mesma, e que possivelmente se revelará útil, talvez até correta.

Colin S. Pittendrigh,
TEMPORAL ORGANIZATION: Reflections of a Darwinian Clock-Watcher
(tradução nossa)

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Resumo Geral

Ritmos biológicos têm um papel importante nos aspectos fisiológicos e comportamentais dos organismos. A compreensão do ritmo diário de insetos sociais nos permite realizar análises desde o indivíduo até o nível da colônia. O estudo dos ritmos diários das formigas, especialmente formigas cortadeiras, tem sido um desafio devido ao tamanho das colônias e ao polimorfismo. O presente trabalho teve como objetivo entender como as colônias de formigas cortadeiras se comportam coletivamente considerando o corte de folhas e o forrageamento, desenvolvendo uma ferramenta de vídeo-tracking para monitorar o fluxo de formigas. Foram utilizadas câmeras para registrar o forrageamento (vídeo) e como a área foliar diminuiu (fotografias). Uma colônia de *Atta sexdens* foi exposta a um ciclo LD 12:12 (500 lux durante a fase de claridade e luzes vermelhas acesas durante todo o experimento; temperatura = 23°C, umidade = 60%). O reabastecimento de folhas (*Acalypha* sp.) aconteceu uma vez por dia usando um protocolo aleatório. Registramos a atividade da colônia durante 30 dias, realizando 2 deslocamentos de fase de 6 horas (atraso e avanço). Observamos que embora a colônia esteja ativa durante as fases de claro e escuro, sua atividade é maior durante a fase de escuro, tanto em relação ao forrageamento quanto ao corte de folhas. O software de rastreamento de vídeo desenvolvido neste projeto nos permitiu contar 143904 formigas após analisar 2133 vídeos. Também analisamos a relação entre o número de forrageadoras e as taxas de corte de folha sob condições sincronizadas e dessincronizadas. Concluímos que esta relação pode ser um parâmetro interessante para discutir estratégias de eficiência a nível individual e coletivo após distúrbios na ordem temporal interna da colônia. Também acreditamos que usando os protocolos e software desenvolvidos neste projeto muitos outros aspectos da organização temporal em formigas cortadeiras podem ser identificados, revelando uma compreensão mais profunda de como a resincronização ocorre após rupturas da ordem temporal interna.

Abstract

Biological rhythms play an essential role in organisms' physiological and behavioral aspects. Understanding daily rhythmicity on social insects allows us to analyze the individual to the colony level. Studying ants' daily rhythms, especially leaf-cutter ants, has been challenging due to the colony sizes and polymorphism. The present work aimed to understand how colonies of leaf-cutter ants rhythmically behaved collectively considering their leaf-cutting and foraging activity, developing a machine learning video tracking tool to analyze the ant's flow. Cameras were used to register the foraging activity (video) and how the leaf area decreased (photographs). An *Atta sexdens* colony was exposed to an LD cycle 12:12 (500 lux during the light phase and red lights on during all the experiments; temperature = 23°C, humidity = 60%). Leaves replenishments (*Acalypha* sp.) happened using a random protocol once a day. We recorded the colony activity during 30 days, performing two 6 hours phase shifts (delay and advance). We have observed that even though the colony is active during both light and dark phases, the colony activity is higher during the dark phase, regarding both the foraging and the leaf-cutting activities. The video-tracking software developed in this project allowed us to count 143904 ants after analyzing 2133 videos. We have also analyzed the relationship between the number of ants foraging and the leaf-cutting taxes under synchronized and desynchronized conditions. We concluded that this relationship might be a sensitive parameter for discussing efficiency strategies at the individual and collective levels after disturbances on the colony's internal temporal order. We also believe that using the protocols and software developed in this project, many other aspects of temporal organization in leaf-cutter ants may be identified, building a deeper understanding of how resynchronization occurs after disruptions in the internal temporal order.

Chapter 1: General Introduction

BIOLOGICAL RHYTHMS

Throughout evolution, organisms have developed the ability to anticipate cyclic environmental changes in physiology and behavior (Pittendrigh, 1993). The development of endogenous rhythmicity generated by biological oscillators and their ability to synchronize with environmental cycles are significant aspects of this adaptive feature, which increases organisms' fitness (Jabbur *et al.*, 2021). In association with the regularity of environmental events, which occurs in the most diverse time scales, several biological rhythms also occur with short duration up to annual scales. In this context, circadian rhythms stand out (with periods near 24h), subject to synchronization by 24h environmental cycles such as light-dark, which synchronize them to daily, 24h rhythms (Aschoff, 1979; Pittendrigh, 1993 and Bloch *et al.*, 2013). Such rhythms are observed in all groups of living things, from single-celled to multicellular organisms (Dunlap, 2004). Considering even a longer time scale, it is worth noting the aspects involved with seasonality. Several polar animals, such as Arctic squirrels and birds, exhibit behaviors related to annual synchronization, such as hibernation and migratory behaviors, respectively (Bloch *et al.*, 2013). Some rhythms are synchronized by the tidal cycle, lunar cycles, and several others (Refinetti *et al.*, 2007; Wagner *et al.*, 2007). Given all this, we can verify the extent of these mechanisms of temporal regulation in nature so that the biological temporal organization occurs through different forms, ranging from cellular mechanisms to behavioral ones (Bloch *et al.*, 2013).

The organism's daily rhythmicity rhythm observed may result from several synchronizers. Among the most common daily synchronizers, the light-dark (LD) cycle, feeding cycles, and temperature cycles are worth mentioning. There is also, for social animals, the presence of a daily social cycle as a significant synchronizer, which is observed in social insects (Bloch, 2013; Lone and Sharma, 2011), bats (Marimuthu, 1981; Marimuthu, 1983), and even humans (Roenneberg *et al.*, 2019) - Figure 1.

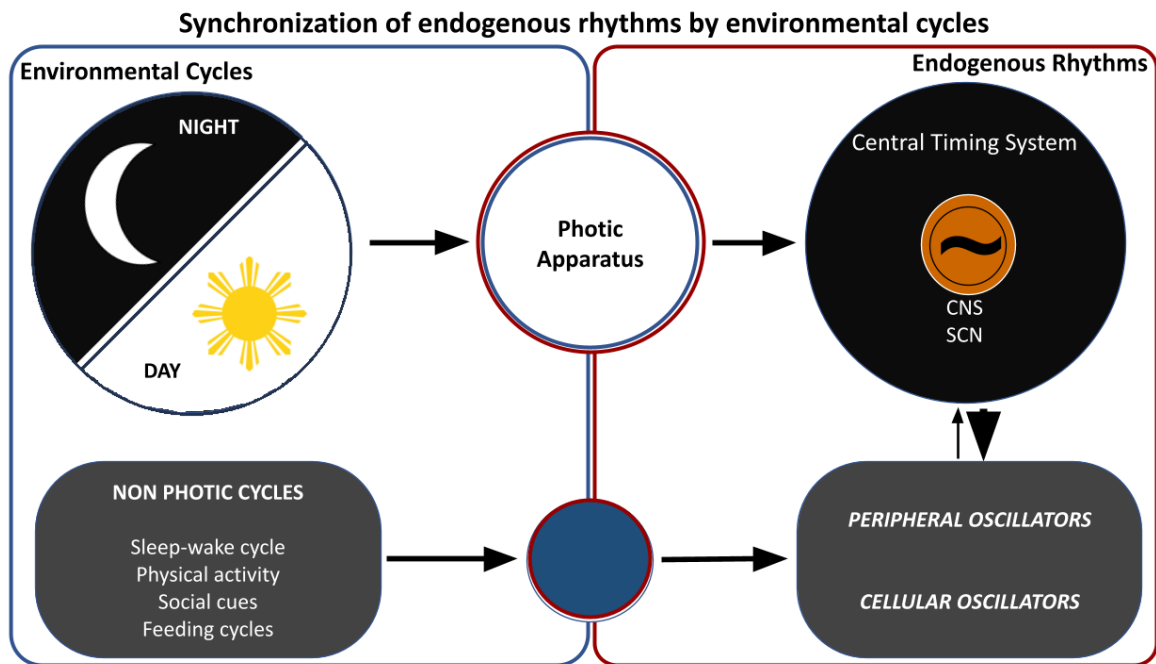


Figure 1) Schematic representation of the synchronization principles of endogenous rhythms by environmental cycles. Environmental cycles are sensed by afferent systems, which transmit timing information to central oscillators that transmit this information to the other peripheral oscillators in the organism, leading to synchronization (adapted from Buttgerit *et al.*, 2015).

The observed daily rhythm can be synchronized through two mechanisms: entrainment and masking. These two have great adaptive value since they provide the temporal organization system with precision and flexibility, respectively (Pittendrigh and Daan, 1976; Page, 1989). Entrainment occurs when the oscillator that generates the observed rhythm starts to exhibit the same period as the environmental cycle (which we call *Zeitgeber*). On the other hand, masking is an acute modulation of the amplitude of the rhythm, either by stimulation or by inhibition, without changing the properties (period and phase) of the oscillator that generates the rhythm. Thus, the activity of an organism can have effects of more than one synchronizer and has both anticipatory and direct response aspects to environmental phenomena (Pittendrigh and Daan, 1976; Page, 1989; Tachinardi, 2012). Another relevant piece of information is that after perturbations on its internal temporal order, such as those due to phase shifts of the LD cycle, an organism takes several days to

resynchronize with the new environmental cyclic conditions. This temporary oscillatory state between two synchronized and steady states is called “transients” and reflects the dynamics of the oscillators during the resynchronization processes (Pittendrigh, 1960).

Biological rhythms on social insects

Social insects are groups of insects that organize themselves in colonies, such as termites, bees, wasps, and ants. In these groups, characterized by eusociality (Wilson, 1971), we find a division of reproductive labor so that a caste is capable of reproducing and another infertile. Those systems also show a complex division of labor corresponding to the other tasks of the colony. Thus, the infertile class (workers) is divided into sub-castes with specific functions within the colony, such as foraging, care of the offspring, defense of the colony, garbage removal, and maintenance of the fungus (Wilson, 1980).

A colony of social insects can have hundreds to millions of individuals. It is worth noting that individual survival depends on belonging to the colony, as the colony's survival relies on the organized functioning of the individuals. Because there are individual and collective aspects, these systems can be considered complex (Boccarda, 2003). In multicellular organisms, not all cells are exposed to light-dark cycle cues; however, a group of cells sensitive to photic information can be synchronized and transmit this information to the whole organism, which can then present a fully synchronized rhythm (Figure 1). Social insect colonies often present groups of individuals exposed to photic environmental cues and can inform them to the rest of the colony that lives in constant conditions (Figure 2). That social interaction within the colony also allows its synchronization, similar to what we observe in many multicellular organisms. Social insect colonies allow us to investigate daily synchronization due to environmental cycles (light/dark) and the internal synchronization due to social interactions (Southwick and Moritz, 1987; Moritz and Sakofski, 1991). In addition, the worker class of a leafcutter ant colony has a high level of genetic similarity, around 75% (Hamilton, 1964). All these features

reinforce the idea of ant colonies as “superorganisms,” making them, therefore, excellent objects for studying the processes involved in the emergence of biological rhythms in colonies and possibly even in other organisms. However, the knowledge built about this topic is mainly resulting only from chronobiological studies conducted in bees (Frisch and Koeniger, 1994; Moore, 2001, Bloch *et al.*, 2001; Toma *et al.*, 2000; Bloch and Robinson, 2001), and few studies have been conducted with ant colonies, and there are still many questions to be explored about their synchronization mechanisms (Figure 2).

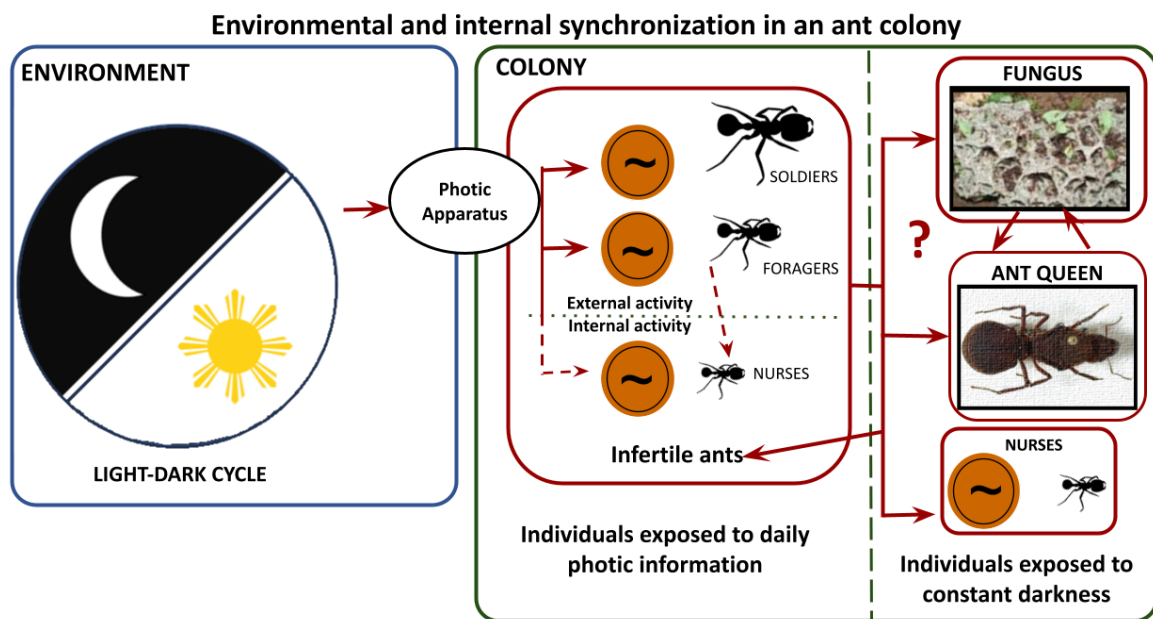


Figure 2) Possible relationships involved in the light-dark cycle synchronization of a leafcutter ant colony. Individual rhythms emerge into rhythms of the subcastes, and these emerge as the colony rhythm. Furthermore, the role of elements such as the fungus and queen in synchronizing the rest of the colony is unknown.

Although leafcutter ant societies, such as *Atta*, offer an excellent opportunity to extend comparative studies (McCluskey 1958; 1965; Roces and Nunez 1996), two main aspects make the task of measuring collective behavior potentially with the support of video recordings difficult. The first is a large number of individuals since

Atta colonies can have up to 8 million individuals. The second is the variety of sizes among ants, since several species show polymorphism (Figure 3), with individuals ranging in size from millimeters to a few centimeters.

Given these challenges, the rare studies of rhythmicity in ants have been carried out exclusively in isolated individuals (Sharma *et al.*, 2004) or small groups (McCluskey 1965, 1967), often deprived of the collective context offered by the colony. Such an approach, although practical, ignores one of the fundamental aspects of colony regulation: the existence of the collective and individual organization, inseparable in regulatory processes.

Recent work has explored ant rhythmicity at the molecular level, from clock genes present in individual circadian oscillators located in the brain (Ingram *et al.*, 2009, Kay *et al.*, 2018). Some studies look at rhythmicity in nurses, showing that the presence/absence of eggs modulates their activity (Fuchikawa *et al.*, 2017). This aspect is fascinating since this sub-caste (and the queen) is not directly exposed to light-dark effects as their activities are restricted to the nest, where there is no light input in a natural environment. In this sense, despite the significant advances made in the study of the spatial organization of ant societies (Clark, 2006), less is known about the temporal organization of their activity and its distinct individual and collective aspects, especially important in these eusocial organisms.

An interesting additional factor in these collective systems is the presence of a symbiont fungus present in some ant groups. The *Neoattini* ants (belonging to the tribe *Attini*), with the genera *Atta* (“saúvas”) and *Acromyrmex* (“quenquéns”), are known as leafcutters and have a high distribution in Neotropical areas. They are characterized by growing a fungus garden, cutting leaves, foraging on a large scale, and colonies that can number in the millions. Particularly, species of the genus *Atta* also exhibit a gradual polymorphism among their subcastes (Weber, 1972 and Wilson, 1980). Thus, larger ants are generally associated with colony defense tasks and are called soldiers. On the other hand, smaller ants tend to be responsible for tasks internal to the nest, such as bringing water to the colony. Foraging is usually related to the intermediary ants (Wilson, 1980) (Figure 3). It is also worth noting the dynamics of this division of labor among the subcastes, which is not deterministic, and the function of an individual may vary according to the colony’s requirements.

Our laboratory also observed that larger and intermediate ants tend to suffer more significant influence from daily environmental cycles (Toledo, 2014) since they perform tasks outside the nest, being more exposed to environmental variations (Bloch *et al.*, 2013). Recent work from our laboratory also shows that leafcutter foragers organize themselves in work shifts, with two distinct states of the colony organization considering the light and darkness phases (Constantino *et al.*, 2021).



<https://upload.wikimedia.org/wikipedia/commons/thumb/7/7d/Atta.cephalotes.gamut.selection.jpg/220px-Atta.cephalotes.gamut.selection.jpg>

Figure 3) Polymorphism in *Atta*. The figure shows the gradual variation in size between the castes. The two most prominent individuals comprise the reproductive caste, representing a queen and a virgin queen. The others correspond to the distinct sub-castes within the workers.

As mentioned, one of the main challenges in dealing with the individuals of a vast colony with a great morphometric variety are methodological aspects. In order to observe the ants in these conditions, computational techniques need to be created, especially considering the number of ants that had to be evaluated (15.000). As discussed later, a method based on machine learning techniques to evaluate large numbers of ants was developed.

A LOOK INTO PRODUCTIVITY, ENERGETIC EFFICIENCY, AND BEHAVIOR

Social insects' literature comprehends a significant number of uses of the terms "productivity" and "efficiency." Sometimes they are used almost as equivalents, but through a more profound look across the decades, we can observe some changes in the guiding ideas that drove the discussion of productivity or efficiency in social insects. In this sense, discussing "productivity" within a social context is not trivial since it can take into account individual aspects (productivity per capita) as well as global aspects of the colony (colony productivity). The productivity of a social insect colony should be thought of within an evolutionary context (Montagna *et al.*, 2010 and Naug and Wenzel, 2006). Keeping in mind the idea of inclusive fitness (or kin selection) within social insects (Hamilton, 1964), the ability to obtain food for the colony should be a key component in determining its productivity (Naug and Wenzel, 2006). Given this, foraging becomes an essential process, as larger foraging areas should imply a greater number of food sources available to the colony (Mailleux *et al.*, 2003, Montagna *et al.*, 2010 and Naug and Wenzel, 2006). The number of individuals recruited to forage is directly related to the colony's foraging area, so the greater the number of foragers, the greater the total area foraged by the colony (Mailleux *et al.*, 2003) Naug, and Wenzel, 2006).

In this way, we have an increased probability of success in finding new food sources while also increasing the number of ants that cannot find new food sources (Mailleux *et al.*, 2003 and Naug, and Wenzel, 2006). Thus, we get two essential concepts for thinking about aspects of colony productivity. Firstly, we have an idea of "Colony Productivity," which can be inferred through parameters such as the total food obtained by the colony, the total number of individuals recruited for foraging activity, in addition to aspects related to colony size, and consequently reproductive parameters associated with the queen, with their particularities within social insect species (Michener, 1964, Mattila *et al.*, 2012, Montagna *et al.*, 2010 and Naug and Wenzel, 2006).

Secondly, we have the concept of "Productivity per capita," which aims to understand how individual components act within the overall colony context

(Modlmeier, 2013). Productivity per capita can be assessed through the efficiency of individual transportation or even the time an individual uses to perform the task (Naug and Wenzel, 2006). The big question associated with these two concepts, brought about by Michener's paradox, consists in the fact that increasing the number of foragers is able to increase the overall productivity of the colony, even if the per capita productivity suffers a reduction (Michener, 1964 and Naug and Wenzel, 2006).

Similar to what we observe with the term "productivity," "efficiency" can also refer to many different aspects of social insects' literature. Efficiency can refer to reproductive efficiency, considering how many individuals can be generated per reproductive female and comparing colony sizes (Connell, 1961; Michener, 1964). Meanwhile, more recent studies focus the "efficiency" discussion in social insects on aspects related to task efficiency (Dussutour *et al.*, 2007; Bernadou *et al.*, 2016; Bouchebti *et al.*, 2015; Yerushalmi *et al.*, 2006; de Toledo *et al.*, 2016). While the first relied on the idea that colonies with higher numbers of individuals were more efficient, task efficiency refers to workers' individual performance, similar to what we observe in the idea of productivity per capita. For example, if a forager cuts and carries more leaves than others, it could be considered more efficient, assuming an isolated parameter (leaves cut per ant). However, it is possible to add more variables considering task performance: ant sizes, speed, distance to the food source, type of source, and time are some of the aspects that can increase the complexity of discussing foraging efficiency. All of those are deeply related to the energetic aspects involving foraging activity. Despite the intrinsic difficulty of defining efficiency, considering that behavior change over time can alter the strategy for obtaining resources in a way that can be observed and described (based on measurable inputs and outputs), it is possible to assume that efficiency has changed being increased or decreased.

Although this idea considering social insects contains several particular features, as discussed above, the main discussion about energetic efficiency is similar to many other species. An organism homeostatic balanced spends less energy to maintain all its activities than their energetic supplies available and, thanks to this optimal relationship, increases its adaptiveness and its fitness (Stewart, 2007). Nevertheless, we are aware that this optimal relationship is constantly being

threatened as natural environments offer a variety of challenges to the organism's survival. Thus, all these aspects also increase the complexity of discussing energetic efficiency since physiological mechanisms and distinct behaviors might offer different advantages in different contexts.

Behavioral plasticity

It is possible to argue that organisms' behavior can be resulted from different strategies to predict and respond to environmental stimuli. In this sense, the ability to anticipate cyclic environmental changes relates to an endogenous component and rhythmic behaviors, while responding to specific cues can be related to an exogenous component, which can involve an acute or even an anticipatory response based on previous experiences, mediated by the cognitive system.

A great yet curious example of these ideas is the octopus *Abdopus aculeatus*. Its habitat involves rhythmic challenges, as it faces diurnal changes of tides. Predators are more present during the high tidal level. During the low tide, they search for food sources (primarily crustaceans) in the intertidal reef, which usually requires several types of locomotion, as these marine animals move from pool to pool walking on land (Huffard, 2005, 2006, 2007). The behavioral set of these animals is vast, as they can quickly adapt from aquatic to aerial environments, besides the constant state of alertness to promote escape from predators. Considering all these challenges, they chose different locomotion strategies that vary from aerobic to anaerobic and have different energetic costs, according to their necessities (Huffard, 2005). Escaping from a predator or attacking a prey usually involves locomotion strategies that require much energy; however, success in these tasks is mandatory for the survival of these octopuses (Huffard, 2005).

Considering this example, we observe the high level of importance to combine rhythmic anticipatory behaviors (endogenous component) and fine-tuning behaviors resulting from the cognitive system (exogenous component). The optimal combination of these physiological components is crucial to an organism's survival, increases its fitness and adaptiveness, and is deeply related to the dynamic variations of behaviors and their specific energetic requirements. The present work

aims to discuss the combination of these physiological components and the dynamic strategies involved in the efficiency of a leafcutter ant colony when facing temporal disruption on its internal temporal order and random feeding events.

MACHINE LEARNING TECHNIQUES APPLIED TO BEHAVIORAL RESEARCH

Machine learning is defined as the study of algorithms that allow computers to perform tasks automatically and improve their performance thanks to previous data. Machine learning uses neural networks and is the basis of artificial intelligence (Naqa and Murphy, 2015; Zhang, 2017). The modern and globalized world uses machine learning techniques and artificial intelligence (AI) to perform several activities, from social media on our cellphones to defining public policies based on big data. Whether we understand it or are aware of this presence, it is everywhere.

Machine learning uses input data to extract features that can “learn” and perform a task based on this learning. Machine learning can be divided into two main groups: shallow and deep learning (Figure 4). The main difference between shallow and deep learning refers to feature extraction. While deep learning involves a more complex architecture and an automatic selection of features, in shallow learning, humans select the features that will be used for training the models. In this sense, deep learning offers an important tool to solve and deal with many biological questions since variability is an intrinsic component of biological data. Selecting the best features for model training may be challenging when researchers do not have enough knowledge about their system of study.

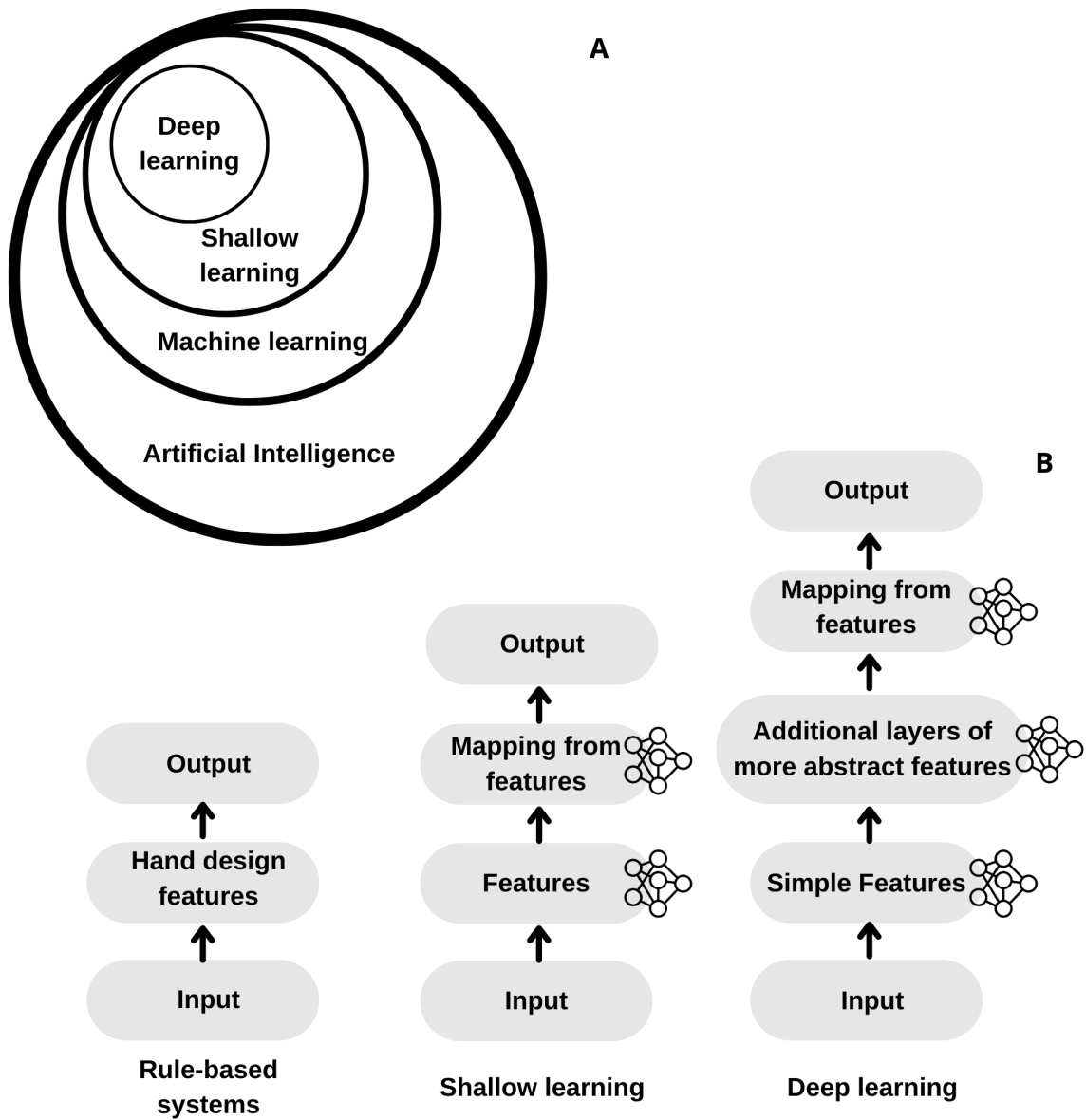


Figure 4) Schematic representation showing machine learning structure. Venn diagram of the relationship between different components of Artificial Intelligence. Flow chart of different AI parts used in machine learning. Small network symbols indicate components that can learn from data (adapted from Goodfellow et al., 2016).

OBJECTIVES

1. Developing a video-tracking software able to analyze the foraging behavior of a leaf-cutter ant colony inside the laboratory.
2. Applying this video-tracking tool to a synchronization experiment using a leaf-cutter ant colony. We also aim to monitor the daily leaf consumption and leaf-cutting rhythm in the same experiment.

Objective 1 is treated briefly below (for more detailed information, please check the Tutorial present in the Annexes section). Objective 2 is the central theme discussed in Chapter 2 of this manuscript.

DEVELOPMENT OF AN OBJECT DETECTION AND COUNTING TOOL FOR ANALYZING ANT'S FORAGING ACTIVITY

Our group has been developing tools for analyzing ant foraging trails through video-tracking, allowing size discrimination, and monitoring a population of about 10-15 thousand individuals. This software uses machine learning and deep learning techniques to automate the counting of high flows of ants on foraging trails, being an essential tool for studies with this type of system, enabling deeper investigation of temporal aspects at the colony level (Toledo, 2014 and 2018). For developing our final video-tracking tool, many steps were involved in the machine learning process (Figure 5).

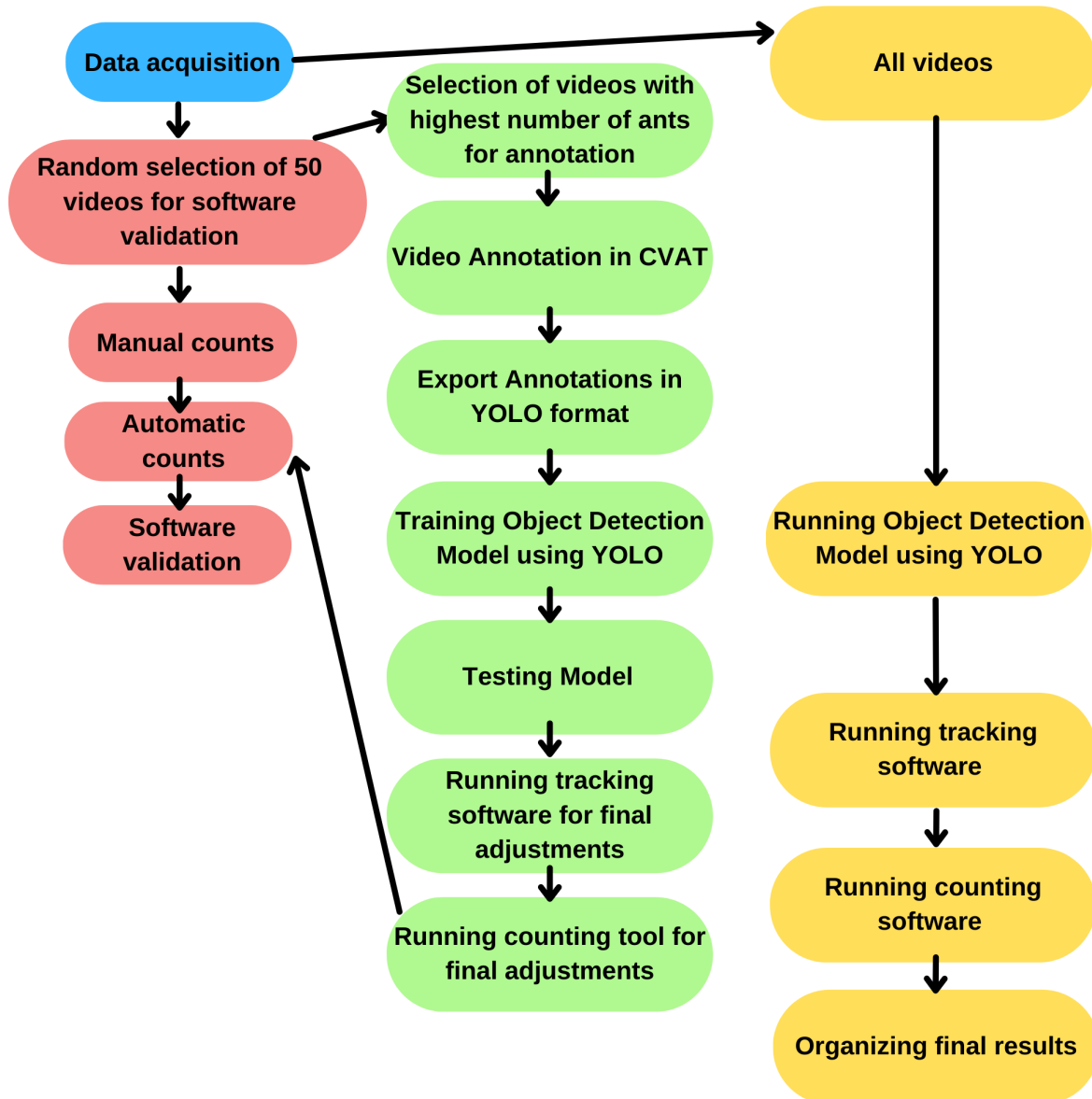
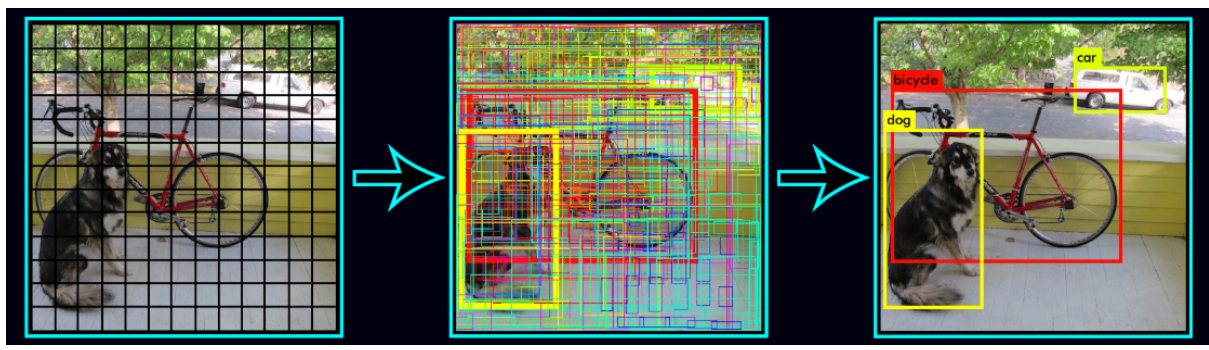


Figure 5) Flowchart of all the steps involved in our video-tracking software development. Blue box involves data acquisition (experiments). Red boxes show all steps involved in the software validation stage. Green and yellow boxes show the deep learning train model stage and run our model results for all our data.

The main challenges regarding using this type of tool involve object detection. In this case, it is necessary to make the computer recognize the object (ant) and follow it throughout the video, accounting for it in the flow counts. However, the foraging trail is a complex environment that involves different elements, albeit in the laboratory context. We have, for example, ants of various sizes and functions, such

as those seeking or carrying leaves, as well as those removing obstacles ("waste") from the trail itself. In addition, the flow of ants can be very high at certain times, especially when the colony is feeding and during the night. Thus, this software needs to be trained to recognize the ants in different frame contexts in the video image. It is precisely in developing this kind of tool that part of the present work took place, making use of powerful object detection techniques such as YOLOv2 (You Only Look Once - versão 2, Redmon *et al.*, 2016) and YOLOv5 (You Only Look Once - version 5, Zhu *et al.*, 2021). When detecting objects in a video, this technique observes each frame once and calculates the probability that each object found in the frame belongs to a previously trained class (Figure 6). This way allows the analysis of a large amount of video data, being an important tool used, for example, for monitoring public security cameras.



<https://towardsdatascience.com/implementing-yolo-on-a-custom-dataset-20101473ce53>

Figure 6: Frame-by-frame object detection mechanism realized by YOLO.

During this project, we developed two versions of this software, and they required several complex steps which are detailed and organized in our supplementary material. The first version used YOLOv2 and counted ants in our pilot experiment with *Acromyrmex* ants (Figure 7). However, in our main experiment using *Atta* ants, the number of ants in the trail became extraordinarily high, reaching hundreds of ants per 2 minutes video. Since our first version was powerful enough to detect and count until 50 ants per video, and the software error increased the higher the number of ants per video, we decided to develop a second version of the

software using one of the most powerful and recent object detection techniques ever developed, which was YOLOv5 (Zhu *et al.*, 2021; Fang *et al.*, 2021).

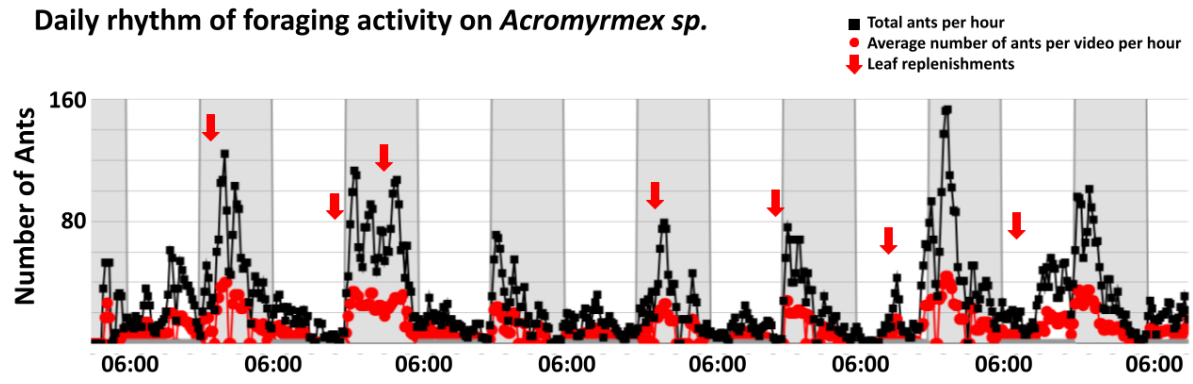


Figure 7: Daily rhythm of foraging. Recordings of 8 days of activity of a colony of *Acromyrmex* sp. exposed to a 12:12 LD cycle (lights on at 06:00h). Humidity was kept at 60% and room temperature at 23°C. Leaf replenishments occurred randomly once a day.

YOLOv5 provided us with great results, as we were able to count over 143.000 ants during the main experiment. Nevertheless, it is essential to acknowledge that developing a second version of the software in such a shorter time (we developed version 1 during a year and version 2 in 4 months) and obtaining these results was only possible due to a recently open-source project for data annotation, called Computer Vision Annotation Tool (CVAT - Intel corporation). CVAT enables video annotation using a tracking mode function and online annotation that can be accessed from different devices simultaneously. Our software was developed using data from our video experiments. A precise object annotation in each image plays a vital role in software performance. Poor data annotation makes machine learning models decrease their accuracy, and it may be computationally hard to boost their performance with optimization tools.

We emphasize the importance of data selection for the annotation step, as it is important for biologists to select data that represents our object of study satisfactorily. In our first version of the software, we faced two issues. First, we transformed all our data in binary and ran algorithms that extracted 10.000 frames (the minimum required to train an object detection model) according to the probability of containing objects that could be identified as ants. Our pilot experiment did not



contain a high number of ants per video (only 16% of the videos contained more than 20 ants), but also many video frames did not contain any objects (27% of the videos had 0 ants). Thus, a significant number of frames extracted by the algorithm had no ants. That was a second issue because a lot of the images used to train the YOLOv2 model could not provide a higher pool of ant annotations, making the model accuracy smaller. When we developed the second version of the software using YOLOv5 and CVAT, we selected a pool of videos with a high number of ants from both light and dark phases. Since we knew how computationally powerful YOLOv5 was, we trusted it would solve videos with smaller numbers of ants and trained our model on the worst-case scenario: hundreds of ants per video. We chose to share this information here because we believe that many ethological researchers using video-tracking software may not understand the importance of clear image data (with high contrast between image background and objects and no unnecessary objects in the video), as machines are not able to solve issues that are easily detected by the expert human eye. Also, we believe sharing this experience may help programmers who collaborate with biologists to develop tools to solve challenging experimental questions such as ours. In our experience, YOLOv2 allowed us to count 5052 *Acromyrmex* ants in our pilot experiment (1152 minutes of video), while YOLOv5 allowed us to count 143.904 *Atta* ants in our final experiment (4320 minutes of video recordings).

Final Conclusions

Considering all the results and ideas discussed previously, we would like to emphasize that an ant colony can be considered a "superorganism" (Wilson and Sober, 1989). Results in this project show that ant colonies can provide an interesting analogy for how synchronization processes occur in multicellular organisms, as ants' individual rhythmicity may be compared to cellular rhythmicity. Through social synchronization, we can observe the coupling process between multiple oscillators. Many are the physiological processes that allow communication and integration between different cells and tissues, and this high degree of cohesion and interdependence are some of the main aspects of multicellular organisms. Although ants individually are also multicellular organisms, social insects' colony context also involves many physiological and behavioral processes that allow its existence in such a high level of eusociality complexity.

Considering the more intimately related aspects to the photic synchronization of multicellular organisms, not all their cells are sensitive to temporal cues obtained from light-dark cycles. In humans, for example, photoentrainment is only possible due to photosensitive cells located in the retina. After perceiving these environmental time cues, this information is processed in the mammalian suprachiasmatic nucleus (SCN). Only then, SCN transmits its information to other organism cells, starting cell synchronization processes, coupling mechanisms, and, finally, the organism's full synchronization (Floessner and Hut., 2017).

Similarly, we believe temporal information from light/dark cycles flows in an ant colony and other social insects colonies. Only a group of ants is exposed to light and darkness in natural conditions, and through social interactions and other physiological processes, this information flows throughout the colony. However, many are the questions that can still be asked to understand synchronization in this social system, and those studies might be able to expand our knowledge about the complexity and the evolutionary importance of living under synchronized conditions. For example, we know the role played by SCN in humans, but ant colonies organize

themselves without central control. Is it possible that the colony possesses some component that plays a similar role to SCN? Individually, ants' brains contain a group of neurons responsible for their control of rhythmicity (Kay *et al.*, 2018). May the queen play a part in this process? Even under constant darkness, the ant queen could receive temporal information and somehow regulate the ants' individual clocks at a molecular level while it similarly lays eggs that regulate many aspects of the colony reproduction (Dolezal, 2019). Does the fungus play any role in the colony's temporal synchronization? Could it be a combination of both the fungus and the queen? Many fungi species present daily rhythms of growth and spore release (Bell-Pedersen *et al.*, 1996). Also, the interdependence between a leafcutter ant colony and its fungus is very high (Schultz and Brady, 2008).

Atta ant's communication occurs mainly through chemical components. Is there a chemical component that plays a similar role to melatonin, allowing this superorganism synchronization? Several studies show that each cell has its own period of oscillation in mammals, and those periods are similar for cells in the same tissue. Ants seem to have a similar relationship when we think about the temporal division of labor between subcastes.

Considering all the remaining questions about colony rhythmicity, we would like to emphasize the complexity of discussing efficiency in these systems, not only regarding rhythmic aspects. As environmental challenges change, colonies' behavioral response changes, and the efficiency measures and the concept of efficiency also require flexibility. An instantly efficient strategy may not be efficient in the long term. This is valid not only for ant colonies and other social insect systems but also for other organisms in their own particular aspects and natural history. Meanwhile, as biological rhythms are present in all groups of living organisms and play an essential role in the organisms' survival by modulating their behavior and physiology, they are likely a major component of efficient strategies over different species.

The present work has made some progress regarding the questions involving biological rhythms in ant colonies, especially as it proposes a new tool to proceed with these studies. We are aware that not all researchers will be able to use the video-tracking software we developed, as it involves its particular challenges.



However, we believe that as technology's advances happen more quickly every day, soon better and more accessible versions of this software will be able to promote many breakthroughs and new insights in behavioral biology.

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Annexes

SUPPLEMENTARY MATERIAL

Automatic Counts Tutorial

SYSTEM REQUIREMENTS:

This tutorial was elaborated for Ubuntu 20.04 LTS.

HARDWARE REQUIREMENTS:

GPU (NVIDIA GTX 1060 in our case)
16GB RAM (we used 32GB)

SOFTWARE REQUIREMENTS:

All codes and Readme.md files are available on GitHub
labcog/Contador repository.

BASIC USAGE (= local copy)###

git tutorial: <http://rogerdudler.github.io/git-guide/>

1. Download the current version:

* if you are downloading it for the first time, you must
clone the repository:

```
#!git
```

```
$ git clone
```

```
https://bitbucket.org/toddy757/labcog/branch/contador
```

```
PS: PRECISA AJUSTAR COMO VAMOS DISPONIBILIZAR O REPOSITÓRIO
```

```
(Não me recordo se ele fica fechado para edição para todo  
mundo que não faz parte do repo do labcog. E que eu me lembre  
precisavamos mover ele pra master ao invés de branch, certo?
```

```
#!git
```

```
$ git pull origin master
```

```
All the alterations that you do will only be saved locally.
```

2. If you want to delete your modifications and download the
current version again:



```
#!/git
$ git fetch --all
$ git reset --hard origin/master

# yolo
v5: requirements.txt from repositior ultralytics/yolov5
v3: opencv (gpu e módulo dnn linkado
pandas

## dataRRANSAC
opencv-python
matplotlib
pyaml
numpy

# dataCountline
numpy
pyaml

# optimization (we do not talk about the optimizers in this
tutorial, but some information is available on the repository
contador)
bayesian-optimization
motmetrics
scipy
```



Figure 1: Flowchart of all the steps involved in our video-tracking software development. Blue box involves data acquisition (experiments). Red boxes show all steps involved in the software validation stage. Green and yellow boxes show respectively the deep learning train model stage and running our model.

1. Data acquisition
2. Software validation
3. Data annotation
4. Training model
5. Testing the object detection model
6. Tracking Tool
7. Counting Tool
8. Analyzing your data:
 - a. Organizing the data
 2. Running Object Detection Model for all videos
 3. Running Tracking tool for all videos



4. Running Counting tool for all videos

5. Some tips and reading suggestions

1. Data Acquisition:

If you plan on using video-tracking tools to analyze your behavioral data, you must record your data in a way that maximizes the probability of the software's success. That means that video quality, recording angle, recording distance, and focus are all essential features. They should not change across your video data. We strongly recommend recording your video data from an angle that does not include unnecessary objects in the images (for example, shadows, reflections, and other objects that will not be analyzed).

Video tracking tools usually contrast the background and the object of interest to identify the object and track it. For behavioral research, that means: if your animal model has light colors, you should use darker backgrounds; if it has dark colors, you should use lighter backgrounds. Also, it would help if you thought about recording time: the longer your videos are, the longer is the time required to analyze them. Also, longer videos usually occupy a lot of memory storage. If you need to compress the video data to save it, you will lose image quality, which can be a problem. We also recommend checking the video formats available to your recordings (we prefer .mp4 or .mkv). Also, the more objects you need to track, the harder it is to obtain a high accuracy from most video-tracking tools. All this information and tips are important because video-tracking programs perform poorly when analyzing video recordings containing noisy images.

Using iSpy for scheduling recordings:

- Download iSpy: <https://www.ispyconnect.com/download.aspx> (it runs only on Windows. Agent DVR is another tool from the same group that runs on other operating systems, but we have not tested it. CHECK IF YOUR VERSION SHOULD BE 32bits or 64bits)
- Install it.
- Run iSpy and add all your recording devices. By right-clicking, you should set all the recording features to that camera and schedule your recordings. Pay attention to which directory your data is being saved.
- iSpy user guide: <https://www.ispyconnect.com/userguide.aspx>



2. Software Validation:

- From all your video data, randomly select a group of videos for software validation (20-50) videos. If you have different recording conditions (in our case, we had videos recorded under light and darkness conditions), make sure that your selected recordings represent all your possible conditions.
- In our case, we manually counted the number of ants in each of these selected videos using the software **contadorManual.py**

contadorManual.py

This program opens the video with a blue line drawn in the middle of the screen and allows manual counting as follows:

The count takes place when the ant crosses the line.

If it crosses the line in the top direction of the video: UP

If it crosses the line in the bottom direction of the videos: DOWN
or

If it crosses the line in the right direction of the video: RIGHT

If it crosses the line in the left direction of the videos: LEFT

To run it (at the directory that contains it): `python contadorManual.py`

Parameters:

`-video` (required)

`[- h]`: optional (help)

`- line [LINE LINE LINE]`: allows you to set 4 parameters to adjust the position of the line (x,y, and x,y coordinates)

COMMANDS:

SHIFT: Play/pause

W: Count Up

S: Count Down

+: accelerate video

-: Decreases the video speed

(you can also set different keys for each command in the software code).

It will save a .txt file with the results.

EXAMPLE:

```
$ python contadorManual.py -video  
/PATH_TO_VIDEO/VIDEONAME.mp4
```




- Run `contadorManual.py` for all your select videos for validation (we recommend saving them in a single directory).
- Organize all `.txt` result files into a single file (`.csv`)
- Here we stop the validation process and proceed with the Data Annotation. We will return to it after training and testing the model.

3. Data Annotation:

- Select the videos manually counted with the highest number of ants (10.000 video frames are the minimum required. It is crucial to select videos from all your experiment conditions. In our case, we selected four videos, two from the dark phase and two from the light phase).
- We ran all our data annotations in <https://cvat.org/>.
- After creating our profile, we created a project, defined the object class for annotation (ants and ants+leaf), and created a task for each video. You can organize the data in other ways.

NOTE: Data annotation is probably the most exhaustive part of this tutorial, but it is also one of the most important. We recommend CVAT because it allows you to have help during annotations and stop and continue your annotations. We took almost a month annotating the data working about 8-10 hours per day, so plan your time accordingly.

CVAT TUTORIAL

CVAT (Computer Vision Annotation Tool) is a tool developed by Intel co. and is open source. In it, you can upload images for annotation and do the annotation directly on video, using one or more classes and with various annotation features. It allows you to download these image annotations in different formats, depending on which one you will use to train your network. CVAT also allows you to start and stop a video annotation at any time.

CVAT can be run in two ways: via the website (cvat.org), and the other is installation via GitHub repository, running through docker with port forwarding. The difference is that the one via the website has an upload limit of 500mb per user.

Note: CVAT via GitHub has access to openVino, an automatic annotation tool, and its docker images occupy a lot of storage (you will need at least 15GB of free disk space).

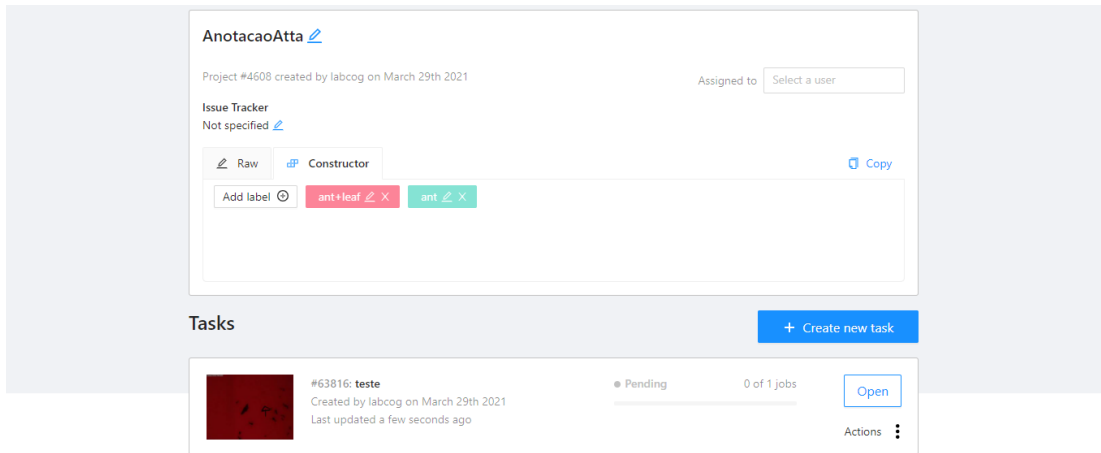
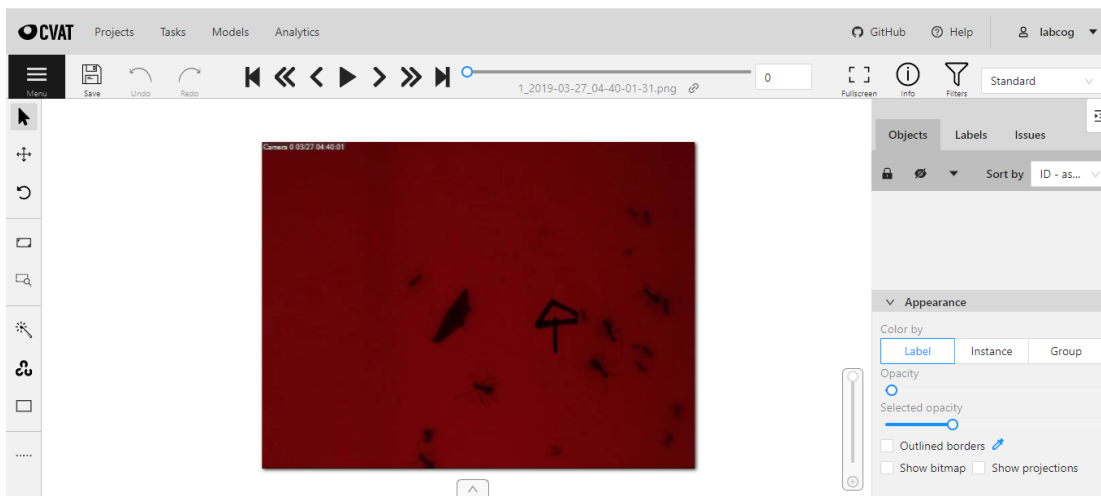


Figure 2: CVAT online.

How to annotate images on CVAT:

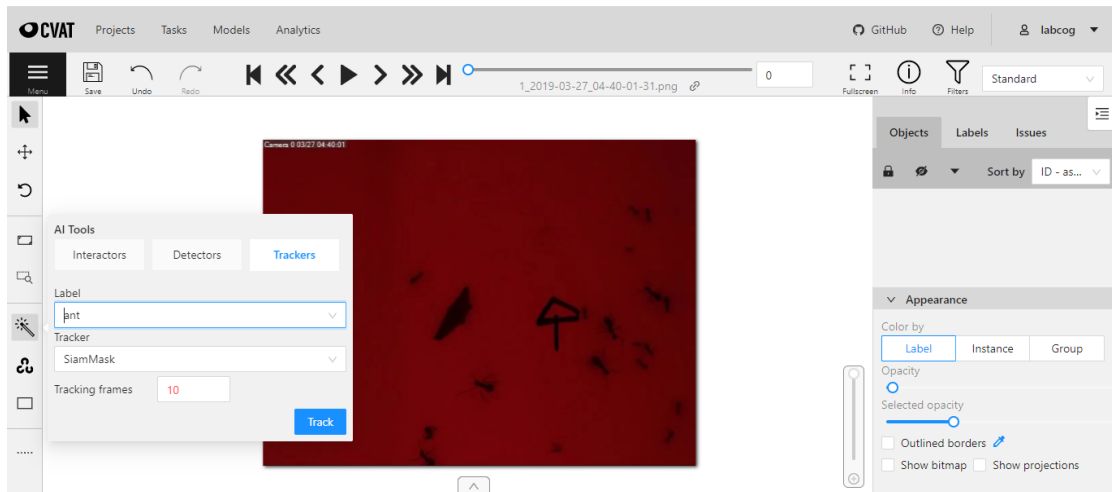
- Click **Open the task** and then click **Job #NUMBER**
- The annotation tool should open with the images associated with that task.



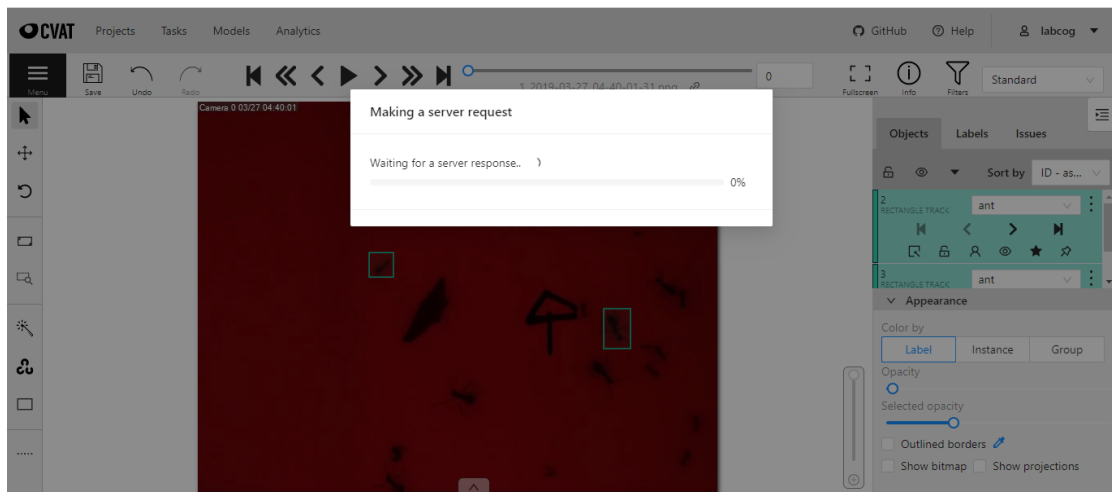
- There are many ways to annotate the images, and the options are in the left corner of the screen. In the right-hand corner, you will see the objects you have annotated in the "Objects" section as you do so. Here we have the automatic annotation recommendation.
- Using annotation with the tracker function.

This function is intended to annotate objects every n frames automatically. To use it, you will annotate the object using the cross-like manual annotation and wait while the system cycles through the following frames and annotates the position of that object as it changes in the video. This process may take a while.

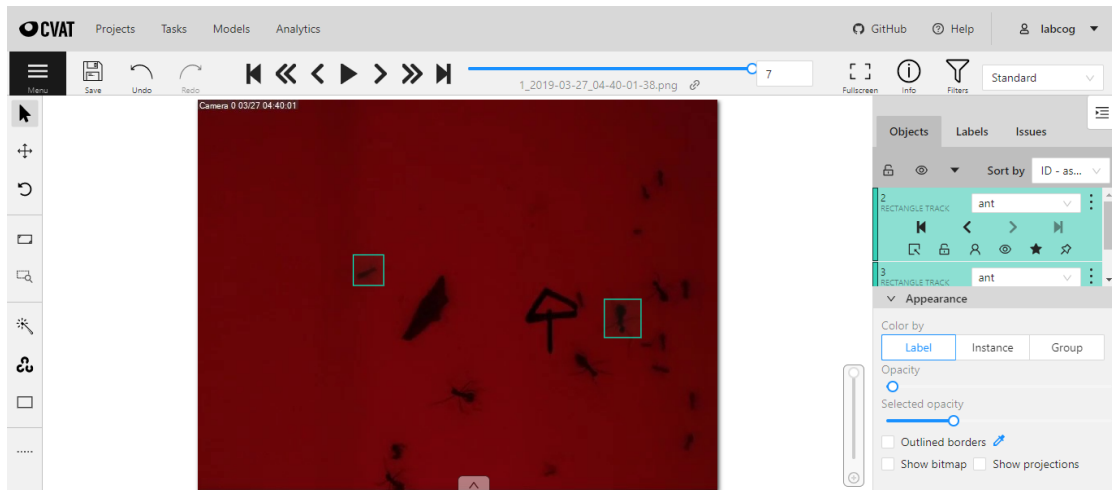
To use this function, click on the "magic wand" button and select the "tracker function":
Select the class you want to annotate, change 10 to 4 frames (it will be faster to process and probably contain fewer errors), and click "track".



To make an annotation, using a cross on the tip of the mouse, you will click on the image and select the area around the ant.
IMPORTANT: SELECT THE WHOLE ANT WITHOUT LEAVING TOO MUCH SPACE OUTSIDE.

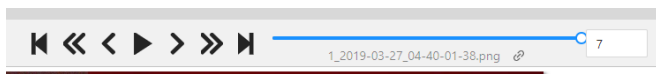


You will see that it has automatically annotated that ant in the subsequent four frames when you finish. Annotate all the ants in that frame.

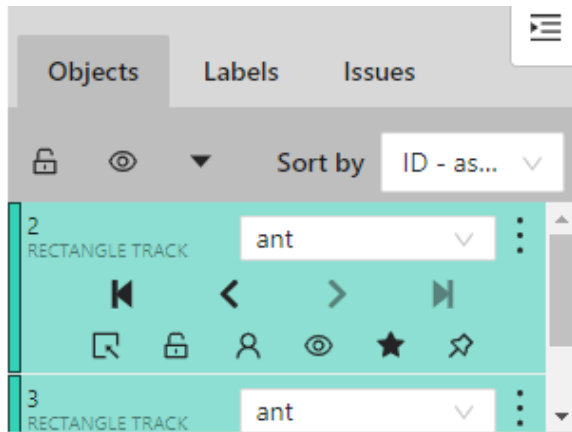


Go through the following image frames correcting the annotation if there are any errors (probably depending on the ant's position, the rectangle may be of inadequate size, so it is worth adjusting or moving it if the ant has moved). Each ant gets an id number (right side of the screen).

To make a new annotation, press the "n" key on your keyboard, and you will see the marking cross on the tip of your mouse again. These little arrows allow you to navigate between the frames of the video.



In the first frame that an ant enters, you should start the annotation, even if it is just partially in the image. As soon as the ant disappears from the image, you should click on the box (see the object's id), go to the Objects section to find the object with that id, and click on the square with a small arrow ("Switch Outside Property"). This function indicates to the image that that object has left the video. If you delete the box, it will understand that that object NEVER EXISTED and delete all annotations for that object.



Note: Remember that the id number says that the object is unique (same ant). If the same ant is not accurately identified (more than one id number for the same object or the same id number corresponding to a different object), the tracking will not work.

Note: Ants interact a lot. Use the zoom mode to make sure where your annotations are accurate.

Note: Save often. I usually save after using the tracker for each new ant and every ten frames by correcting/adjusting the boxes (there is also an option on CVAT that saves your annotations automatically, but I also recommend manually saving it to be safe).

4. Training Model:

- We used YOLOv5 (a state-of-the-art real-time object detector system) to train the object detection model.
- Download all the annotations from CVAT in the format YOLO to train the YOLOv5 network.
- You will also need to download all the annotations in the CVAT video (.xml) format for adjustments in the tracking mode (RRANSAC - will be treated later).
- All the following steps will require GPU to run. Our graphic card model was NVIDIA GTX 1060
- Install the NVIDIA driver (**some of these steps might change according to your NVIDIA model**)

```
$ wget -c
https://us.download.nvidia.com/XFree86/Linux-x86\_64/450.80.02/NVIDIA-Linux-x86\_64-450.80.02.run
```

```
$ wget -c
https://developer.nvidia.com/compute/cuda/10.0/Prod/local\_installers/cuda\_10.0.130\_410.48\_linux
```

1) \$ `chmod +x` for the first file

2) \$ ls Downloads

3) Run: `sudo ./NVIDIA..(name) --dkms`

4) Follow the instructions on the screen

a) If an error occurs:

i) `$ more /etc/X11/default-display-manager` (it returned -gdm3, but it could other display manager)

`$ sudo systemctl stop gdm3`

`$ sudo ./NVIDIA-Linux-x86_64-450.80.02.run --uninstall`

b) Reboot

c) Repeat the installation process

5) Check GPU status

`$ nvidia-smi`

It should return something like this if everything is working.

```
mila@mila-System-Product-Name:~$ nvidia-smi
```

```
Wed Jan 15 11:55:05 2020
```

```
+-----+
+-----+
| NVIDIA-SMI 435.21   Driver Version: 435.21   CUDA
Version: 10.1   |
+-----+-----+-----+
+-----+
| GPU Name          Persistence-M| Bus-Id        Disp.A | Volatile
Uncorr. ECC |
| Fan  Temp  Perf  Pwr:Usage/Cap|      Memory-Usage | GPU-Util
Compute M. |
+=====+=====+=====+
+-----+
|   0  GeForce GTX 106...  Off  | 00000000:01:00.0 Off  |
      N/A |
|  0%   47Cp5   7W / 120W | 169MiB /  3019MiB |   2%
Default |
+-----+-----+-----+
+-----+
+-----+
+-----+
| Processes:
      GPU Memory |
| GPU      PID  Type  Process name
Usage      |
+=====+=====+
+-----+
|   0      2223      G   /usr/lib/xorg/Xorg
      96MiB |
|   0      2411      G   /usr/bin/gnome-shell
```



```

70MiB |
+-----+
-----+

```

- You might also need to install the Cuda toolkit:

```

Installation (item 3.8 Ubuntu):
https://docs.nvidia.com/cuda/cuda-quick-start-guide/index.html#ubuntu-x86\_64
$ sudo sh cuda_<version>_linux.run
1. Create a file at /etc/modprobe.d/blacklist-nouveau.conf
with the following contents:

blacklist-nouveau
options nouveau modset=0

2. Regenerate the kernel initramfs:

$ sudo update-initramfs -u
3. Reboot

```

- YOLOv5 Repository (please check the Readme.md file for instructions): <https://github.com/ultralytics/yolov5>
- 10000 training cycles are sufficient for small data sets, but the ideal, especially for larger data sets, is 100000 cycles.
- You can find all the scripts described here in: <https://drive.google.com/drive/folders/1PBiS48w303iVhtvE3M0aBI7ZqWX06c9s?usp=sharing>
- Run the exp.sh
 - It is a bash script that generates all the other files. In this case, it takes images in 4 folders (each one corresponds to an annotated video), mixes the images, and separates them between training and validation so that all videos are represented in both the training and the validation set. In the end, it generates a list of images and labels with this training set and another one with the test set. Output of exp.sh: shows for each folder (video) how many images are used in training and in validation

```

##exp.sh##

#!/bin/bash
DATAPATH="/home/marcelo.arruda/Projetos/M/datasets/videosComplicados/"
#DATAPATH=""
V1="1_2019-03-27_04-40-01"

```



```
V2="1_2019-04-04_23-00-00"
V3="1_2019-04-05_06-40-00"
V4="1_2019-04-08_12-00-01"

echo "
# ReTreinar Yolo em VideosComplicados
"

echo "
## Dados
  1_2019-03-27_04-40-01 (treino)
  1_2019-04-04_23-00-00 (validação e teste)
  1_2019-04-05_06-40-00 (validação e teste)
  1_2019-04-08_12-00-01 (treino)
"

printVideoDSInfo () {
  video=$1
  datapath=$2
  echo "- ${video}:
  - images: $(ls "${datapath}${video}"/*.png | wc -l)
             $(ls "${datapath}${video}"/*.png | head -1)
  - txts:   $(ls
"${datapath}${video}"/[0-9]*[0-9].txt | wc -l)
             $(ls "${datapath}${video}"/[0-9]*[0-9].txt |
head -1)
  - list:   $(cat
"${datapath}${video}/list_${video}.txt" | wc -l) lines
             $(cat "${datapath}${video}/list_${video}.txt" |
head -1)
             $(cat "${datapath}${video}/list_${video}.txt" |
tail -1)
  "
}

printVideoDSInfo $V1 $DATAPATH
printVideoDSInfo $V2 $DATAPATH
printVideoDSInfo $V3 $DATAPATH
printVideoDSInfo $V4 $DATAPATH

echo "
## Treino:
  ${V1}: $(cat "${DATAPATH}${V1}/list_${V1}.txt" | wc
-1) +
  ${V4}: $(cat "${DATAPATH}${V4}/list_${V4}.txt" | wc
-1) "
cat "${DATAPATH}${V1}/list_${V1}.txt"
"${DATAPATH}${V4}/list_${V4}.txt" > list_train.txt
echo "    -> list_train.txt $(cat list_train.txt | wc
```




```

-1)"

echo "
## Validação e teste:
  ${V2}: $(cat "$DATAPATH"${V2}/list_${V2}.txt" | wc
-1) +
  ${V3}: $(cat "$DATAPATH"${V3}/list_${V3}.txt" | wc
-1) "

cat "${DATAPATH}${V2}/list_${V2}.txt"
"${DATAPATH}${V3}/list_${V3}.txt" > list_testval.txt
shuf list_testval.txt -o list_testval.txt
head -n 1000 list_testval.txt > list_validation.txt
tail -n +1001 list_testval.txt > list_test.txt
echo "    -> list_validation.txt $(cat list_validation

```

- Run the fullTrainingVComplicados.sh
 - YOLO training script on the lists listTrain.txt and listVal.txt. The execution of this script will actually produce a trained model.

```

#fullTrainingVComplicados.sh#

rush=/usr/local/bin/rush

find "$1" -name "*[0-9].txt" | $rush "python
multilabelsYoloAnnotation.py {} {}"

```

- fullTrainingVComplicados.yaml: contains all especifications from the YOLO model

5. Testing the object detection model

- Install docker:
 - <https://docs.docker.com/v17.09/engine/installation/linux/docker-ce/ubuntu/>
- Building the dockerfile and open a new image: (tutorial <https://www.youtube.com/watch?v=LQjaJINKQ>)

```

$ make cudnn_tensorflow_opencv-10.2_2.2.0_4.3.0
$docker images

output example:

```

REPOSITORY	TAG	IMAGE ID	CREATED	SIZE
datamachines/cudnn_tensorflow_opencv	10.2_2.2.0_4.3.0-20200615	d58af4a715a4	14 hours ago	5.86GB
nvidia/cuda	10.2-cudnn7-devel-ubuntu18.04	2b8bb5f68029	6 days ago	3.82GB

```
$ xhost +local:docker
$ docker run -ti --rm --gpus all -e DISPLAY=$DISPLAY -v
/tmp/.X11-unix/:/tmp/.X11-unix --ipc host --device /dev/nvidia0:/dev/nvidia0
--device /dev/nvidiactl:/dev/nvidiactl --device /dev/nvidia-uvmm:/dev/nvidia-uvmm
-v /home/:/home/ d58af4a715a4 /bin/bash (the image id varies according to your
computer)
```

if an error occurs:

```
"docker: Error response from daemon: could not select device driver ""
with capabilities: [[gpu]]."
```

```
$ sudo apt install nvidia-container-runtime
```

PS: you might need to install some packages inside the docker container:

```
$ apt-get update
$ apt-get upgrade
$ apt install python3
$ apt install python3-pip`
$ python3 -m pip install --upgrade pip
$ python3 -m pip install numpy`
$ python3 -m pip install opencv-python
$ python3 -m pip install opencv-python==3.2.0.8 (this version may change)
$ python3 -m pip install pyaml
```

PS: --GPU is the option that makes the computer run using the GPU instead of the CPU. You can check the GPU and CPU usage while yolov5Detect.py is running to verify "how much it is taking from the computer." If you do not use the --GPU parameter, yolov5Detect.py will run using only the CPU, which will be slower and require a lot of computer power.

to check CPU usage on Ubuntu:

```
$ htop (shows CPU usage, if the CPU memory usage increases a lot while you
run yolov5Detect.py, you are probably using only the CPU.
```

```
$ nvidia-smi (if the GPU memory usage does not change while you run
yolov5Detect.py, you are probably using only the CPU)
```

- Inside the container:

Choose some videos to test the YOLO network
Run a test:



```
$ python3 yolov5Detect.py --source  
../../yolov5/1_2019-04-04_23-00-00.mp4 --weights  
../../yolov5/weights/yolov5l_best_50.pt --conf-thres  
0.1 --view-img --nosave --yaml teste.yaml --yolov5_path  
../../yolov5
```

ps: check all the filenames. The command above is an example.

```
$ xhost -local:docker
```

6. Tracking tool

- For tracking the ants, we run our implementation of the RRANSAC, which reads the .yaml files generated by the yoloVideoDetect.py and creates a new .yaml file with the tracks. For more information see the Readme.md file on /labcog/contador/dataRRANSAC/.

Options:

```
- data: .yaml input file  
- out: .yaml output file with the generated tracks  
- outfw: .yaml output file with the generated tracks  
separated by vectors (frames, ids, position)  
- display: shows the video while it runs (optional - it takes  
more time to run)  
- RRANSAC params: (must have)  
-M (int) Number of stored models  
-U, (float) Merge threshold  
-tau_rho (float) Good model quality threshold  
-tau_T, (int) Minimum number of time steps needed for a  
good model  
-Nw, (int) Measurement window size  
-ell, (int) Number of RANSAC iterations  
-tauR, (int) Inlier threshold (multiplier)  
-Qm, (float) Q multiplier  
-Rmx, (float) Rx multiplier  
-Rmy (float) Ry multiplier
```

```
$ ./dataRRANSAC -data exemploInput.yaml -M 50 -U 5 -tau_rho  
0.1 -tau_T 3 -Nw 100 -ell 200 -tauR 2 -Qm 2 -Rmx 5 -Rmy 17
```



```
-display PATH_TO_VIDEO -out exemploInput_RRANSAC.yml
```

All these parameters were manually adjusted. They change according to the YOLO model quality.

7. Counting tool

- For counting the ants, we run the COUNTLINE, which reads the .yaml files generated by the dataRRANSAC and creates a .txt file containing all the counts. For more information see the Readme.md file on /labcog/contador/dataCOUNTLINE/.

```
$ python3 dataCountline.py -yaml {} -cline 0 239 640 241 > {^_RRANSAC.yaml}_count.txt'
```

PS: -cline defines where the line is positioned on the video frame. The counts occur when the object crosses that line.

8. Analyzing your data:

a. Organizing the data

- If everything is working well, you should proceed to analyze all your data. However, it is crucial to organize all the data and programs well. We organized all the output files for each step (video data, detection, tracking, and counting) on different directories. Also, as we had more than 2000 videos to analyze, we divided them into "days," allowing us to run all the steps for every day recorded. That is a good option because all the steps will run faster, and it makes it easier to find errors (basically, if the number of output files is different between the directories, an error occurred, and you should run that step for the missing file again).
- Do not move the programs from their original paths! Many programs used here access other program files to run, and if you change the directories, they will not find them, returning errors.
- Example of organization:
 - Directory Day_1:
 - DATA: 72 video files (.mp4)
 - YOLO: 72 .yaml files
 - RRANSAC: 72 _RRANSAC.yaml files

- COUNTS: 72 .txt files + Day1allcounts.txt file (will be discussed later)

9. Running Object Detection Model for all videos:

- You will use a cross-platform command-line tool for executing jobs in parallel to run the detection for all the videos (as well as for the tracking and counting steps) called RUSH.
- Install RUSH: <https://github.com/shenwei356/rush>
- You must run this command for every directory containing the video data. That step will take much time according to the number of files that will be analyzed and the computer settings.

```
# 1. run the YOLO detector
find /PATH_TO_DIRECTORY_CONTAINING_THE_VIDEOS_FROM_THAT_DAY
-name "*.mp4" | rush "python3 yolov5Detect.py --source
../../yolov5/1_2019-04-04_23-00-00.mp4 --weights
../../yolov5/weights/yolov5l_best_50.pt --conf-thres 0.1
--view-img --nosave --yolov5_path ../../yolov5 -out
{^.mp4}.yaml"
```

10. Running Tracking tool for all videos:

- You must run this command for every directory containing the YOLO detections.

```
# 2. run dataRRANSAC
find
/PATH_TO_DIRECTORY_CONTAINING_THE_DETECTIONS_FROM_THAT_DAY
-name "*.yaml" | rush "./dataRRANSAC -data {} -M 50 -U 5
-tau_rho 0.1 -tau_T 3 -Nw 100 -ell 200 -tauR 2 -Qm 2 -Rmx 5
-Rmy 17 -out {^.yaml}_RRANSAC.yaml"
```

11. Running Counting tool for all videos:

- You must run this command for every directory containing the YOLO detections.

```
# 3. run Countline
find
```



```
/PATH_TO_DIRECTORY_CONTAINING_THE_TRACKINGS_FROM_THAT_DAY  
-name '*_RRANSAC.yml' | rush 'python3 dataCountline.py -yml  
{ } -cline 0 239 640 241 > {^}_RRANSAC.yml}_count.txt'  
  
cd /PATH_TO_DIRECTORY_CONTAINING_THE_COUNTS_FROM_THAT_DAY  
# 4. join all counts  
tail $(find  
/PATH_TO_DIRECTORY_CONTAINING_THE_COUNTS_FROM_THAT_DAY -name  
"*_count.txt") | grep -Po "^==>\s+(\S+)|(\d+)$" | tr '\n'  
';' | tr -d '=' | tr '>' '\n' >> Day1allcounts.txt
```

- **PS: PAY ATTENTION TO FILE NAMES AND PATHS.** You might need to change something from our command lines. Remember that if you do not change the name of the output files, they will be overwritten.
- After running these steps for all data, we recommend organizing all the .txt files containing the counts for each day on a single file (.csv, .xml, or .txt)

12. Some tips and reading suggestions

YOLO:

<https://pjreddie.com/darknet/yolo/>
<https://towardsdatascience.com/yolo-v5-is-here-b668ce2a4908>
<https://medium.com/deelvin-machine-learning/yolov4-vs-yolov5-db1e0ac7962b>
<https://blog.roboflow.com/yolov5-improvements-and-evaluation/>

DOCKER:

https://www.youtube.com/watch?v=sYr4frA_1d8
<https://djangostars.com/blog/what-is-docker-and-how-to-use-it-with-python/>

NVIDIA-DRIVER:

<https://medium.com/@rosdyanakusuma/how-to-install-nvidia-driver-in-ubuntu-18-04-307c25f73259>

If something returns an error, do not hesitate to google the error. StackOverflow and other online forums contain many different solutions for almost every error.

