Panlab
An Affiliate of Harvard Bioscience, Inc.

USER MANUAL

ACTITRACK 2.7.13

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1. Introduction

ACTITRACK is a program designed to control the IR Motor Activity Monitors. Using ACTITRACK it is possible to add to the standard features OFFERED by the IR Motor Activity Monitors the capability of determinate the position along time of an experimental subject within the IR frame (tracking). Therefore, by means of ACTITRACK, it is possible determinate parameters like distance travelled, speed, time spend in a predefined area of interest, etc.

The complete ACTITRACK system consists of the following elements:

- One or several (up to 16) IR Motor Activity Monitors.
- Each monitor is composed by a Control unit and one or two IR sensor frames attached to each control unit.

1.1. The ACTITRACK program

Experience has shown that the configuration of all the parameters involved in the conduct of a specific experiment is just as important as the revision of the findings and the statistics obtained from the findings: the team of experts that designed this system attached great importance to addressing this question satisfactorily. Thus, using the data obtained in the experiment ACTITRACK can generate a myriad variety of statistics quickly and efficiently, which will allow the researcher to focus on the experiments he or she is carrying out at the time, without having to attend to purely computing-specific questions, which are evidently far from being even a partial endpoint. While we believe that this system covers the most important requirements that an experimenter may pursue, in terms of achieving results, neither did we want to "burn our bridges" with regard to the possible computing tools which the ACTITRACK user may be used to working with. Thus, all data obtained by ACTITRACK can be exported to a wide range of spreadsheets and statistics programs.

Moreover, and to make the researcher's work easier, the data acquired can be checked either in the lab (where the data acquisition will be obtained in real time) or on any computer (including laptops). So ACTITRACK can be installed in as many computers as may be required, and the user will be able to check data, generate statistics, print out results and obtain graphics.

To finalize with this brief overview of ACTITRACK, we would like to remember that the user would require a minimum knowledge of the Microsoft® Windows® environment, since ACTITRACK has been designed on this operating system. Thus, a basic knowledge of how to carry out operations such as opening, closing or rearranging windows, use of the mouse or any other main operations that may be carried out with files will be necessary: this manual is not addressed to this aspect.

2. Installation Overview

2.1. Requirements

ACTITRACK needs the following equipment:

- A fully compatible computer with at least:
- 2,2 GHz Pentium® CPU (Celeron CPU excluded)
- 2 Gb of RAM
- 150 MB of free hard disk space
- Graphics: 1024x768 pixels and 32-bit true colour
- 1 free USB port for the protection key
- Connection interface
- 1 free USB port for a USB-Serial adapter included in the software pack.

Operating system supported:

- Microsoft® Windows® 11 64 bits.
- Microsoft® Windows® 10 32 bits / 64 bits.

External software needed:

- Microsoft Excel ®.
- Microsoft Word ® (only for reports in Word format).

If external software is not available, some analysis reports could not be generated. Please contact your IT staff in order to install the external software before analysing sessions.

- Printer (advisable):

At least one "virtual printer" must be correctly installed. Please refer to 2.6 for more details.

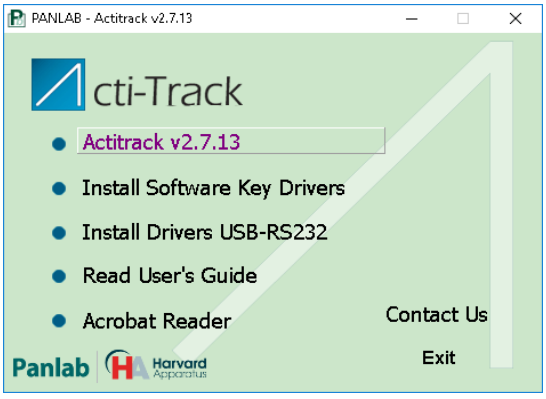
2.2. Installing the software

ACTITRACK software is delivered within a single USB flash drive. The USB flash drive contains the software installation tool, this User's Manual in PDF format and other components required to work in specific conditions.

Due to security reasons of the Windows® operating system, a user with administrative rights is required to install the software and other components. Please contact your IT staff before installing the software.

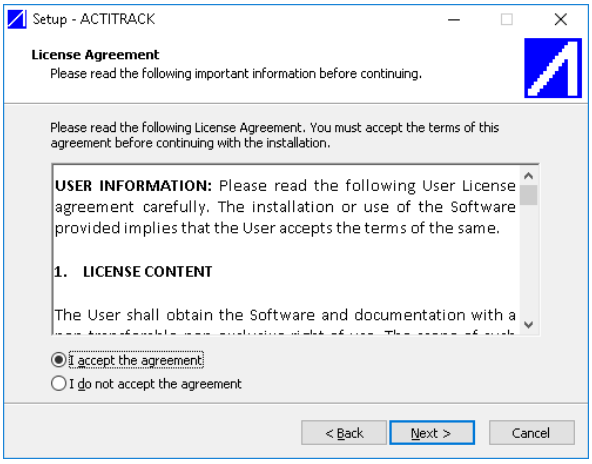
Once you get the administrative rights to install the software, please follow these steps:

- Plug the USB flash drive in a free USB port of your computer and wait until Windows® installs it as a new removable drive.
- Access the new removable drive detected and execute the PANLAB.EXE file. A window will be shown, as below:

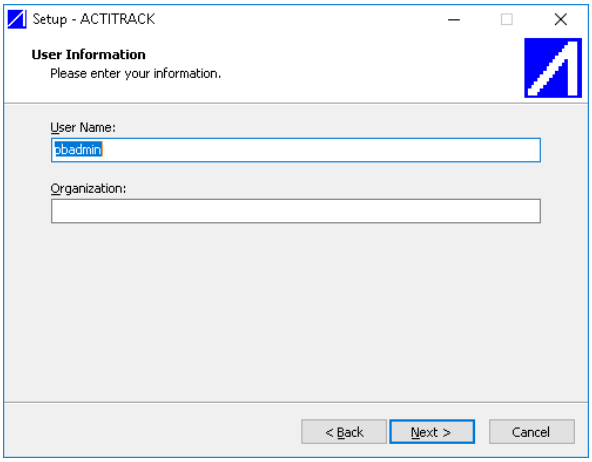


- Press the [Actitrack v2.7.13] button to start the software installation.
- An installation wizard will appear. Press the [Next] button to start the software's installation.

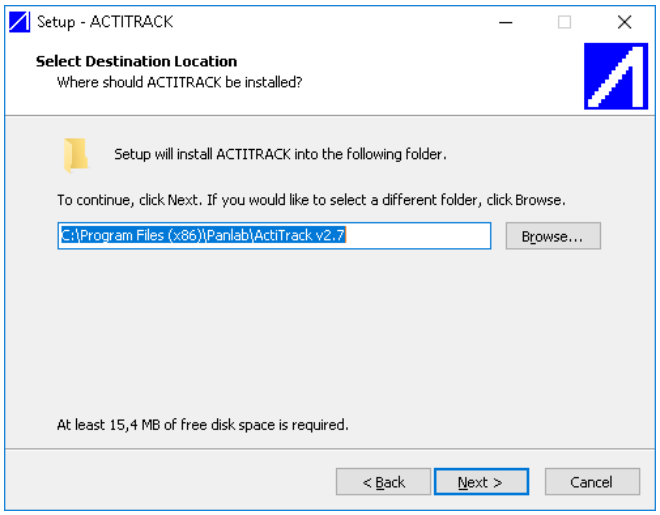
- Read carefully the License Agreement statement and select the "I accept the agreement" option to continue the installation of ACTITRACK. Then press the [Next] button to start the installation.



- In the next windows introduce the name of the user and the company in the correct field. After this, press [Next] button to continue.



- During the installation process, the software is installed in a new folder called [Panlab\ACTITRACK v2.7\] created under the Programs Files folder. If desired, the installation program allows you to choose another folder to locate the software. The location of the software is independent of the data folder, which is defined by the user using the corresponding options of the program.

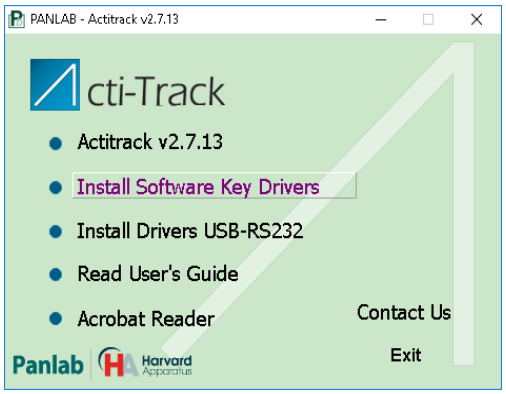


- Press the buttons [Next] and [Install] following the Install Shield Wizard until reaching the [Finish] button.
- A new shortcut will appear on your desktop. Use it for executing the program later.

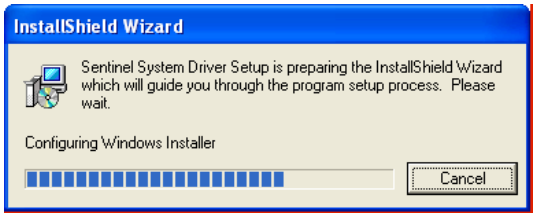
2.3. Installing software protection key

ACTITRACK software is delivered with an USB protection key that avoids from fraudulent use of the application in a computer which does not have it installed.
Before installing the drivers, all applications must be closed, and all USB SuperPro keys must be removed.
In order to do a correct USB key protection installation, please follow the steps below:

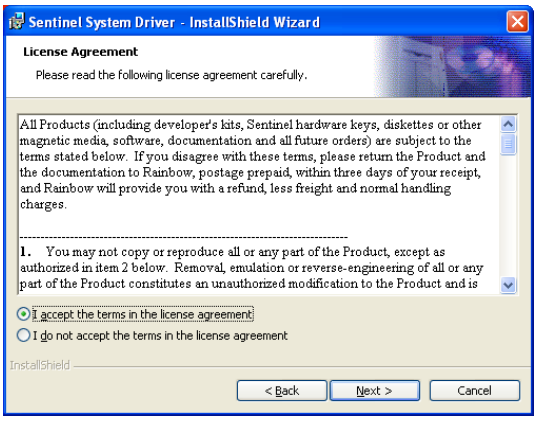
- Press the [Install Software Key Drivers] button to start the driver's installation.



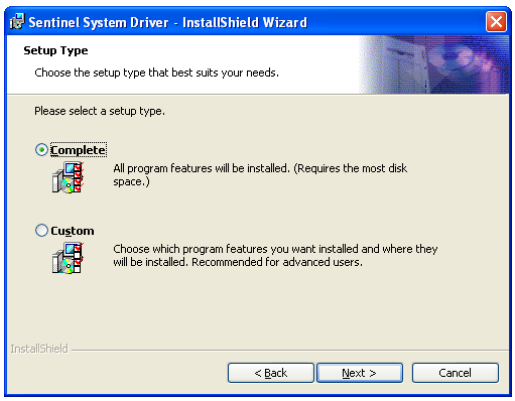
- Automatically a USB key wizard installation will be shown. When the Welcome screen appears, click [Next] to continue.



- Chose [I accept the terms in the license agreement] and click [Next] button to continue.

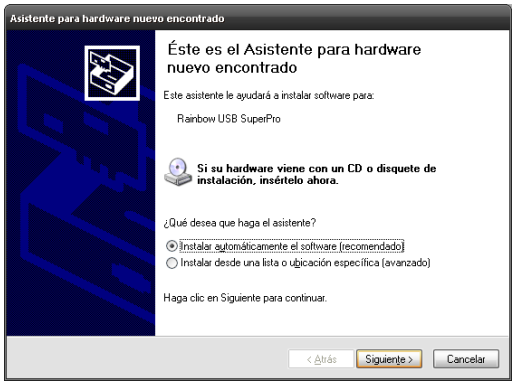


- On the incoming windows please select the [Complete] option of setup type and click [Next] and [Install] buttons to continue.

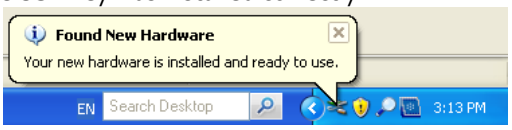


- After pressed [FINISH] button you must reboot the system. Thus, your computer will recognize the USB security key.
- Did you reboot the computer? If you answer yes, please connect the USB key. In the lower right corner of the screen will appear the next message.

- The wizard for installing the drivers will run when your computer detected correctly the USB key. This process will need some minutes depending on your PC.
- Choose [Automatic Installation] and press the [NEXT] button.



- Wait while the wizard looks for the drivers until it asks you to press the [FINISH] button.
- Finally, a new message will appear in the lower right corner of your screen. The USB key was installed correctly…



Important remark: This step has to be repeated for each USB port of your computer.

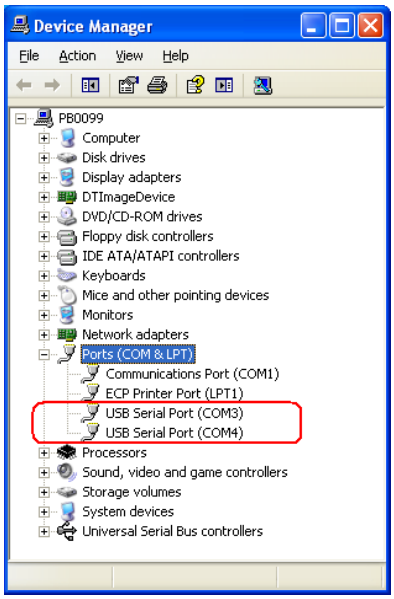
2.4. CONRS232USB-HS converter (high speed mode)

ACTITRACK requires the use of the high-speed converter from RS232 port to USB port

WARNING: do not use direct connection between the LE8825 data loggers and the computer RS232 serial port (if any).

The RS232/USB converter is needed for converting a USB port to a serial port valid for communications between hardware and software. It allows the use of two serial ports in your PC or laptop. We recommend the use of a specific model of converter. We cannot guarantee a correct functioning of the system with any other USB-serial converter. The converter includes an extension cable just in case.
To Install the converter:

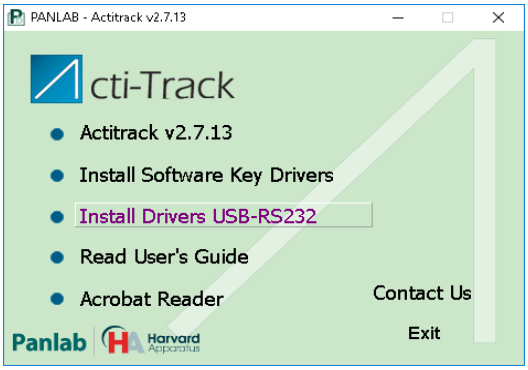
- Connect the converter to the computer.
- Windows 8, 10 and 11 will automatically install the drivers.
- If working with a Windows 7 or previous, please refer to the notice provided in the box of the converter.
- Once connected and installed, two serial ports will appear into the [Device Manager] window on the Windows Operative System. Usually, the numbers assigned by Windows are sequential.



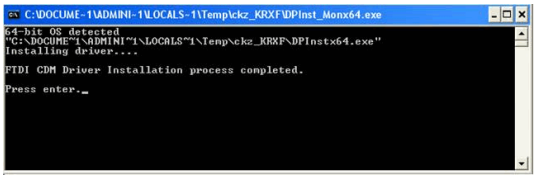
2.5. CONRS232USB (blue) converter (Legacy mode)

The blue RS232/converter was included in older ACTITRACK software packages.
ACTITRACK is still compatible with the use of the system in a Legacy mode (not high-speed). In case you need to re-install this device, please follow the below procedure:
In order to make this device operational in your computer, please follow the steps below:

- You need to have administrator privileges to install any new drivers. Please contact your IT staff in order to clarify this issue before continue installing the device.
- The drivers should be installed prior to hardware installation. Do not connect the blue adapter to the USB port of your computer before you finish driver installation.
- Insert the ACTITRACK software USB flash key into a free USB port of your computer and access its content.
    - If your PC is running Windows XP, a manual installation is required: go to folder Files\USBCom and execute file USBCom-CDM_20824.exe.
    - Otherwise, for the rest of Windows versions, execute the installation assistant (Panlab.exe). The following installation window will be shown. Press the [Install Drivers USB-RS232] option to start the software installation process.



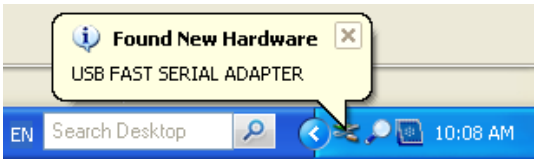
- The USB COM installation program will auto-detect the OS type and install the driver automatically. In some operating systems, it might appear a dialog box asking to press [ENTER] at the end of the installation.



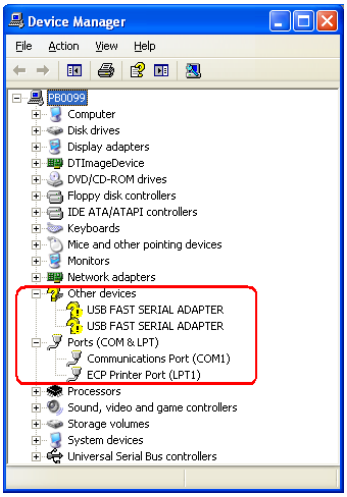
- After the message "FTDI CDM Driver installation process completed" appears, press [Enter] to complete the driver installation.

- Plug the blue adapter in any USB port of your computer. Windows will finish the installation of the driver files.

- In the lower right corner of the screen the next message will be automatically shown:

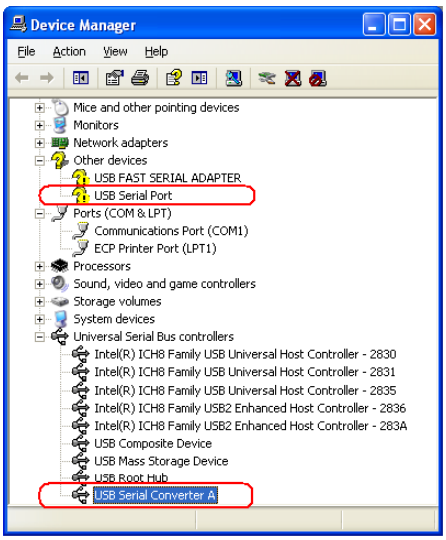


- At the same time, two devices will appear into the [Device Manager] window. The ports provided by the new [USB FAST SERIAL ADAPTER] will be shown under [Other devices] with a warning sign attached.

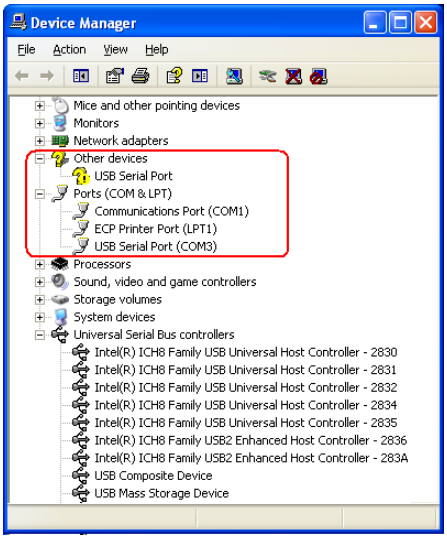


- Please, wait while the wizard locates the drivers installed previously. This process may require some minutes depending on your PC.

- The process of the correct activation of the device (that is, when the PC or laptop recognizes the new serial port), is done one by one.



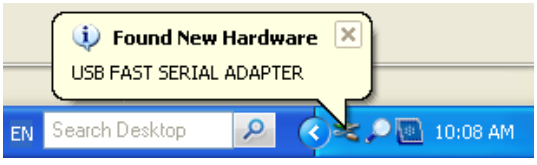
- The next picture shows how the number of the port is finally assigned by the system.



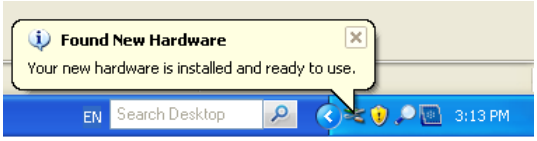
- When the wizard finishes will ask you for pressing the [FINISH] button.

Important remark:

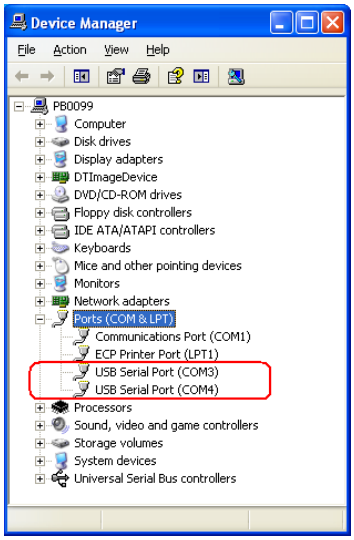
Until now, only one serial port has been correctly installed. The process must be repeated for the second port. Please, wait while your PC or laptop found another COM port. Once again, the next message will appear in the lower corner of the screen:



- The adapter will be correctly installed when all previous steps had been repeated. Finally, the message will appear in the lower right corner of.



- At the same time, the two serial ports will appear into the [Device Manager] window. Usually, the numbers assigned by the system are sequential.

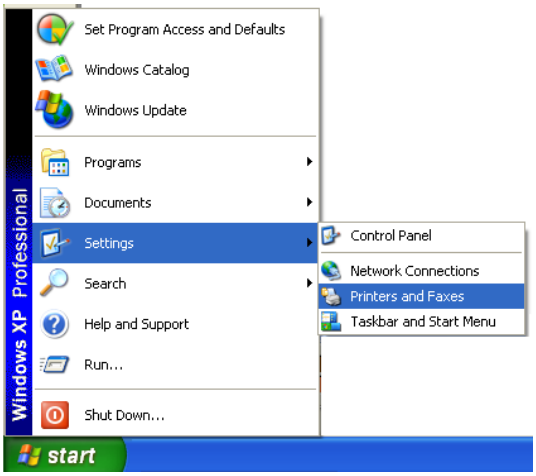


A yellow label with the text [Port 1] is attached to the adapter device to identify the first port recognized for the computer system. That means that if [Device Manager] shows two ports (COM3 and COM4), then that label [Port 1] corresponds to COM3.

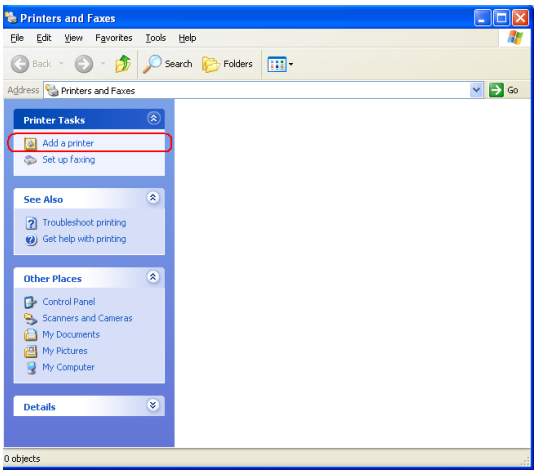
2.6. Installing a printer by default

If there is not a printer installed in your PC, one virtual printer must be installed by default. If your PC is running Windows 10 or superior, there are two virtual printers installed by default (Microsoft XPS Document Writer and Microsoft Print to PDF) and no additional action is required; but for earlier Windows versions, the next steps must be followed to fulfil the system requirements:

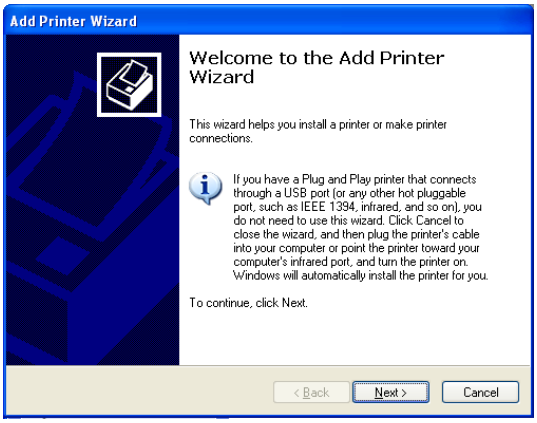
- Go to [Printers and Faxes] option of your system. The access is possible by clicking on [START – Settings]



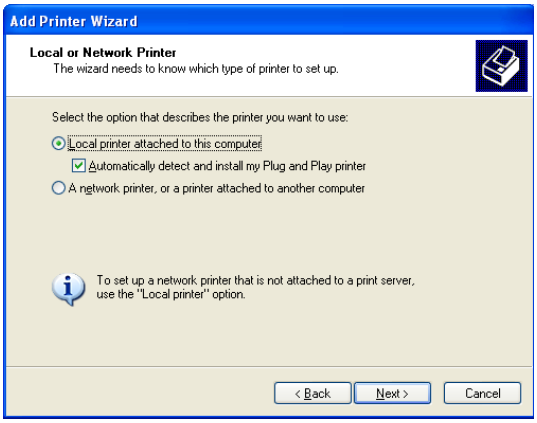
- In the [Printers and Faxes] window press on the [Add printer] button.



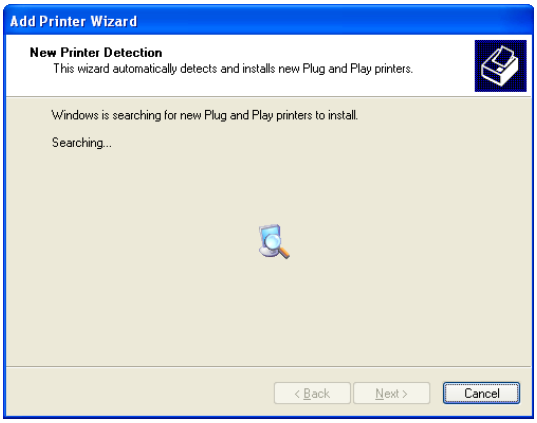
- The Welcome to the add printer wizard appears, click [Next] to continue.



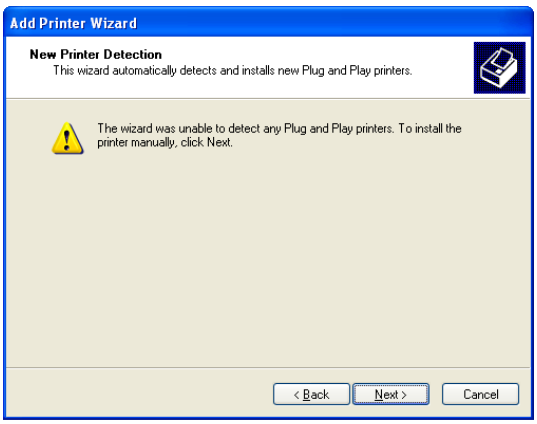
- As this procedure is for installing a virtual printer, the options must be selected as is shown in the next window. Press [Next] button to continue.



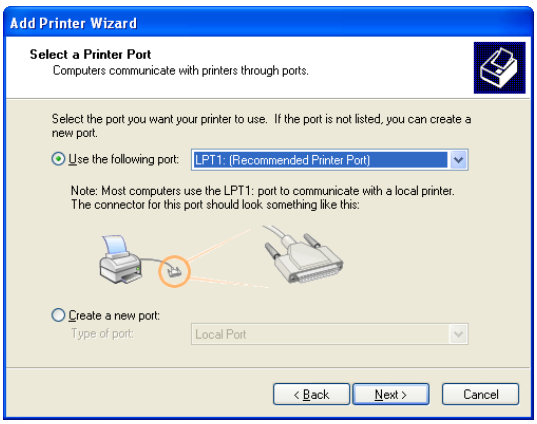
- The wizard will search the drivers for the virtual printer. This process will need some minutes depending on your PC.



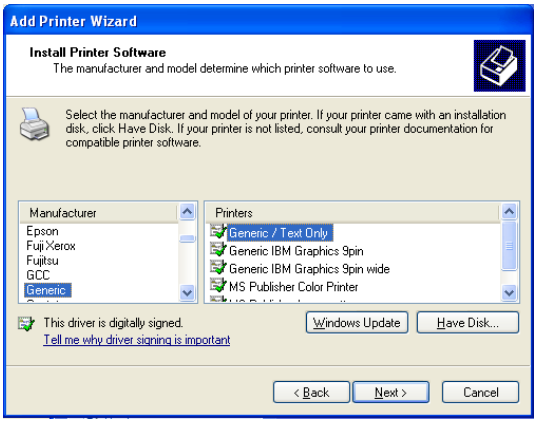
- The wizard notifies that there is not a real printer present. Press [Next] button to continue



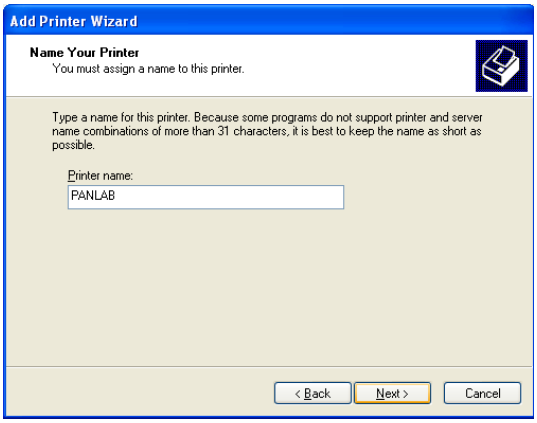
- Obviating the previous message, the options for the next window must be selected as is shown in the next picture before pressing the [Next] button



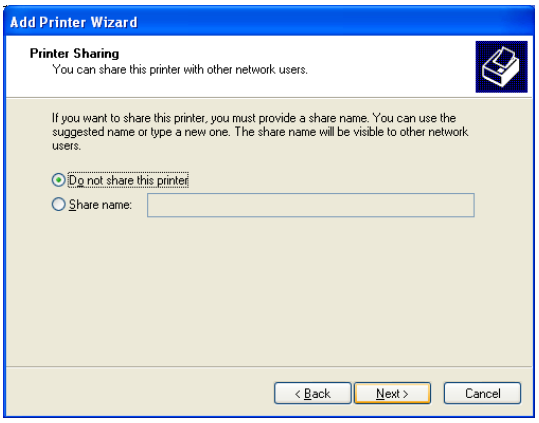
- The virtual printer will be a [Generic / Text Only] thus these options must be selected in the next window before press the [Next] button



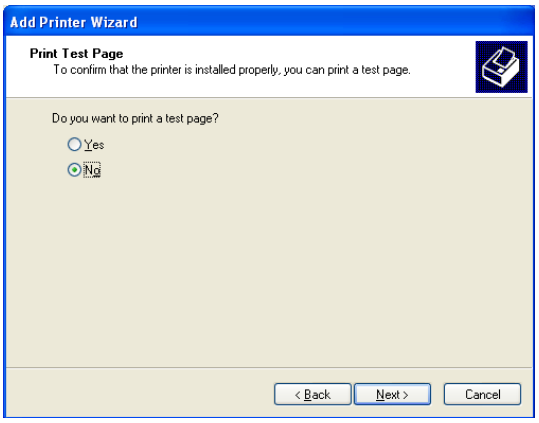
- This virtual printer can be called [PANLAB] so that it can be easily identified when a real printer is connected to the system. The option [No] must be selected before pressing the [Next] button.



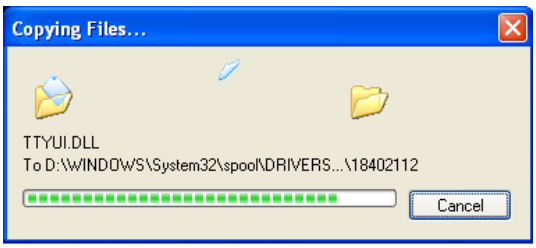
- Of course, it is not necessary to share the PANLAB printer so select the [Do not share this printer] option and press the [Next] button to continue.



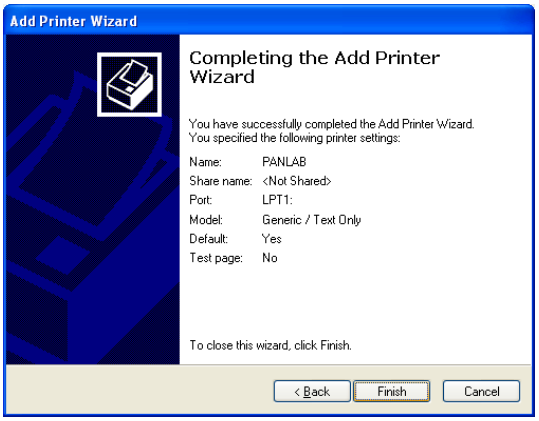
- It is not necessary to print a test page either so select the [No] option for answering to the wizard and press the [Next] button to continue.



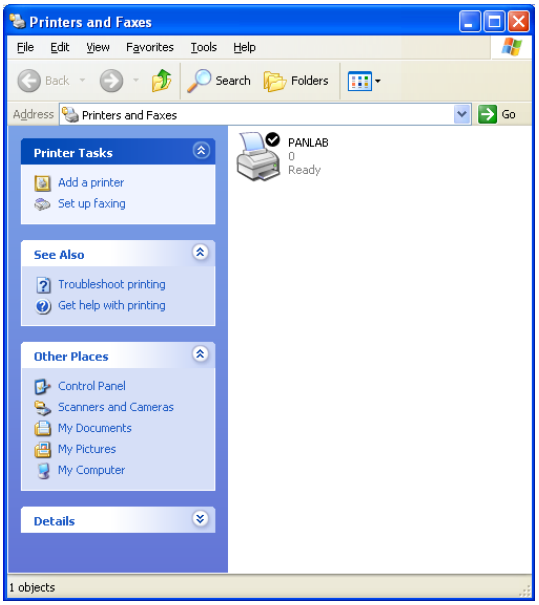
- The wizard will copy the drivers for the virtual printer. This process will need some minutes depending on your PC.



- When the virtual printer is successfully installed the wizard shows you a window as the next picture. Press [Finish] button



- A new icon will appear into [Printers and Faxes] window called [PANLAB]. Close the window and launch the software.



2.7. Data communication

ACTITRACK uses a serial port to communicate between the PC and the LE8825 Activity Monitors, even if more than one monitor is being used.
A cable should link the PC with the first LE8825 (to the "RS 232 Main" connector of the LE8825 Control unit). The rest of the monitors should be chained using a link between the "RS 232 Remote" of the first unit to the "RS 232 Main" of the following and so on until the last (see the LE8825 User Manual for details).
When no RS232 serial port is available, a RS232/USB adapter has to be used. For its installation follow the instructions "2.4 Installing serial ports for the cages".
Before running the program ACTITRACK, the following configurations in the SETUP-PC menu of the LE8825 have to be done for ensuring a correct communication with the ACTITRACK software (see detailed instructions about these two points in the LE8825 User Manual):

- Module ID (Identification)
  It is indispensable that each LE8825 monitor is correctly identified. Each one of them must have a distinct ID number.
- SEND DATA
  The "SEND DATA" option has to be activated. This option allows the transfer of the IR frames information from the LE8825 to the PC through RS232/USB communication.

Once the installation has been completed, the reader may access the ACTITRACK program and begin to learn how to use it.

3. Getting Started with Actitrack

Whenever experimenters start to work with a new system for research, they are always advised to read the manual before embarking upon experiments with the system. Therefore, and to get the ball rolling, we will start off with a series of tips on how an experiment should be conducted with ACTITRACK: the reader will thus obtain an overview before going on to focus on each one of the parts in greater detail.
An experimental session normally consists of the following steps:

- Cage detection
- Cage activation and checking
- Timing configuration
- Defining or retrieving the zone files to be worked with.
- Track registering and saving
- Track analysis and Data report generation

These 6 points are given merely by way of guidance and should be regarded as such by the reader.

4. Configuring Actitrack

4.1. The Main Menu

This is the menu the user will see on entering ACTITRACK.

This screen shows a menu at the top for accessing the different parts of the program, and which can be accessed either by the mouse or by the keyboard (typing "Alt", to activate it, followed by the letter underlined in the menu). So, if you want to access configuration, type "Alt" and then "C".
The following is a short explanation of the functions carried out by each of the options of the Main Menu:

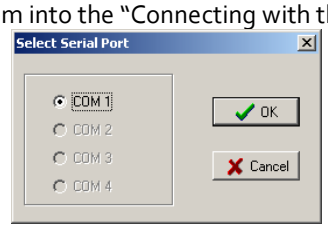
- **File**
  Allows for the configuration files to be open or saved. The configuration files provide the appropriate environment for each different experiment.
- **Zones**
  This option provides to tools necessaries to define the different zones in which the full tracking area will be divided, according to the needs of each different experimental situation.
- **Tracking**
  This option allows for the data acquisition from the LE8825 Activity monitors. Data acquisition is carried out according to the setting of the configuration options (see below).
- **Analysis**
  Allows for the analysis of data, carried out either on the basis of a single or multiple files at the same time.
- **Configuration**
  Using this option, data acquisition timing and the RS 232 to be used for data transmission can be configured.
- **Help**
  Allows for the opening of a help file with basic information about the program

4.2. The “Configuration” option

This option allows for the setting of the timing of the data acquisition as well as the selection of the serial port used for communications with the IR monitors.

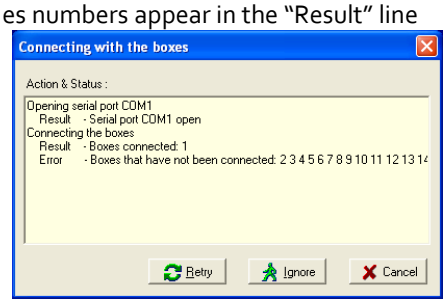
4.2.1.Serial port – Cage detection

Communications between ACTITRACK and the IR monitors LE8825 is done through a serial port. The *Select Serial Port* panel allows the selection of the port to be used from the ones available in the computer.
Select the serial port number on the left section of the panel and press the *OK* button. The software will scan the available cages connected to the system and display them into the “Connecting with the boxes panel”.



The “Connecting with the boxes panel” shows information about the Serial port used and the status of the connections to the cages.

The detected cages numbers appear in the “Result” line



The undetected cages numbers appear in the “Error” line.

Press the 

button to continue

Press the 

button to retry the detection test

Press the 

to exit for the connection panel

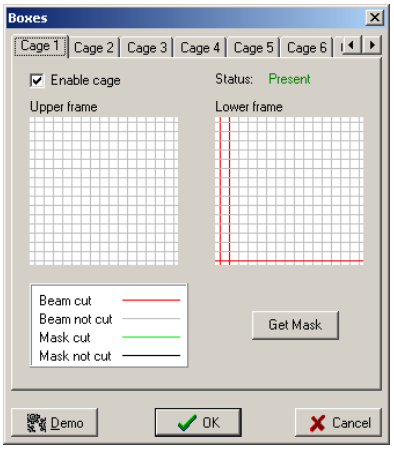
This scanning is also performed when entering into the Acquisition module of the software.
When the connection is set with ACTITRACK software, the message “Mode ACTITRACK” should appear in the LE8825 display.
If no cages are detected during the scanning process, another port should be selected, and the detection test performed again.

4.2.2. Cages activation and checking

From the "Boxes" panel available from the "Configuration" menu, the User can review the different available boxes by clicking on the corresponding tabs or using the arrows.
For each box, the following operations can be made:

4.2.2.1. Cages activation/deactivation

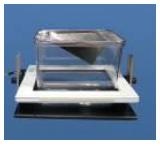
The Status indicates the status of detection of each Cage/LE8825. Up to 16 LE8825 devices can be connected to the system.
By default, all the detected cages are "activated". If there is no aim to use all of them for data acquisition, the cages can be disabled individually by deselecting the corresponding "Enable cage" box.



4.2.2.2. IR frame checking

The "Upper frame" and "Lower frame" panel allow the User checking the photocells of the IR frames connected to each LE8825 devices. To do this:
Select the tab of the cage to be checked
For checking the Lower frame, the User has to cut the photocells one by one in the cage by passing a finger or an object in front of each infrared beams and checking whether a red line appears for all the lines of the grid of the frame on the "Lower frame" panel of ACTITRACK. Each red line indicates a correct activation of the IR beams. This operation has to be for each X and Y axes of the frame).
Repeat the same process for the "Upper frame" of the cage if there is any connected.
Repeat the same process for all the cages

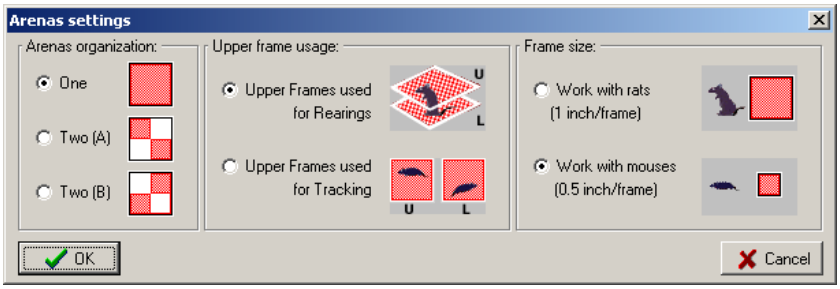
4.2.2.3. "Get Mask" option.



"Get Mask" button should be used (i) when the system is used with a home cage placed in the centre of the IR frames or (ii) when some static objects are continuously present in the arena so that some infrared beams are always cut. Pressing the "Get Mask" button allow the system to discard the photocell that will be continuously cut by the edges of the home cage or by the objects in the field of detection of the infrared beams.

4.2.3. Arenas configuration

The "Arenas setting" panel allows the User setting the configuration of the IR frames/LE8825 devices.



4.2.3.1. Arenas organization:

Red zones indicate the available zones for trials and the white zones indicate the unavailable zones. The IR frame can be configured in several way ways:
One: One animal by frame
Two(A) / Two(B): Two animals by frame (need the Arena divider option for separating the animals in different working areas). The animals have to be placed *in diagonal* areas for being able to tracks them without interference of the IR beam breaks made by one animal on those of the other. This option cannot be combined with the mice frame size.

4.2.3.2. Upper frame usage

Two frames can be connected to each LE8825 devices. Each Frame can be configured to be used for Activity measurement or Rearing/Nose poke measurements. Two configurations can be set:
"*Upper frame used to Rearings":* The two IR frames are associated to the same area in which the lower frame is used for Activity and the upper frame is used for Rearings/Nose poke.

"*Upper frame used for Tracking"*: The two IR frames are associated to different areas in which the frame is used for evaluating Activity.

Note: When using the IR actimeter for the Hole-board test (lower frame for nose-poking and upper frame for tracking), the option "*Upper frame used to Rearing*" has to be selected and the frame connections to the LE8825 have to be inverted: upper frame to the LE8825 Lower slot and lower frame to the LE8825 Upper slot.

4.2.3.3. Frame size

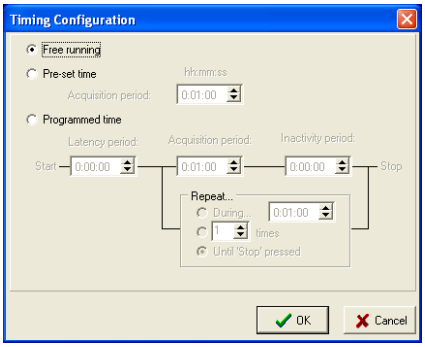
User has to select the frame type to be used.
The *"Work with rats (1 inch/frame)"* option has to be selected when the LE8815 IR frame (45 x 45 cm) is used.
The *"Work with mice (0.5 inch/frame)"* option has to be selected when the LE8816 IR frame (25 x 25 cm) is used. The mice frame size does not allow working with 2 mice as described in the *Two(A) /Two(B)* arenas organizations.

4.2.4. Timing Configuration.

This section describes the way in which the duration and time-structure of the experiment is carried out in ActiTrack. There are three ways of acquiring data: Free Running, Pre-set and Programmed.
Note: It is important to remember that when Actitrack is being used, the time protocol selected in the LE8825 control unit will not be taken into account. Actitrack fully controls all timing.

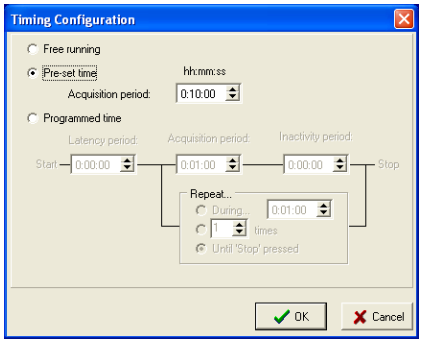
4.2.4.1. Free Running.

In this case, the duration of the data acquisition time is entirely controlled by the user. Acquisition begins as soon as the user presses the "Start" button and last until he or she presses "Stop". This configuration is the one by default for the program, which means that it will always selected run unless the user chooses other option.



4.2.4.2. Pre-set

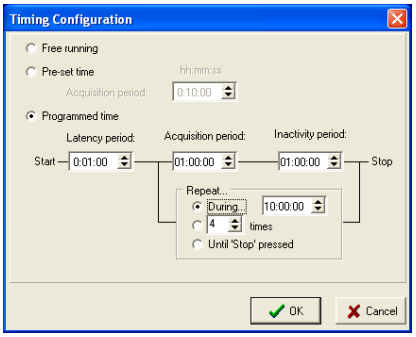
Using this option, the duration of the data acquisition time is pre-defined. Once the user presses "Start", data acquisition begins and stops when the pre-defined data acquisition period ends; a "Time is over" message will appear. Obviously, if this option is used, the duration entered by the user must be other than zero.



4.2.4.3. Programmed

If this option is used, it is important to define three different time periods:

- Latency period:
  It is an optional "waiting time" during which no data is taken. This period is only used once, at the beginning of the data acquisition. During this period, the program remains no active no matter the behaviour of the subject being tested.



- Acquisition period:
  During this period of time the program is actively capturing data. This period is followed by the inactivity period (when the "Inactivity period" has a value other than zero) or is reinitiated (when the inactivity period is zero).
- Inactivity period:
  Once the acquisition period has elapsed the user can define a certain waiting time interval. Thus, this time is very similar to "Latency period"; the difference being that unlike the latter, the "Acquisition" and the "Inactivity" periods can be repeated cyclically. It may be zero, although this would not make a great deal of sense.

If the programmed option is used, the total duration of the experiment is determined not only by the settings of the time periods defined above, but also by the "Stop" settings. The Acquisition and the Inactivity period are chained cyclically and repeated until the "Stop" criterion is reached. There are three ways to stop data acquisition in the "Repeat..." section:

- During... HH hours MM minutes SS seconds:
  The acquisition + inactivity cycle will be repeated during HH hours MM minutes and SS seconds. Logically, this time must be greater than the sum of the "Acquisition" and "Inactivity" periods. If the user enters a lower time, he will be warned so on pressing the "Ok" button of the timing configuration form.
- n times
  In this case the acquisition + inactivity periods will be repeated n times and the session will finish after the end of the last acquisition period.
- "Until "Stop" key pressed"
- This option allows the system applying the programmed protocol during an unlimited period of time until the user presses the "stop" button

In all the cases, the session can be interrupted manually by the user by pressing the "stop" button available from the "Start/Stop Control" panel of the acquisition window.

4.3. Configuration files

All the ACTITRACK settings can be saved in a "configuration files", thus helping in carrying out repetitive experiments without having to re-select all the parameters. As many different configuration files as different experimental set-ups can be defined, stored and recovered when wanted.
The configuration file contains the following parameters:

- Timing settings
- Name of the zones
- Serial port being used
- Speed thresholds
- Name of the last configuration file on use
- Name of the files used when tracking (zones, data files)
- Status of the data acquisition windows
- Header used in text files

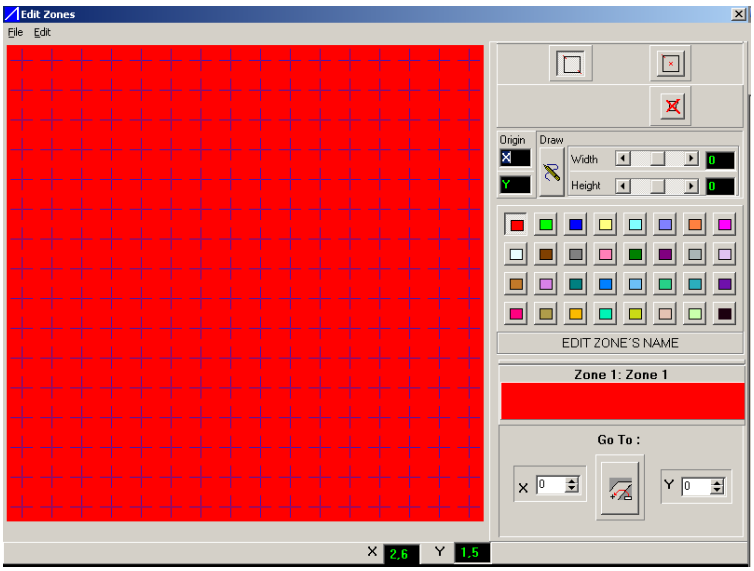
Parameters selected for multiple track analysis (in all the analysis modalities)
Setting of the report coverage in multiple track analysis
Configuration files can be saved and recovered by using the *File/Save* option in the main menu.
Configuration files are stored using ".ini" as extension. The *Actitrack.ini file* is used as default configuration file at the first launch. After that, the last configuration used before closing the application is loaded each time ACTITRACK is launched.
The configuration files are ASCII coded files and any external modification using text-processor programs is strongly proscribed to keep the system in good working order.

5. Zones Definition

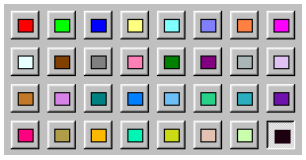
Selecting Zones/ Definition in the main menu will prompt the Zones Editor Window, which allows definition, description and categorization of zones and arenas.
A "Zone" can be understood as a user-defined set of co-ordinates that have some specific meaning from the behavioural point of view and for which a defined number of parameters of interest aim to be calculated.

5.1. The Edit zones window

When the "Zones/Definition..." option is selected on the main window, the following window will appear:



In the editor, a representation of the IR frame is found at the left panel, with a cross showing the position of each intersection between beams of the "x" and "y" axis.
Evidently, the form and the number of definable zones in an image will be determined solely by the experimenter's criteria. A zone, in a few words, is a slice of image that can be treated statistically. Before explaining the zone formation process on a specific image, we shall tersely comment on a series of considerations that are highly important for the proper understanding of the Zone Editor philosophy.
The left side shows the part of the screen used to select the colour to draw a zone. When the mouse passes over the different buttons a sign (with a blue background if it has not been used or red if it has already been defined on the drawing) will appear offering the identifier of the zone it defines. Thus, if it is placed over the first button the name "Zone 1" will appear, if it is over the second it will be Zone 2", etc. These are the names or identifiers ACTITRACK takes as default. We shall see how the user can define them later.



There are up to 32 definable Zones that correspond directly to the 32 buttons that appear grouped together on the right of the editor screen.
We are now going to provide a series of rules that will help the ACTITRACK user to understand the zone editor in depth.
Each zone has a colour univocally assigned to it: therefore, red has been assigned to zone 1, green to 2, etc. We have tried to assign colours that are clearly different from the rest, to each zone.
More than one area can be defined as "Zone 1" in a given image, even not being contiguous. ACTITRACK will regard all of them as a single zone (and it will therefore be treated as such in the statistics).
The zones are mutually excluding, which means that a given point of an image belongs to one zone or another, but not to two at the same time.
The zones defined in an image need not be consecutive. Thus, a file may contain zones 21, zone 3 and zone 14.
One same image can have as many associated zone files as the experimenter wishes.

5.2. Drawing Zones

We shall now address how several zones can be defined on the image. The user of ACTITRACK must choose one of the drawing tools located top right of the screen and then press one of the 32 keys that define the different zones. The user will now be ready to draw it.
In general, to do so, place the cursor on the target point of the image and holding down the left mouse button move it until it reaches the target point; release the button and the zone will be drawn superimposed on the image.
To draw correctly you must have selected a zone button (colour) and a drawing tool, and the selection can be made in any order. The reader will see that if he or she has a zone defined on a determined image and decides to draw another on it, the latter will be superimposed on the former: this is the result of the aforementioned fact that the zones mutually exclude each other.
Note that even the cursor can be freely moved throughout the frame, when drawing a zone only some co-ordinates are allowed. For a better understanding of these limitations, it is important to briefly discuss about the way in which the position of the experimental subject is obtained.
When a subject is entered into the IR frame, some beams are interrupted. When only one beam is interrupted, it is not possible to derive the position, as only one parameter is known (either an "x" value or a "y" value). Thus, the minimum information necessary to obtain a valid position is when two beams are interrupted, one in the "x" axis and other in the "y" axis. If so, the subject position will correspond to the (x, y) values of the beams being interrupted.
Nevertheless, it is possible than more than one contiguous beam is simultaneously interrupted (2, 3...). In this case, the subject's position is computed as the "mean" position: the arithmetic means of the two extreme beams being cut. So, supposing the subject is cutting beams 2 & 3 in the "x" axis and beams 5 & 6 in the "y" axis, the subject's position will be computed as x= (2 + 3) / 2 and y= (5 + 6) / 2, that is x = 2,5 and y = 5,5.
As a general rule, the subject's co-ordinate can only be represented by an integer if the number of beams being cut is odd or a half fraction (1,5; 2,5; etc.) if the number of beams being cut is even. The extreme positions are (1, 1) and (16,16).
To avoid a zone limit to be coincident with a possible co-ordinate (and thus making the decision about where the subject is impossible), the zones are forced to be limited to 0,25 or 0,75 above or below a (x, y) intercept.

5.2.1. Drawing Tools

The full screen available for drawing goes from (0.5, 0.5) up to (16.5, 16.5), from up to down and from left to right. In the lower left corner, there are two boxes labelled "X=" and "Y=", in which the current cursor co-ordinate is shown.
ACTITRACK provides the user with a series of tools designed to make it possible to draw different zones easily. Let us have a look at what type of drawing tools ACTITRACK offers the experimenter.

- **Square (End-End)**
  Dragging the mouse with the right button pressed from one end (the upper-left) draws a square shaped zone to the other (the lower-right).

- **Square (Center-End)**
  This tool operates as described for the previous one but using the centre and one of the ends to define the limits.

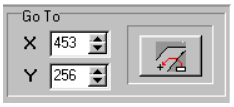
5.2.1.1. Erasing the image

If you press this button, all the zones created until the moment will be erased. By way of precaution, and before this action is taken, a dialogue window will appear prompting for confirmation.

5.2.1.2. Drawing with Co-ordinates.

Drawing with the mouse is not the only way the program has for defining zones or Scenario. If you prefer to work with co-ordinates use this tool. It works as follows: Once you have selected a drawing tool and a colour, fill in the 4 fields that appear in green on a black background. Remember that depending on the drawing tool you select the meaning of the fields will change. Once you have completed the fields, press the "Draw" button to add the figure to the image.

5.2.1.3. Placing the cursor over specific co-ordinates of the image



Fill in the boxes for X and Y and press the button on the right to set the cursor on the chosen co-ordinates.
There are also tools that give help or guide the user in the drawing process, namely:

DISPLAYING THE CURSOR POSITION.

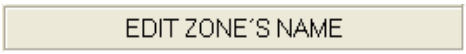
While the cursor is in the drawing zone of these windows the user will be kept informed as to its position. The range of values goes from X=0, Y=0 (top left-hand corner) to X=16, Y=16 (bottom right-hand corner).

MODIFYING A PREVIOUSLY DEFINED ZONE FILE

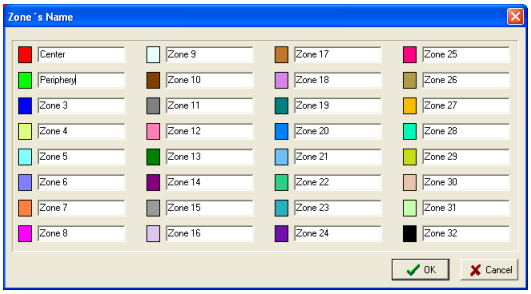
In the same way, the user may modify a zone file already saved to disk. Simply open the file through the menu and the File/Open Zones option to load the required file. You can now save the relevant modifications and save it again if necessary.

5.2.2. Rename zones

Besides their colour, zones can be also identified using their names. ACTITRACK uses a default name definition, assigning to each zone the "Zone 1", "Zone 2" and so on until "Zone 32".



By using the option "Edit/Zones..." a window appears in which the user can enter a new name to identify a zone, more appropriate for the description of some particular behaviour.



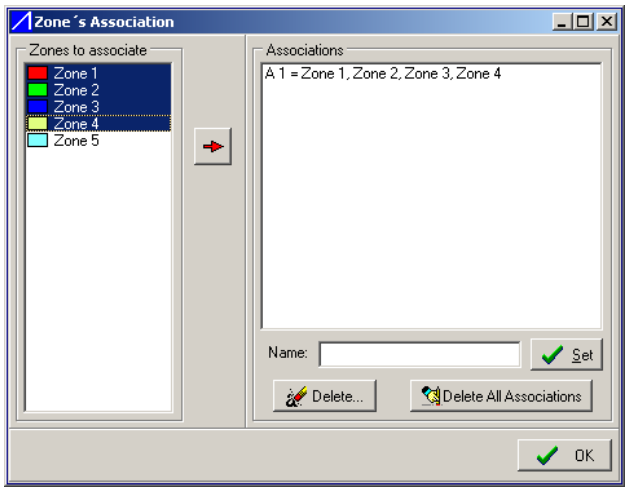
5.3. Defining Associations

Once the different areas on an image have been drawn it is possible to make associations. For practical purposes, an association may be regarded as another zone. Possibly the best way of explaining an association is by example. An experimenter may wish to obtain statistics on the four corners of a frame and its centre zone. Thus, he or she will draw 4 zones on each one of the corners on the image of the frame (Zone 1, Zone 2, Zone 3, Zone 4) and one for the centre (zone 5). However, what happens if he or she requires statistics on when the animal goes to any of the corners, whatever the zone is? This is where the concept of association comes in. In fact, the experimenter can create an association with the 4 zones required (Association 1=Z1+Z2+Z3+Z4) and will thus obtain results for each one of the 4 zones, and for all of them jointly.
Let us see some ideas about associations.
It is only possible to create associations with zones already defined (already drawn on the image).
Each association may be formed by up to a minimum of 2 zones and a maximum of 31.
A given zone may belong to more than one association. Thus, you may have A1=Z1+Z3+Z25, A2=Z3+Z5 and A3=Z1+Z2+Z3 created in the same zone file.

5.3.1. Create Associations

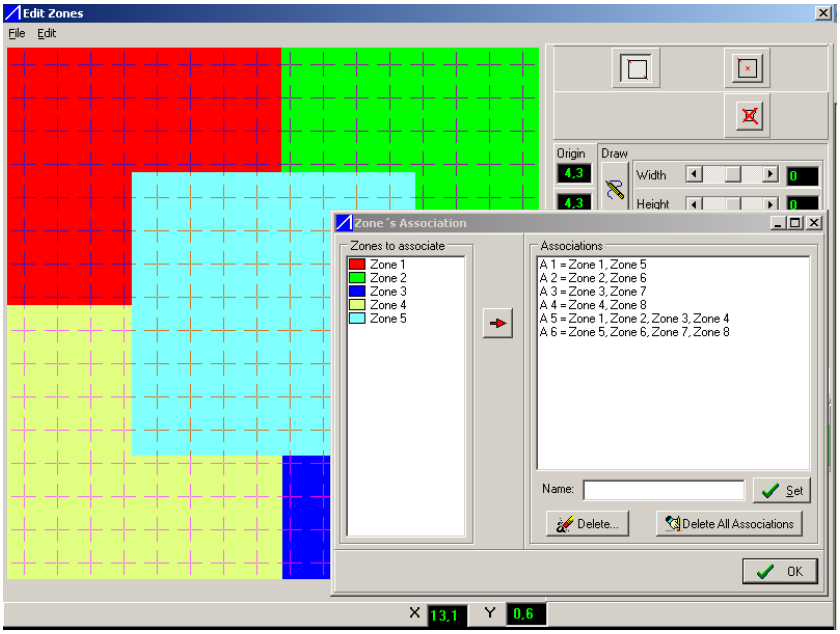
The process to be followed for the creation of associations is as follows:

- Define different zones
- Select the option "Edit\Associations..." on the main menu
- Select the zones to be associated in the left "Zones to associate" section of the "Zone's Association" panel:



- Press the "red arrow button" to validate the created association that will appear in the right "Associations" section
- Create as many associations as wanted

- Press OK when finished



5.3.2. Rename Associations

By Default, the associated will be named with the following name: A1, A2…An.
To set user-defined name, the next steps have to be followed:

- Select the Association to be renamed in the "Association" right section.
- Enter a new name in the "Name" section.
- Press the [Set] button to register the new name.

5.3.3. Delete Associations

All the associations defined to date can be deleted: to do so, press the "Delete All Associations" key and answer "Yes" to the dialogue window that appears.

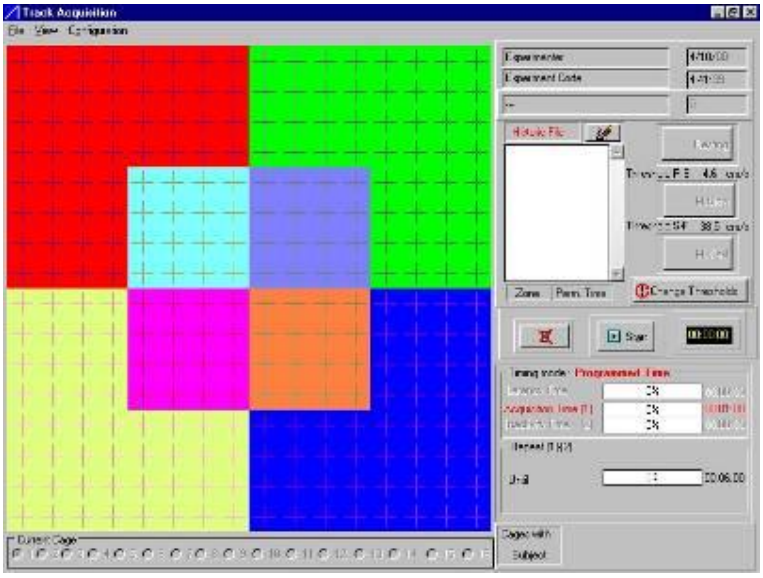
5.4. Zone definition saving

It is worth examining a series of considerations on the way ACTITRACK stores and treats the files created in the zones editor.
The zones will be saved through the menu (option File / Save) with the extension "zne".
Associations are saved automatically within the zone file.
Note: Previous versions of ACTITRACK managed two files, one for zone definition with 'zac' extension and another for zone associations with "ass" extension. These zone files can be directly opened without any problem from the Acquisition and Analysis panels of the present ACTITRACK versions. Nevertheless, when these zone files are opened from the zone editor of the present ACTITRACK version, they can be saved only saved in the new compact format using the "zne" extension.

6. Track Acquisition

6.1. The Acquisition main window

When opening the "*Tracking*" option of the main menu, the next window is opened.



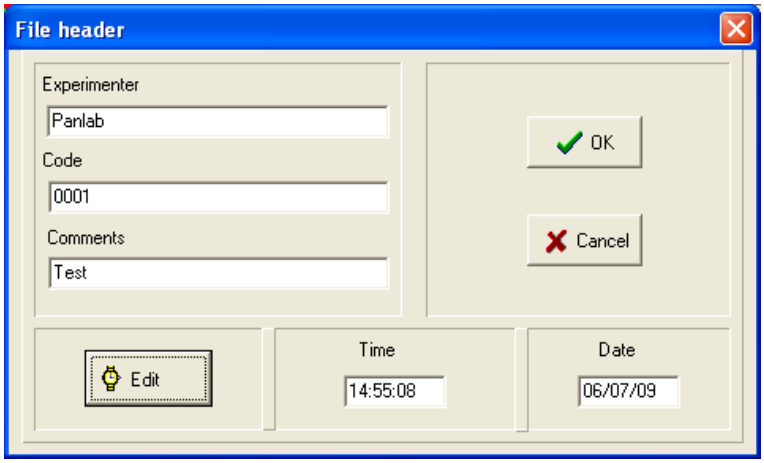
In this window, the area representing the IR Frame display is shown on the left side. On the right side, some tracking controlling tools are displayed, as well as information about the current timing and track status.
The "File" option in the new main menu allows for files management, including the opening of zones files, already existent data files (for appending new tracks) or creating new files.
The "View" option allows for the opening of specific information windows giving further details about the current track status.
The "Configuration option should be used for editing the file identification header and to set the timings that will affect data acquisition.
Before addressing how Tracking works in depth, it should be mentioned that the data file saved with the track will allow ACTITRACK to conduct the subsequent analysis of the data and the generation of statistics.

6.2. The File menu

File/New: Create a new track file. Name and location will be asked.
File/Open/Tracks: Open an already existing Track file. The new registered tracks will be added to the tracks contained in the Track file.
File/Open/Zones: Chose a different zone definition from the already defined zone files.
Exit: Close the Acquisition window and come back to the ACTITRACK main menu

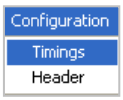
6.3. Header

The "File header" panel allows the user to register the track file with additional information about the "*Experimenter*" name, experiment "*code*", "*comments*", "*Date*" and "*Time*".



Pressing the "*Edit*" button allows the User freely editing the Time and Date
Pressing the "*Update*" button set the Time and Date given by the computer.

6.4. Timing configuration



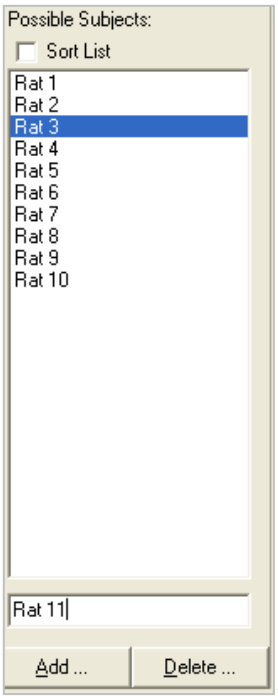
Timing configuration can be set directly from the acquisition window by selecting the "*Timing*" option of the "*Configuration*" menu. This option gives a direct access to the timing panel described in the chapter 4.2.4.

6.5. Subject List

Subject Lists are commonly used during the execution as they provide an easy source to select the animals to run a session.
The edition of Subject Lists IS NOT MANDATORY given that name of the subject associated to the current registered track can be entered when the track recording finishes.

6.5.1. Subject list source

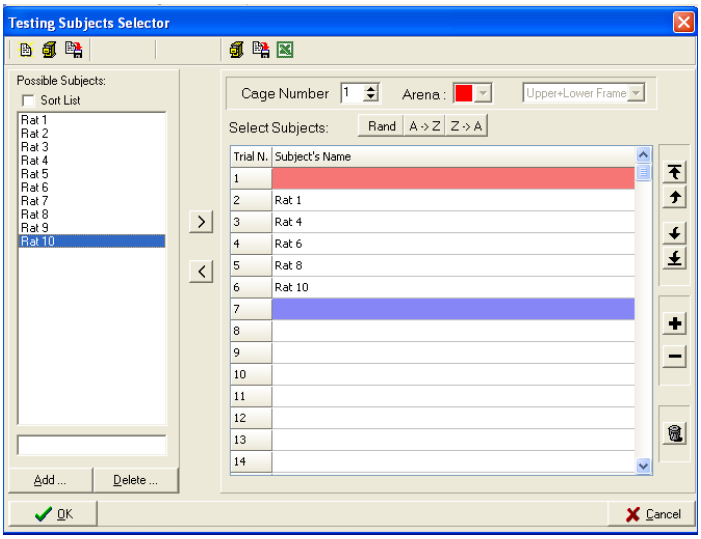
The left panel allows the creation of subject lists that will be used as a source of available subject.



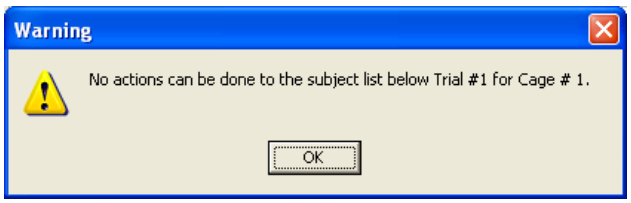
Press the "*New Subject list* "to create a new list of subjects.
Add a Subject by filling the dedicated case with the subject name and press the "*Add*" button to add this name to the list of "*Possible subjects*"
Delete a Subject by selecting a subject in the list of "*Possible Subjects*" and press the "*Delete*" button.
Check the "*Sort List*" box to sort the animal using alphabetical order.
Save the list by pressing the "*Save Subject List*" button
Open a registered list by using the "*Open Subject List"* button"

6.5.2.Subject list selection for Trials

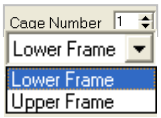
The right panel allows the creation of a list of subjects in the same order they will be used for track acquisition. This list will then allow associating automatically the registered trial with the name of the subject indicated in the list.
To select the subjects that will be used for the trials, move them one by one from the left panel to the right panel list by using the > button.
Use the < button to remove a subject from the right panel list.



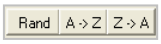
Note: If the first line of the selected subject list is red, trying to displace a subject from the left panel to the right panel will display the following message.



This message can be avoided by selecting first an empty line before pressing the > button.
If several cages are used, select the cage to which the list will be applied.
If each LE8825 device is connected to two activity frames (for monitoring 2 animal at the same time in different arenas), select the frame to which the list will be applied.



Use the "*Rand*", "*A->Z*" and "*Z->A*" to randomize the subjects of the list or to put them into the "alphabetical" or the "inverse alphabetical" order, respectively



Use the ⤒ and ⤓ buttons to displace the subject to the top and to the bottom of the list

Use the  and  buttons to displace the subject one line above or one line bellow.

Use the  and  buttons to insert or delete a line in the list

Use the  button to delete a selected subject.

Save the Selected Subject List by pressing the "*Save Selected Subject List*" button

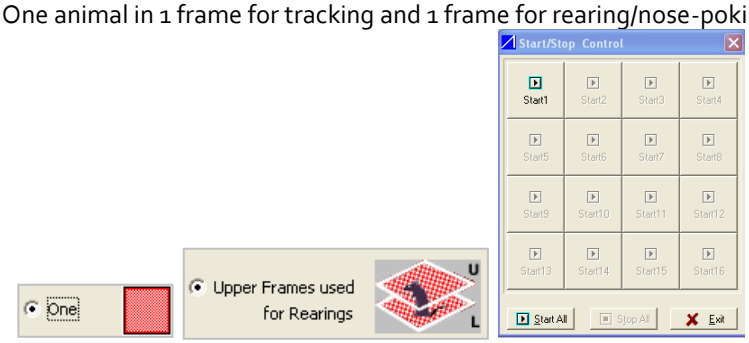
Open a registered list by using the "*Open Selected Subject List"* button"

Export the Selected subject list to Excel by pressing the "*Export to Excel"* button"

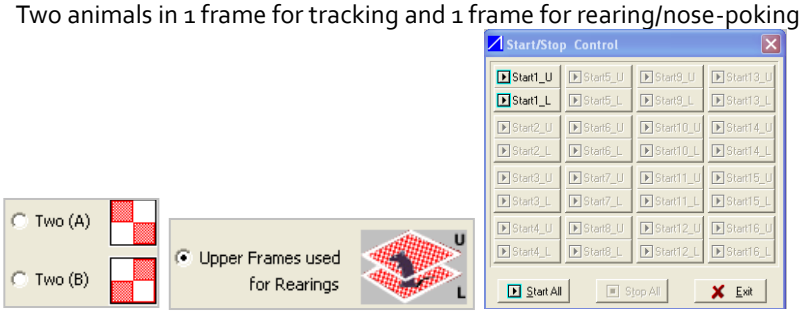
6.6. Start/Stop track acquisition

When the "Start" button is pressed a window like those shown at the left appears.
This Start/Stop control box allows the user to select the LE8825 device to which the Start/Stop orders apply. In this way, the LE8825 devices can be independently controlled, and the timing of each one of them is carried out without affecting the rest.
The design of the Start/Start button of the Start/Stop control panel may vary depending on the configuration chosen in the panel "Arena Settings".

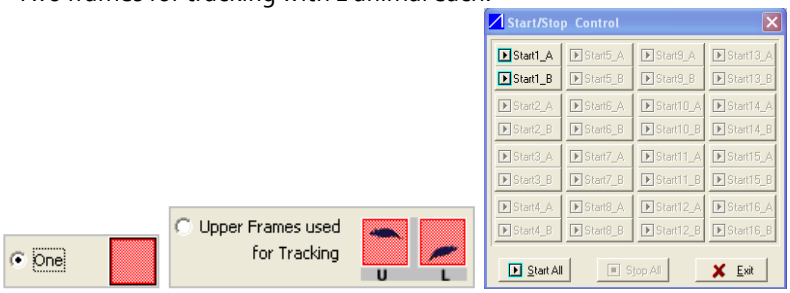
- Configuration 1:
  One animal in 1 frame for tracking and 1 frame for rearing/nose-poking.



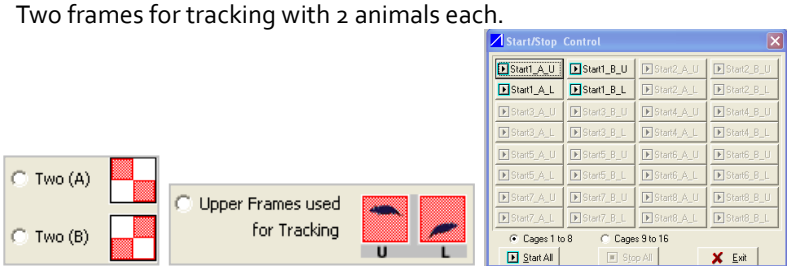
- Configuration 2:
  Two animals in 1 frame for tracking and 1 frame for rearing/nose-poking.



- Configuration 3:
  Two frames for tracking with 1 animal each.



- Configuration 4:
  Two frames for tracking with 2 animals each.



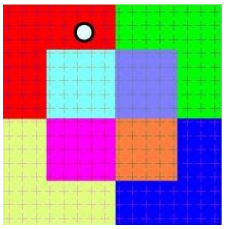
All the cages can be started and stopped simultaneously by using the next buttons:

Note: The tracking process will ONLY start when an animal is detected: if there is no animal in the scenario, ACTITRACK will do nothing (it will neither capture data nor count time). If there is one, ACTITRACK will start to count time and pick up data as soon as the "Start Tracking" button is pressed.

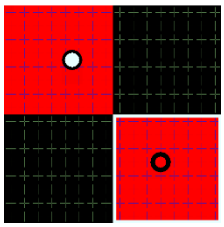
6.7. Data shown during acquisition

6.7.1. Visualization of animal position

Once a subject is detected in one cage, a white circle drawn in the current x-y co-ordinate shows the current position of the experimental subject in the corresponding IR frame display.



Each cross represents the interception of the IR beams.
In the configuration in which 1 frame is used for two animals, the "discarded" areas appears in black.

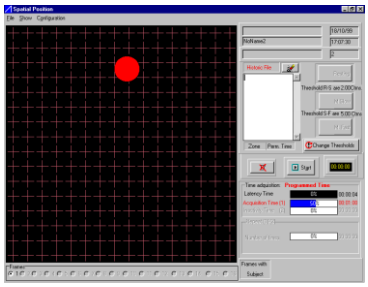


- How ACTITRACK calculates the spatial position:
  The presence of a subject in the experimental arena is detected as the breaking of one (or several) IR beam both in the x and y-axis, produced in the lower IR frame. The position co-ordinate is evaluated according to the following rules:

If a single beam is broken in each axis, the co-ordinate corresponds to the broken beam.
If more than one beam is broken, the co-ordinates correspond to the mean between the most extreme broken beams. That is, if in the x-axis the beams number 2 and 3 are broken, the x co-ordinate is (2+3)/2=2.5; if the 2,3,4 and 5 are broken, the x co-ordinate is (2+5)/2=3.5 and so on.
Only the beams that are together are considered. If a beam separated from the others is cut, the beams further away from the last known position will be ignored. For example, if beams 2, 6 and 7 are cut, and the last known co-ordinate was between 6 and 7, then the beam 2 will be ignored. Another case would be if the last known position was near beam 2, then the beams 6 and 7 would be ignored. In case there is no last known co-ordinate, as for example at the beginning, the largest group of beams will be considered.

6.7.2. Visualization of rearing and nose-poking

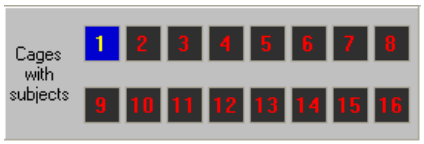
In ACTITRACK, the detection and visualization of rearing and nose-poking behaviours requires the combination of the IR Frame for Rearing/Nose-poking with the IR Frame for activity. Indeed, the activity frame is need for determining the position (x-y co-ordinates) of the detected Rearing/nose-poking.
When the rearing/nose-poking is detected, the circle representing the animal is filled with red colour.



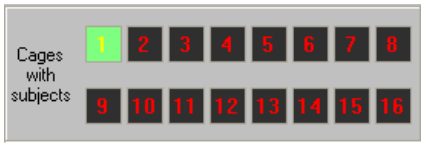
6.7.3. Cage number visualization and selection

The "Cage with subjects" section indicates the state of activation of the cages used in the experiments.
The yellow number on grey background indicates that the cages which are ready to be used.

The red number on grey background indicates the cages which are "enabled" in the "Box configuration" section but not detected.
The grey number on grey background indicates the cages which are disabled in the "Box configuration" section.
The yellow number on blue background indicates the cages in which a session has been started but the subject not yet detected. In this cage, the tracking process is then in stand-by.



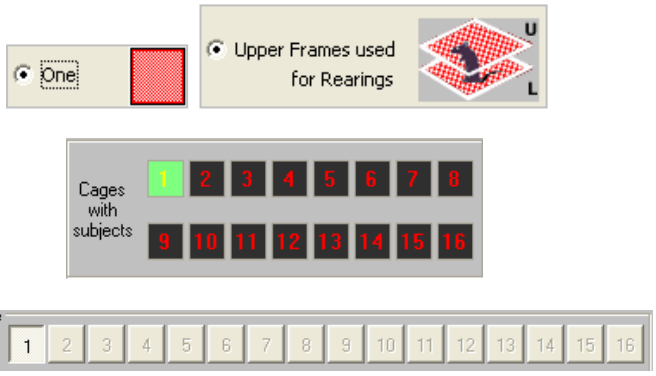
The yellow number on green background indicates the cages in which a session has been started and the subject detected. In this cage, the tracking process is running.



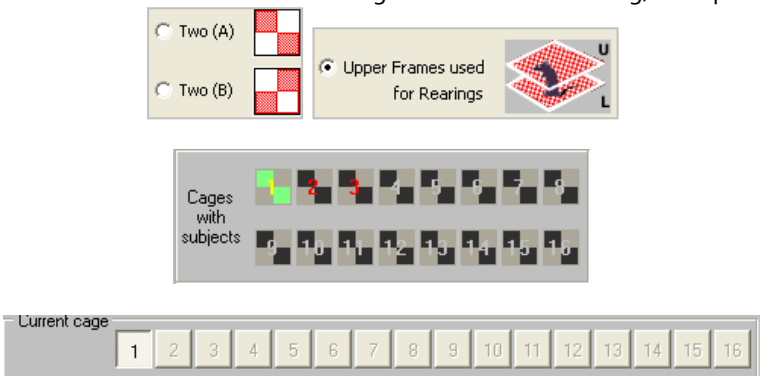
Below the IR Frame display, the "Current cage" section displays a set of buttons indicating the number of the LE8825 units in which experimental subjects have been detected. These buttons can also be used to select the LE8825 device whose data is represented (from 1 up to 16).

Here again the design of these two sections may vary depending on the configuration chosen in the panel "Arena Settings".

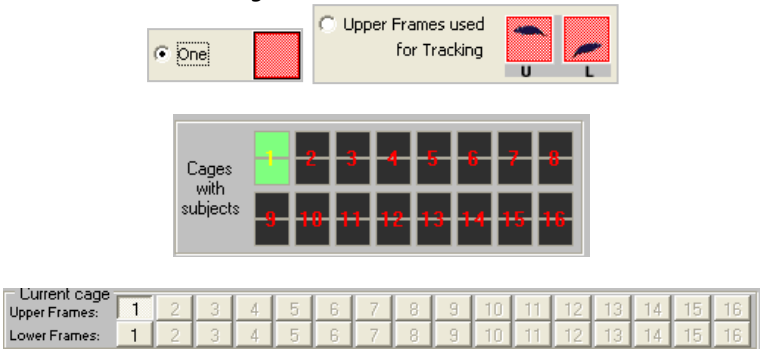
- Configuration 1:
  One animal in 1 frame for tracking and 1 frame for rearing/nose-poking.



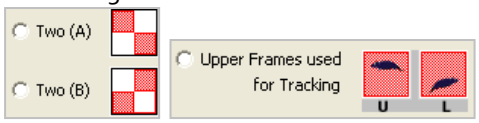
- Configuration 2:
  Two animals in 1 frame for tracking and 1 frame for rearing/nose-poking.

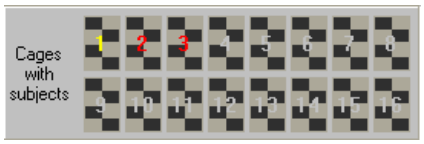


- Configuration 3:
  Two frames for tracking with 1 animal each.



- Configuration 4:
  Two frames for tracking with 2 animals each.





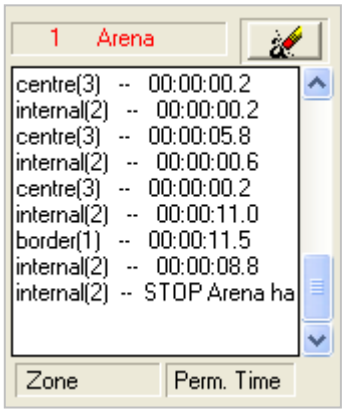
6.7.4. Track information

This section displays information about the session:

- Experimenter name
  (Experimenter 1): entered in the Header configuration panel. This area is empty when the Experimenter name of the Header configuration panel has not been filled by the user.
- Date
  (31/03/09): data of track registering (DD/MM/YY)
- Experiment code
  (Exp 1): Experimental code entered in the Header configuration panel. This area is empty when the Experimenter name of the Header configuration panel has not been filled by the user.
- Clock Time
  (08:51:23): hour of track registering (HH:MM:SS)
- Name the subject
  (Mouse 1-2): name of the subject used in the session. When a subject list is used, the name of the subject indicated from the list is shown; when no subject list is used, the system gives a by-default name that can be changed by the user when the track is saved.
- Session number:
  Number of the current session.

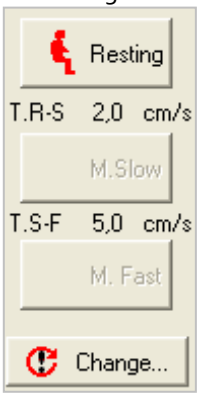
6.7.5.Track History

The user can also view, in a section located on the right of the screen, permanence time in each zone; this information is merely for guidance and is not saved with the track. Evidently, the main reason is that the information will be generated again in the analysis phase.
The erase button can be used to reset the information contained in the section between two track acquisitions.



6.7.6.Moving fast/slow, resting

ACTITRACK evaluates movement speed and accumulates separately the time during which the subject is moving fast or slow or it is resting.

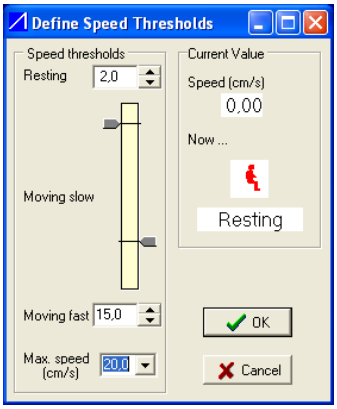


A current view of the movement state and threshold set for defining these movements is displayed in the acquisition main windows.

6.7.6.1. Defining movement thresholds

ACTITRACK uses two thresholds for differentiating the different kind of movements whose values should be set by the user empirically.
The first threshold refers to the limit speed to be considered as separation between slow and fast movements; the second threshold separates the slow movements from the resting time.

By pressing the "Change threshold" button, a window is presented (see above) in which the user can change the current thresholds by moving up/down the sliding bars. The current value of the threshold is shown in the upper and lower extremes of the bar.
The maximum upper value is determined by the current selection in the "Max. Speed" box. The user must select a maximum speed coherent with the experiment.
In the "Current value" box, the current speed and the current movement state are shown:



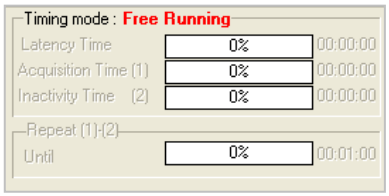
6.7.7. Time elapsed

A timer gives the current time elapsed in the session (HH:MM:SS). This timer only begins when the subject is detected in the frame area and includes the latency time when the Programmed mode is used in the time settings.

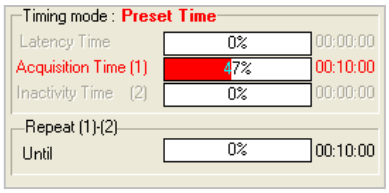
6.7.8. Timing mode section

The time acquisition section allows visualising the evolution of the acquisition period as defined by the user is the "Timing configuration" panel.

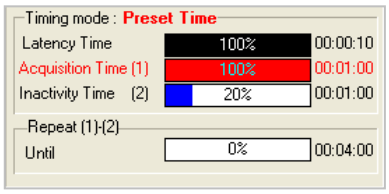
- Free running:
  In this timing configuration, the Timing mode section is inactivated. The time elapsed of the session can be visualised in the chronometer section.



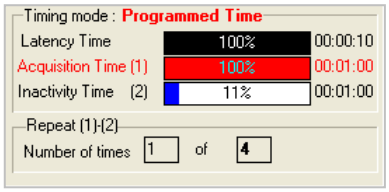
- Pre-set time:
  In this timing configuration, the duration of the acquisition is displayed in red, and a progression bar shows the evolution of the time elapsed.



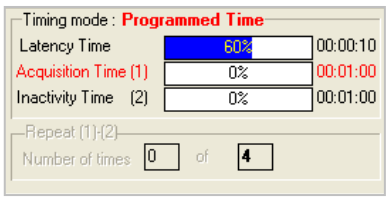
- Programmed time – Repeat during HH:MM:SS:
  In this timing configuration the evolution of the latency time, acquisition time and inactivity time can be followed through independent progression bars. The total duration of the acquisition time set by the user is shown in the "Repeat (1)-(2)" section.



- Programmed time – Repeat X times:
  In this timing configuration the evolution of the latency time, acquisition time and inactivity time can be followed through independent progression bars. The remaining number of cycles is shown in the "Repeat (1)-(2)" section. The "Number or times" value is actualized each time the acquisition time of each cycle is elapsed.



- Programmed time – Until "stop" pressed:
  In this timing configuration the evolution of the latency time, acquisition time and inactivity time can be followed through independent progression bars. The "Repeat (1)-(2)" section remains inactivated.



6.7.9. Other current parameters

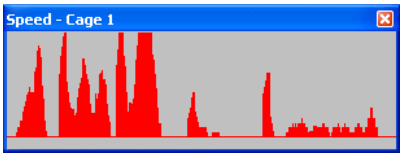
Other parameters available from the "View" main menu can be visualised in real-time during the track acquistion process. Each option of the menu opens an independent window which can be placed wherever desired by the user.

- Time
  Creates a window inside which, the time elapsed since the "Start" key was pressed appears in larger size.

- Cage
  Shows the number of the cage under visualisation

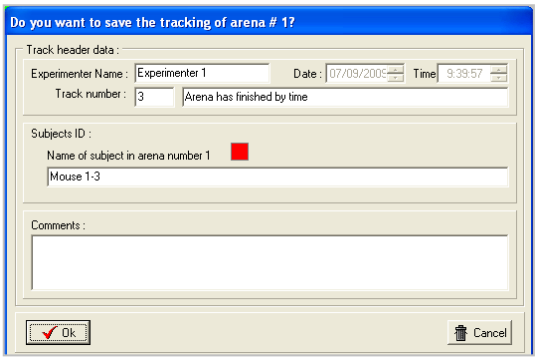
- Zone
  Shows the current position of the subject in the zones.

- Speed
  Shows a graphic with the instantaneous velocity. It is given by way of orientation only, which means that for greater accuracy the experimenter must save the file with the track and study it in the analysis part. This graph is just a current view of the speed and is not saved.

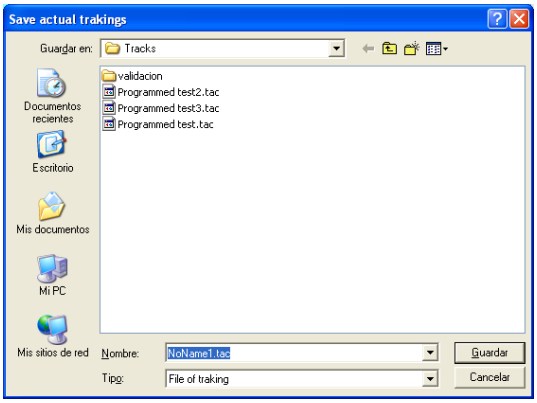


6.8. Track saving

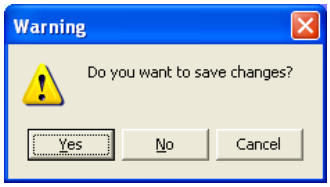
Once the session is finished a saving windows appear allows the user saving or discarding the track. When using a subject list, the subject information displayed on the saving panel are already filled but can be changed by the user.



When a track file has been loaded before acquisition the new registered tracks will be added to the list of the track contained in this file.
When no track file has been loaded before acquisition, the user can save the tracks in a new track file by using the "Save" option of the "File" main menu.



When the user closes the acquisition panel without saving the registered track in an experimental file a warning signal will ask whether the user want to save change and open a new saving window for allowing the user creating a new experimental file containing all the registered tracks.

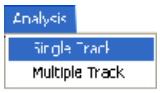


Note: all the registered tracks that are not saved into a Track file will be lost.

7. Track Analysis

The registered tracks can be analyzed in two different ways:
Single-Track Analysis: for an individual track visualization and basic analysis preview.
Multiple Track Analysis: a saving-time section allowing the simultaneous analysis of a set of tracks and the generation of advanced data reports.

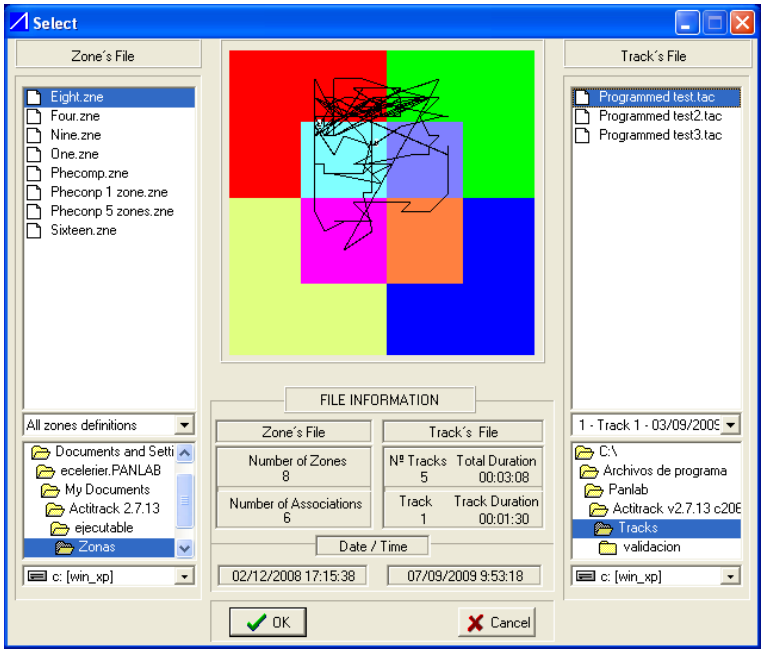
7.1. Single track analysis



The Single-Track Analysis section is only used when the user wants to process to the individual analysis of a given track; for instance, for reviewing the shape of a track combined with the shape of the zone used and/or for previewing the standard data given.
To generate same data reports (and others additional advanced ones) simultaneously for a set of user-selected tracks, the Multiple track analysis mode has to be used.

7.1.1. Combine Track and Zone definition

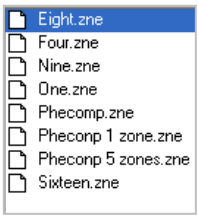
The first step necessary to carry out the analysis of the Track is to combine a Track file with a zone file.
The window presented below allows doing so.



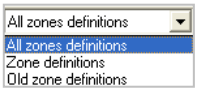
This window displays, besides other tools, an image viewer (in the centre) and two file explorers (one on each side). The aim of this screen is simply to select a track and a zone file for their subsequent analysis.

7.1.1.1. Select a zone file

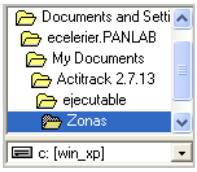
Select a zone file in the "Zone's file" section of the "Select" panel. Below the window showing the zone files, there is a sector for choosing the zones (*.zne format). A view of the zone design contained in the file and other additional information (date of file creation, number of zones and associations defined) will be displayed in the central part of the "Select" panel.



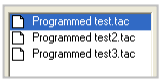
A specific list allows the user filtering the type of zone files shown above: All zones definitions (displays all zone formats), Zone definitions (displays the zone files created using the more recent zone format: *.zne) and old zone definitions (displays the zone files created using the old format: *.zac).



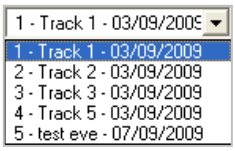
When a folder is selected in the Explorer window of the "Zone´s Files" section, all the files previously created in this directory with ACTITRACK will be shown.



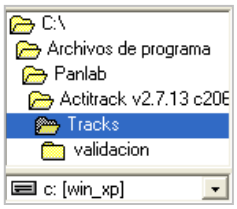
7.1.1.2. Select a track file



Select a track file in the "Track's file" section of the "Select" panel.
A view of the first track registered in the selected track file and other additional information (date of file creation, number of tracks it includes and their duration) will be displayed in the central part of the "Select" panel.
Below the list of track files, the user can choose the track to be analyzed between all the tracks associated to the file.

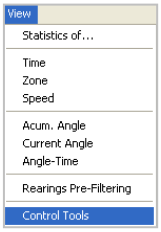


When a folder is selected in the Explorer window of the "Track´s Files" section all the files previously created in this directory with ACTITRACK will be shown.

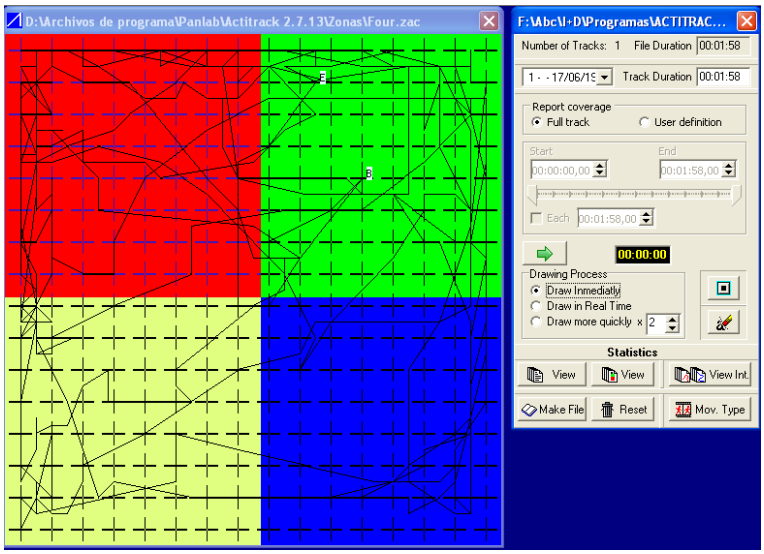


Once the zone and track files have been selected for combination press the "Ok" button to enter into the main single track analysis module.

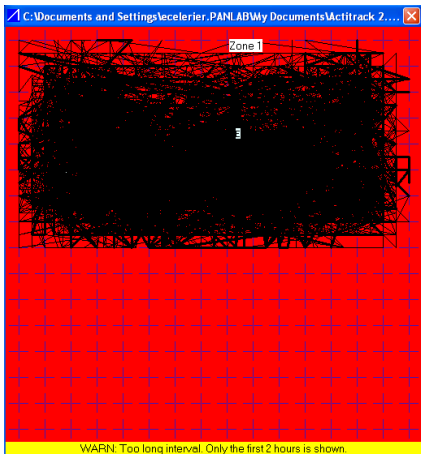
7.1.2.IR Frame display



The analysis main window consists in a left panel containing an IR frame display and a right control tools panel for analysis configuration.



The IR frame display superimposing the image of the select zone with the image of the selected track. Each cross represents the interception of the IR beams.

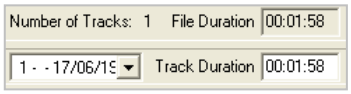


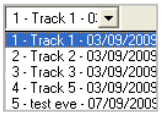
When the duration of the current track or of the user-defined interval time is longer than 2 hours, only the 2 first hours are displayed in IR frame display and the following message is shown in yellow: "WARN: too long interval. Only the first 2 hours are shown.

Since many views, reports or windows can be shown in the analysis section; the control tools panel can be closed and opened again by selecting the "Control Tools" option of the "View main menu".

7.1.3. Track selector

The top section of the control tools panel provides information about the track file selected and allows the user selecting which track of the file will be analyzed.





Select the track from the list displayed in this section.
The information provided is:

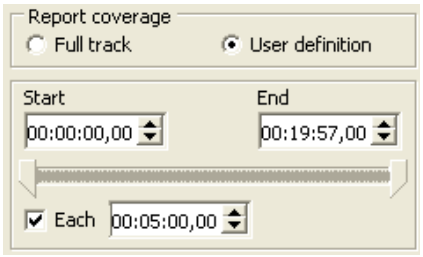
- Number of tracks:
  Number of tracks contained by the track file.
- File Duration:
  Total duration of the file, consisting of the sum of the durations of all the tracks.
- Track Duration:
  Duration of the current track displayed

Furthermore, track to be analyzed in the list located on the left of the track duration information.

7.1.4. Report coverage

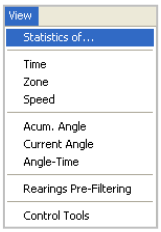
The user can define a time interval of analysis in the "report coverage section":

- Full track:
  Analysis of the whole track.
- User definition:
  The "User Definition" option allows the user changing the starting and End time of the analysis window as well as subdividing the analysis in various time intervals (using the "Each" option).

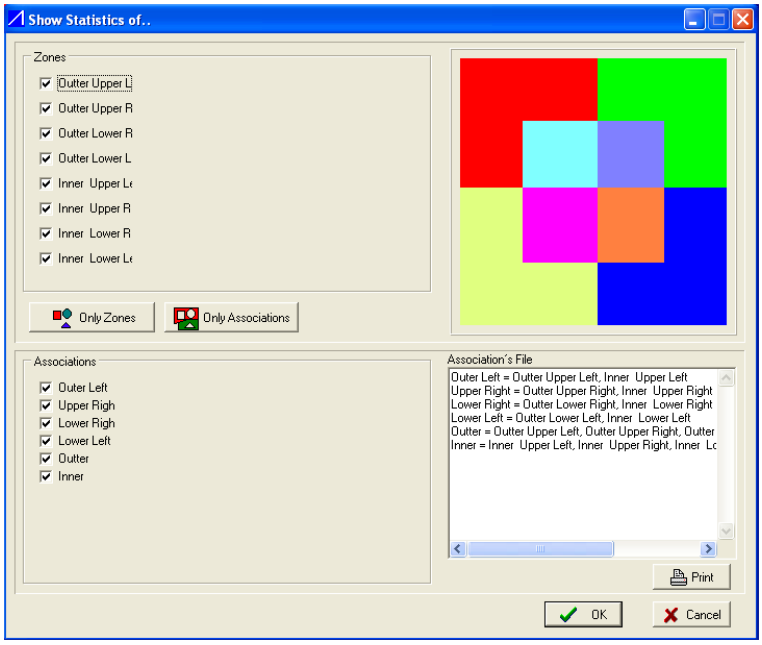


Note: The subinterval duration has to be inferior to the track analysis duration defined in the "Start" and "End" boxes. The maximum number of sub-intervals that can be processes by ACTITRACK for each track is 100.
The view of the track in the IR frame displayed in left is actualised for showing only the portion of track defined by the interval time of analysis.

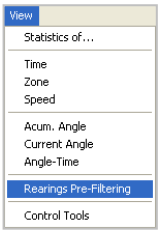
7.1.5. Select zones and associations



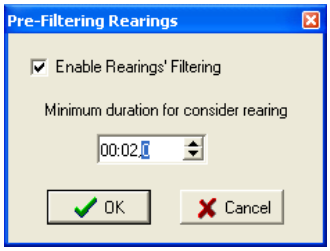
By pressing the "Statistics of" option of the "View" main menu, the user can select the zones and associations from which statistical calculations will be calculated. Clicking the respective check-buttons should activate/de-activate the selection. By default, all associations and zones are selected.



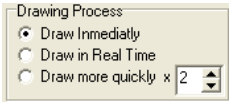
7.1.6. Define a Rearing duration filter



The duration of rearing episodes can be filtered by the user. In the "Pre-filtering rearing" window, a duration filter can be activated by checking the option "Enable Rearing's Filtering". Then, the user has to enter the minimal duration for considering a rearing. In other terms, when the rearing filter is activated, the rearing episodes with duration strictly inferior to the value entered by the user will not be considered as a rearing episode for all the calculation related to rearing given by the system.



7.1.7. Drawing process and analysis speed



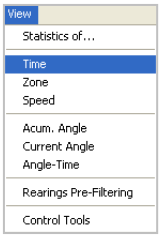
Track drawing (for replay visualization) and analysis are performed simultaneously.
The "Drawing process" section defines the speed of track drawing in the IR frame display and of the concomitant analysis process.

- Draw Immediately:
  The analysis process is done instantaneously. Note: in this mode, the system draws the different intervals immediately, which means that if the computer used is fast the user will not be able to view them in the IR frame display.
- Draw in Real Time:
  The analysis process is done in real-time. In other terms, its duration will be the same than the duration of the experiment.
- Draw more quickly x X:
  The analysis process is done in a speed depending on the value defined by the user which is a multiple of the real-time value (default value = 2).

7.1.8. Start/stop drawing and analysis processes

Start the drawing process concomitantly with the analysis process by pressing the "Start Analysis" button
When using the "Draw in real time" and "Draw more quickly" options, the actual time in the track is displayed in the Timer.
The drawing process can be interrupted at any moment by pressing the "Stop Drawing" button.
The "Clear" button reset the view of the track on the IR frame display.

7.1.9. Current views during analysis process



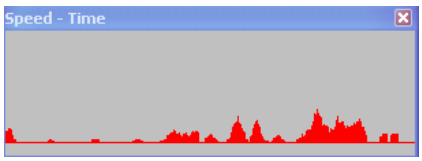
When using the "Draw in real time" and "Draw more quickly" options, the evolution of the following parameters available from the view main menu can be visualized in real time.

- Time
  Open a window that shows the elapsed time from the beginning of the track.

- Zone
  On revising the track, a window will appear showing the name of the zone being currently visited by the subject (in mode "Draw in real time" or "Draw more quickly").

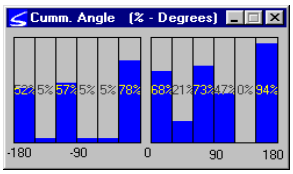
- Speed

  Shows a graphic with the average speeds in a graphical format.



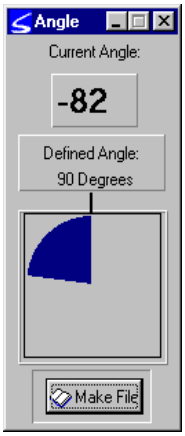
- Cumm. Angle

  Shows in bar chart form, the direction of the angle of the track with for reference, the beginning of the representation. The cumulated angle is shown in percentage form with regard to the maximum value of the angle (which is assigned to a value of 100 %).

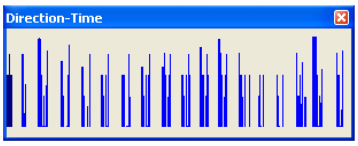


- Current Angle

  Shows the current angle (in mode "Draw in real time" or "Draw more quickly"). The following criteria used: a totally vertical bottom-to-top track is given a value of 0 degrees. On this basis, and going clockwise, angles up to 180 degrees will be positive; in the other direction, negative angles are those up to –180 degrees. Thus, the range will be from zero to 180 degrees and from zero a –180 degrees, as was the case of the window shown in the previous section. Finally, by pressing the button "Make File", when the track is gradually drawn, creates a file with the times and the angles according to the criteria described above. This file can be printed or saved for subsequent analysis on spreadsheets.



- Angle-Time
  Gives information about the direction of the animal. Every sample time, every displacement angle comprised between -90º y +90º is represented as a bar as its absolute value. Angles inferior to -90º and superior to +90º are not represented. This tool allows discriminating displacements forward (represented in the chart) from the displacements backward.



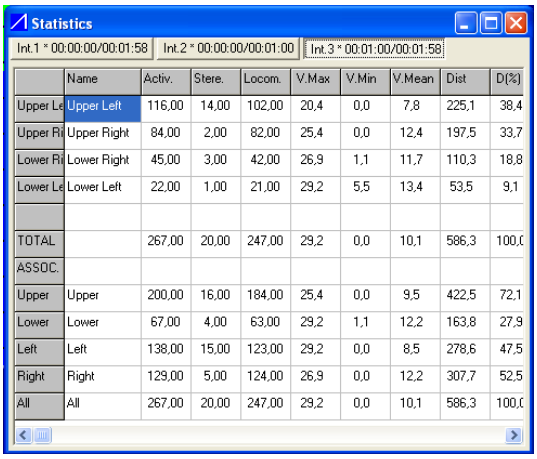
Moreover, the bars can be painted with two different blue: dark or clear. The dark bars indicate that the current speed of the animal associated to the angle was considered as slow (previously defined by the user with the speed thresholds) and the clear ones are reefer to fast displacements.

7.1.10. View data calculations and graphs

After the end of the analysis process, the numeric values are not directly presented. The user can choose between different possibilities of views.

7.1.10.1. Displaying Data in Numerical Format

Once the numeric calculations have been requested by pressing the green arrow button, the user can press the "View (data)" button, located at the bottom on the left of the Control window displaying in a spreadsheet format the different calculations provided by the single-track analysis module.
Note: This report appears empty until the "Start analysis" button is pressed (as described in previous sections of this Manual).



	Name	Activ.	Stere.	Locom.	V.Max	V.Min	V.Mean	Dist	D(%)
Upper Le	Upper Left	116,00	14,00	102,00	20,4	0,0	7,8	225,1	38,4
Upper Ri	Upper Right	84,00	2,00	82,00	25,4	0,0	12,4	197,5	33,7
Lower Ri	Lower Right	45,00	3,00	42,00	26,9	1,1	11,7	110,3	18,8
Lower Le	Lower Left	22,00	1,00	21,00	29,2	5,5	13,4	53,5	9,1
TOTAL		267,00	20,00	247,00	29,2	0,0	10,1	586,3	100,(
ASSOC.									
Upper	Upper	200,00	16,00	184,00	25,4	0,0	9,5	422,5	72,1
Lower	Lower	67,00	4,00	63,00	29,2	1,1	12,2	163,8	27,9
Left	Left	138,00	15,00	123,00	29,2	0,0	8,5	278,6	47,5
Right	Right	129,00	5,00	124,00	26,9	0,0	12,2	307,7	52,5
All	All	267,00	20,00	247,00	29,2	0,0	10,1	586,3	100,(

Each time the "Start analysis" button is pressed for the current track a new tab is added in the "Statistics" table. The title of each table shows the actual track period of time considered for analysis.
In each tab, a numeric data table contains columns with the parameters calculated for each zone and associations defined by the user.

The following parameters y calculations are provided:

- **Name**
  Name of the zone or association.
- **Activ**.
  Global Activity: Subjects global activity during the analyzed interval, number of samples where the position of the subject is different from its position during the previous sample (sum of Stereotypes and Locomotion).
- Stere
  Stereotypes: Subjects stereotyped movements during the analyzed interval, number of samples where the position of the subject is different from its position during the previous sample and equal to its position during the 2nd sample back in time.
- **Locom**.
  Locomotion: Subjects locomotion during the analyzed interval, number of samples where the position of the subject is different from its position during the previous sample and different to the position of the 2nd sample back in time.
- **V.Max**
  Maximum Velocity: Maximum speed (cm/s) reached by the experimental subject. If its value is zero, it means that the animal has not moved throughout the whole time interval. In such case, both the minimum and mean speed will also be zero.
- **V.min**
  Minimum Velocity: Minimum speed (cm/s) detected in the corresponding part of the track.
- **V.Mean**
  Mean Velocity: Calculated as the distance travelled divided by the time the animal stayed in a given zone or association.
- **Dist**
  Distance: Total distance (cm) travelled into the corresponding zone or association during the interval.
- **Dist (%)**
  Distance (%): Distance travelled in the zone expressed as a percentage over the total zones or associations.
- **P. Time**
  Permanence Time: Total time (sec) spent in the zone or association, no matter the number of entrances.
- **PT (%)**
  Permanence Time (%): Permanence time expressed as a percentage of the total.
- **Res. T.**
  Resting Time: Time period (sec) during which the speed of the subject is below the resting threshold.
- **RT (%)**
  Resting Time (%): Resting time expressed as a percentage of the total duration.
- **Mov. S.**
  Time moving slow: Time period (sec) during which the subject speed is above the resting threshold and below the fast threshold.
- **MS (%)**
  Moving Slow (%): Moving slow time expressed as a percentage of the total duration.
- **Mov. F**
  Time moving fast: Time period (sec) during which the speed of the subject is above the fast threshold.

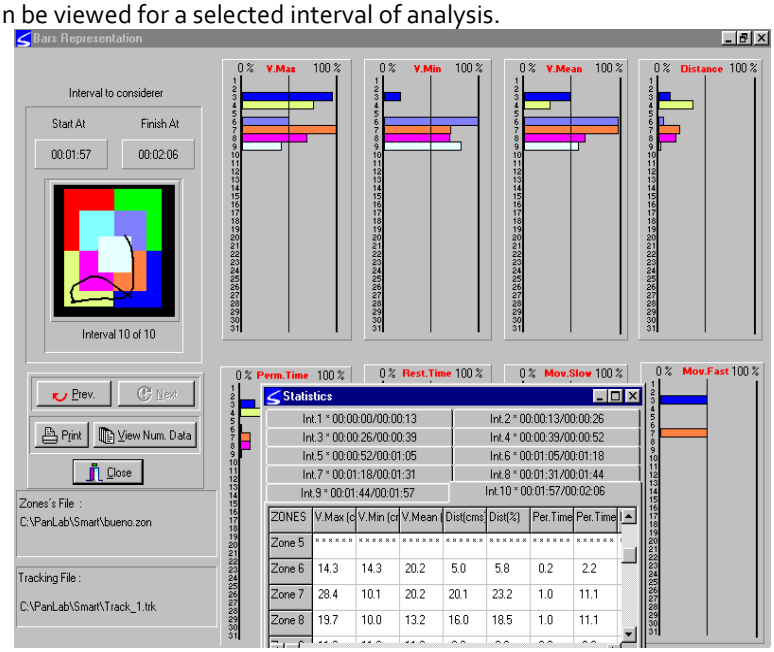
- **MF (%)**
  Moving Fast (%): moving fast time expressed as a percentage of the total duration.
- **N. Rea**
  Number of rearing episodes.
- **M. Rea**
  Mean duration of rearing episodes (sec).
- **N.I. Rea**
  Number of rearing episodes initiated in the corresponding zone or association and time interval.
- **M.I. Rea**
  Mean duration of the rearing episodes initiated in the corresponding or association and time interval.
- **Lat. T**
  Latency Time: Time period (sec) before the first entrance into a zone or association.
- **N. Ent**
  Number of entrances: times the subject enters into a zone or association.
- **B/E**
  Begin / End: zone or association in which the subject begins and ends the track.

All distances are expressed in cm; time is expressed in sec; speed is computed as cm/sec. No data is represented as "*****".
The Zone and Association tables are completed by a "TOTAL" and "All" rows respectively. The values in the raw "TOTAL" consider all the zones for the calculations of the analyzed parameters. The values in the raw "All" consider the whole experimental area as a unique zone for the calculation of the parameters.
Note: A few comments should be made about the part of the table dedicated to associations. Of all the values described, only "Number of Entries" deserves special mention.
If the zones belonging to an association are un-joined (they do not touch each other), or if it enters zones belonging to another association, the resulting value will be the sum of the entries into each one of the zones. However, if the animal exits a given zone and goes into another one that belongs to the same association, ACTITRACK, as is logical, supposes that there has been no change of Association and will not increase the number of entries. Therefore, not all cases will be a simple addition.
The other fields (V. Max, V. Min,...) have the same significance or meaning as the "TOTAL" row, which has already been dealt with, but logically only related to the zones belonging to a given Association.

When required by the user, the content of the table can be reset by using the "Reset" button of the "Control Tools" panel.

7.1.10.2. Displaying data with Bar´s Representation

By pressing the "View (Bar)" button, numeric data results as well as bar charts can be viewed for a selected interval of analysis.



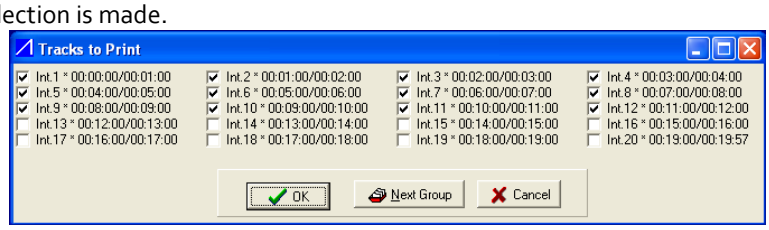
Note: This report appears empty until the "Start analysis" button is pressed (as described in previous sections of this Manual).

By pressing the "Prev." or "Next" buttons, it is possible to accede to the previous and next interval respectively, according to the order of the interval's data tabs in the numeric results window (see previous sections in this manual).

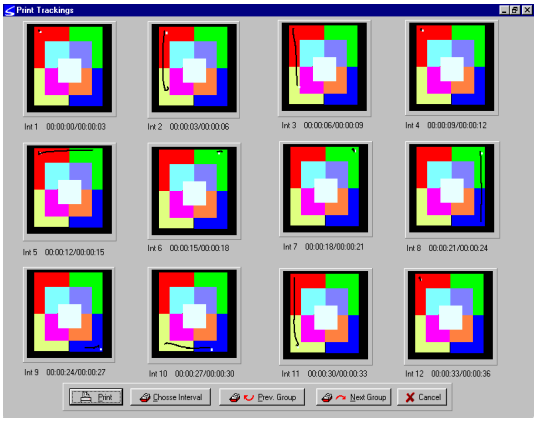
The user can visualize the numerical calculation of the analysis parameters by pressing the "View Num. Data" button. A new window appears with a table identical to those given by pressing the "View (data)" button in the Control's tool window.

7.1.10.3. Visualizing tracks by intervals

By pressing the "View Int." button, a "Tracks to print" panel appears. The user can select up to 12 intervals, corresponding to the tabs included in the "Numeric Results" window, to be shown and printed. Press OK once the selection is made.

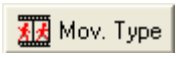


The image presented below shows the aspect of the "Print Tracking" window.

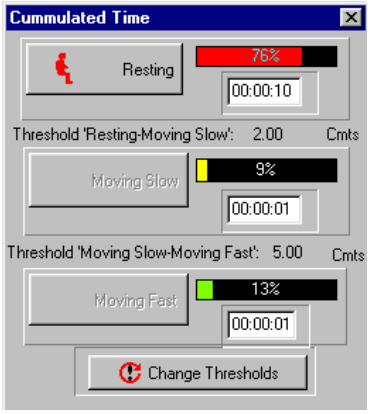


When the "Next intervals" button is pressed the next 12 intervals are shown (if there are any) and so on.
Similarly, the "Prev.Interval" button gives access to the previous 12.
"Choose intervals" button allows selecting the intervals to be shown on the same screen (with a maximum of 12).
Going back to the control tool, a button allows deleting all the time intervals analyzed (all the tabs from the corresponding window) for calculating statistics anew.

7.1.10.4. Movement pattern view



A view of the movement pattern can be obtained by pressing the "Mov. Type" button of the "Control Tools" box
Using the subject average speed (the last second samples), ACTITRACK can classify the speed according to two user-defined thresholds separating resting from slow and fast displacements.
If the current subject speed is below the "Resting/Moving slow" threshold, the movement is classified as "rest".



If it is between the "Resting/Moving slow" and the "Moving slow/Moving fast" thresholds, movement is classified as "moving slow". Otherwise, movement is classified as "moving fast". For a given interval (or for the full track), time spent moving fast, moving slow and resting is calculated and expressed both in time units (sec) and as a percentage of the total duration of the interval.

The speed thresholds can be modified either by moving up/down the cursors of the speed bar or by changing the numeric value of the fields with the associated up and down arrows. The "Max. Speed" field allows the selection

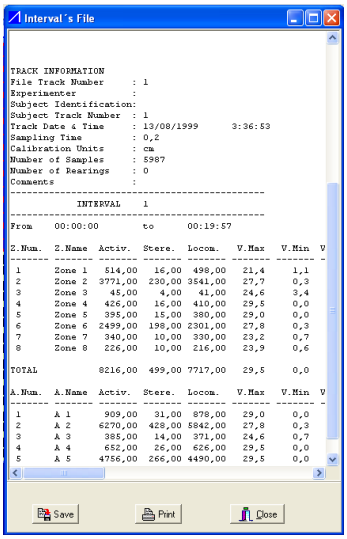
of the expected maximum speed, to allow permit an adequate precision of the threshold values.
Note: It is important to note that the threshold settings affect the numeric results of the "View (data)" table (the columns referred to Resting, Slow & Fast movement, Distance, and Velocities). This implies that the threshold settings must be carefully decided and once a proper value is reached, numeric statistics re-generated accordingly.

7.1.11. Creation of data files

Once the data tables have been obtained, the researcher might wish to print or save them into a file for further revision or analysis.

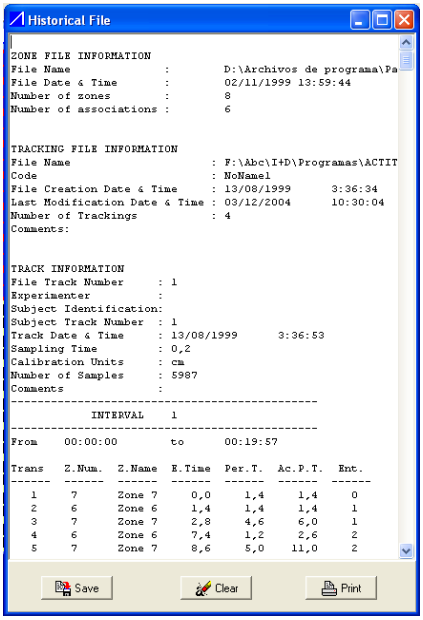
The operation involving the transfer of the statistical data to a file is carried out by the "Make File" button. Once pressed, the button generates a panel with two options: "Interval´s File" and "Historical File".

- Interval´s File
  This option transfers the data in the numerical table directly into a data file with *.txt format. It can be saved or printed. A header is inserted at the beginning of the file with information about its creation, the zone definition used, the track analyzed and the analysis parameters configuration (speed, rearing filter...).



- Historical File

  The historical file report is a chronological list of all the zones changes made by the animal. It also inserts a general info header about the track and the zone definition at the beginning.



The following parameters are contained in the historical file:
Trans
Transition: Each time the subject goes from a zone to another. Every zone change is considered as a transition and is associated to a number. Therefore, this field reports the number of zone changes.

- **Z. Num**

  Zone Number: Number of the zone into which the subjects entered.
- **Z. Name**

  Zone name: Name of the zone into which the subject entered.
- **E. Time**

  Entrance time: Time period (sec) elapsed from the beginning of the track until the zone transition that occurred.
- **Per. Time**

  Permanence time: Duration (sec) of this before the next transition (time spent during this visit).
- **Ac.P.T.**

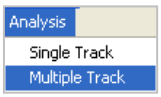
  Accumulated permanence time: Total duration (sec) of all the visits made in the corresponding zone (total time spent in this zone from the beginning of the track).
- **Ent.**

  Number of entries: Number of times the subject entered into the corresponding zone. It should be remembered that the beginning of the track in a determined zone is not considered as an entry. Thus, if the user only defined one zone in the experimental area, the number of entries will be zero.

Note: The data txt files can be opened directly by the Excel application for facilitating further analysis.

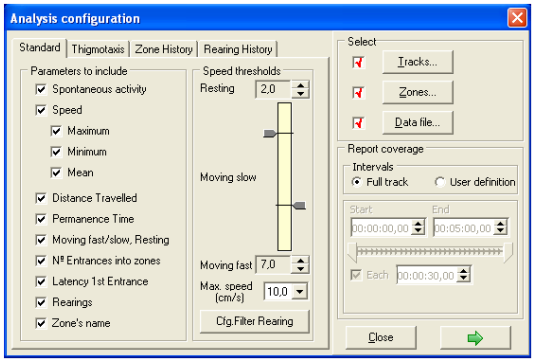
7.2. Multiple-track analysis

This option allows for the carrying out of the analysis of as many files as wanted in a single stroke.
Multiple-track analysis is designed to manage large amounts of data. Thus, it does not provide visual utilities as in single-track analysis. Its strength is the capability of analyzing multiple tracks jointly. With multiple-track analysis, it is also possible to export some standard analysis data directly to Excel file for further analysis.
This analysis is fully controlled by the window shown below that it is acceded by selecting the "Analysis/Multiple-track" option in the main menu.



7.2.1. The analysis configuration panel

The analysis configuration panel is divided into two parts:
On the left, four tags allow the selection of the reports to be included in the analysis report modes.
On the right, the section "Select" allows the choice of the tracks to be analyzed, the zone definition and the file in which the data will be saved. The section "Report coverage" allows the setting of the track analysis time interval (start, end and repeated time interval).



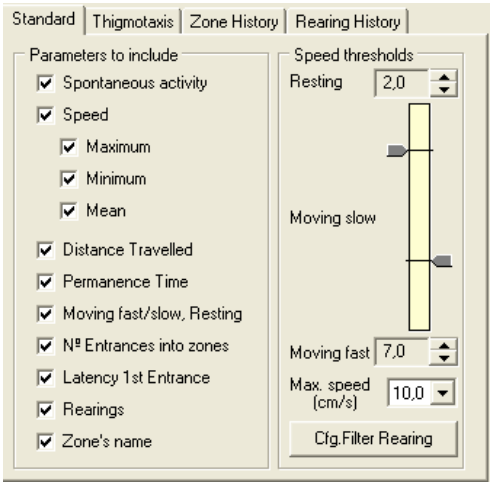
7.2.2.Select data to be reported

Four reports are available: The Standard and Zone History analysis reports give data available in the single-track analysis. The Thigmotaxic provides a specific analysis mode in which the track is analyzed in terms of permanence into sidewalls corridors (which is an index of anxiety) and left/right side walking into them. The "Rearing history" report gives a chronological list of the rearing.

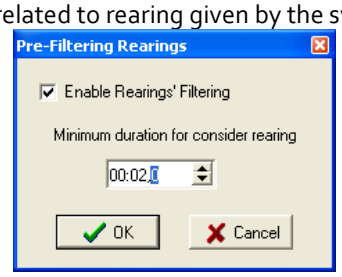
7.2.2.1. Standard Analysis report configuration

This report allows for the selection of the calculations that the user wants to report. The calculations provided in this table are exactly the same than the ones provided in the numeric table of the single-track analysis (see details about these calculations in the "Single track analysis" chapter).
The advantage of the multiple track analysis is the possibility to select the parameters that will be included in the reports, by checking them or no.

The speed thresholds settings can be adjusted for classifying the displacements of the subjects according to its speed (resting, slow or fast) (see previous related sections).



As in the single subject tracking analysis, the duration of rearing episodes can be filtered by the user. In the "Pre-filtering rearing" window available by pressing the "Cfg.Filter Rearing" button, a duration filter can be activated by checking the option "Enable Rearing's Filtering". Then, the user has to enter the minimal duration for considering a rearing. In other terms, when the rearing filter is activated, the rearing episodes with duration strictly inferior to the value entered by the user will not be considered as a rearing episode for all the calculation related to rearing given by the system.



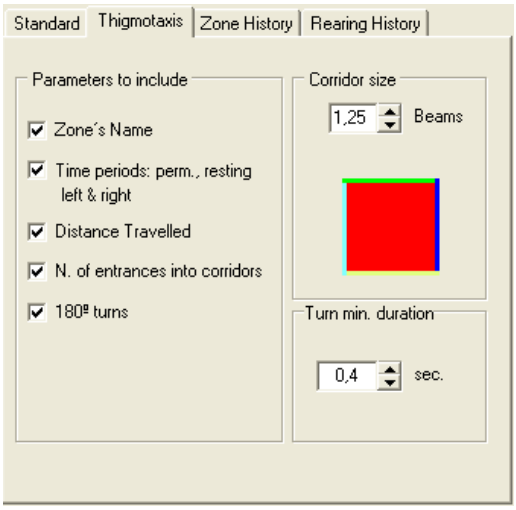
7.2.2.2. Thigmotaxis report configuration

This analysis mode is available exclusively from the multiple tracks analysis module.

Thigmotaxis behaviour is defined as the tendency of rodents to spend more time circling around the peripheral sector of the experimental area and is commonly considered as an index of anxiety.

Even that most of the information provided by the "Thigmotaxis" could be also derived from the Standard analysis, this section has been implemented in order to give the user a higher degree of convenience in doing a much more specific analytical approach.

Most of this analysis has to do with the way in which the experimental subjects interact with the walls. In order to implement this, the user can define "corridors". A corridor is an aisle with one of his sides contiguous to a wall. In this way, there are four corridors and a centre. The corridors are situated North, South, East and West respect to the centre (see next image).



The minimum corridor size is 1,25. This means that when evaluating if the experimental subject is or not within the corridor, only one co-ordinate is possible (x or y = 1,0): the subject is breaking the most external IR beam. If the corridor's size is increased up to 1,75 (the next available step), a second possible co-ordinate is added: the subject is breaking the two most external IR beams (and thus the co-ordinate is x or y = 1,5). When the subject is breaking only the second IR beam, the x or y co-ordinate will be 2,0 and will be evaluated as being out the corridor.

The second parameter evaluated is the direction of movement, as derived from the slope trend obtained after calculating the equation of the line joining successive co-ordinates. When the slope is "around" 0º, the subject is moving south to north; if the slope is about 180º, moves from north to south. A slope close to 90º should indicate displacement from west to east; finally, when the slope is about 270º, the subject is moving east to west.

The direction of movement, as described above is used to decide if the subject moves "clockwise" or "anticlockwise". The subject is moving clockwise when the slope is around 0º and the co-ordinates are close to the west corridor. The same occurs when the slope is about 90º and the position is close to the north corridor, or close to the east corridor and 180º as slope and for the south corridor and 270º as slope.

The anticlockwise movement is derived from the opposite: the slope is 180º and the co-ordinates close to the west, slope 270º and north corridor, slope 0º and east corridor and slope 90º and the south corridor.

If the subject moves clockwise and it is moving within the corridor, the "Left time" and "Left distance" will be increased accordingly. The opposite will be done with the "Right time" and Right distance" if the subject moves anticlockwise and it is also moving within the corridor. When the subject is not in the corridor, the direction is irrelevant to the analysis.

This approach implies that the experimental subject does not move in a 45º-angle respect the proximal wall. When the subject moves from the location with the coordinates (1, 8) to (2, 8), the slope is 90º, but the displacement is perpendicular to the closest wall and the decision "moves clockwise or anticlockwise" is not possible.

A third parameter included in this analysis is the "turns" detection. From the evaluation of the direction of movement it is possible to decide when a 180º-turn have been produced. At this respect, it is important to clarify that a 180º-turn cannot be evaluated only based on the evaluation of changes in the direction of movement. In fact, a subject walking along the walls of the IR monitor gives a 180º-turn when complete a have-circle after being in touch

with three of the walls (the turn thus having a about 8" radium). This is why in order to allow for the separation between a real 180°-turn, produced with small turn radium, a time criterion is implemented.
A 180°-turn must be completed within a maximum time, whose value is also included into the analysis parameters. The default value is 1.0 sec, meaning that the full 180°-turn must be completed in no more than 1 sec. Once the threshold time is over, the angle of turns is zeroed again.
If the turn radium is different from zero, the turn direction can be evaluated (the subject turns from right to left or left to right). This is not possible when the turn radium is zero, as it is if the coordinates are like (x_1, y_1) -> (x_2, y_2) -> (x_1, y_1).
In the table below, a sample of the results of this analysis can be found. The table contents are self-explanatory, and data is derived from the "sample.tac" file (included in ACTITRACK as a sample), using 1,25 as corridor size.

Zone		Permanence Time		Resting Time		Distance	
Num.	Name	(sec)	(%)	(sec)	(%)	(cm)	(%)
2	North	13,4	11,3	0,8	6	106,6	8,3
3	East	6	5,1	1	16,7	81,1	6,3
4	South	***	***	***	***	***	***
5	West	11,2	9,5	0,6	5,4	112,7	8,8
1	Central	87,8	74,2	10	11,4	986	76,6
Total		118,4	100	12,4	10,5	1286,3	100

Zone		Left Time		Left Distance		Right Time	
Num.	Name	(sec)	(%)	(cm)	(%)	(sec)	(%)
2	North	1,6	11,9	20,4	19,2	4,8	35,8
3	East	1,2	20	42,2	52,1	1,6	26,7
4	South	***	***	***	***	***	***
5	West	2	17,9	35,1	31,2	4,4	39,3
1	Central	0	0	0	0	0	0
Total		4,8	4,1	97,8	7,6	10,8	9,1

Zone		Right Distance		Centre Crosses		180° Turns		
Num.	Name	(cm)	(%)	to	from	Total	L->R	R->L
2	North	72,2	67,7	15	15	5	4	1
3	East	31,9	39,3	9	9	3	2	1
4	South	***	***	***	***	***	***	***
5	West	69,6	61,8	16	16	4	2	2
1	Central	0	0	40	40	53	21	25
Total		173,7	13,5	80	80	65	29	29

Definition of the available parameters to include in the report:

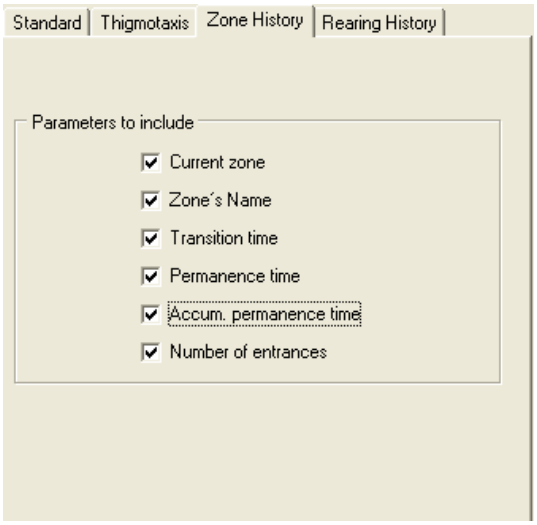
- **Zone's name:**
  information about the zone configuration (number of the corridor, name of the zones)
- **Time periods:**
  information about time spent into the zones, time spent in resting state and time spent in clockwise and counterclockwise trajectories.

- **Distance travelled:**
  information about distance covered into the zones, distance covered in clockwise and counterclockwise trajectories.
- **N. of entrances into corridors:**
  information about number of crosses between the centre and the corridors
- **180º turns:**
  number of 180º turns

To generate the analysis, a track (or a set of tracks) must be selected in the "select" area of the "Analysis configuration panel".
Note: it is not necessary to select a zone file, as the analysis uses as zone definition those coming from the "corridor size" settings.

7.2.2.3. Zone History report configuration

This analysis modality allows generating information about the location of the subject into the zones during the experiment. Basically, this report is a chronological list of all the zone changes made by the animal.
This information is the same than those obtained from the one available in the single-track analysis (see Single Subject Tracking chapter for further details).
In the "Zone History" tag, the user can select the parameters to be included in the analysis report by clicking in the checking box beside each of them.



Definition of the available parameters to include in the report:
- **Current Zone:**

number of the current zone
- **Zone's Name:**

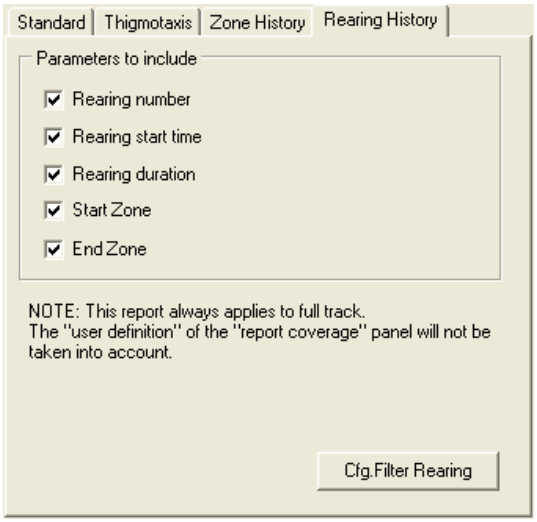
name of the current zone
- **Transition time:**

Time in seconds in which the subject enters into the zone
- **Permanence time:**

time spent into the zone
- **Accum. permanence time:**
  total duration of time spent into the zone since the beginning of the analyzed interval
- **Number of entrances:**
  Number of entries into the zone

7.2.2.4. Rearing History report configuration

This analysis modality allows the generation of a historic report about all the rearing occurring during the experiment.
In the "Rearing History" tag, the user can select the different parameters to be included in the report by clicking on the corresponding checking boxes.



The user can configure the filter of rearing. This filter allows setting a minimal duration for considering a rearing episode. To do so, press the *Cfg. Filter Rearing* button. In the pre-filtering rearing window that appears, click on the checking box for activating/inactivating the filter and, if activated, enter a minimal duration for which the episodes will be taken into account for the calculation of the rearing-related parameters to be analyzed.
Note: the rearing historic report is analyzed independently of the time analysis setting defined by the user. It always applies to the FULL TRACK duration, without taking into account intervals of analysis.

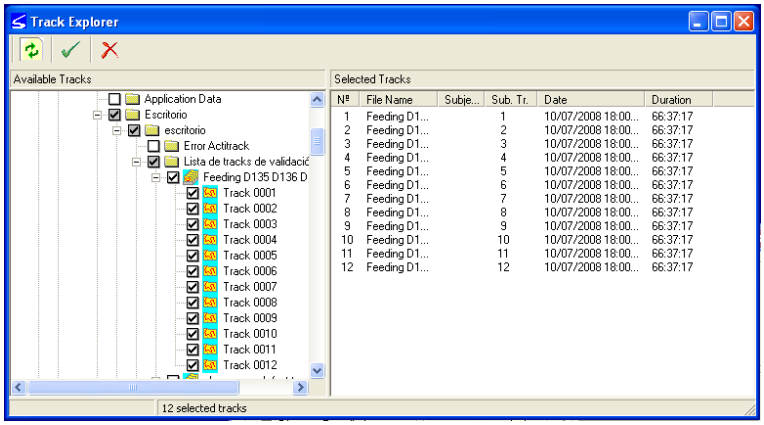
Definition of the available parameters to include in the report:

- **Rearing number:**
  Number of detected Rearing
- **Rearing start time:**
  Time in which the rearing episode started
- **Rearing duration:**
  Rearing episode duration
- **Start Zone:**
  Zone into which the rearing started
- **End Zone:**
  Zone into which the rearing ended

7.2.3. Select track, zone and data files

7.2.3.1. Track selection

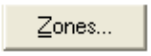
The tracks to be analyzed are selected by pressing the button "Tracks" in the "Select" panel.



In the left side of this window, a panel presents the full list of tracks available in the folders of the computer. By checking elements of the list, the corresponding element is selected or de-selected. Note that it is possible to select a complete folder, an entire file or a single track. Each time an element is selected, the corresponding track(s) is/are added to the right-side panel and in the status line at the bottom of the window appears the information about the number of tracks already selected.

The upper row buttons should be used for re-filling the tracks list, accepting the selection or cancelling it. Once tracks have been selected, the checkbox besides the "Tracks" button appears as selected.

7.2.3.2. Zone selection



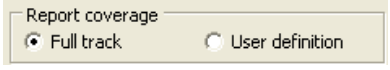
By pressing now, the "Zones" button, a standard file-selection window is open allowing the selection of a common zone file to be used for analysis with all the selected tracks. Once the zone definition has been selected, the checkbox besides the "Zones" button is checked.

7.2.3.3. Data file selection

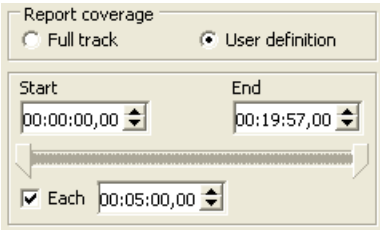
The last step to be completed is to create a new *.txt file in which the data will be save or to open an existing file (appending or overwriting existing data). Once the destination file has been selected, the checkbox besides the "Data file" button appears as selected.

7.2.4. Report Coverage

For analyzing tracks, the user has to define a time interval that can be the entire track duration or a defined interval.
For an analysis of all tracks duration, the user has to select the option "Full Track".



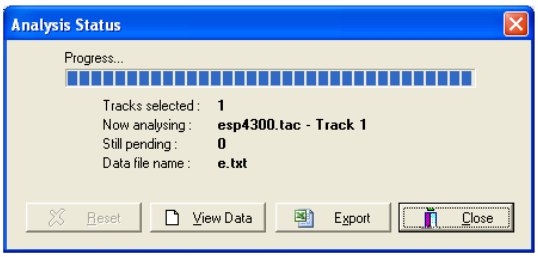
For a track analysis of an arbitrary time interval, the user has to select the option "User Definition". Then, the beginning and the end of the track time interval can be defined by entering the values in the corresponding "Start" and "End" cells or by displacing the cursors.



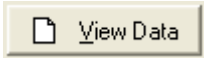
Moreover, the user can choose to subdivide the analysis in various time intervals. To do so, the option "Each" has to be selected and the repeated time interval to be defined for the track analysis. This time interval duration has to be inferior to the track analysis duration defined by the "Start" and the "End". When various tracks have been selected for the analysis, the value in the "End" field corresponds to the higher duration between the tracks.

7.2.5. Start Analysis Report generation

Once the parameters to be included in the report have been set, press the button containing the green arrow for generating the analysis.
An "Analysis Status" window appears, with an evolution bar showing the progress of the calculation process.



7.2.6. Data visualization and saving in *txt.format



When the process has finished, the report can be visualized in .txt format, by pressing the button "View Data".

7.2.6.1. The Txt Data Window

The Txt "Data Window" is divided two different sections:
Header: a header is inserted at the beginning of the file with information about its creation, the zone definition used, the track analyzed and the analysis parameters configuration (speed, rearing filter…).
Reports: The reports are then organized by starting with the Standard Analysis, then the Zone History, the Thigmotaxis, and the Rearing History.

The "open text file" button allows opening a different text file for visualization and further saving

The "Save in file" button allows saving the txt file with a different name

The "print" button allows printing the content of the file

The "Copy to clipboard" button allows saving the content of file into the clipboard

The "Exit" button closes the "Data Window" panel and leads to the "Analysis Status" panel.

7.2.6.2. Standard report description

List and definition of the parameters displayed in the standard report table:

- **Zone Num:**
  Number of the zone/association.
- **Zone**
  Name: Name of the zone/association.
- **Activ.**
  Global activity during the analyzed interval, number of samples where the position of the subject is different from the position of the previous sample (sum of Stereotypes and Locomotion activity).
- **Stere**
  Stereotyped movements during the analyzed interval, number of samples where the position of the subject is different from the position the previous sample and equal to the position of the 2nd sample back in time.
- **Locom.**
  Locomotion during the analyzed interval, number of samples where the position of the subject is different from the position of the previous sample and different of the position of the 2nd sample back in time.
- **V.Max:**
  Maximum Velocity/speed (cm/s) reached by the experimental subject. If its value is zero, it means that the animal has not moved throughout the whole time interval. In such case, both the minimum and mean speed will also be zero.
- **V.Min**
  Minimum Velocity/speed (cm/s) detected in the corresponding part of the track.
- **V.Mean**
  Mean velocity/speed calculated as the distance travelled divided by the time the animal stayed in a given zone or association.
- **Dist**
  Total distance (cm) travelled into the corresponding zone or association during the interval.
- **D(%)**
  Distance travelled in the zone expressed as a percentage over the total zones or associations.
- **P. Time**
  Total time (sec) spent in the zone or association, no matter the number of entrances.
- **PT(%)**
  Permanence time in the zone or association expressed as a percentage of the total.
- **Res.T.**
  Time period (sec) during which the speed of the subject is below the resting threshold.

- **RT(%)**
  Resting time expressed as a percentage of the total duration.
- **Mov.S.**
  Time period (sec) during which the subject speed is above the resting threshold and below the fast threshold.
- **MS(%):**
  Moving slow time expressed as a percentage of the total duration.
- **Mov.F.:**
  Time period (sec) during which the speed of the subject is above the fast threshold.
- **MF(%):**
  Moving fast time expressed as a percentage of the total duration.
- **N.Ent.:**
  Number of entries into the corresponding zone/association.
- **B/E.:**
  name of the zone or association in which the subject begins and ends the track.
- **Lat. T.:**
  time in seconds before the first entry into the corresponding zone/association.
- **N. Rea:**
  Number of rearing episodes detected in each zone/association taken independently.
- **M. Rea:**
  Mean duration of rearing episodes (sec) detected in each zone/association taken independently.
- **N.I. Rea:**
  Number of rearing episodes initiated in each zone or association and time interval.
- **M.I. Rea:**
  Mean duration of the rearing episodes initiated in each zone or association and time interval.

7.2.6.3. Thigmotaxis report description

List and definition of the parameters displayed in the Thigmotaxis report table:

- **Zone Num:**
  Number of the corridor.
- **Zone Name**
  Name of the corridor.
- **Permanence Time sec**
  Time spent in the corresponding corridor, in seconds.
- **Permanence Time %**
  Percentage of time spent in the corresponding corridor.
- **Resting time sec**
  Time spent in resting in the corresponding corridor, in seconds.
- **Resting time %**
  Percentage of time spent resting in the corresponding corridor.
- **Left Time sec**
  Time during which the animal moves clockwise and within the corresponding corridor.
- **Left Time %**
  Percentage of time during which the animal moves clockwise and within the corresponding corridor.

- **Left Distance cm:**
  distance in cm traveled within the corresponding corridor when the subject moves clockwise.
- **Left Distance %**
  Percentage of distance traveled within the corresponding corridor when the subject moves clockwise.
- **Right Time**
  sec: Time in seconds during which the animal moves anti-clockwise and within the corresponding corridor.
- **Right Time %**
  Percentage of time during which the animal moves anti-clockwise and within the corresponding corridor.
- **Right Distance cm**
  Distance in cm traveled within the corresponding corridor when the subject moves anti-clockwise.
- **Right Distance %**
  Percentage of distance traveled within the corresponding corridor when the subject moves anti-clockwise.
- **Distance cm:**
  Distance traveled in cm within the corresponding corridor.
- **Distance %**
  Percentage of the distance in cm traveled within the corresponding corridor.
- **Centre crosses to**
  Number of transitions from the center to the corresponding corridor.
- **Centre crosses from**
  Number of transitions from the corresponding corridor to the center.
- **180º turns Total**
  Total number of the 180º turns within the corresponding corridor.
- **180º turns L → R**
  Number of 180º clockwise turns.
- **180º turns R → L**
  Number of 180ºanti-clockwise turns.

7.2.6.4. Zone History report description

List and definition of the parameters displayed in the Zone History report table:

- **Trans**
  Number of the zone entrance.
- **Z. Num.**
  Number of the zone.
- **Z. Name**
  Name of the zone.
- **E. Time**
  Time in seconds when the subject enters the zone.
- **Per. T.**
  Time in seconds spent in the zone during the corresponding stay.
- **Ac. P. T.**
  Accumulated time in seconds spent in the corresponding zone since the beginning of the analyzed interval.
- **Ent.**
  Number of entries in the corresponding zone.

7.2.6.5. Rearing History report description

List and definition of the parameters displayed in the Rearing report table:

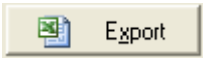
- **N. Rear**
  Number of rearing.
- **S. Time**
  Time of the rearing episode starting.
- **Durat.**
  Rearing episode duration.
- **Z. Start**
  Zone into which the rearing started.
- **Z. End**
  Zone into which the rearing ended.

7.2.6.6. Data saving

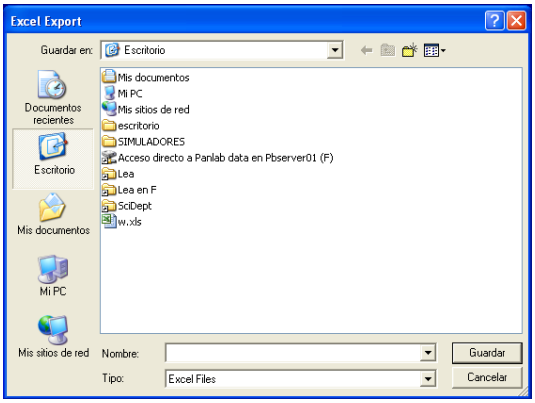
The report can be saved in text file format, by pressing the corresponding button on the upper part of the window.
Be aware that the analysis given for the Thigmotaxis, and the Zone History are not exported directly in Excel and so, can only be saved first in text file format report.

All the data txt files can be opened afterward directly by using the Excel application.

7.2.7. Data direct exportation to Excel



ONLY the Standard and Rearing History reports can be directly exported in Excel format.
To do so, go to the Analysis Status panel (accessible by closing the Data Window if it has been opened), press the "Export" button and name the Excel file. If none of the parameter of Standard or Rearing History modalities has been selected, the "Export" button remains inactivated.



The data columns of the two reports will be preceded by 9 new columns containing the following information:

- **File name**
  Explorer address of the track file analyzed.
- **Track No**
  Number of the track in the file
- **Subject**
  Date and clock time of session registration.

- **Duration**
  Total duration of the session.
- **Session Date**
  Date of session registration.
- **Start Time**
  Analysis window Start time.
- **End Time**
  Analysis window End time.
- **Int. Start**
  New Start time when setting the analysis window start time to zero.
- **Int. End**
  New End time when setting the analysis window start time to zero.

8. GLP fulfillment facilities (optional)

ACTITRACK allows an optional module called GLP which enables several functionalities targeted to facilitate laboratories to fulfil some basic Good Laboratory Practices (GLP) requirements.
To be precise, ACTITRACK GLP module enables the following characteristics:

- User access control
- Audit trail
- Data integrity

Whether you require any of these functions, please do not hesitate to contact your dealer in order to extend your current ACTITRACK installation.
The rest of this section is only applicable to those ACTITRACK sites with GLP module active.

8.1. User access control

User access control is a tool which restricts the usage of ACTITRACK application to those users that had been registered previously.

8.1.1. User login

The first time ACTITRACK starts, a login form is shown requesting user name and its personal password:

- As no user is registered, the first usage of ACTITRACK GLP module only allows login by mean of **Administrator** user name. Its password is **administrator01**. It is very important that this password is changed just after the first login operation.
- Please note that passwords are case-sensitive, this means, capital and lower-case letters are considered as different letters when checking the validity of the password.
- Due to security reasons, a user will be automatically blocked when it has accumulated up to three failed login trials. Please refer to section 9.1.5 in this chapter for enabling a blocked user.

When the login process is successful, the user name is shown in the status bar of ACTITRACK application, located in the lower border of the window.

8.1.2. Users capabilities

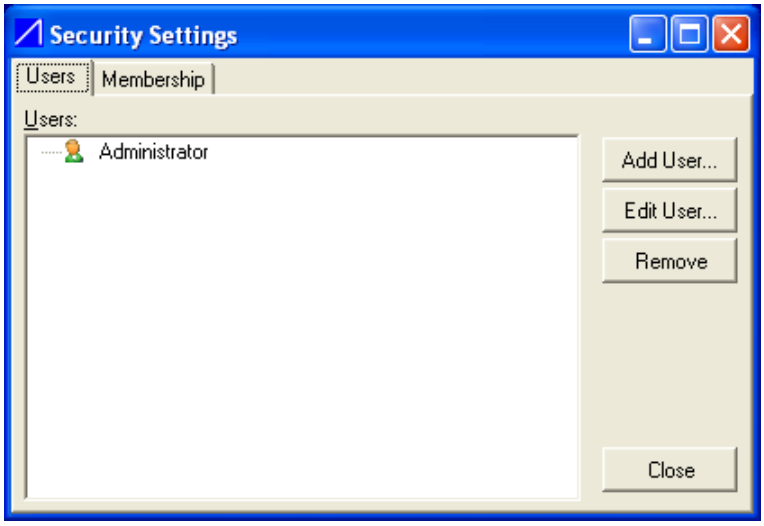
Users are categorized in two different groups with different capabilities:

- Administrators
  Users in this group can manage the users list by adding, changing and enabling / disabling other users. Administrators may delete entries in the audit trail for maintenance purposes. Administrators cannot create nor open experiment files.
- Experimenters
  Users in this group are the only ones enabled to manage experiment files (creation, opening, saving, starting data acquisition, analysis, etc.). Experimenters cannot manage users list.

Administrator user is created automatically by the ACTITRACK application and assigned to the group **Administrators**.

8.1.3. Creating experimenter users

Once logged in with **Administrator** user (or other user of the <Administrators> group), a new **Configuration – Users - Users Management** menu option is enabled in the main window of **ACTITRACK** application.



This **Security Settings** dialog allows Administrator to:

- View the list of allowed users
- Create a new user
- Assign a user to a membership
- Change user properties (such as its password)
- Enable and disable users

To add a new User:

- Press Add User... button
- Enter the user name (this is the name or nick used to log in to application)
- Enter the full name of this user (the real name)
- Request the user to enter its personal password (it must be entered twice to check that it is correct)

Password length must be at least of 8 characters, and it must not match to the user name.

- Be sure that Enabled checkbox is checked to allow this user to access the application.
- Press Ok button.

8.1.4. Changing user membership

All new users are automatically associated to Experimenters membership, but an Administrator user can change it by mean of the Security Settings dialog.
To do that:

- Enter Security Settings dialog through the Configuration – Users - Users Management menu option.
- Select **Membership** tab in the dialog.
- Select the user from the **Groups** list and press  button to remove from its current group.
- Select the user from the **Users** list and the new group from the **Groups** list.
- Press  button to assign the user to the new group.
- Press **Close** button to finish the process.

8.1.5. Changing user properties

User real name and password can be changed any time by an Administrator user.
To do that:

- Enter Security Settings dialog through the Configuration – Users - Users Management menu option.
- Select the user from the **Users** list and press **Edit User...** button.
- Change the properties (real name and/or password).
- Check or uncheck the **Enable** checkbox to enable or disable user access rights. This checkbox is needed to re-enable access to a blocked user.
- Press **Ok** button to finish the process.

Due to security reasons, the new password must be different from the previous 24 passwords used by that user.
Due to security reasons, passwords expire 60 days before the user creation or password modification. Use this process to set the password whenever it occurs.

8.1.6. Disabling a user

A User can be permanently or temporally disabled. To do that:

- Enter Security Settings dialog through the Configuration – Users - Users Management menu option.
- Select the user from the **Users** list and press **Remove** button.
- Confirm the disabling of the user.

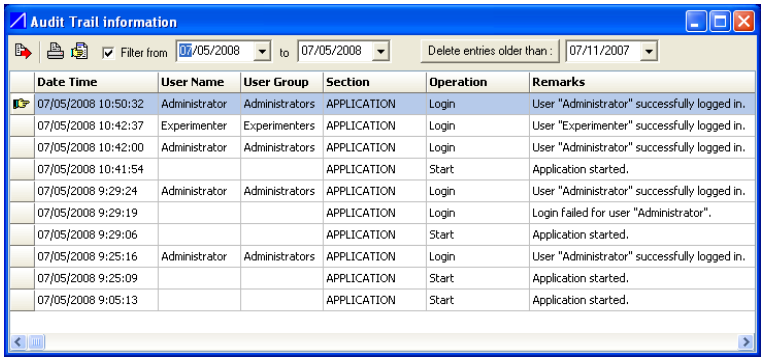
To recover a disabled User, select the user from the **Users** list and press **Edit User...** button, check the **Enable** checkbox and press **Ok** button to finish the process.

8.2. Audit trail

ACTITRACK GLP module is designed to automatically record the most important actions carried out by the User during the execution of the experiment, facilitating the traceability of the obtained results.
Actions that are registered into the audit trail are:

- Application starting and termination.
- Track creation and openings.
- Logged user changes.
- Configuration changes (Timings, Arenas, Serial Port and Boxes).
- Zone files opening and saving.
- Zone definition changes.
- Zone association changes.
- Zone name changes.
- Track files opening and saving.
- Track acquisition acceptance and rejection.
- Subject list file opening and saving.
- Trial list file opening and saving.
- Subject list changes.
- Trial list changes.
- Track header changes
- Data acquisition starting and stopping.
- Speed thresholds changes.

Audit trail can be analyzed by mean of the **View — Audit trail** menu option:



Each line of the audit trail report corresponds with an action carried out by the user shown in the **User** column, which was part of the group shown in the **Group** column. **Date/Time** column allows the sorting of the lines by clicking of its title. **Section, Operation** and **Remarks** columns contain the information related to the action.
The toolbar of the Audit Trail information dialog allows the following operations:

- Exportation of the report to a Microsoft® Excel® sheet.
- Previsualization of the printed audit trail report.

Obtain a printed version of the audit trail report.

These controls allow viewing a range of entries of the audit trail. If the check box is checked, only those entries which occurred between the configured dates will be shown.

These controls are only visible if the logged user is an Administrator. The button "Delete entries older than" will delete all entries that are older than the date selected at the right.

8.3. Data integrity

In order to avoid external undesired data modifications, ACTITRACK GLP module automatically protects the experiment files when they are closed by the User.

9. Contact Information

We are available to help you with your questions and concerns. Should you hit a roadblock or need some additional training, please feel free to visit the HBIO Behavioral Support Center at https://support.behavior.hbiosci.com to find articles and helpful information in our knowledge base or submit a ticket. We are happy to help!

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