Tracker - Manual

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The purpose of this documentation is to give you a review of the Tracker software used to run and analyze behavior experiments.

Every experiment done by the Tracker has three parts:

- **Setup**
- **Running the experiment**
- **Analysis**

1 Setup

In the setup, you set all the experiment properties such as the experiment paradigm, duration of the experiment, targets, size and type of the experimental arena, type of the subject you are going to track, control of external devices, application appearance etc.

After the configuration is set, it can be saved on your hard drive so you can load it every time before running an experiment without the need of creating the configuration from scratch every time you want to run a specific experiment.

To create new configuration, click **File – New** in the main application menu.
To open existing configuration, click **File – Open**.
To save the active configuration or save the configuration into a different file click **File – Save** or **File – Save as**.

Let's go through the main parts of the experiment setup. Click **File – Edit – Options**.

The options window has 6 tabs:

- **Video**
- **Experiment**
- **Tracking**
- **Devices**
- **File Output**
- **Window**

1.1 Options - Video

In the video options we setup the video source of the tracking system and its properties.

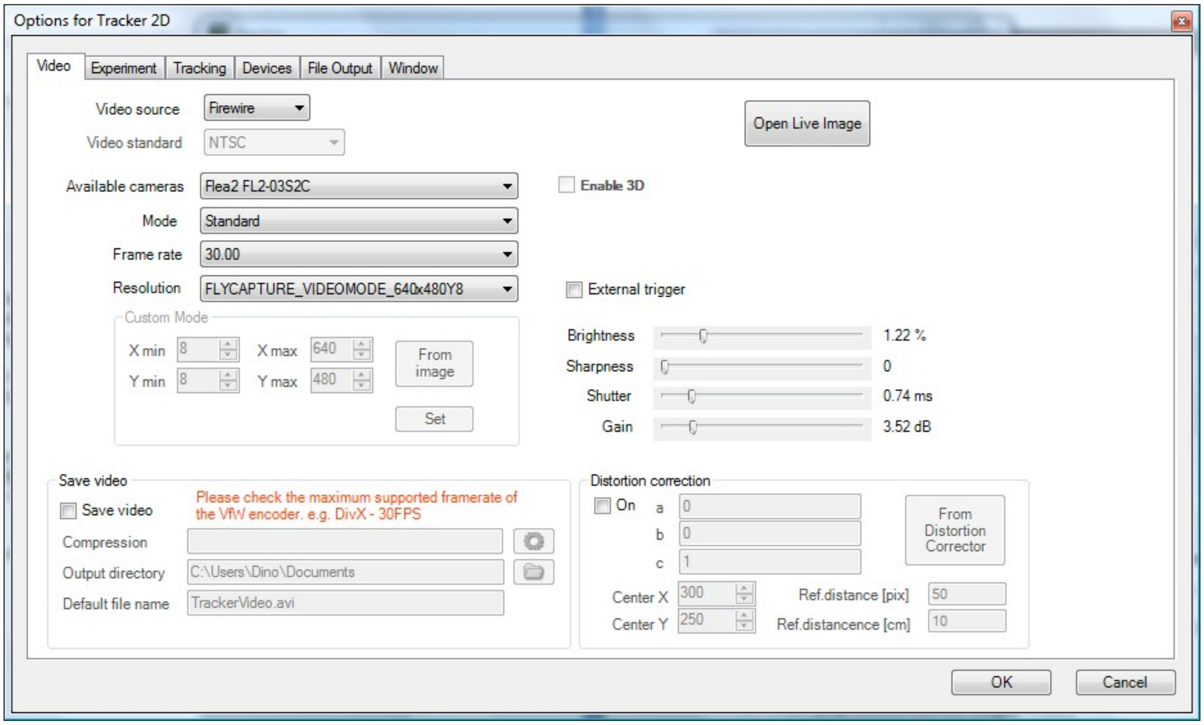


Figure: Options-Video

The **Video source** property sets the video input of the Tracker. Generally there can be three inputs:

- AVI file (640x480 / 30FPS)
- DT3155 Framegrabber (analog CCTV video)
- Pointgrey firewire camera (digital video)

Video standard applies only for the analog video (DT3155) and sets the TV standard of the video signal. There are two video standards used in the world:

- **PAL** – this video standard has the frame size of **768x576** pixels and the frame rate (tracking speed) of **25** frames per second. This standard is used in most European countries, China, Hong Kong, India, Israel, South Africa, etc.
- **NTSC** – this video standard has the frame size of **640x480** pixels and the frame rate of **30** frames per second. This standard is used in countries like Canada, Japan, Mexico, Taiwan, USA etc.

The following options are related only to firewire cameras (Pointgrey Flea2, Grasshopper, etc.).

Available cameras lists the cameras present in the system. If your camera is not listed, check the camera power and connection and try to select the **Firewire** video source again.

Firewire camera has two modes of operation: Standard and Custom. **Standard mode** allows us to use the Standard Image Formats specified by the IEE1394 digital video specification. For example with the Flea2 camera, we can only use a single resolution of 640*480 pixels and frame rates of 1.875, 3.75, 7.5, 15, 30 or 60 FPS.

The **Frame rate** and the **Resolution** set the properties of the Standard mode.

Enable 3D switches the camera into 3D mode (if supported by the camera).

In the **Custom mode** we could specify a Region of Interest, which is a subset of the image used for the image processing (e.g. searching for the subject location). Since the camera doesn't transfer the whole image, it allows it to use the remaining bandwidth to send more images through the bus which results in a higher frame rates in the custom mode.

Custom mode properties (region of interest and the custom frame rate) are set in the Custom mode box.

Basic optical properties (iris, zoom and focus) can be adjusted directly on the camera lens. However, some of the image properties can be set also in the configuration:

- **Brightness** (luminance of the image)
- **Sharpness** (digital sharpening of the image)
- **Shutter** (shutter speed – the time period for which the camera shutter is exposing the image)
- **Gain** (digital image gain)

In order to check the video settings, we can start the **Live Image** application (click on the **Open Live Image** button). If the firewire camera is used, we can modify the video settings when the Live Image is running and thus see the immediate effect.

External trigger sets the camera to the trigger mode (as opposed to the standard free-running mode). In this mode the camera acquires frames based on TTL pulses provided on the PIN2 at the GPIO connector.

By default the camera provides **strobe** pulses at the PIN1 of the GPIO connector. This allows synchronizing acquired video frames with other streams, e.g. the electrophysiology data. After each strobe the camera starts the image integration, which is set by the Shutter time. After closing the shutter, image data are time-stamped and transferred to the computer through the firewire bus. For precise synchronizing results it is important to understand that the strobe corresponds to the beginning of the image integration (i.e. opening of the shutter) and the timestamp embedded in each frame corresponds to the end of the image integration (i.e. closing of the shutter). Since we know exactly the shutter time, we may synchronize the video stream to any other stream.

Save video allows the user to record the video of the experiment for further analysis. DivX codec is necessary for saving the AVI file (http://www.divx.com).

After pressing the button next to the Compression box, the Video Compression dialog will appear. If the DivX codec is present in the system, it should appear between available compressors as "DivX ® 6.8.4 Codec (2 Logical CPUs).

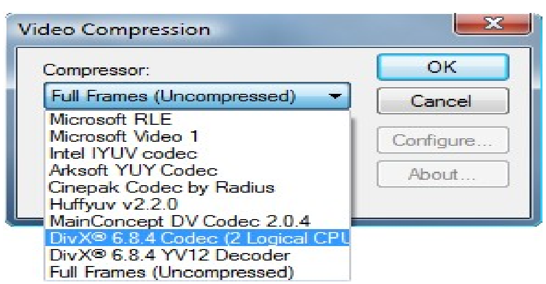
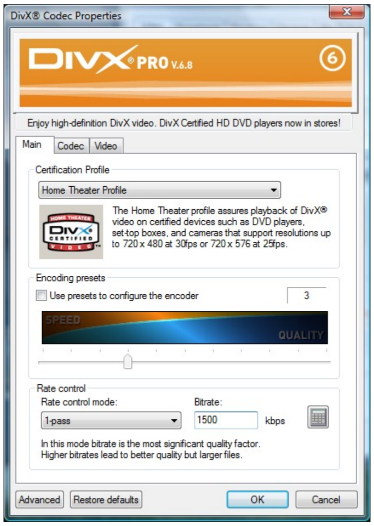
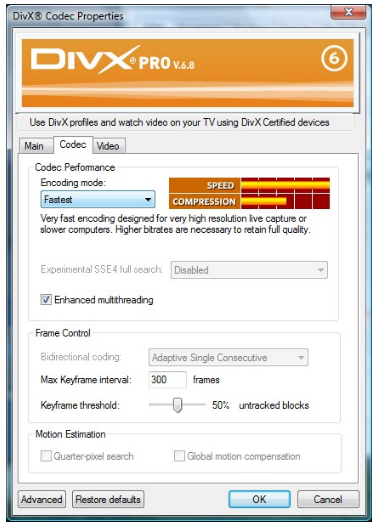


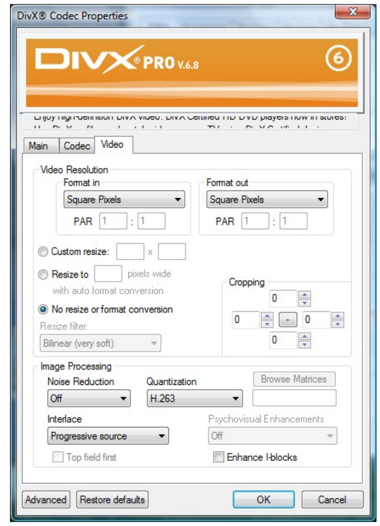
Figure: Video Compression

After selecting the DivX codec, click the Configure button, advanced dialog for the codec will appear.

Setting the codec properties requires knowledge about video compression mechanisms. Recommended settings are shown below.







Output directory sets the directory where output video files are stored.

Default file name sets the default name of the video file.

Note: *Since video files can grow very quickly even if they are compressed, video files are automatically partitioned after approximately 900MB. A description file is then saved together with the AVI file so it is clear which frame numbers are stored in which AVI file.*

1.1.1 Distortion correction

Every lens introduces a radial distortion of the image. The distortion is smaller when higher focal length of the lens is used (lens zoom) and it is higher when the focal length is decreased. In other words, the higher field of view the lens has, the higher the distortion. Lens with the highest field of view (fish-eye lens) have the highest distortion.

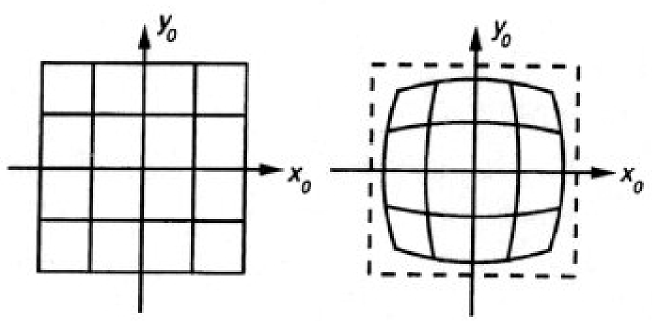


Figure: Radial distortion

If using a lens with low focal length is unavoidable (e.g. because of the low ceiling height), we can use the distortion correction algorithm to correct the distortion. Based on the settings we provide in the distortion correction wizard, the software rectifies the subject's position.

The distortion correction is optional, it has to be used only when the low focal length lens is used and/or high precision of the subject's position is desired.

To open the distortion correction wizard, enable it by checking the **On** box and clicking **From Distortion Corrector**.

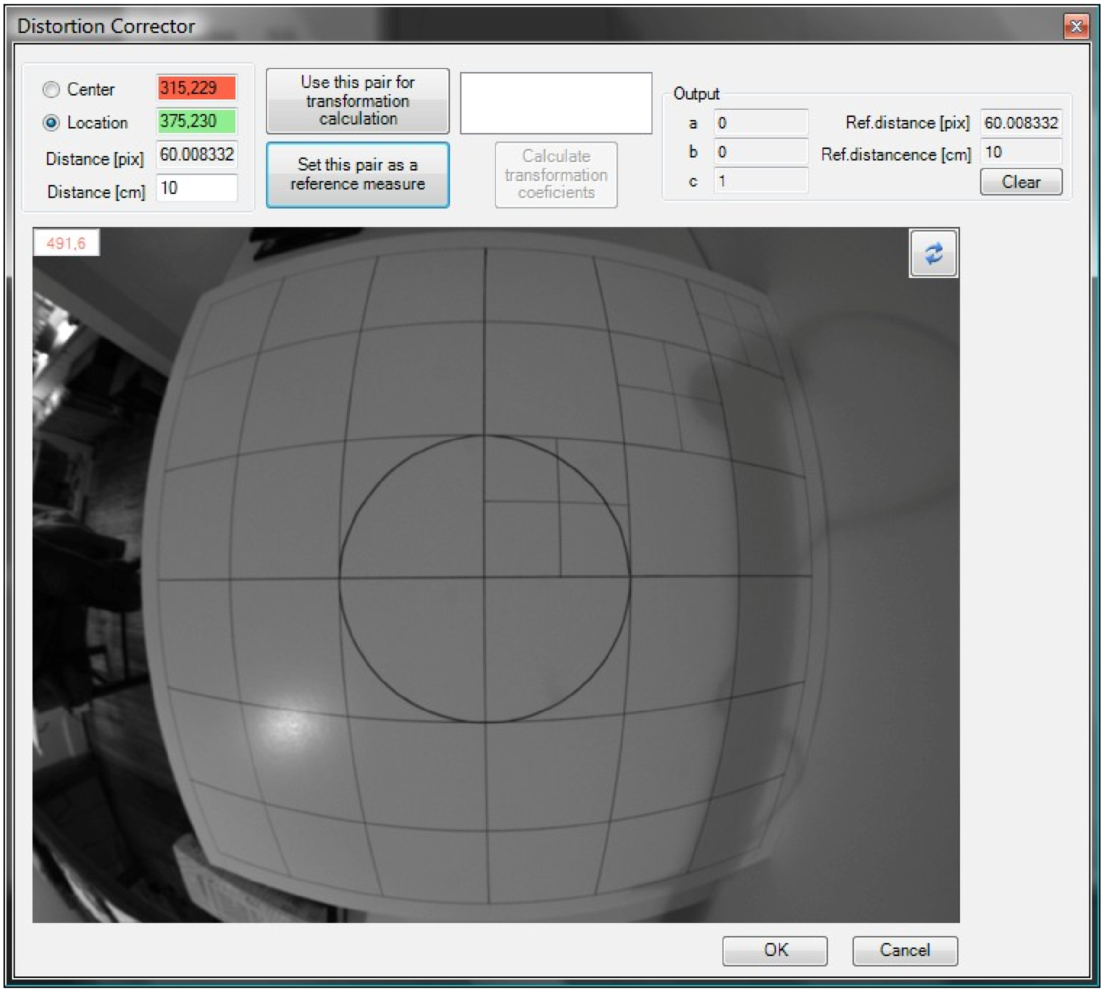


Figure: Distortion correction

On the assumption that the distortion is symmetrical around the camera's optical center and the camera's optical center matches the center of our experimental environment, we can create a set of pairs of measures between the pixel distances (distances between two pixels on the camera image) and the real world distances (distances between two points in the real world in centimeters). After finding at least three such pairs, we can find the distortion function, giving us the relationship between the distance from the camera's center and the corrected distance from the camera's center (as if there would be no distortion).

The best way to find the pairs between the pixel coordinates and the real world coordinates is to create a calibration grid as shown on the following figure. The grid is made of parallel lines distant 10cm from each other. The grid's center has to be placed exactly to the camera's center. We can use the Live Image to match the grid position (Edit – Options – Video – Open Live Image).

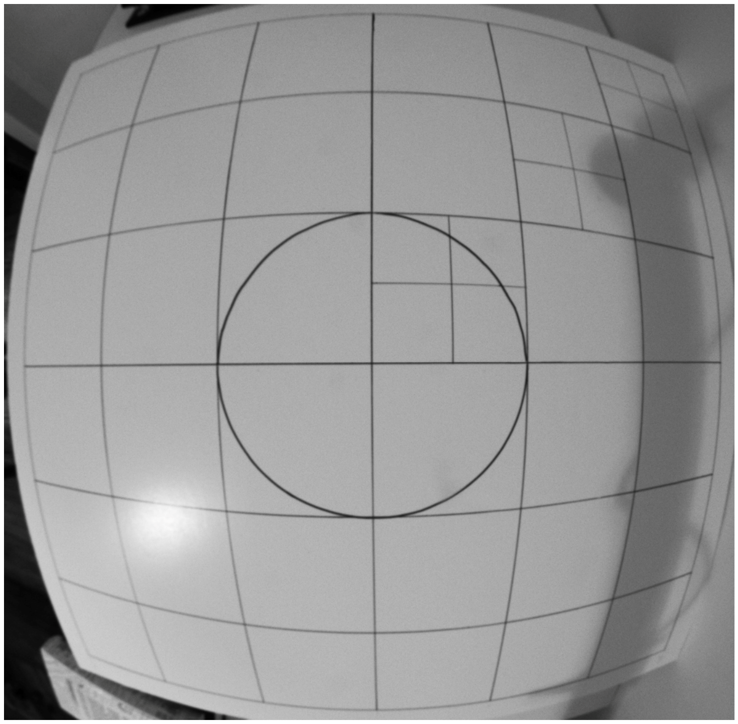


Figure: Calibration grid

Follow these steps to set the distortion correction:

1) Refresh the camera image.
2) The first step is to select the image center. Check the **Center** box at the top left corner of the window and then click where you see the image center. Pixel coordinates of the center would appear in the red box.
3) Now we need to set the **reference pair**, which is the **reference distance in pixels** and **reference distance in centimeters** so the corrected coordinates can be again converted to pixels.
   Check **Location** box and click to some point in a known distance from the center. The reference point should not be too far from the camera's optical center since this would introduce distortion. The smallest distortion is around the camera's optical center. After you choose your reference pixel coordinate, the distance between this pixel and the camera's optical center is shown in **Distance [pix]** box.
4) For the reference pixel distance you selected, set a corresponding reference distance in **Distance [cm]** box. The example of good reference points is shown on the following figure.

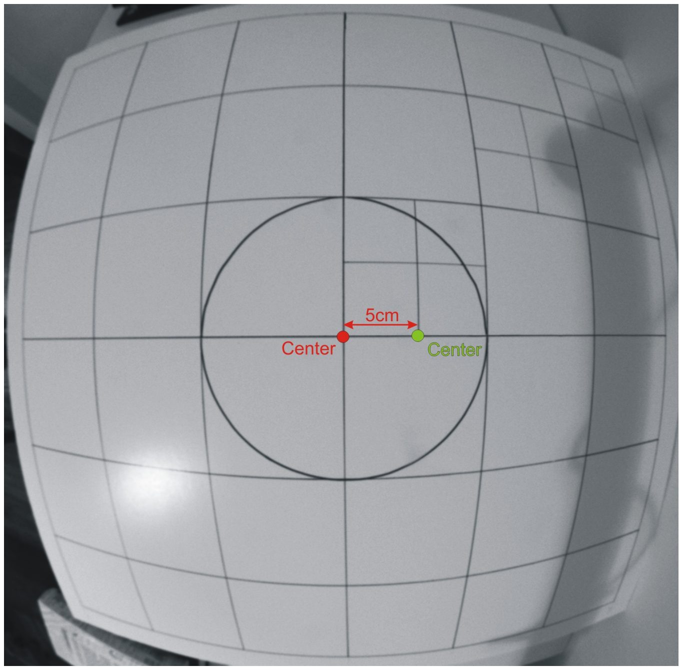


Figure: Reference distance

5) Once you are happy with the reference distance in pixels and centimeters click the **Set this pair as a reference measure**.
6) The next step is to provide 3 pairs of distances in pixels and centimeters for calculating the distortion correction. Make sure the center is properly set and click the new location at a known distance from the center. An example of good points (marked by a green spot) is shown in the following figure. After setting each new pixel location, the corresponding pixel distance is shown in the **Distance [pix]** box. For each pixel distance you have to provide the corresponding distance in centimeters **Distance [cm]**. After you select the pixel and the centimeter distances, you can add the pair into the set of pairs by clicking the **Use the pair for transformation calculation**.

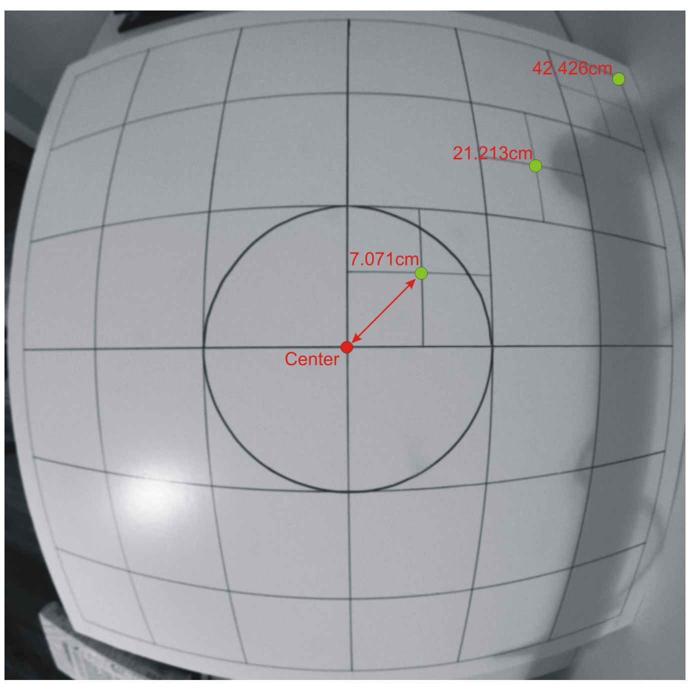


Figure: Calibration spots

7) After providing 3 points necessary for calculation the distortion correction coefficients, you can click **Calculate transformation coefficients**. This would calculate the coefficients and show them in the **Output** box as **a**, **b** and **c** coefficients.
8) The easiest way to check the precision of the correction coefficients is to click at known distances from the camera's center and see the calculated centimeter distance. If you are not happy with the precision, start over by clicking the **Clear** button.

Note: *Precision of the distortion correction largely depends on several things:*

a) Precision of matching the center of the camera with the camera's optical axis
b) Precision of mounting the camera (the camera has to be precisely vertically aligned)
c) Precision of the calibration grid
d) *Precision of reading the calibration points*

1.2 Options - Experiment

Under the Experiment tab you set your experimental paradigm and its main properties.

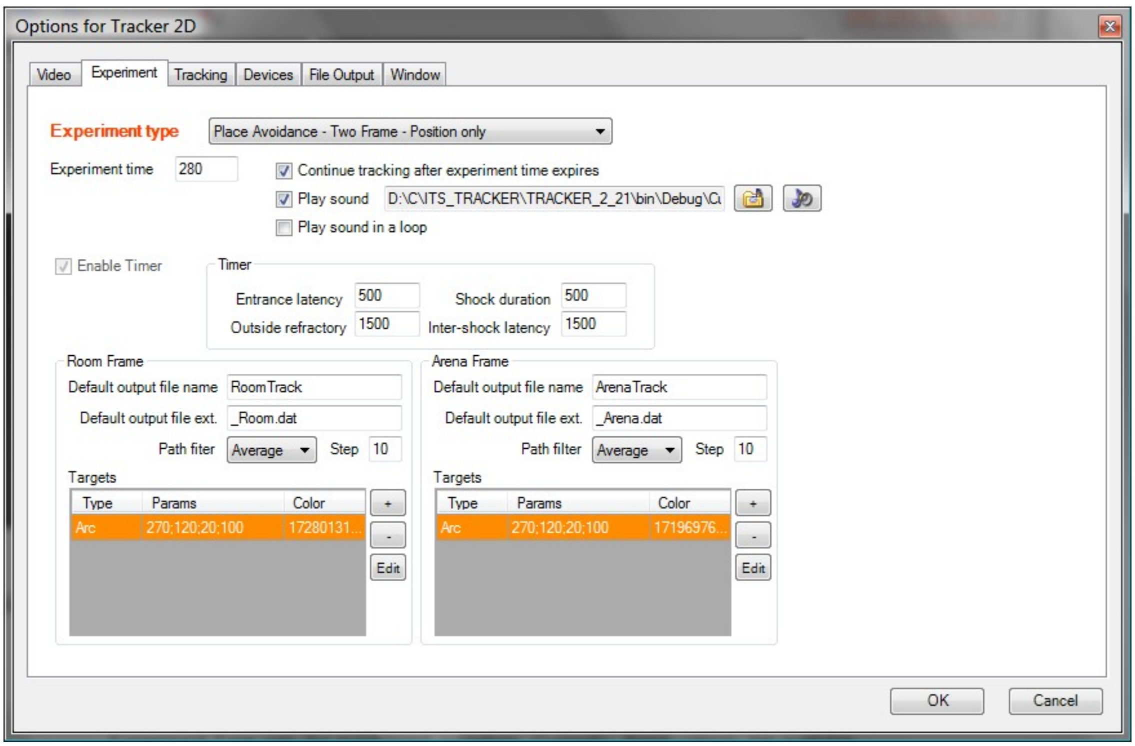


Figure: Options-Experiment

Experiment Type sets the experiment paradigm. Currently, these options are available:

- **Tracker – Position only** – general position tracking of a single spot
- **Tracker – Position and Angle** – general position tracking of a single spot and a reference angle spot tracking
- **Place Avoidance – One Frame – Position only** – One frame (Room Frame) Place Avoidance
- **Place Avoidance – One Frame – Position and Angle** – One frame (Room Frame) Place Avoidance with reference angle spot tracking
- **Place Avoidance – Two Frame – Position only** - (Room Frame and Arena Frame) Place Avoidance
- **Place Avoidance – Two Frame – Position and Angle** - Two frame (Room Frame and Arena Frame) Place Avoidance with angle tracking
- **Plus Maze**

Experiment time sets the duration of the experiment in seconds.

By enabling **Continue tracking after experiment time expires** the experiment will continue after the experiment time elapses. The experiment has to be stopped manually then.

By enabling **Play sound**, alarm sound can be played when the experiment time expires. You can find a couple of sound samples in the program directory under Sounds. If there is no sound card present in the PC, the computer will beep using the built-in speaker. **Play sound in a loop** keeps playing the sound continuously.

Each paradigm in Tracker has its specific configuration. In the following chapters we are going to describe all of the paradigms present in the Tracker.

1.2.1 Place Avoidance

Place Avoidance has been designed to reinforce a moving subject to avoid a to-be-avoided sector. Reinforcement (usually aversive stimulation from a current source) is based on user-defined to-be-avoided sectors (arc, circle or polygon shape). The to-be-avoided sectors are independent for each spatial reference frame. Timing the delivery of the reinforcement is set by the user-defined parameters: Entrance Latency, Shock Duration, Inter Shock Latency and Exit Latency.

1.2.1.1 Place Avoidance Timer

Timer is a virtual device which controls the output devices such as the **Constant Current Source** or the **Pellet Feeder** based on the temporal and spatial relationship between the subject location in time and space and a target which can be defined in the experiment. The Avoidance timer used in the Place Avoidance experiments activates the Current Source when the subject enters the target.

Avoidance timer has 4 properties:

- **Entrance Latency** – time between subject's entering into the avoidance target and the first shock
- **Shock Time** – duration of shock
- **Inter Shock Latency** – time interval between two shocks
- **Outside Latency** – time after leaving the target, when the state is set to "outside"

In addition to that the timer itself might be disabled so no action commands are sent into the output devices even if the subject is present in the target.

For a better understanding of how the Avoidance timer works, let's analyze its state diagram.

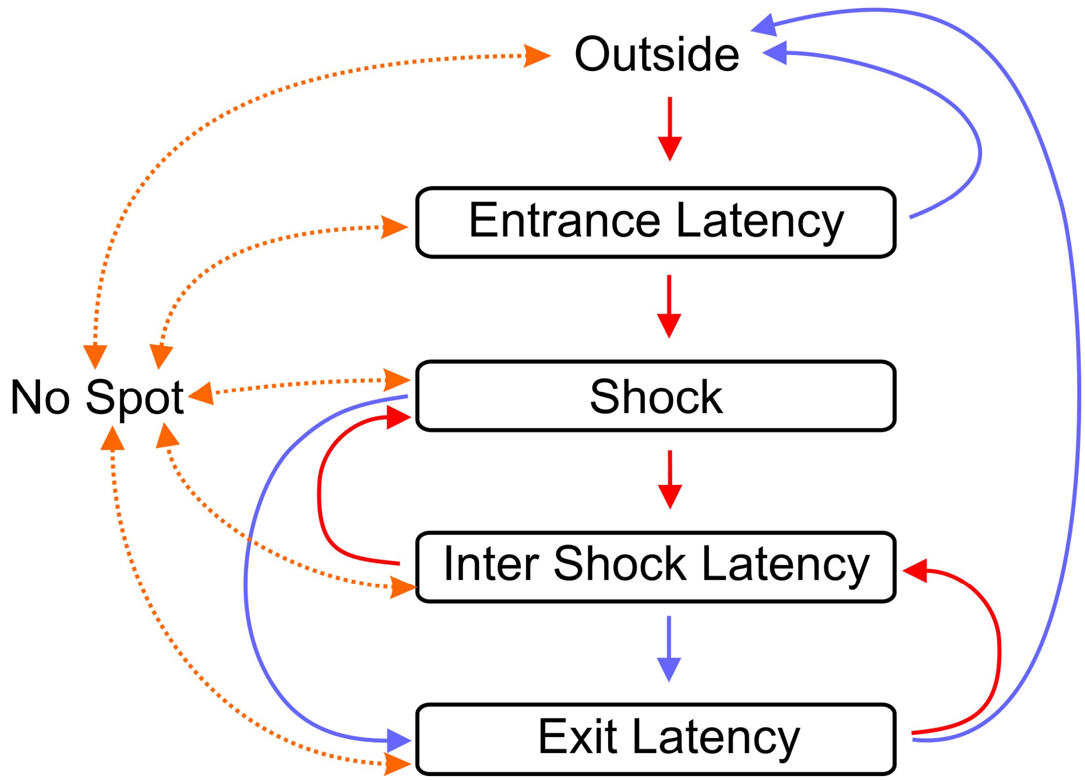


Figure: Avoidance Timer

Basically, there are five states of the Avoidance timer: **Outside**, **Entrance Latency**, **Shock**, **Inter Shock Latency** and **Exit Latency**. In addition to that, there is one more state called **No Spot**. When the Tracker loses the information about the subject's position, it would set the timer state to that state, remember the last known state and after the subject's location is known again, return to the state before losing the subject's location.

The red color in the scheme means that the subject is **inside** the target; the blue color means that the subject is **outside** of the avoidance target. Orange color indicates that no information about the subject's coordinates available.
All arrows which begin at the bottom of some state mean that the next state follows only when the specified time of the state expires. When the state changes before the time specified, arrows begin at the side of the state.
First timer state is always **Outside** (even if the subject is located inside the target when the experiment begins). When the subject comes into the avoidance target, **Entrance Latency** time is counted. If the subject leaves the avoidance target before the Entrance Latency time expires, the state is again Outside, if the subject stays in the avoidance target, the state is changed to the **Shock**. In this state the Current Source is activated for the time specified as the **Shock Time**. If the subject escapes from the avoidance target before the Shock Time expires, the state is changed to the **Exit Latency**. If the subject remains inside the avoidance target until the Shock Time expires, the state is changed to **Inter Shock Latency**. In this state, the Current Source is turned off. When the subject leaves the avoidance target during the Inter Shock Latency period, the state is changed to Exit Latency; if not, a new Shock state is started. This means that if the subject remains in the target, two states – Shock and Inter Shock Latency will be alternate until the subject leaves the target. When the subject leaves the Avoidance target during the Shock Time or Inter Shock Latency, the state is changed to Exit Latency; if the subject returns into the Avoidance target before the Exit Latency expires, the state is changed to the Inter Shock Latency; if the subject remains out of the avoidance target for a period longer than the Exit Latency, the state is changed to Outside.

If you found the previous explanation of the avoidance timer function confusing, perhaps the following figure can make things more transparent. Let's imagine an experiment with some big Arena and the circular avoidance target. The next figure shows the movement of the subject across the Arena and the activation of the avoidance states from the overhead perspective.

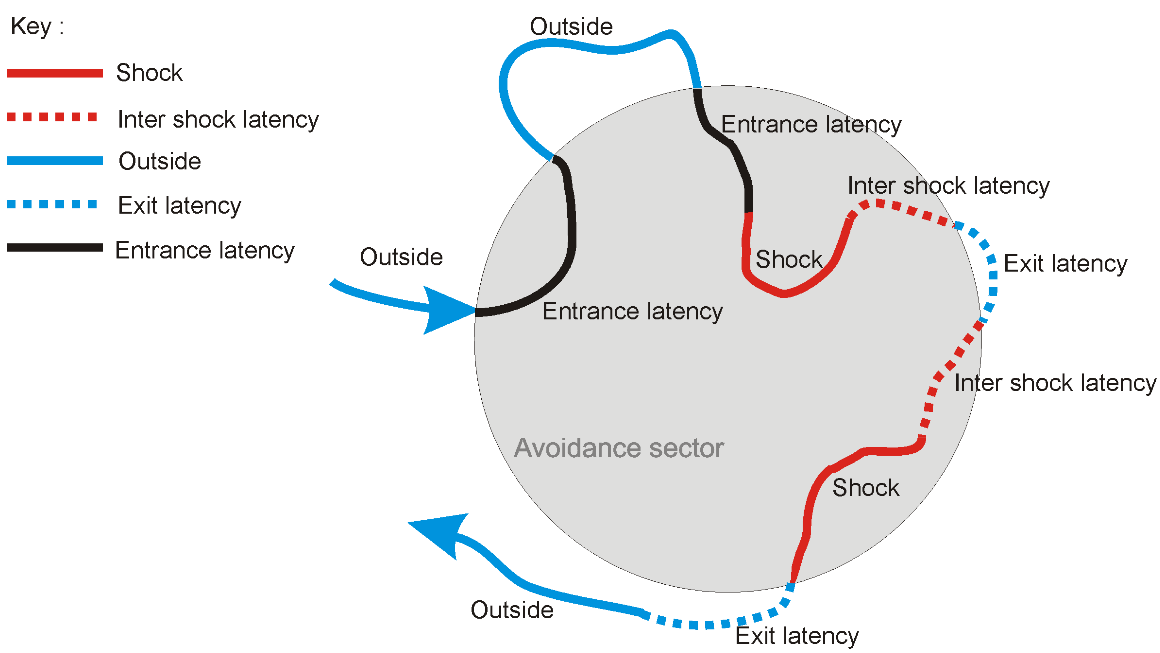


Figure: Avoidance Timer

Entrance to the avoidance target is marked with the arrow. When the subject first enters the target, the Entrance Latency is being counted; because the subject leaves the target before the Entrance Latency expires, Outside state is activated again. When the subject enters the target for the second time, again Entrance Latency is being counted, when it expires, subject is still in the target, which means, that the state is changed to the Shock. In this state, the Current Source is activated and the subject receives the shock for the Shock Time. After this state finishes, the subject still stays in the target, so the next state is the Inter Shock Latency. Subject leaves the target during this state, and the state is changing to the Exit Latency. Because the subject returns into the target before the Exit Latency expires, state is changed to Inter shock latency, with the following Shock (subject stays in the target). Now, subject leaves the target, Exit Latency expires, Outside state is activated.

The next graph shows the avoidance states changing in the time perspective for the virtual experiment described in the previous paragraph. On the vertical axis, there is the current at the output of the Current Source; on the horizontal axis, there is the time.

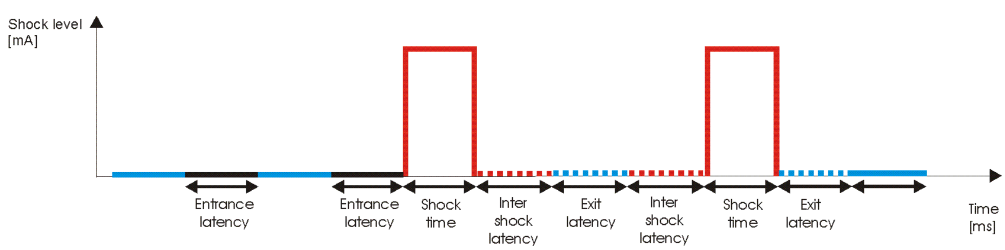


Figure: Avoidance Timer

Note: *If more than one frame is defined (e.g. the Room and the Arena frame), each frame controls its own timer. The shock is triggered on the basis of both timers independently.*

1.2.1.2 Frames

The Tracker can operate in two reference frames – **Room Frame** and **Arena Frame**. Frames are basically independent views to the experiment. ITS is designed to run the frames in parallel mode, response with events (Shock, Feeding) for each single frame and provide the output data for each frame separately.

Room Frame represents the view from the camera perspective.

Arena Frame represents the view as you would see it if you stood on the Arena's surface.

For each frame you have to specify several properties:

- **Default output file name** – default pattern for the output file name
- **Default output file extension** – default pattern for the output file extension
- **Path Filter** – specifies the filter which is used to calculate the elapsed path of the subject
- **Targets** – lists targets used in the experiment

Let's talk about Path Filters and Targets in more detail.

1.2.1.3 Path Filter

Path Filter sets the filtering type for the distance traveled by the subject during the experiment. Three filters are available:

- **None** – path is calculated without filtering
- **Interval** – filter skips constant number of positions specified by **Step** - and for the path calculation reflects only the ones which were not skipped.
- **Average** - filter simply averages the coordinates based on value edited in the **Step**. Those averaged values are then used for the path calculation

1.2.1.4 Targets

Target is a specific place in the experimental arena which might trigger some actions when the subject is present in it. In the Place Avoidance task, targets trigger Current Source.

Note: *All target sizes are defined in a relative way (percents). To set the transformation between the absolute (pixels) and relative coordinates, you have to set the **Region of Interest** either under the **File Output** tab or when the target is defined by dragging the boundary. The region of interest also sets the origins of the relative coordinates used in the output files.*

Generally there are three types of targets:

- **Arc**
- **Circle**
- **Polygon**

Arc target has four properties:

- **Minimum Radius** [%] - lower curve of the arc (100% is the size of the Region of Interest)
- **Maximum Radius** [%] - higher curve of the arc
- **Place Angle** [deg] - angle between the center of the arc and the x axis
- **Angle Width** [deg] - width of the arc in degrees

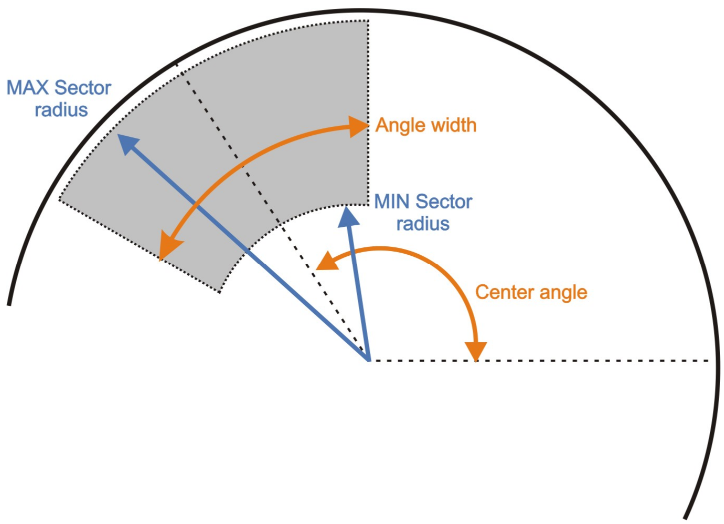


Figure: Arc target

Circle target has three parameters:

- **Place angle** [deg] - angle from the centre of the circle to the x axis
- **Place radius** [%] - distance from the center of the arena to the center of the circle target in percents
- **Target radius** [%] - radius of the target in percents of the region of interest

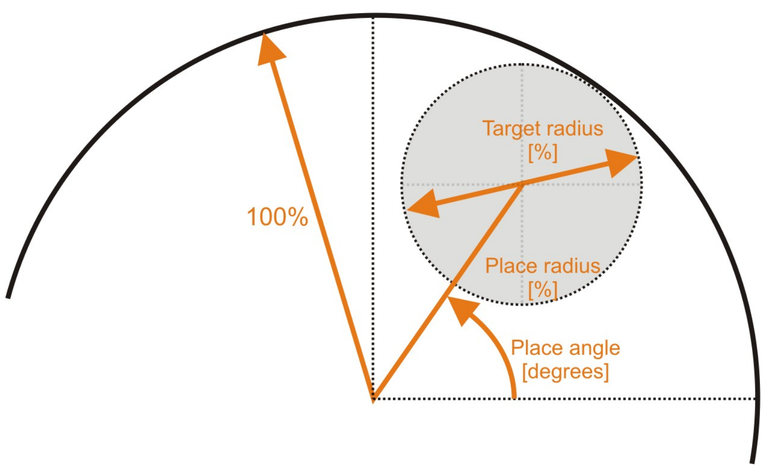


Figure: Circle target

Polygon targets are defined by the list of their vertexes (X1 Y1 X2 Y2…), when the coordinates are at percentage of the unit circle, defined by the boundary rectangle.

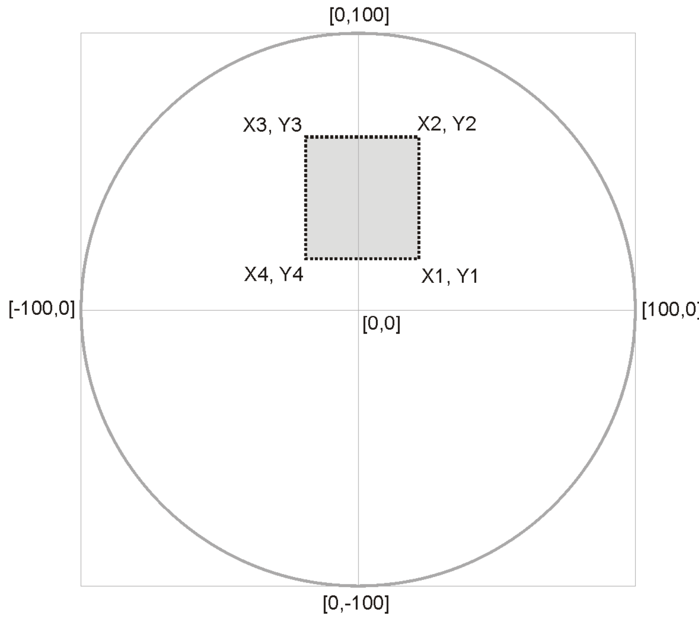


Figure: Polygon target

To add the target for each frame, click ‘ **+**’ to **add** the target, ‘ **-** ‘ to **remove** the target and ‘ **edit**’ to **edit** existing target. After you click the ‘+’ or ‘edit’ button, the target dialog appears.

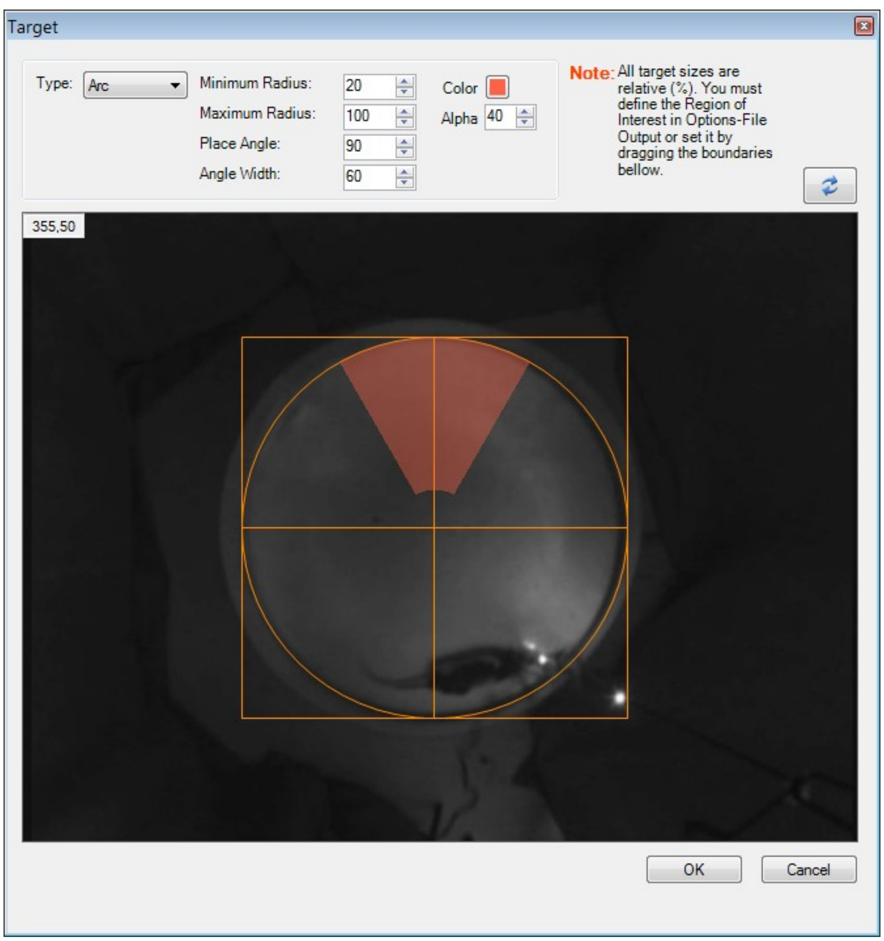


Figure: Target Properties

You select the Target type in the **Type** box. For **Arc** and **Circle** you just edit their properties by typing into the property boxes. If you want to refresh the background image, click the **Refresh** button.

When you select the **Polygon** target type, you simply draw the polygon by pointing the mouse and clicking the Arena image. If you want to delete the last point, click the right button on your mouse. To close the polygon, double-click the place where you want to insert the last point. If you want to clear the polygon and start the drawing again, click the **Clear** button.

For each target you may choose a different **color** and **alpha** value (transparency).

1.2.2 Elevated Plus Maze

The Elevated Plus Maze experiment is an anxiety model to assess different types of emotional states in the animal.

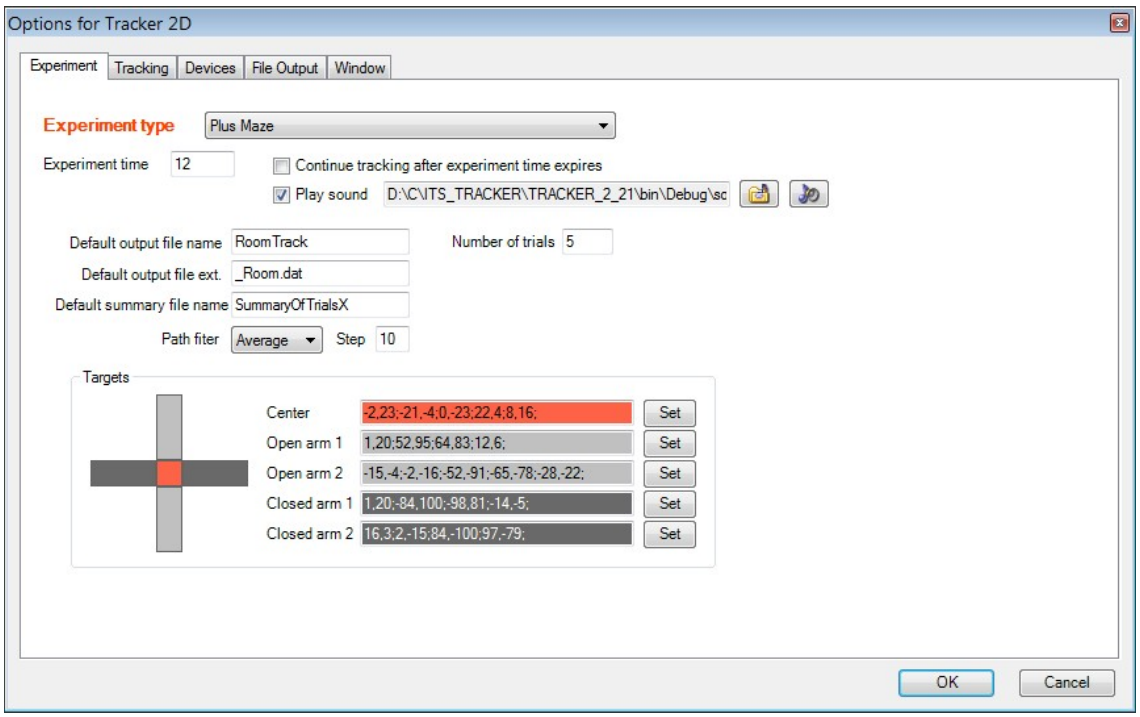


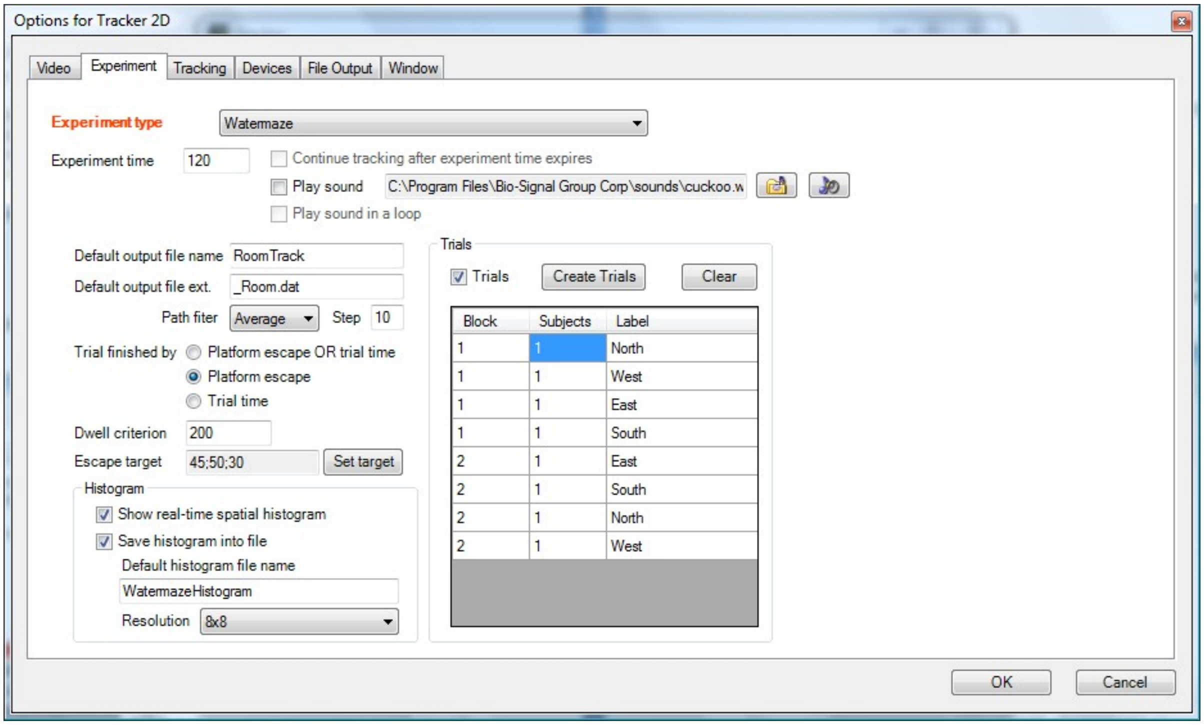
Figure: Elevated plus maze options

Several options are available for the Elevated Plus Maze experiment:

- **Trials** – number of trials for each experiment (for each experiment a single file with the tracking information and single summary file is provided)
- **Default output file name** – default pattern for the output file name
- **Default output file extension** – default pattern for the output file extension
- **Default summary file name** – default pattern for the experiment summary
- **Path Filter** – specifies the filter which is used to calculate the elapsed path of the subject See chapter 1.1.1.3 for more details about the path filters.
- **Targets** – five standard polygon targets have to be defined for the Elevated Plus Maze experiment. You can select each target by clicking the schematic figure on the left side of the targets panel or by clicking on the Set button on the right side of the target coordinates. See chapter 1.1.1.4 for more details about polygon targets.

1.2.3 Watermaze

The classic experimental procedure first described by Morris (1981). A subject is tracked while it attempts to escape onto a platform in a swimming pool.



Several options are available for the Watermaze experiment:

Trial finished by handles the end of experiment. Experiment can stop either by the expiration of the Trial time, reaching of the platform or both.

Dwell criterion sets the entrance latency for the platform.

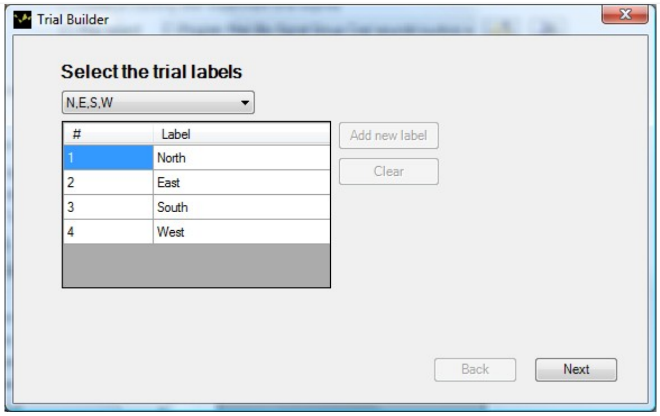
Set target allows to specify the target.

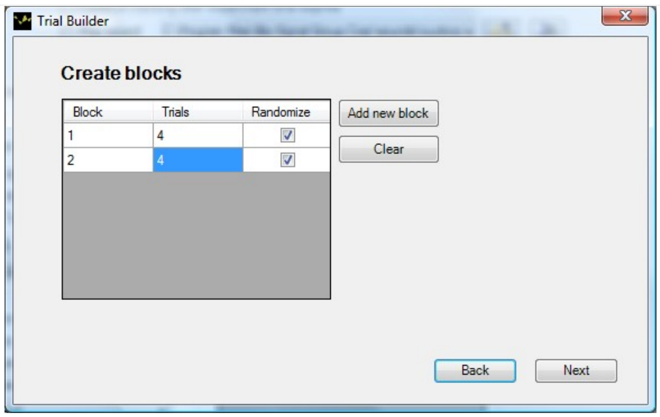
When the experiment runs, spatial **histogram** can be shown and/or saved into output file in Excel-compatible format.

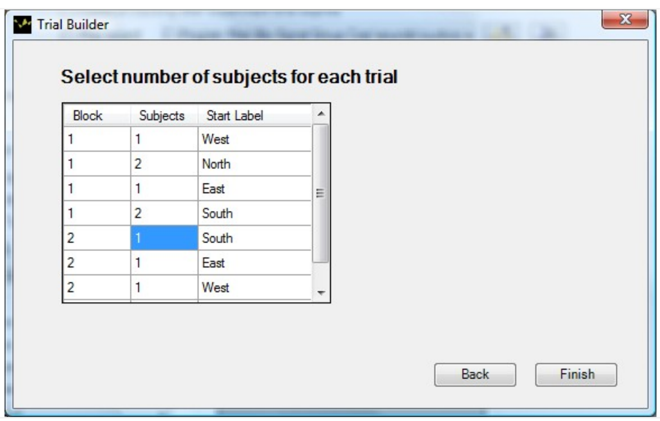
Experiment can be organized in **trials**. Click **Create Trials** button to launch a wizard which will guide you through the setup of trials.

In the first step you define trial labels (starts). Then you create groups of trials (blocks). One block contains all trial labels defined in the first step. In the last step you can assign number of subjects for each trial.

Next figures show an example of the setup of trials.







1.3 Options - Tracking

In the Tracking tab you set up the properties of the image acquisition and spot searching algorithm, namely what are the properties of the subject, angle reference spot (if used) and angular displacement reference spot (if used).

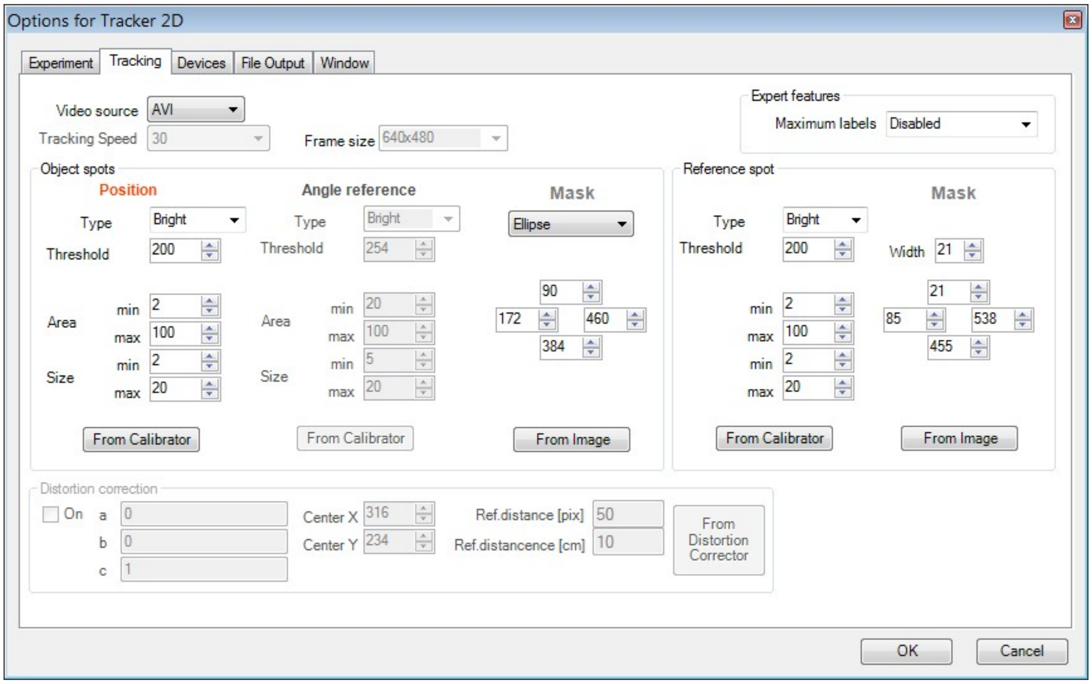


Figure: Options-Tracking

The **Maximum labels** (Expert features) option sets the maximum number of 'labels' which are the isolated spots present in the image. Since the theoretical number of labels can be half of the width of the image multiplied by the half of the height of the image, it may take a huge amount of time for the algorithm to process such frame. This might happen only because of a very bad calibration but when it happens, the spot searching algorithm might stop processing of the frame and thus prevent the application from freezing. If the feature is enabled, the location is shown as TMS (Too Many Spots) in the GUI. The value is usually determined by trial and error but the standard values can be 500-1000.

Video tracking is based on image processing. When the subject has a different brightness according to the background (note, that it can be either a bright object on a dark background or dark object on a bright background), you can subtract the image information of the background and see if any continuous bright or dark regions remain.

There are three main properties, which characterize each spot:

- **Threshold**
- **Area**
- **Size**

1.3.1 Threshold

In the following picture you can see the real Image acquired from the camera.

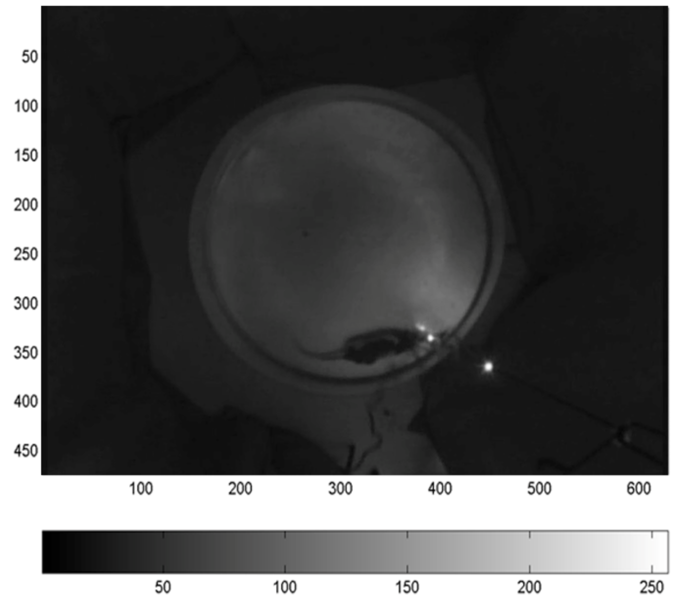


Figure: Camera image

You can recognize the outline of the Rotating Arena, its Reference LED (right side of the picture) and the subject LED. The image has 640 Columns and 480 Rows.

The picture is called a **bitmap** because every point (**pixel**) with its coordinates (**Columns** and **Rows** or **X** and **Y**) has its bit representation of color (**level of gray**). Because we use 256 levels of gray, each pixel's color is represented by a number at the interval of 0-255, where "0" means Black and "255" means white.

If you imagine a figure which shows the number of pixels (vertical axis) per each level of gray (horizontal axis) in the camera picture (we call it **histogram**), it will look as shown at the following figure.

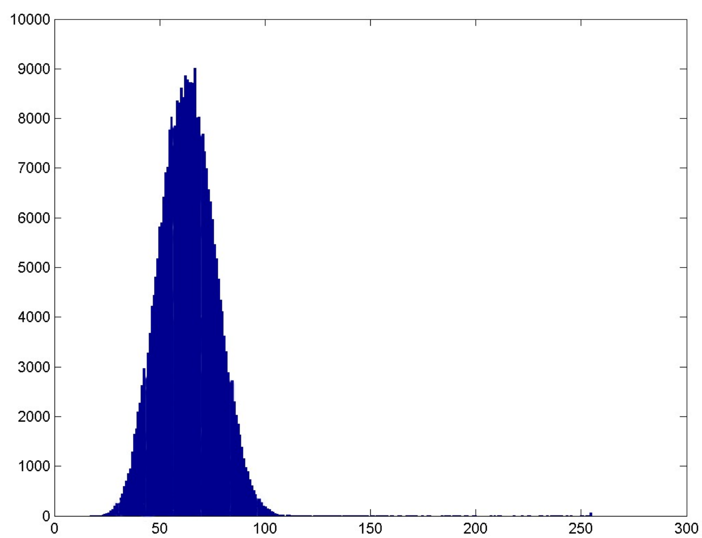


Figure: Image histogram

You can see, that the most of the pixels have their levels of gray between 30-100.

In your setup, you are looking for the bright points (LEDs) whose gray levels are around 150-255. This means you can „cut" all the image information in which you are not interested so that you can recognize the spots. The point which specifies the value from which all the levels are subtracted from the image is called

Threshold. If you are interested in the **Bright** spots, you use **positive thresholding** which means that you set all pixels bellow threshold to “**0**” and all pixels above the threshold to “**1**”. This step converts the monochrome image into a binary image.

Similarly, If you are interested in the **Dark** spots, you use the **negative thresholding** which means that you set all pixels with **higher** gray value than the threshold to “**0**” and all pixels with lower gray value than threshold to “**1**”.

If you want to track all spots between two threshold values, you use **interval thresholding**.

Let’s see what happens with the previous picture if you use the positive thresholding and cut all the gray levels from 0 to 200.

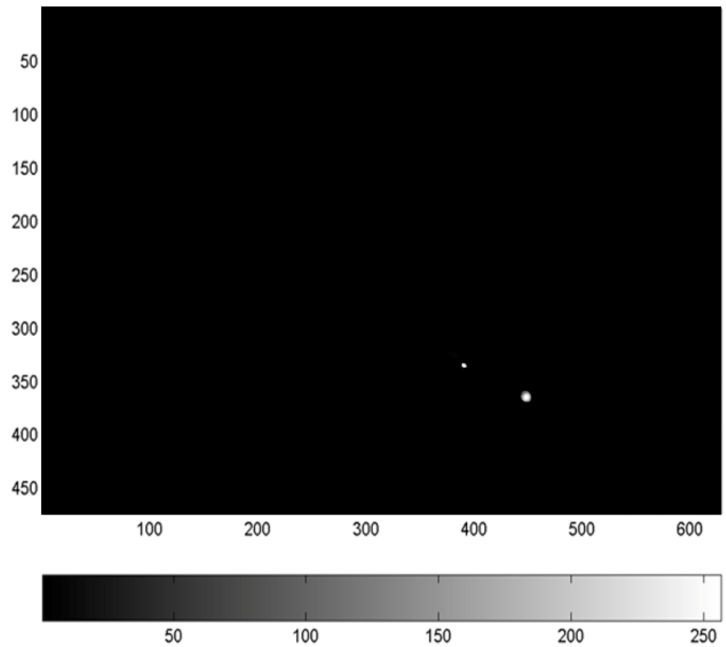


Figure: Camera Image after filtering

Now you can see, that all the “midlevels“ are gone from the picture. The spot searching algorithm goes through all areas present on the image and filters out all which doesn’t meet the criteria of **Area** and **Size**.

1.3.2 Area and Size

Now, when you have isolated interesting parts of the picture, you need to take one step further, and that is to say which spots have the properties you expect.

You can imagine using the LED, attached on the subject, as it is shown on the previous figures. This spot has some typical size even when the subject is moving on the arena. However, it may happen that the light from the LED is being reflected to the camera from some reflective object (e.g. the plastic wall around the arena, another object on the arena, etc.) so the system may recognize multiple objects and can’t be sure which one belongs to the right object.

For this reason you find subject’s typical **area** and **size**, so you can distinguish the subject from all other spots.

If you zoom one of the spots from the previous figure, it will look like this :

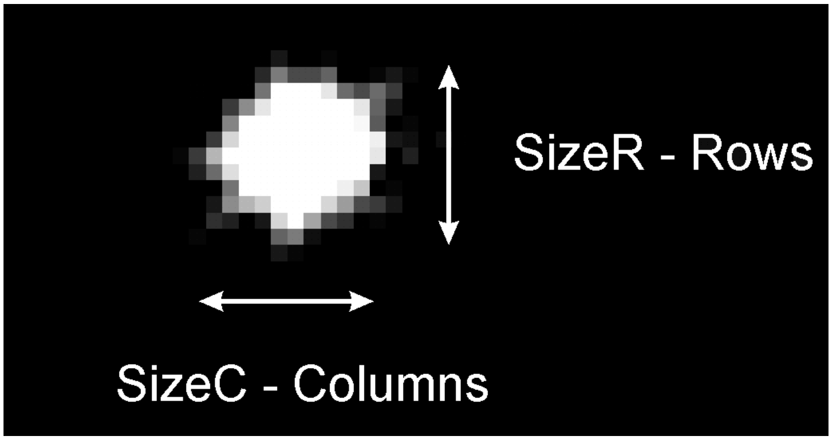


Figure: Spot detail

You can count that the spot occupies roughly area of 70 pixels and about 11 columns and 11 rows.

1.3.3 Finding correct values for the Subject

In the most cases, your experimental arena covers only a part of whole the camera image and therefore the rest of the camera image is not interesting for us. To save the CPU time in searching for your spots outside the **Region of Interest**, the **Mask** is introduced. The mask is basically a filter, which enables only a subset of the image for spot searching. The mask can be either **disabled**, **rectangle**, **circle** or **polygon**.

Let's setup the mask for the subject spot.

- Select the **Circle** mask from the **Mask** box
- Click the **From Image** button, the mask configuration dialog will be open
- In the mask configuration, click the refresh button in the top left part of the window
- Now adjust the size of the mask either by dragging the mask by mouse or by typing into the boxes

The mask should appear as shown as the following figure

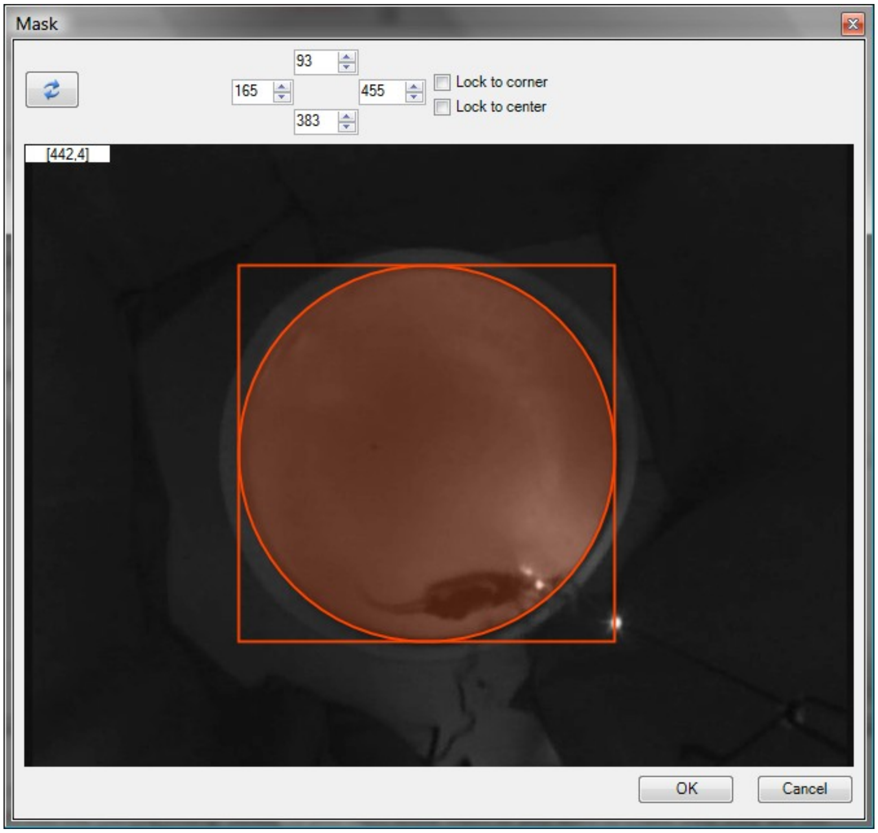


Figure: Mask

Now it's time to set the subject's tracking properties. If you already know the properties, you might directly type them into the corresponding boxes. If you don't know them or you want to make sure they are still accurate, you use the **Calibrator**.

Click the **From Calibrator** button.

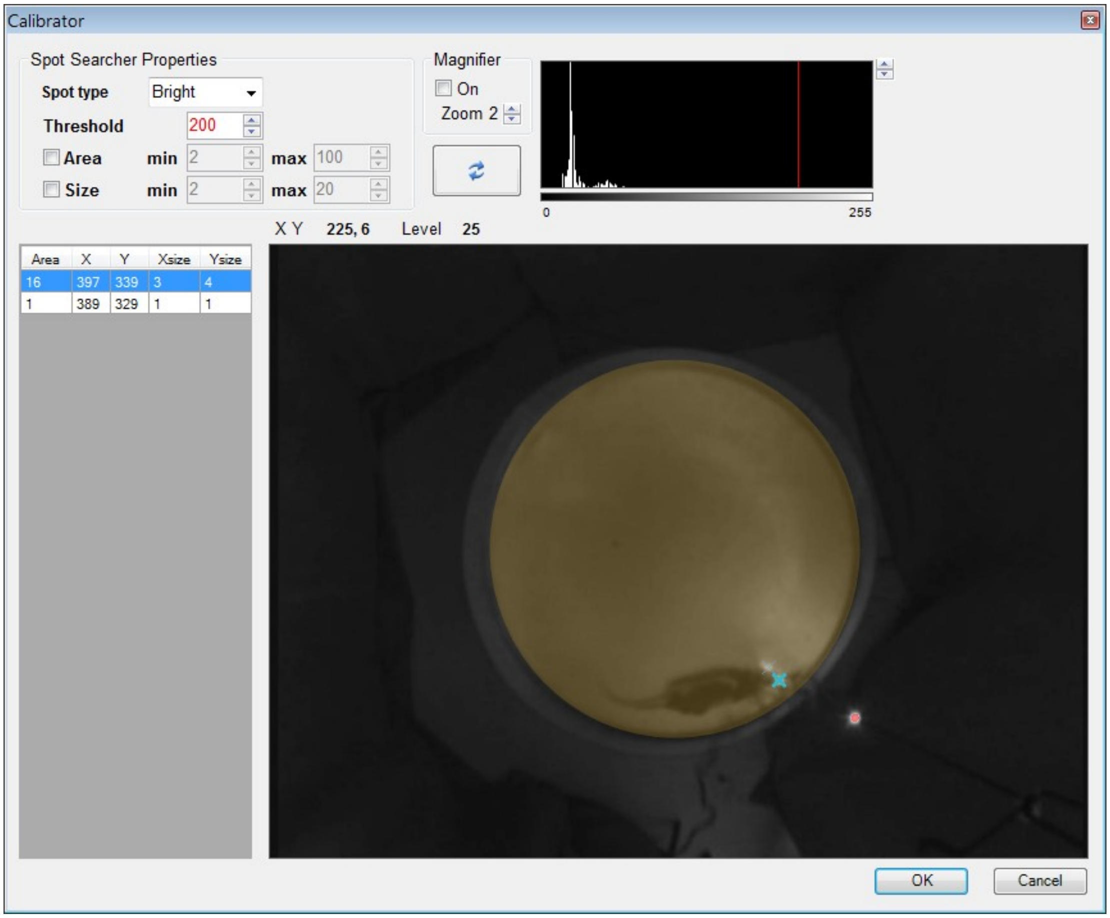


Figure: Calibrator

If you specify the Mask before running the Calibrator, the mask appears as a yellow circle (or rectangle). When the Mask is specified, the spot is searched only within the mask.

Let’s go through the calibration process now.

Spot calibration is the most sensitive part of the configuration process. This step must be done with precision, otherwise you tracking results may be poor.

As you have read in the previous chapters, tracking is based on the difference of the contrast between the subject and its background. By specifying some contrast level – **Threshold** -- which corresponds to the tracked subject contrast level, we may cut all the background image information so that ideally the only remaining information on the image (our subject) remains.

See **Spot Searcher Properties** box in the Calibrator. If you are looking for a dark spot on a bright background, select the **Dark** button. In case of a bright spot on a dark background, choose the **Bright** button. To track within some interval of the grey levels, choose **Interval**.

Now you have to specify the **Threshold** value. You may do it either by trial and error by pressing the **Refresh** button and checking if your subject is selected or by a more sophisticated way which is closer investigation of the tracked subject. After you click the **Refresh** button, you should be able to see the image from your camera. Areas which are marked by red color lie below or over (depends on if you track the bright or the dark spot) the actual threshold. When you move the mouse above the camera image, some numbers above the top left corner of the camera image are changing. The first two numbers provide the X and Y coordinates of the cursor on the camera image (in pixels), the next number provides the gray level of the actual pixel on which you point with the cursor. This is exactly the information you are looking for. If you point your cursor above the subject you want to track, you should be able to read the typical gray value of the object. If you want, you may turn on the **Magnifier** in the Magnifier box for an even closer look.

The Threshold value might be also changed by dragging the red line in the image histogram.

The point of this step is to eliminate as much as possible image information except our subject. The following example shows a wrong value of the Threshold (Threshold = 60). You may see that there is more than the subject spot marked. This would increase the error in your tracking and make the tracking inaccurate and unreliable or even impossible.

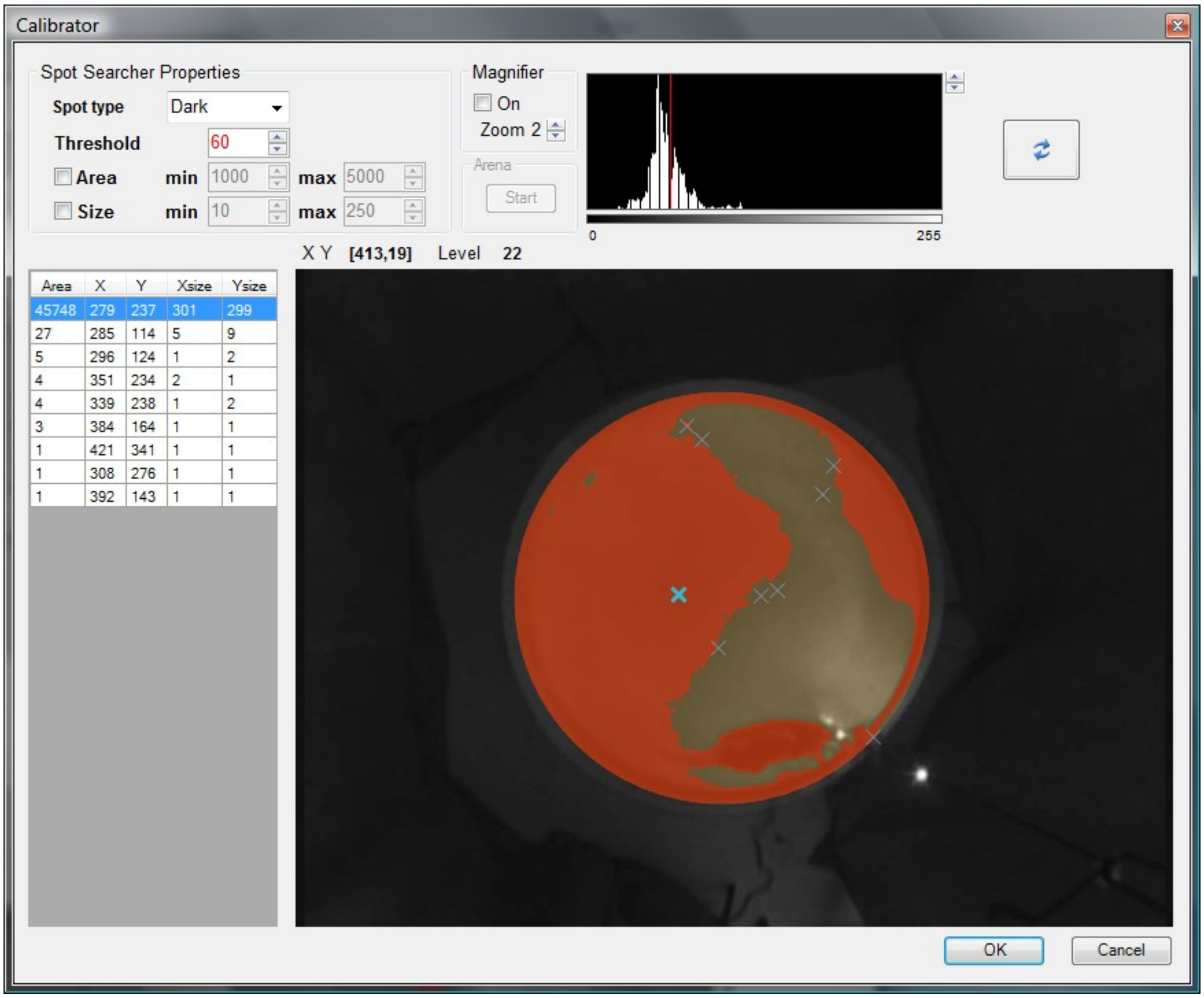


Figure: Bad threshold

When you move the Threshold down more, you can get nicer separation of the background and the subject as you may see at the following figure (Threshold = 33).

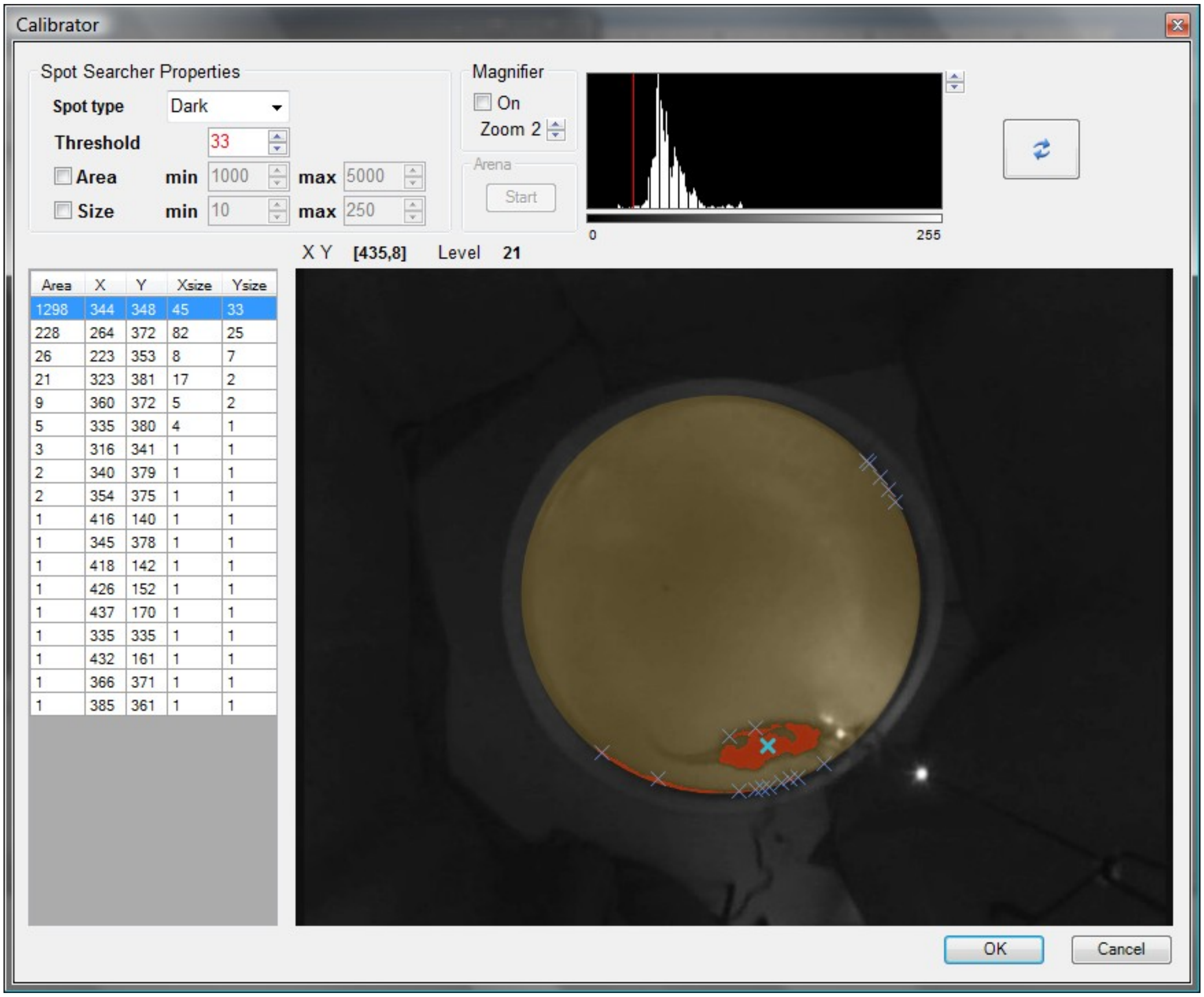


Figure: Good threshold

You may see that now there is only your subject marked and also couple of smaller spots, which may be eliminated by other filtering.

After you eliminate as much as possible background image information when your subject is still marked, you can move to the next step, which is to find the tracked spot (subject) measures – **Area** and **Size**.

When you click the **Refresh** button, areas which lie below or over the specified threshold are marked. This creates closed areas (spots), which are then analyzed. On the left side of the application you can see the table with found spots and their properties (the number of spots is limited by the size of the table). For each spot you can find its Area, X and Y coordinates and Size in X and Y dimensions. In addition to that, each spot is drawn as a blue cross over the camera image. The first spot (or the spot you select in the table) is marked in the camera image by a bigger cross.

Now you can find what area the subject spot has in the spots table. You can either find the subject spot according to its X and Y coordinates or by sequential selecting of each spot in the table, after the subject spot is marked by the bigger cross. In the figure above, the subject spot has the area of 1298 pixels, the X size 45 and Y size 33 pixels. When you know this information, you may eliminate all of the other spots in the camera view by providing an area **interval** in which the subject spot should be found. Check the **Area** box, which enables the **Min** and **Max** boxes and specify the values in some larger interval. For example, 800 pixels for the minimum area and 2000 pixels for the maximum area. Then click the **Refresh** button to check, if all the spots with the areas smaller than Minimum Area or bigger than Maximum Area were filtered from the list.

By increasing or decreasing the Minimum and Maximum Area values you can subtract all the non-interesting spots, so the only one remaining is the subject spot.

Using the **Size** property of the spot is similar to using the Area. By checking the box on the left side of the Size label, you enable the boxes for entering values. Then you set the intervals of the desired sizes of the spot and check if they are correct by capturing the frame using the **Refresh** button.

The goal of the Spot Search is to find 5 properties for the spot search (Threshold, Minimum and Maximum Area, Minimum and Maximum Size) which would make it possible to track the subject spot. You should press the Refresh button multiple times to make sure you find the spot each time even if you place the subject into other place of the arena.

1.3.4 Reference spot

In **Place Avoidance** paradigm which uses the **Rotating Arena** you also have to specify the reference spot so you have the information about the angular displacement of the Arena. You get this by setting up another spot called **Reference spot**. The calibration of the Reference spot is exactly same as the Subject spot. You only have to set another type of mask – the annulus mask (see the following figure).

In the Calibrator, you may rotate the Arena by clicking the **Start** button in the **Arena** box. This allows you to move the Arena around so you can set the mask and reference spot properties based on various locations of the reference spot.

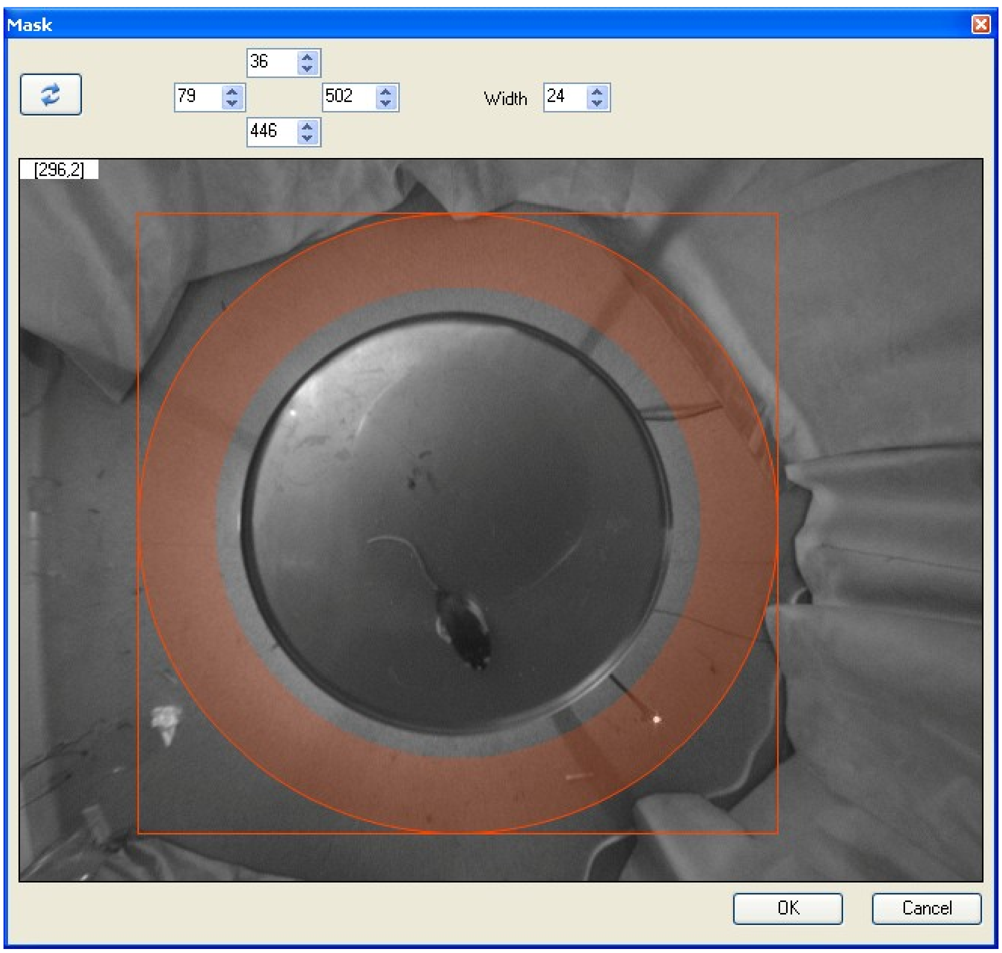


Figure: Reference calibration

Note: *Make sure that your light conditions in the room are constant. It is better to use the artificial light rather than the daylight which changes significantly in its intensity during the day.*

Note: *Most important objective of the Spot Calibration is to set the intervals wide enough so the system can find the spot, even if it became a little bit different in size (e.g. when the subject changes its posture but still small enough, so it doesn't interact with the false spots. It's important to understand that ITS's accuracy is directly affected by the calibration. If you calibrate the system poorly, you can't expect good results in tracking. We suggest that you take calibration seriously, because it is a crucial part of the system usage and if it is done precisely, you usually don't need to do it again for a longer period of time.*

Note: *For troubleshooting purposes, you may save the Camera Image picture into a PNG file. Right click to the Camera Image window and choose **Save Image to File**.*

1.4 Options - Devices

Under the **Devices** tab you set all parameters for the external devices controlled by the ITS. External devices are not controlled directly from the computer, but through a special device called the **Control Box**. This device converts TTL control signals from the computer to the high power control signals for the devices.

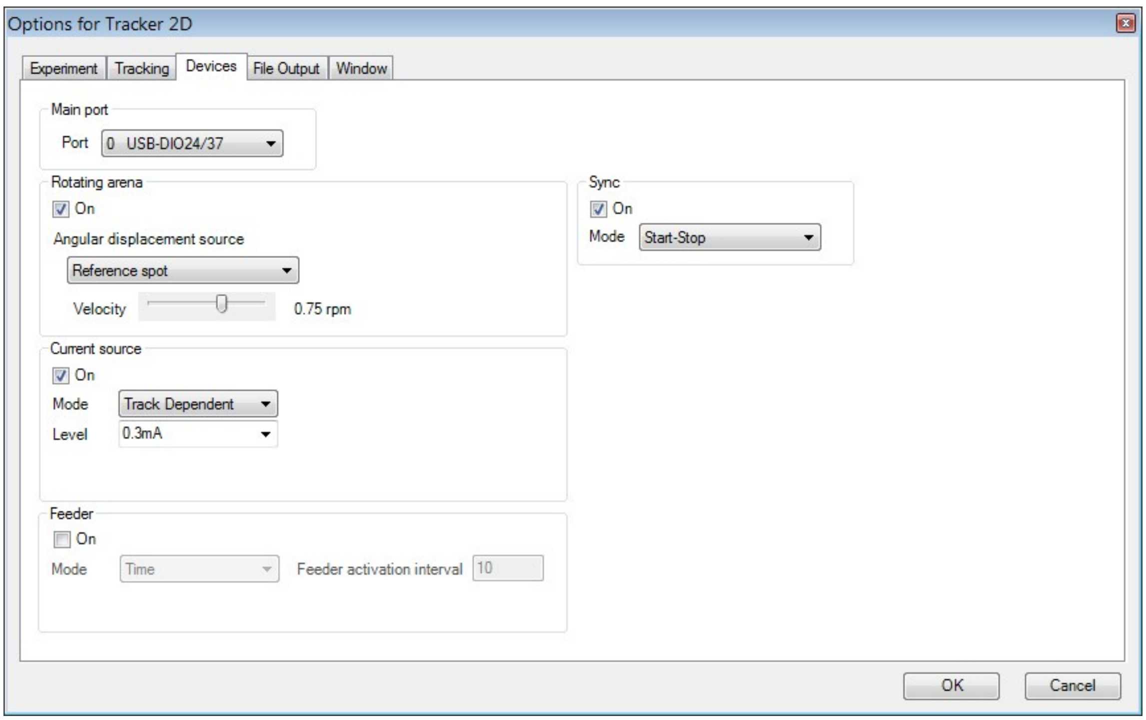


Figure: Device Properties

1.4.1 Rotating Arena

Rotating Arena is used in such experiments which require rotation of the experimental space, such as the 2 frame Place Avoidance experiment.

Since the software has to record the angular displacement of the arena you have to select the **Angular displacement source** which can be of two types:

- **Reference spot** (usually a LED mounted on the Rotating Arena; the tracking properties of the LED are set under the **Options – Tracking – Reference spot** tab.
- **DIO-24 card** (card connected with Rotating Arena's Incremental Encoder)

Velocity sets the rotating speed of the arena in clockwise/counterclockwise directions.

1.4.2 Current Source

There are three modes for the constant current source:

- **Track dependent** – Current Source is activated on the basis of the avoidance targets (when the subject is present in the target)
- **Time** – Current Source is activated periodically, based on **Inter-shock latency** and the **Shock duration**
- **Random** – Current Source is activated randomly, based on user specified **Minimum inter-shock latency**, **Maximum inter-shock latency** and **Shock duration.**
- **Manual** – Current source is activated manually by **F11** (ON) and **F12** (OFF) keys.

Level of the current source sets the initial level of the Current Source (0 – 0.7mA).

1.4.3 Feeder

The Feeder has three modes:

- **Track Dependent** - Feeder is activated on the basis of the preference targets (when the subject is present in the target)
- **Time** – Feeder is activated in constant intervals, based on **Feeder activation interval**
- **Random** – Feeder is activated randomly, based on **Minimum activation interval** and **Maximum activation interval**
- **Path Triggered** – Feeder is activated after the subject crosses the **Minimum distance** during the **Testing time**

1.4.4 Main Port

In order for the computer to be able to control the external devices, you have to set the **Main Port** which is the address of the DIO-24 card in the PC.

Note: *before the Main Port property can be set, we must install the DIO-24 card and i's drivers and run the **Instacal** tool to register the card in the system.*

1.4.5 Sync

For the synchronization with other systems, e.g. an electrophysiology recording system, the Tracker has a feature of the output synchronization signal. Two types of the sync signal are available:

- **Start-Stop -** when the experiment starts, sync goes high, when it stops, sync goes low
- **Frames -** when the experiment starts, first even frame sets the sync high, odd frame sets the sync low.

1.5 Options – File Output

Under the **File Output** tab you set the properties related with the file output.

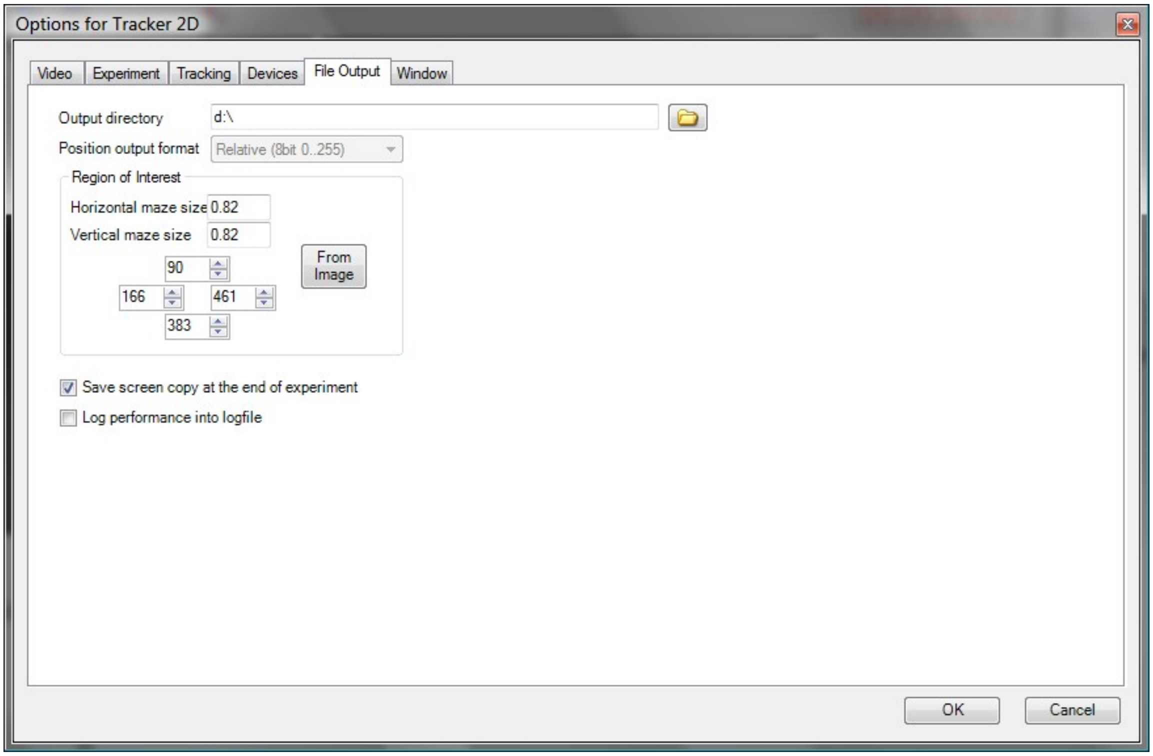


Figure: File output properties

Output directory sets the default directory where the output files are stored.

Position output format sets the way how the output coordinates are stored in the output files. Two types are available:

- **Relative (8bit 0..255)** - in this mode the **Region of Interest** is scaled into the relative coordinate system 0..255, where the top left corner of the region is [0,0] and the bottom right corner is [255, 255]
- **Absolute (camera pixels)** - in this mode spot positions are recorded in the camera pixel coordinates

Region of Interest has two roles:

- defines the region which is used to transform the pixel coordinates into the relative coordinates
- defines the vertical and horizontal 'measures' of the arena in order to calculate the elapsed path etc.

Note: *when the Tracker can't recognize the subject's location, it's coordinates are [0,0].*

To define the Region of Interest graphically, click **From Image** button.

Log performance into file lets you log some of the Tracker's performance into files so it can be reviewed if there are some problems appearing.

Save screen copy at the end of experiment saves the graphical representation of the subject's path (as seen on the Tracker's main window) into PNG files. The files are saved into the same directory as the DAT files and they have the same name (except the extension).

1.6 Options – Window

Window tab provides settings for various options related to the program appearance.

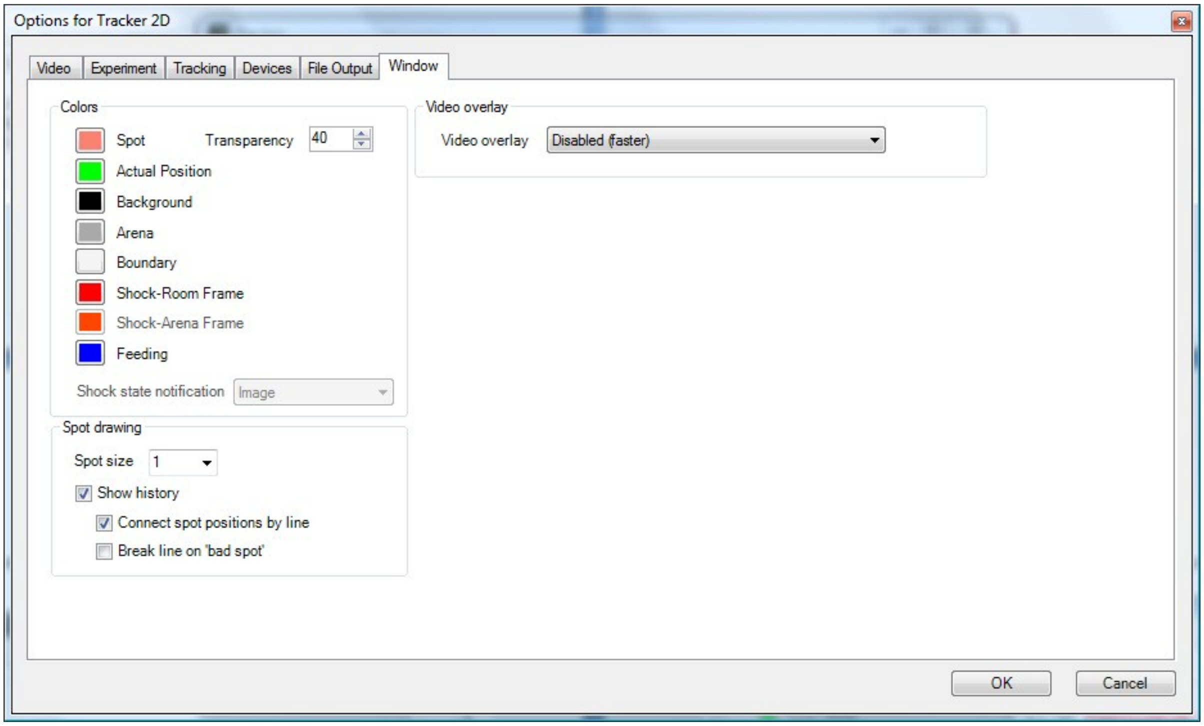


Figure: Window properties

Colors set the colors of the drawing into GUI.

Shock state notification sets the way of displaying the active Current Source in the GUI. It can either display an icon in the center of the active target - **Image** or flash the target border - **Flashing target**.

Spot sets the color of the subject's path.
Actual Position sets the color of the actual position of the subject.
Background sets the color of background (behind the arena).
Arena sets the color of the arena.
Boundary sets the color of the Region of Interest.
Shock-Room Frame sets the color of the subject's coordinates in the room frame when the shock is triggered.
Shock-Arena Frame sets the color of the subject's coordinates in the arena frame when the shock is triggered.
Feeding sets the color of the object coordinate when the Feeder is triggered.

In the **Spot drawing** box you set the properties related to the spots drawn in the GUI.

Spot size sets the size in pixels of the spot drawn in the GUI.

When **Show history** is checked all tracked spots would be drawn in the GUI from beginning till the end of the experiment.

When **Connect spot positions by line** is checked, subject positions would be connected by a line.

When **Break line on 'bad spot'** is checked, the connecting line would be broken (not continuous) when there would be a bad spot (spot searcher can't find the spot in the camera image).

Video overlay sets if the video image from the camera would be drawn on the background of the experiment screen. Three options are available:

- **Disabled** – no video overlay is drawn
- **Flexible size** – video overlay is drawn in the size of the application window

2 Running the experiment

After you create the experiment configuration and save it you may start the experiment. Before the experiment can be started, you have to set the output file names. You might either click **Acquire – Set Filenames** or click the shortcut button in the main window as shown in the following figure.

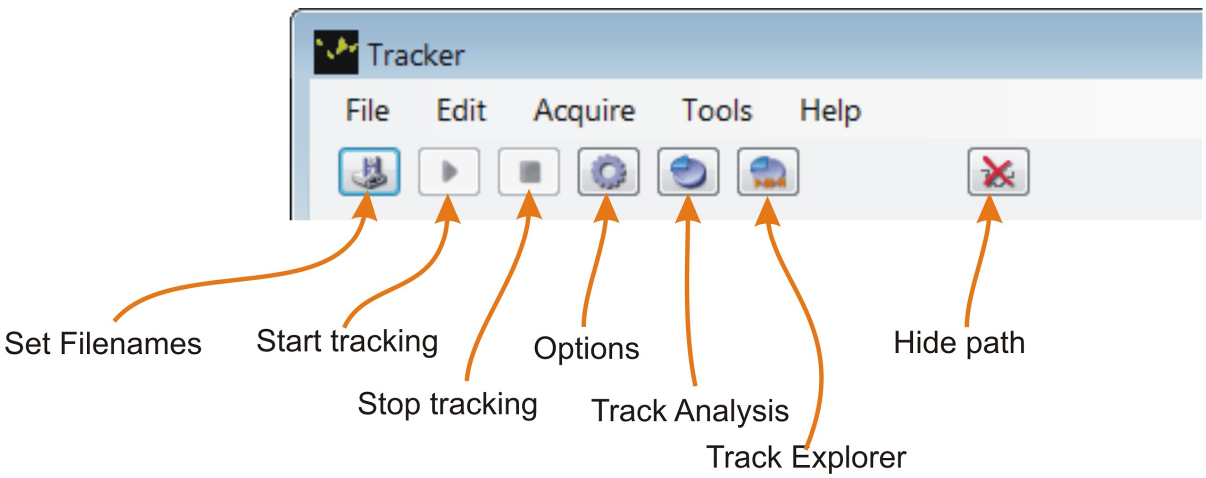


Figure: Shortcuts

Set Filenames dialog presents you the default settings (pattern) for the output files which you set in the configuration. In this step you might still change it e.g. to reflect the actual subject you are going to experiment with (RoomTrack_Room_Day1_Trial2_123.dat).

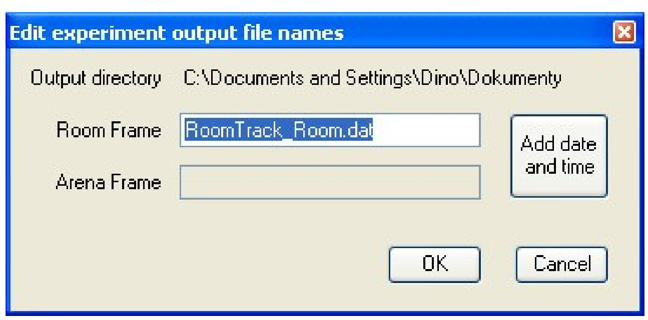


Figure: Edit file names dialog

After you set the file names, you might start the experiment straight from the main menu as **Acquire – Start** or using the shortcut button.

You may stop the experiment at any time by **Acquire – Stop** or by pressing the shortcut button.

If you don't end the experiment manually, it will stop after the **Experiment time** expires unless you've set the **Continue tracking after experiment time expires** in the experiment configuration. When this feature is active, a small notifying dialog will appear and optional sound alarm would sound.

When the acquisition is running, Tracker shows various types of online information, as shown in the following figures for different kind of experiments.

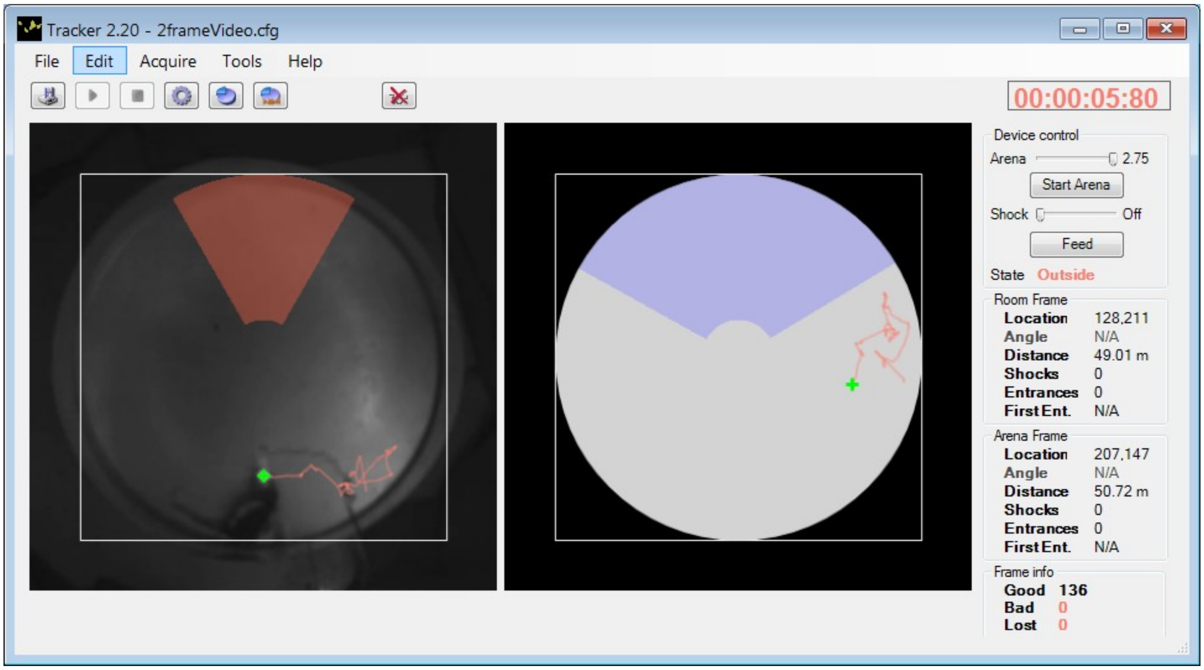


Figure: Tracker main window – Place Avoidance

Device control allows the control of the output devices during the experiment. Start Arena button can be used to manually start the arena rotation before the experiment is started. When this way of starting the rotation is selected, the arena is not stopped after the experiment ends and has to be stopped by pressing the button again. **Arena** sets the Rotating Arena speed and direction. **Shock** sets the level of current used for shocking. **Feed** button might be used for custom activation of the Feeder.

In the **Room Frame** box you can see the actual subject's **Location** in relative coordinates. If the **Angle** is being tracked (tracking of 2 spots), its value is also presented. Distance shows the total distance traveled by the subject from the beginning of the experiment.
Note: *When the subject location is calculated in relative coordinates [0..255] and the Tracker can't recognize the subject's location, it's coordinates are saved into the output file as [0,0] and they appear in the Room Frame box as **N/A**. If the Maximum labels feature is enabled and there are more labels than the specified maximum level, location is shown as **TMS** (Too Many Spots). See chapter 1.2 for more details about this feature.*

The same information is displayed for the **Arena Frame** when you run the 2 frame experiment.

Frame Info shows results from the tracking. **Good Frames** are all frames where the Tracker was able to find all predefined spots. Bad Frames are all frames where the Tracker can't find at least one spot (e.g. the subject spot and/or the reference spot). **Lost Frames** are all frames which were dropped (usually caused by loading the computer with some CPU time-demanding tasks other then tracking).

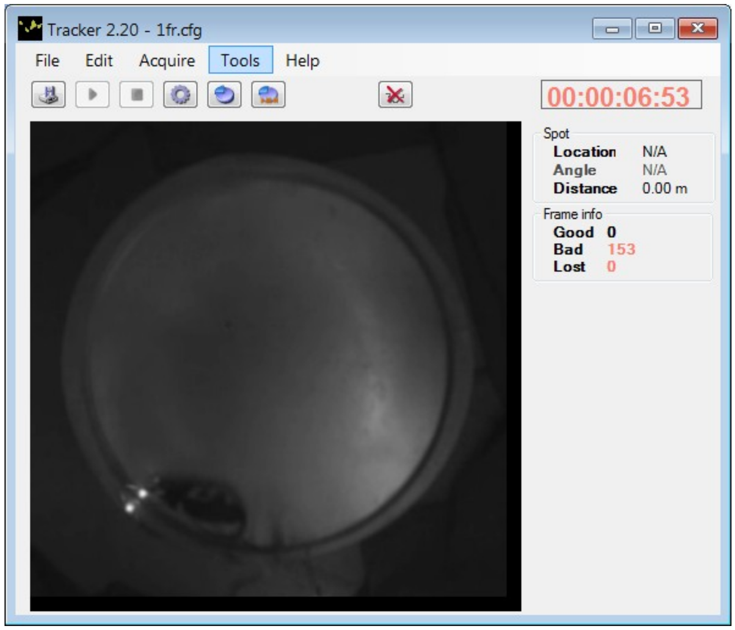


Figure: Tracker main window – Open Field

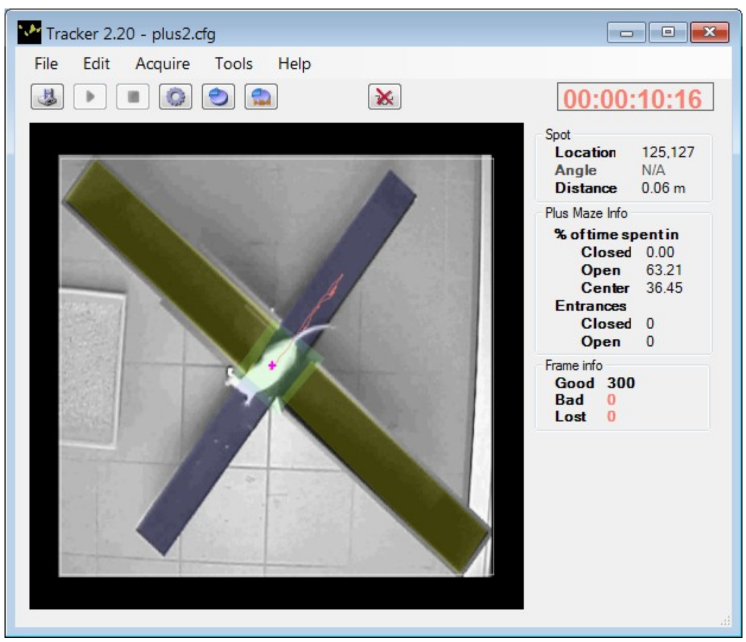


Figure: Tracker main window – Elevated Plus Maze

3 Analysis

There are two basic tools available for the Analysis

- **Track Analysis**
- **Track Explorer**

3.1 Track Analysis

TrackAnalysis analyses the track from a data file. The parameters it calculates depends on which experimental **paradigm** is defined in the **.dat** file header, and which options are enabled. TrackAnalysis was designed for **batch processing** of .dat files, which contain a time series of the coordinates of the tracked subject, events, and states of various devices.

TrackAnalysis requires the user to design their **analysis** by specifying which .dat files will be analysed together. The data corresponding to the list of files in an analysis are collected into a tabular format file (.tbl) for importing to spreadsheet or statistical software.

Each analysis is called a **Task** in the Track Analysis application.

3.1.1 Output Files

Three types of output files are generated by the TrackAnalysis. They are suffixed by **.ps**, **.tbl** and **.sum**. Each file contains essentially the same information but the data are organized in different formats. Each is considered in turn.

Examples of these files are provided. For simplicity, the same file was analyzed repeatedly as if it were several files.

3.1.1.1 PostScript files (.ps)

These are postscript files containing graphical depictions of the data in one file. The data are represented as vector graphics and can be imported into standard graphics software for further editing and figure construction.

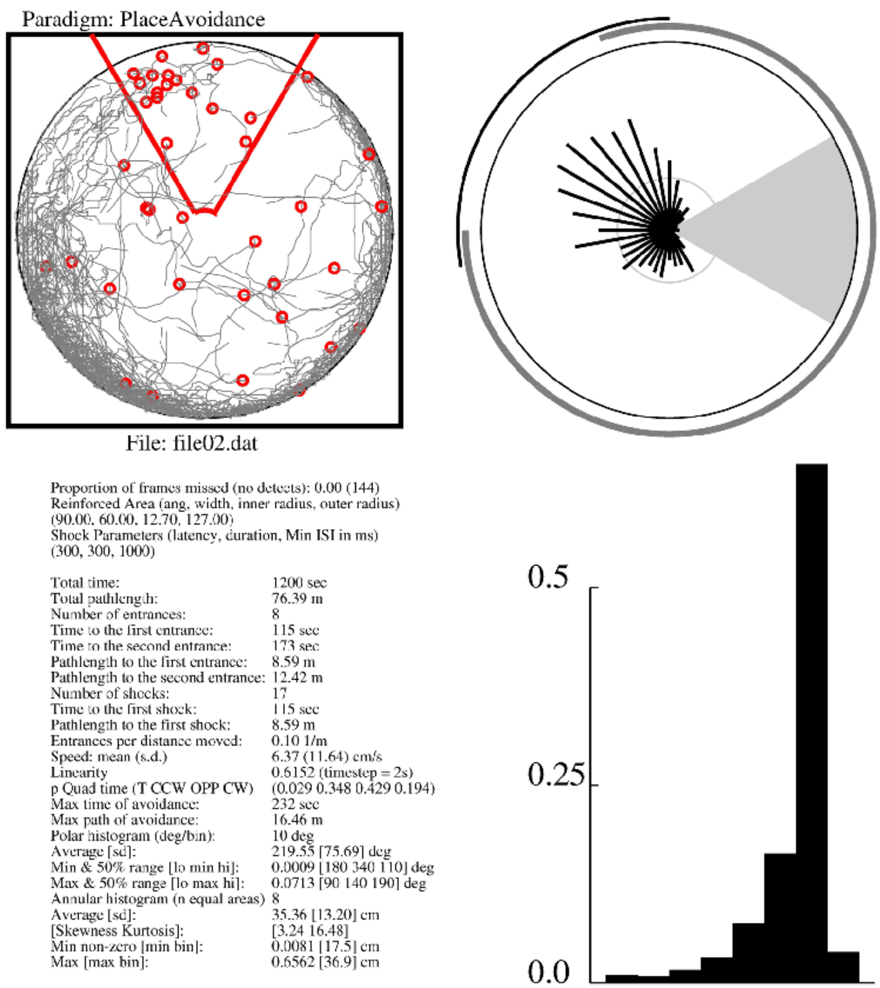


Figure: PostScript output from TrackAnalysis

The file has four parts :

1) A 2-D plot of the subject's track (top left on the figure above). If one or more "**sectors**" (arbitrarily defined regions of the environment) was defined in the .dat file then these are drawn too (an annulus-sector was defined in the example that is provided). If **events** were registered in the .dat file, then they are marked in the track representation (red circles in the example provided). Events are user-defined and will depend on the particular experiment. For example, shock, food delivery, lever presses, user-defined key presses etc.

2) Polar histogram of the subject's locations (top right on the figure above). The **histogram** is plotted in a standardized coordinate system such that if a sector was defined, then its angular center is the origin of the **polar coordinate system**. The sector's angular location is represented as a gray region. The environment is divided into **10° bins**. The proportion of time the subject was in each bin is represented by the length of the corresponding radial black line. Chance (on the assumption of homogeneity, i.e. 1/36) is marked by the thin gray circle in the center of the plot. The thin black circle represents the indicated proportion of the time (in the example provided it is 0.1). Two additional arcs are on the figure. The black arc represents the more preferred region and the gray arc represents the less preferred region. These regions are determined as follows: To find the more preferred region, the most visited bin is identified. Then the adjacent flanking bins are compared. The larger of the two is selected and added to the previously selected preferred bin. Then the two bins that flank the preferred set of bins are compared and the larger is selected again. This construction of the preferred region continues until the region accounts for 50% or more of the recording time. The analogous process is performed for the less preferred region. The least visited bin is selected and at each iteration, the lesser of the two flanking bins is selected until 50% or more of the recording time is accounted for.

3) Histogram of the annular distribution of the subject's locations (bottom right on the example above). The environment is divided into **8 equal area concentric annuli**. The central region is represented at the left of the histogram and the peripheral region at the right.

Various experimental parameters and measures are also provided on the page. Most are self explanatory, but some need further explanation.

Measures from the 2-D track:

- **Path length**: The path length measures are calculated by measuring distance between successive 1 second samples.
- **Speed:** Speed is calculated as the distance traveled in each 1 second interval. The average and s.d. are given.
- **Linearity:** linearity is the average ratio of the linear distance / integrated distance calculated each 2 seconds. The linear distance is the distance between coordinates sampled 2 seconds apart. The integrated distance is the integral of the distance between the successive coordinates in the 2 seconds. The successive coordinates were sampled each 16.7 ms. When Linearity = 1 the subject moved in a straight line. The closer to zero the more crooked the path.
- **P Quad time**: These are the proportions of time in each quadrant of the environment. When a sector is defined within one quadrant, then the proportion represents the proportion of time in the sector compared to the corresponding regions centered in each quadrant. The target (T), clockwise (cw), counter clockwise (ccw) and opposite quadrants are defined with respect to the sector, if it is defined.
- **Max time of avoidance**: Maximum time without entering the sector.
- **Max path of avoidance**: Maximum path moved before entering the sector.

Measures from the polar histogram:

- **Deg/bin**: Indicates the resolution of the plot.
- **Average [s.d.]**: The average vector and the angular s.d. of the histogram.
- **Min & 50% range [lo min hi]**: These values describe the less preferred region. Min is the value of the least preferred bin, which is 'min'. 'lo' and 'hi' are the bins that define the limits of the less preferred region.
- **Max & 50% range [lo max hi]**: These values describe the more preferred region. Max is the value of the most preferred bin, which is 'max. 'lo' and 'hi' are the bins that define the limits of the more preferred region.

Measures from the annular histogram:

- **Average [s.d.]**: The average annulus and the s.d. of the distribution. The skewness and Kurtosis of the histogram are given.
- **Min non-zero [min bin]**: 'min' is the proportion of time spent in the lowest non-zero bin. The location of the center of the bin is given by 'bin'
- **Max non-zero [max bin]**: 'max is the proportion of time spent in the largest non-zero bin. The location of the center of the bin is given by 'bin'

3.1.1.2 Table files (.tbl)

These files contain the **quantitative data in tabular form** for import into **spreadsheet** or **statistical software**. Each data file is represented by entries in one row. The various measures are in columns. By organizing which files are processed by TrackAnalysis and in what order, the organization of the data for analysis of an experiment can be accomplished in batch mode with a single command.

filename	frame	total time [s]	# of entr.	time to first entr. [s]	path to first entr. [m]	entr. per dist. [1/m]	# of shocks	time to first shock [s]	path to first shock [m]	speed [cm/s]
file01.dat	?	1200	12	40	3.35	0.19	29	40	3.35	5.31
file02.dat	?	1200	8	115	8.59	0.1	17	115	8.59	6.37

(**Note:** table has been truncated on the right side)

3.1.1.3 Summary files (.sum)

These files contain the **quantitative data as text**. The data from all the data files submitted to TrackAnalysis for analysis are represented in the file. These files summarize an experiment and are convenient for inserting into laboratory notebooks.

3.1.2 Running Track Analysis

The main concept of the TrackAnalysis is based on setting up the **Tasks**. **Each task is an 'analysis' comprising a list of files to analyze**. Each task is specified by a single output directory, where the output files from analysis are stored as well as the list of files for analysis. This way you can set up multiple tasks for each experimental day, each with several analyzed files (e.g. different experimental objects).

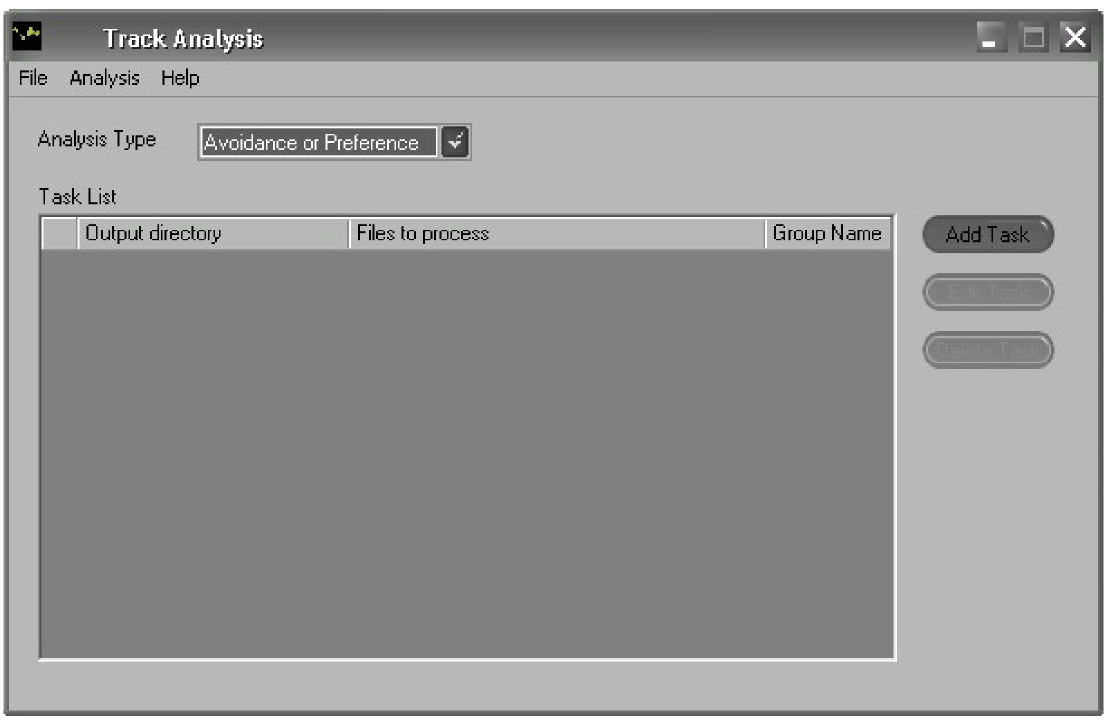


Figure: Track Analysis main window.

In the previous figure you can see the main application window with several controls :

- **Analysis Type** – sets type of the analysis (paradigm), choose “Avoidance or Preference” for Place Avoidance or Place Preference type of experiments, “Open Field” for Open Field experiments and “Watermaze” for Watermaze experiments.
- **Add Task** – adds single tasks with single or multiple analyzed files
- **Edit Task** – edits selected task on the cursor position
- **Delete Task** – deletes selected task on the cursor position

3.1.2.1 Adding a Task

When you press the **Add Task** button, a new window is opened :

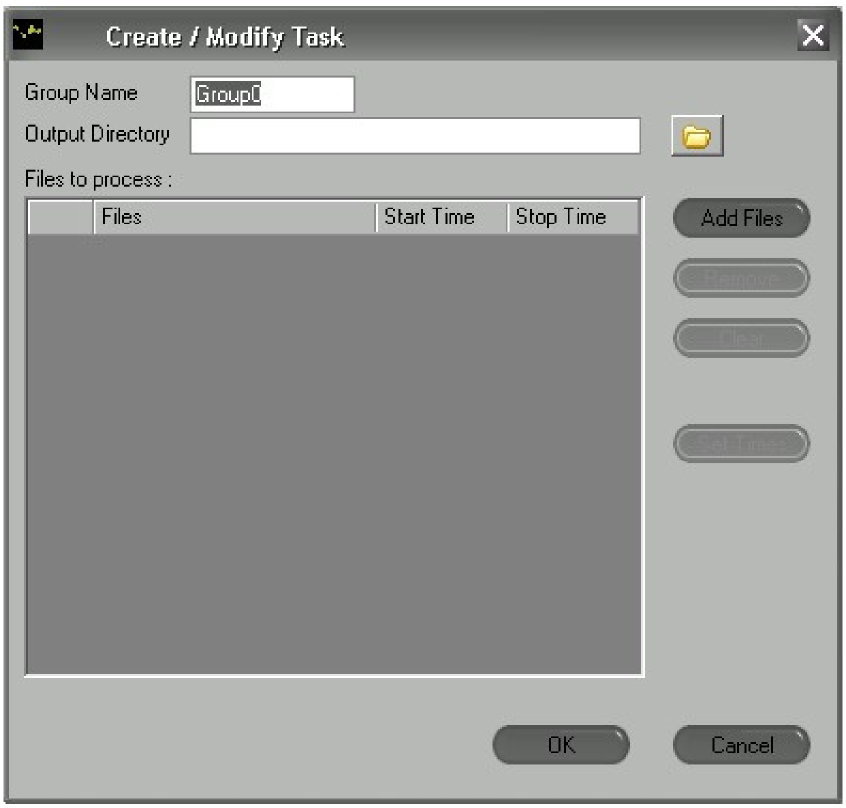


Figure: Adding Task

First you edit the **Group Name** if you don’t like the one being automatically set. Each task needs to have its name which helps you navigate between output files, when you process multiple tasks. You may choose any string with small or big letters and numbers (e.g. “day1”). Please do not use special characters as (/ < > . ? : ; ‘ “ \ | [{] } ` ~) to avoid problems with analysis.

Now you need to set the **Output Directory** for the output files from the analysis (PostScript files, SUM files, TBL files and LST files with list of files being analyzed).

You may choose any directory in your computer (or at a network volume); you should only make sure that you have **permissions** to write to such a directory. After analysis is started, multiple folders will be created to hold different types of the output files.

Click the Folder icon to choose the output directory. Browse for the existing directory or click Create New Folder icon to create a new folder for the output files.

Now you are ready to add file(s) for the analysis. Click **Add Files** to browse for the files and select one or many of them.

Note: *you may choose multiple files by drawing a selection rectangle above those files when holding the left mouse button. Another possibility is choosing only some of the files by holding the CTRL key on your keyboard and clicking those files. Another option is to hold the CTRL key on your keyboard, clicking the first file you want to add, then pressing also the SHIFT key on your keyboard while still holding the CTRL key and click the last file you want to add. All files between the first and the last files will be added into the task.*

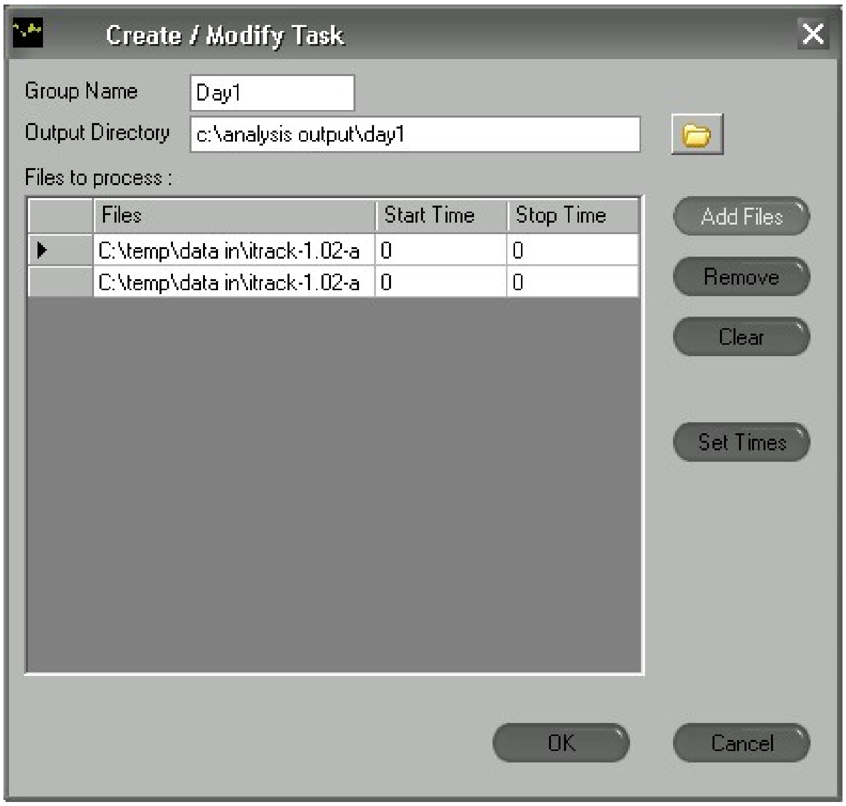


Figure: Adding files

You can set the **Start Time** and the **Stop Time** for each file, where the analysis is made. E.g. when you set Start Time as "0" and Stop Time for "600" for the file, only the first 600 seconds (10 minutes) will be analyzed from this file. If you wish to set global Start and Stop Times for all the files in the task, press **Set Times** button to set them.

If you set the Start Time and Stop Time both for 0, no limitation will occur and files will be analyzed completely.

After you press the OK button, the task is added into the **Task List.**

3.1.2.2 Running Track Analysis

After you set all the tasks you want to run in a batch job, choose **Analysis – Run Analysis** from the main window to start the analysis.

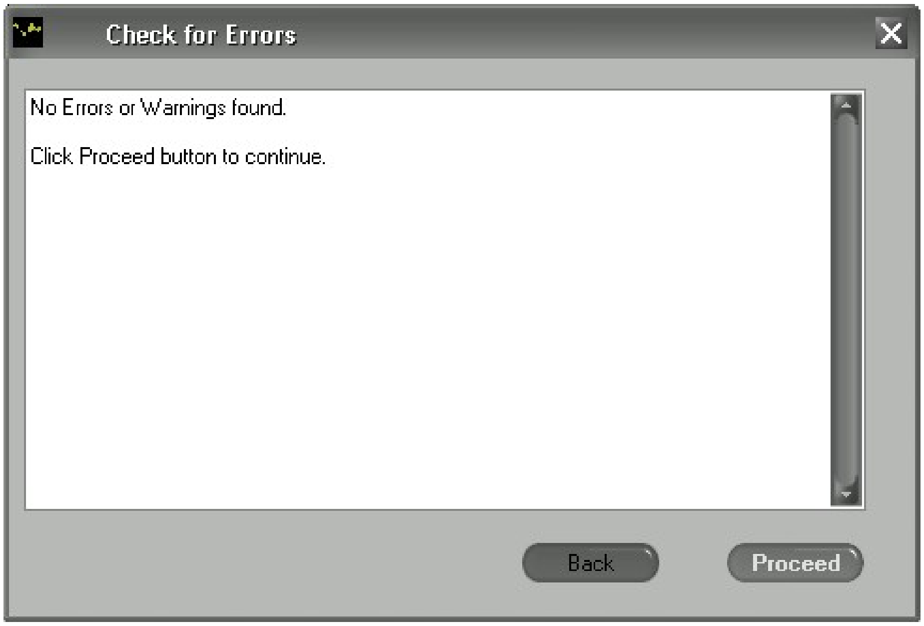


Figure: Running the batch job

Before you can proceed with the analysis, several tests are done to avoid the possible errors; those tests include checking for the existence of the files and folders, checking the writing permissions and checking for the file overwriting.

If there is some serious problem e.g. the output directory doesn't exist, it is not writable or the input files don't exist, it is reported as an **error**. In this case, you are not able to continue running the task, and you need to solve the issue first and then run the analysis again.

If only some files are being overwritten, they are reported only as **warning**, so if you don't care about files being overwritten, you may continue running the batch.

If no errors are reported, you may continue to run the analysis by clicking the **Proceed** button. TrackAnalysis will proceed to all the tasks and create the outputs exactly as you set.

3.1.2.3 Storing analysis configuration

Sometimes it may be useful to store the analysis configuration (tasks you set up for the analysis). One example may be adding some new files to the analysis at a later time and then running the analysis again or changing analysis properties (i.e. how much of the files are analyzed, etc.).

Functions for opening and saving the analysis configuration are located under the File bookmark in the main application menu. Configuration is stored in **.tac** files (which stands for "Track Analysis Configuration").

3.2 Track Explorer

The Track Explorer is an analysis tool designated for time-lapse analysis of the ITS output data. Based on the information about the subject's position at a time it is able to reconstruct the path, play it in real time and calculate various properties such as the 2D histogram, total path or subject's presence in a target of arbitrary shape.

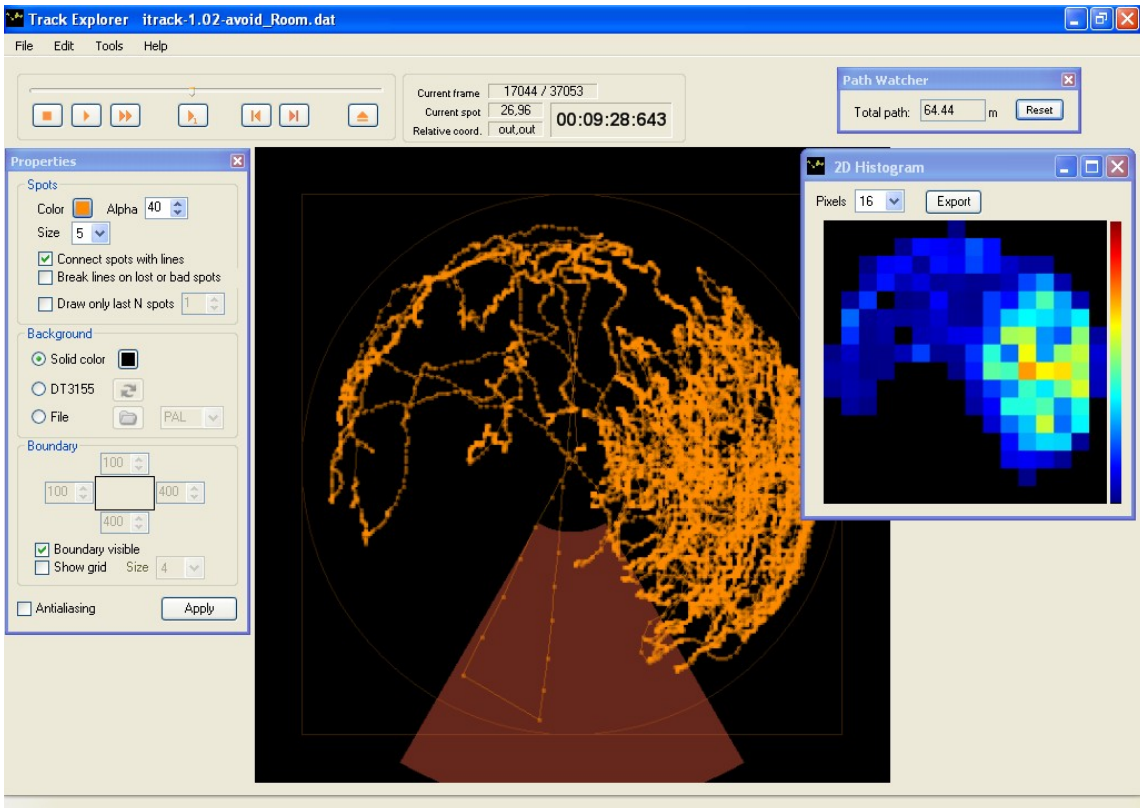


Figure: Track Explorer main screen

3.2.1 Main Window

Track Explorer is basically controlled as a standard VCR. You open an ITS data file (.dat) either by the menu function **File – Open Data File** or by pressing the Open file button at the navigation bar.

After the dat file is selected, it gets loaded into the application and some basic information is shown on a screen.

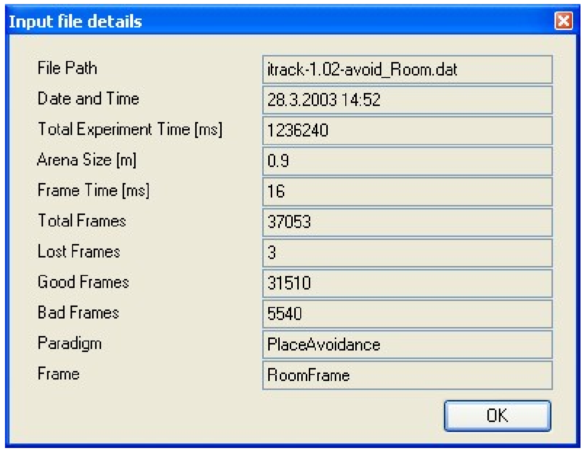


Figure: File information

This informs you about the properties, which were set before the data were acquired (i.e. the Arena Size) but also about the quality of the data, i.e. the number of lost frames (Lost Frames), number of frames with a subject detected (Good Frames) or a number of frames without the subject detected (Bad Frames).

You may recall the File information window at any time by clicking **File – File information**.

For the navigation through the file you use the Navigation bar.

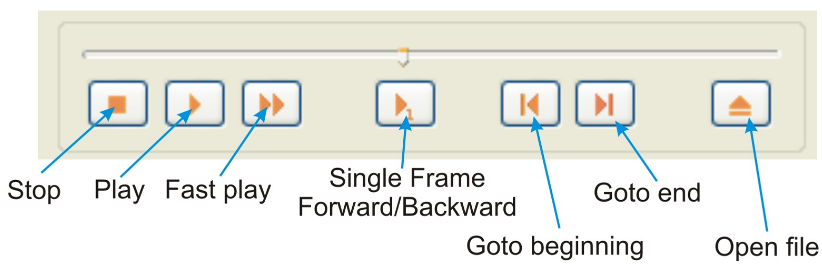


Figure: Track Explorer navigation bar

You may also drag the scroll bar above the controls for easy navigation across whole the file.

Notice, that when you move through the file, information in the status window changes.

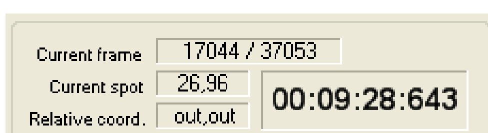


Figure: Status bar

Current frame shows the actual frame position in a file. **Current spot** shows the actual spot location in relative coordinates. **Relative coordinates** shows your mouse position converted into the relative coordinates. This might be useful i.e. when the precise polygon targets are drawn.

Note: *you may export the actual track into a file by right clicking the graphics area and choosing **Save Image.***

3.2.2 Properties

3.2.2.1 General Properties

General application properties are available under **Edit – Properties**.

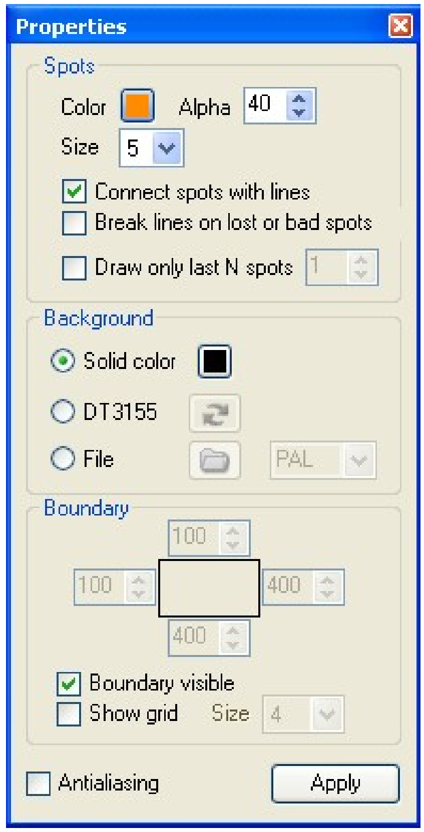


Figure: Drawing properties

Spots box sets how the reconstructed path is drawn. The following properties are available:

- **Color** – sets the color of the reconstructed spots and path.
- **Alpha** – sets the alpha (transparency) of the spots and path. When there is a lot of data to draw over the screen, it may be useful to set the lower alpha so that another track drawn across the existing one would add an intensity to the previous so you are still able to distinguish the path orientation.
- **Size** – sets size of the drawn spots.
- **Connect spots with lines** – when checked, all drawn spots are connected with a line.
- **Break line on lost or bad spots** – when checked, the line connecting the spots is broken when there is no information about the subject position for the particular video frame.
- **Draw only last N spots** – enables to draw only last N spots.

Background box sets the background properties.

- **Solid color** lets you set the solid background for the main window.
- **DT3155** lets you grab actual image from the PC's DT3155 framegrabber.
- **File** lets you load a background from an image stored in a file.

Note: *For both DT3155 and File you need to choose appropriate video format (NTSC or PAL)*

Boundary box allows you to match the relative positional data to the absolute pixel coordinates of an image from the framegrabber or a file. You may use the same numbers as you used in the ITS for specifying the region of interest.

- **Boundary visible** hides the boundary when unchecked.
- **Show grid** enables drawing of a square grid over the analysis window. Size property sets the number of pixels in each grid cell.

3.2.2.2 Target Properties

To be able to run place specific analysis of the trial, you have to setup the targets. You access the window by **Edit – Targets**.

There are three basic target shapes which you might choose in **Target Properties** box – Arc, Circle or Polygon. Every shape has its specific parameters. Arc and circle properties are set using available boxes, polygon is drawn in a main window using a mouse. To add the active target to the setup, press the **Add** button. To remove target from the set of targets, press the **Remove** button. To update target's properties, press the **Update** button.

Each target may have a specific **Color** and **Alpha** to be easily distinguished from the others when drawn on the main screen.

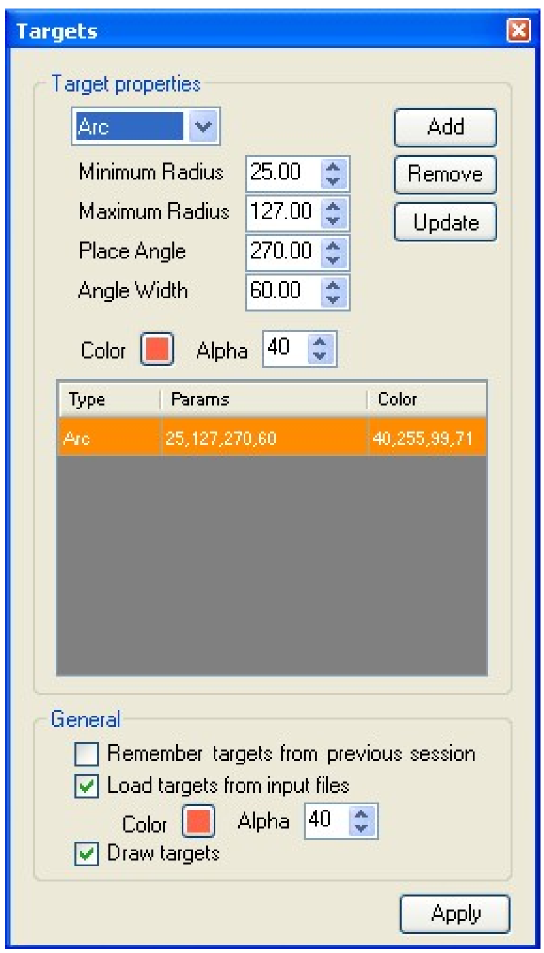


Figure: Target properties

The General box sets some of the general properties of the targets:

- **Remember targets from previous session** – when checked, application stores all the targets you set and uses them when the application is restored next time.
- **Load targets from input files** – because ITS files store also the information about the targets used during the experiment, the application may load these targets if the box is checked. You may choose specific color.
- **Draw targets** – unchecking this box disables drawing of targets in the main window.

3.2.3 Tools

Tools are basically application plugins which integrate specific function. All tools can be find under the **Tools** menu in the main application window.

3.2.3.1 Target Presence Watcher

Target presence watcher is designed to log the subject's presence in a particular target. You are free to setup as many watchers you want, i.e. each for a single target you create.
Each watcher has a box at the top, which specifies the actual target with its properties listed. You also may edit the Entrance and Exit latencies to avoid counting very short target entering or escaping as a new event.

Entrance latency is basically a time constant which is counted when the subject first appears inside the target. When the subject escapes the target before the latency expires, the event is not logged. If it remains inside the target after the latency expires, the event is being logged (with a time of the first target entrance).

Exit latency is a similar time constant, but it is active when the subject leaves the target, i.e. it is being counted when the subject first leaves the target. If the subject returns inside the target before the latency expires, the event (subject presence inside the target) is not finished and the presence time continues counting. When the subject stays outside the target after the latency expires, the first time when subject left the target is logged as the exit time.

Setting the entrance and exit latency to 0 disables them.

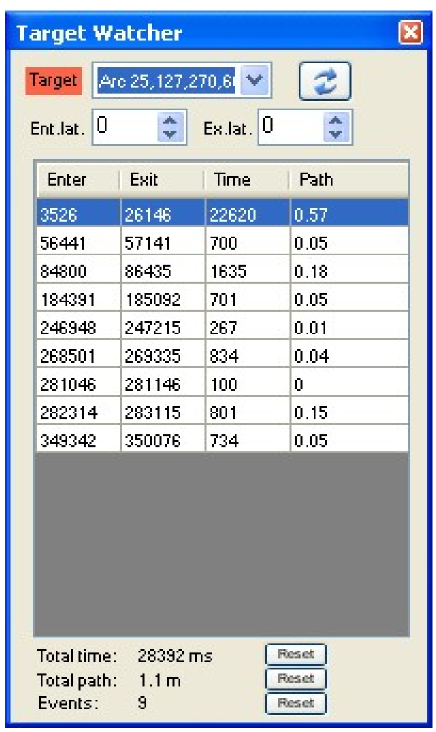


Figure: Target presence watcher

As you may see on the figure above, all events are logged in a table, including the entering time, exit time, time spent in a target and a path crossed inside the target.

In addition to this, some global parameters are counted which include all the events related to a specific target – total time, total path and number of events. All counters may be reset as needed.

3.2.3.2 Target Path Watcher

Path Watcher is a simple path integrator, which integrates the path crossed by the subject.

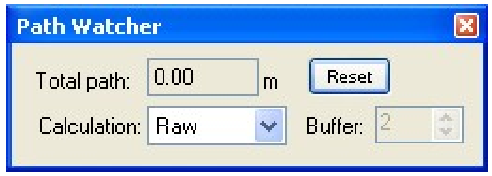


Figure: Path Watcher

There are three modes for path calculation:

- **Raw** – in raw mode, every position is used for the path calculation
- **Skip** – in skip mode, a number of positions set by a **Buffer** property is skipped so the path is calculated only from some positions
- **Average** – in average mode, all locations are used for the path calculations, but they are averaged based on the **Buffer** property.

3.2.3.3 Histogram

2D Histogram is a tool designed for monitoring a spatial distribution of the subject's location inside the arena.

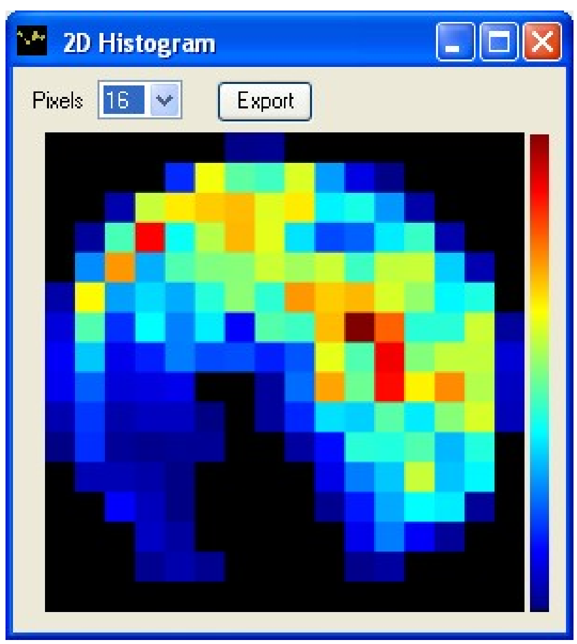


Figure: 2D Histogram

You may change the resolution of the histogram by changing the **Pixel** property.

If you need the exact values of the subject presence inside a specific pixel, you may click the **Export** button and save the values in a tab-delimited file.

Note: *you may also save the histogram as an image into a file by right-clicking the graphics area and choosing **Save Image.***

4 Tools

4.1 Port Tester

ITS Port Tester is used to troubleshoot the output devices. **Current Source**, **Rotating Arena**, **Feeder** and **Sync** might be set on and off in order to check their functionality.

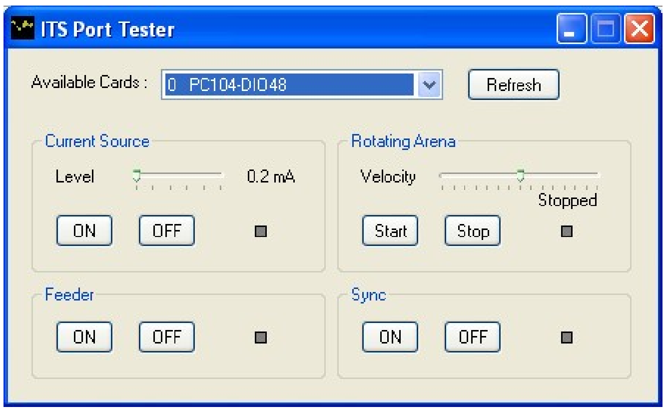


Figure: Port Tester