

Tracking and Analyzing Behavior with CineLyzer™

動物行為影像分析軟體

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Agenda

- 基礎介紹
- 應用原理
- 數據收集與分析
- 實做

Cinelyzer是?

- 自動化的動物軌跡追蹤及行為分析軟體
- 所有迷宮都適用（Water maze, Plus maze, radial arm maze, Open field…）
- 自動/手動排程＝自由度高的實驗設定
- 多種追蹤方式（全身追蹤,Color marker,Head）
- 精準的物件判別技術（動態背景功能）

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適合實驗種類

- Alternating T-Maze（T型迷宮）
- Radial Arm Maze（8爪/放射狀迷宮）
- Elevated Plus Maze(高架十字迷宮)
- Open field（開放空間）
- Water Maze（水迷宮）
- Novel Object Tasks（物件辨識）
- Place Preference（偏好實驗）
  - Real-Time Place Preference with optogenetics
- Pavlovian Conditioning
- Tracking in Bussey Touchscreen Chambers

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Cinelyzer功能

紀錄影片/Recording	軌跡追蹤/Tracking	分析數據/Analyzing
Features	Features	Features
Record Digitized video of freely-behaving animals in light or dark conditions	Define specific arenas, zones, and zone sequences – **flexibly** alter placement **between** trials and post-hoc!	Track trajectory visualizations displayed on arena/zone configurations
Record to .AVI files with MPEG compression for reduced file size	**Tracking via three methods**: whole body (contour), LED, or (up to 12) color markers	Automatic computations for arena, zone and zone sequences: time, distance traveled, speed, latency to zone/sequence entry
Start/Stop recording manually or from external devices	**Dynamic Zones:** track areas around markers (LED and Color Marker) – useful for social interaction tasks!	**Overlays Feature** – apply settings from a different experiment to the current experiment for quick comparisons
Comprehensive Database – store subject ID and experimental variables with video	Behavior Events: speed, location, head direction, point to point angles of motion	Compare individual sessions or group based on experimenter-defined variables – graphical outputs
Offline frame-by-frame playback	Use behavioral data to trigger external devices with TTL pulse	Mean, SD computed and displayed in table format – Export to .csv

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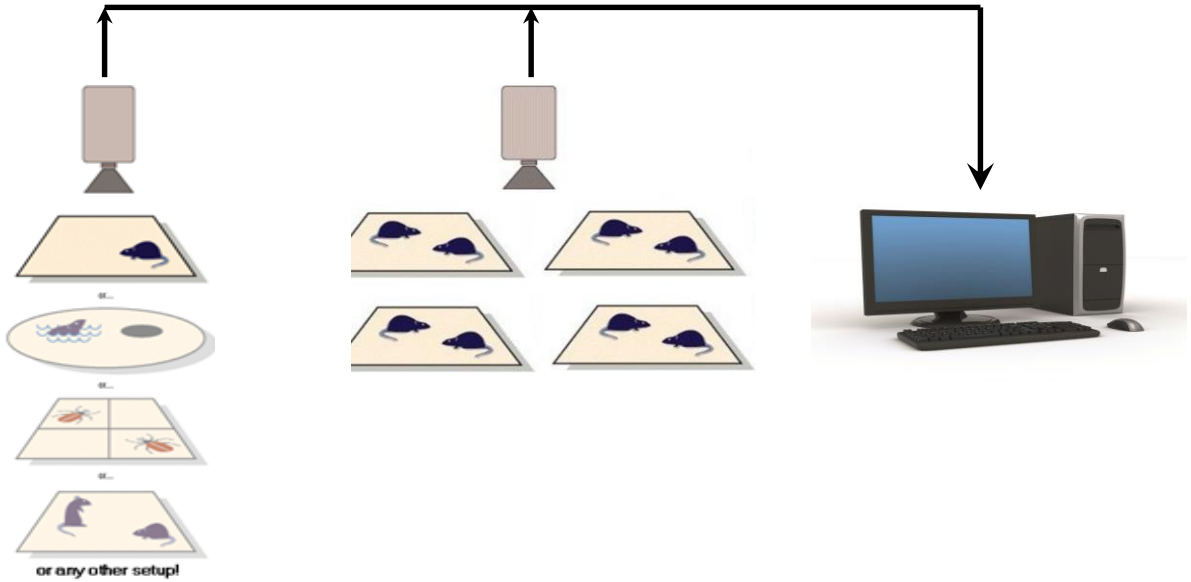
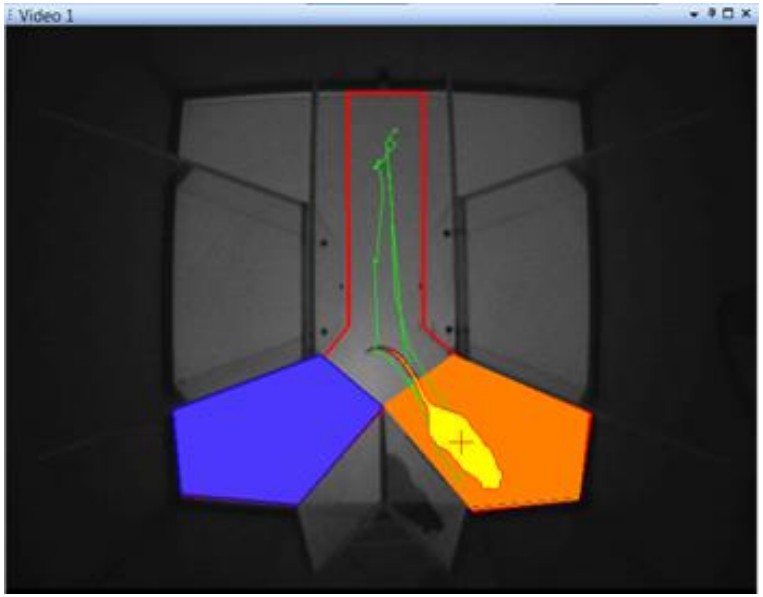
基本架構

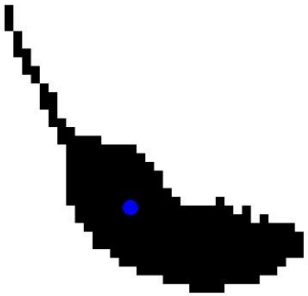


- Firefly® cameras
  - Model FMVU-03MTC-CS (for color imaging)
- All cameras pre-configured with a 1/3" High-Resolution varifocal lens (3 to 8mm)

如何抓取影像特徵?



- 物件偵查
  - 利用像素原理
- 物件判別
  - 移除多餘雜訊干擾
  - 抓取物件面積
  - 也可辨識多個物件
- 物件特徵分析
  - X, Y 位置 (per sample)
  - 計算區域停留時間，移動距離，速度(平均速度)，進出次數



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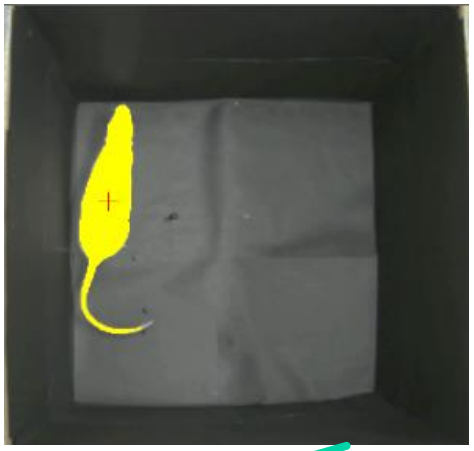
追蹤模式

Advanced Color Markers (up to 12)

Color Markers (up to 5)

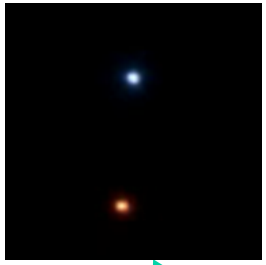
Whole body (Contour)

Object Contour Tracker	
Background Image	Ready
Use Background	☑
Threshold	33
Close Contour	0
Detail Filter	0
Min Object Size	5
Motion Measure	☑ PLX



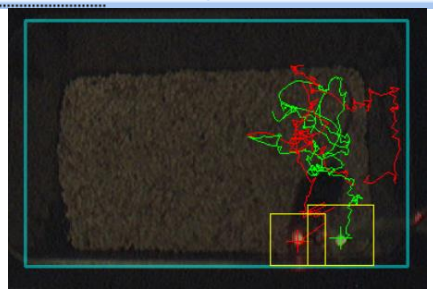
LED (up to 3)

LED Tracker	
Pure Colors	☑
Min Object Size	5
Filter Details	☐
Threshold	130
LED 1	☑ PLX
Track	
Cent.Grav.	
Contour	
Fill	
LED 2	☐ PLX
LED 3	☐ PLX



Tracking 1

Color Markers Tracker	
Pure Colors	☐
Min Object Size	5
Filter Details	☐
Marker 1	☑ PLX
Mrk 1 Threshold	230
Marker 2	☑ PLX
Mrk 2 Threshold	230
Marker 3	☑ PLX
Mrk 3 Threshold	230
Marker 4	☐ PLX
Marker 5	☐ PLX



Tracking in both normal and infra-red (IR) illumination

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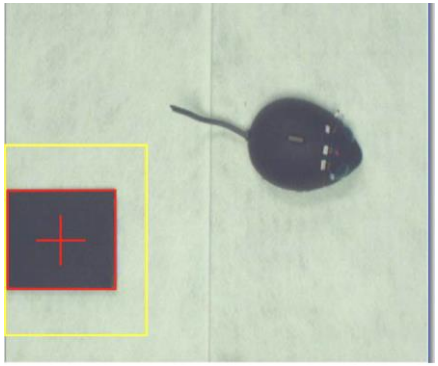
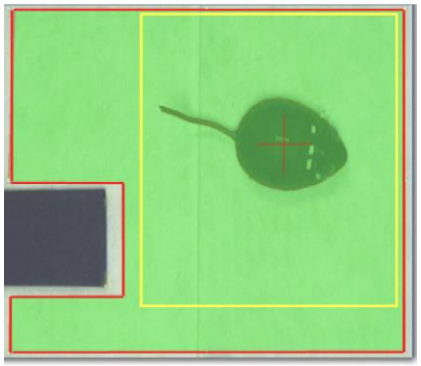
動態背景功能





底圖 － 即時影像 ＝ 物件

前一張影像 － 即時影像 ＝ 物件

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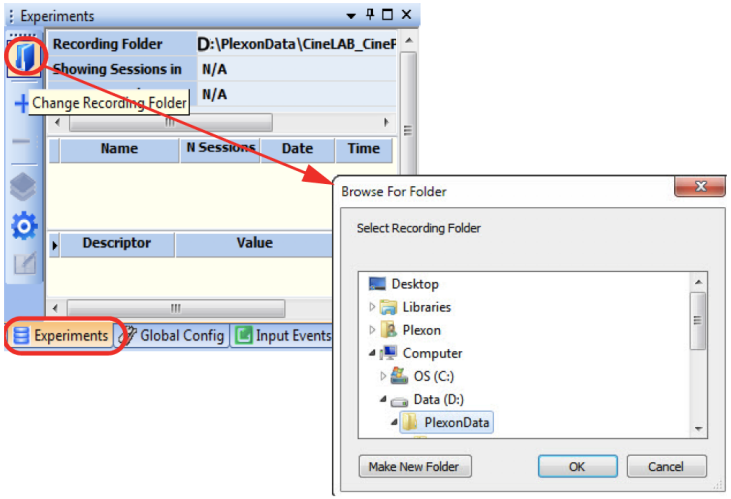
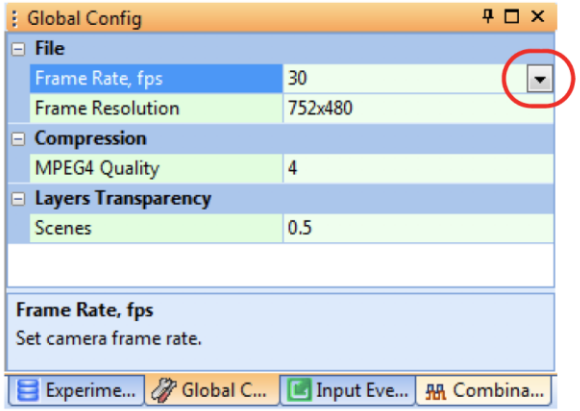
軟體設定

先點選左上角選單，選擇攝影機

確認儲存資料夾



確認攝影機設定



Dropdown lists are available for **Frame Rate, fps** and **Frame Resolution**

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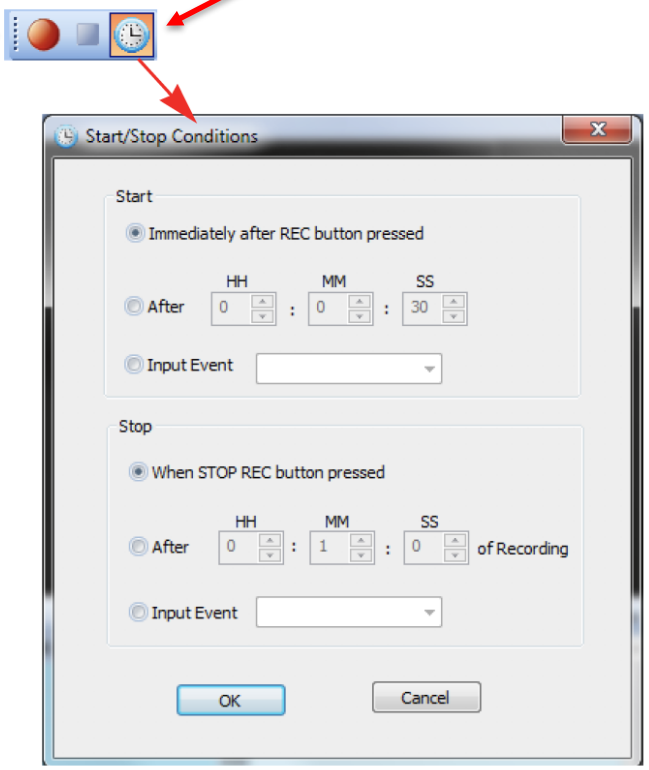
軟體設定

可手動或排程開始/停止實驗

開始/停止紀錄按鈕

排程紀錄按鈕



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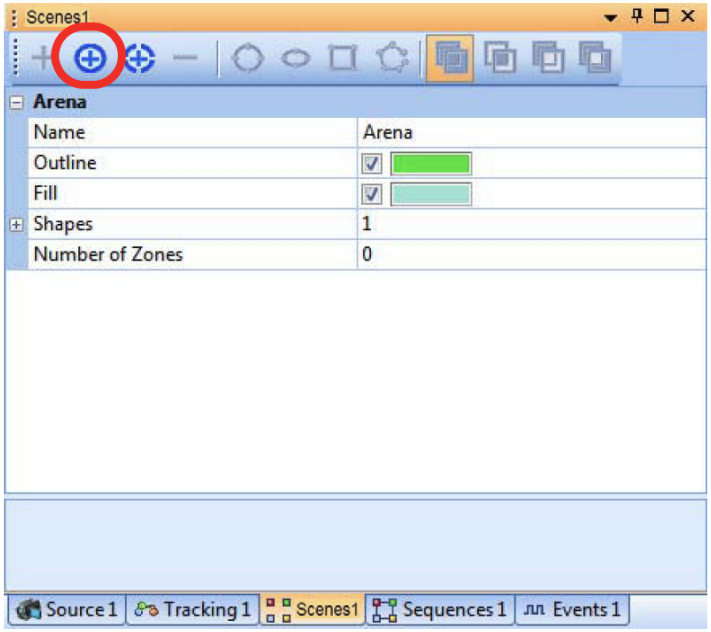
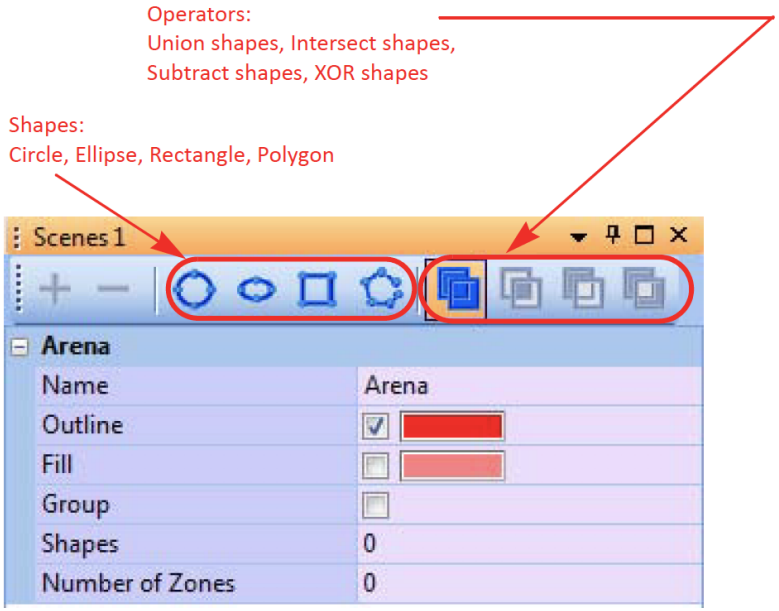
畫追蹤區域

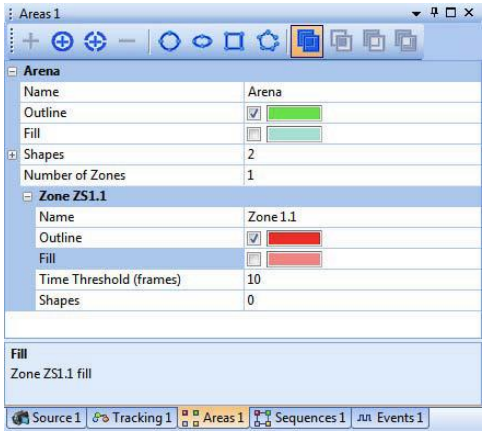
Adding a Static Zone

先點選 加上Arena(分析範圍)



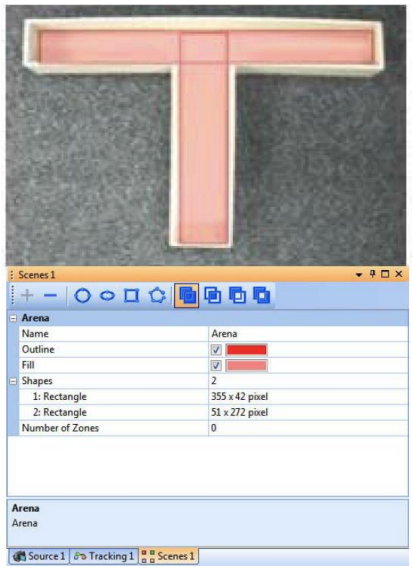


選形狀符號畫範圍

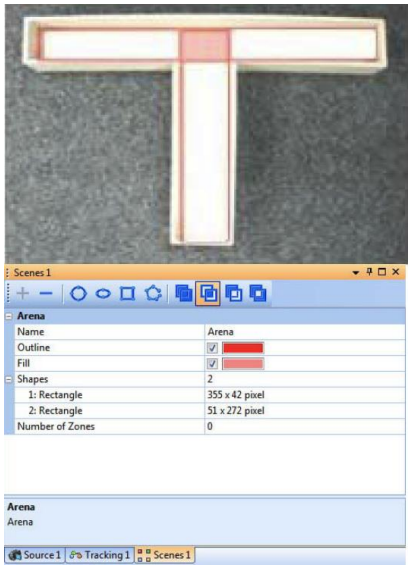


Zone定義

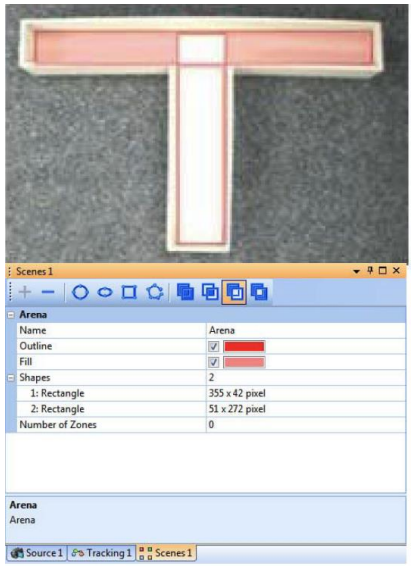
Union shapes



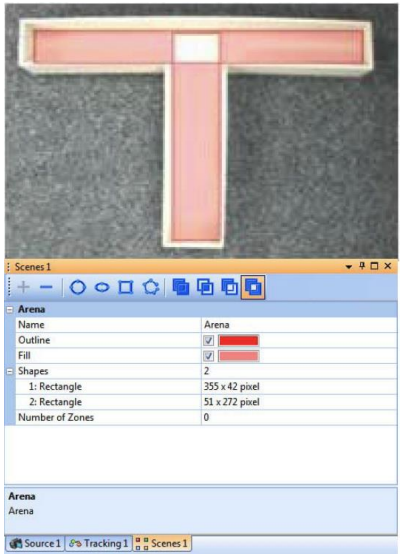
Intersect shapes



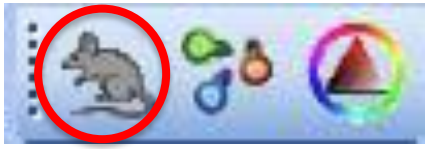
Subtract shapes

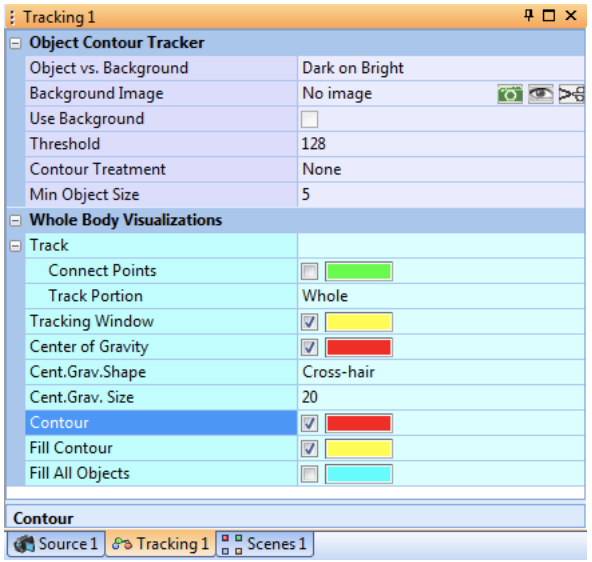


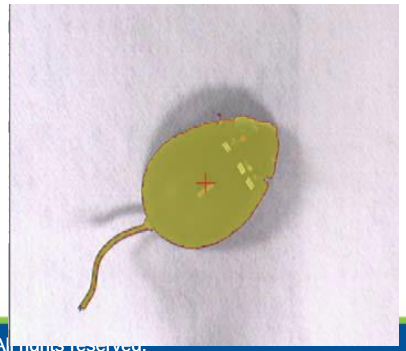
XOR Shapes



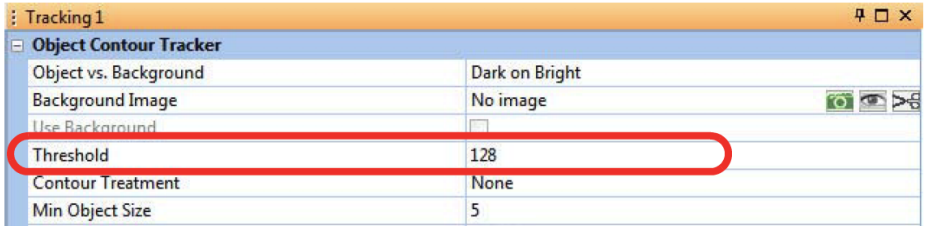
對比追蹤設定
Object Contour Tracking





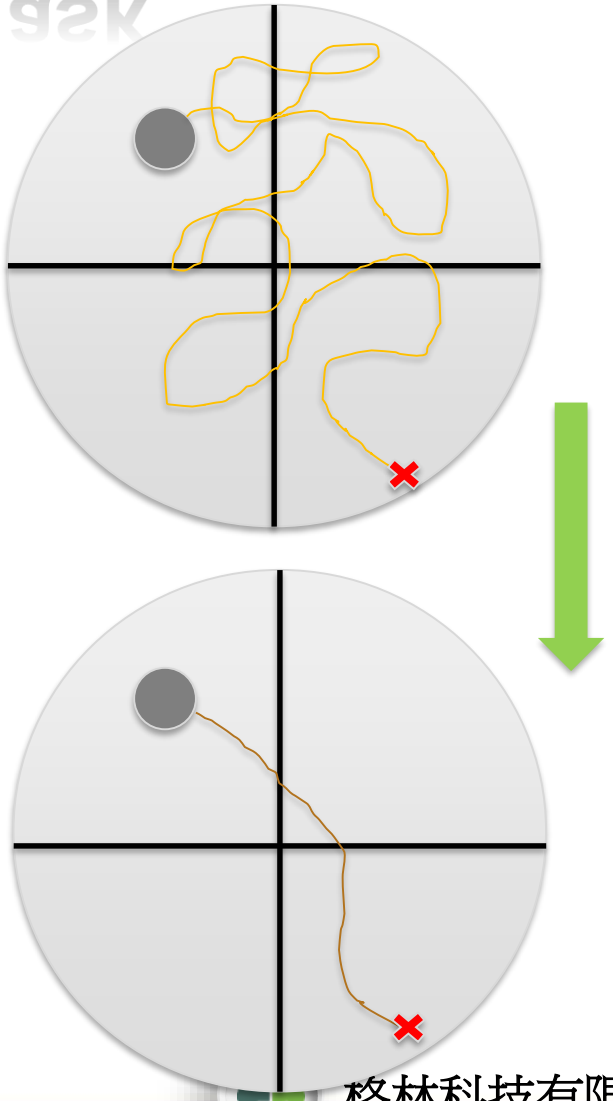


調整參數
(1)黑鼠(Dark on Bright)
or
白鼠(Bright on Dark)
(2)閥值 (Threshold)偵測靈敏度
(3)Mini Object Size:最小追蹤物件大小
(5)Background Image:建議使用



水迷宮
Water Maze Task

- 空間記憶功能測試
- 大鼠/小鼠不喜歡水
  - Place in circular “maze”
  - Swim to escape
  - Escape platform in one of 4 quadrants
- 動物利用周邊的提示標籤/物件去更快的找尋並抵達平台



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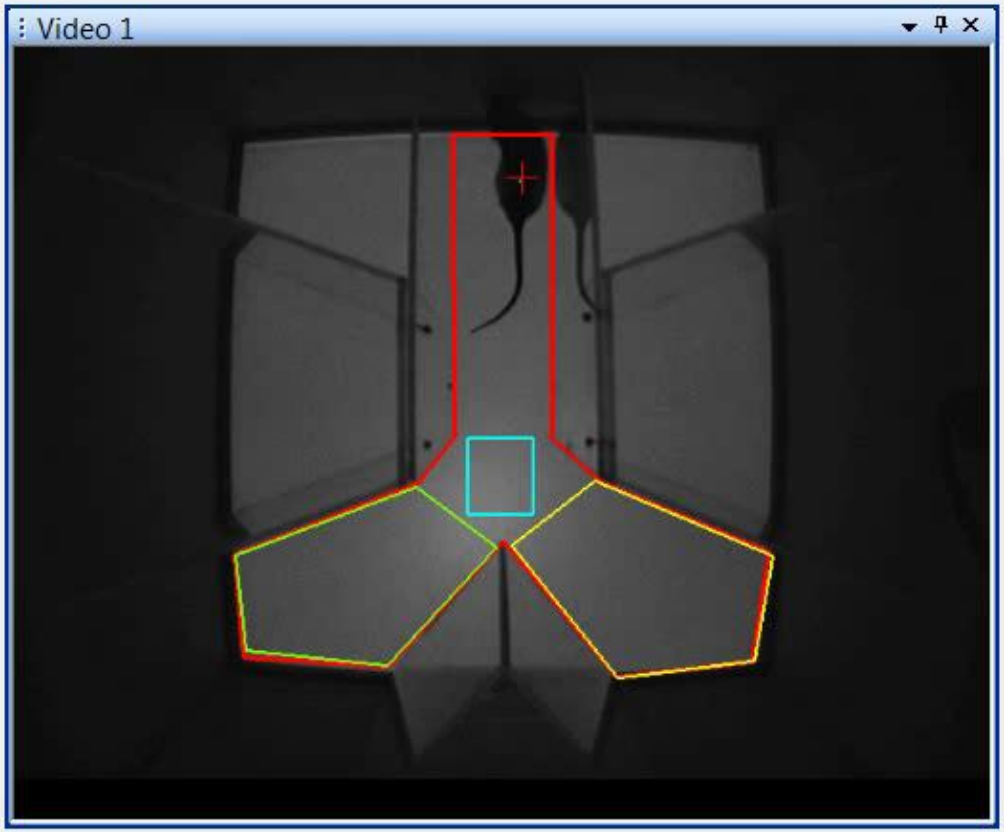
Zone Events

Scenes 1

Arena	
Name	Arena
Outline	
Fill	
Shapes	1
Number of Zones	2
Zone ZS1.1	
Name	Left
Outline	
Fill	
Time Threshold (frames)	10
Shapes	1
1: Polygon	174 x 122 pixel
Zone ZS1.2	
Name	Right
Outline	
Fill	
Time Threshold (frames)	10
Shapes	1
1: Polygon	164 x 121 pixel

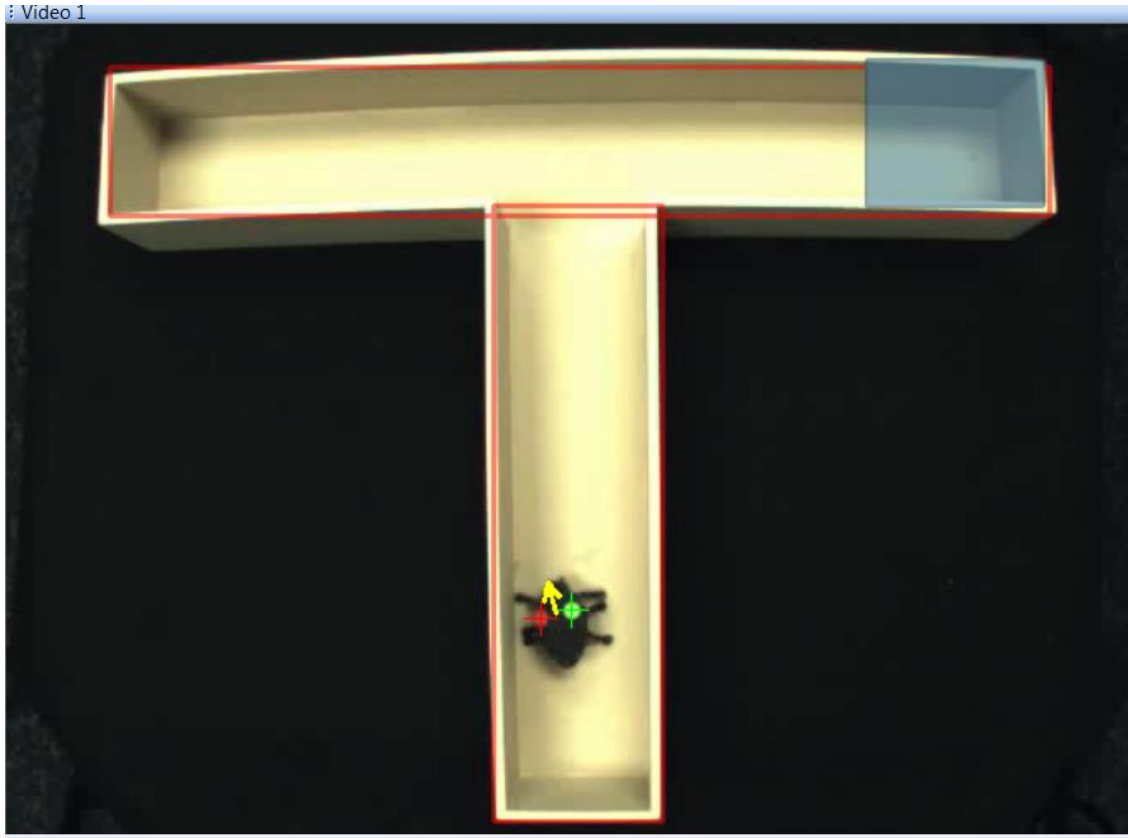
-Static Zones

-Zone Events – enter/exit zone



T Maze Task
T型迷宫

- Test of working memory
- Alternate between left and right arms to receive a reward
  - Must remember the previously-visited arm to make correct response on subsequent trial



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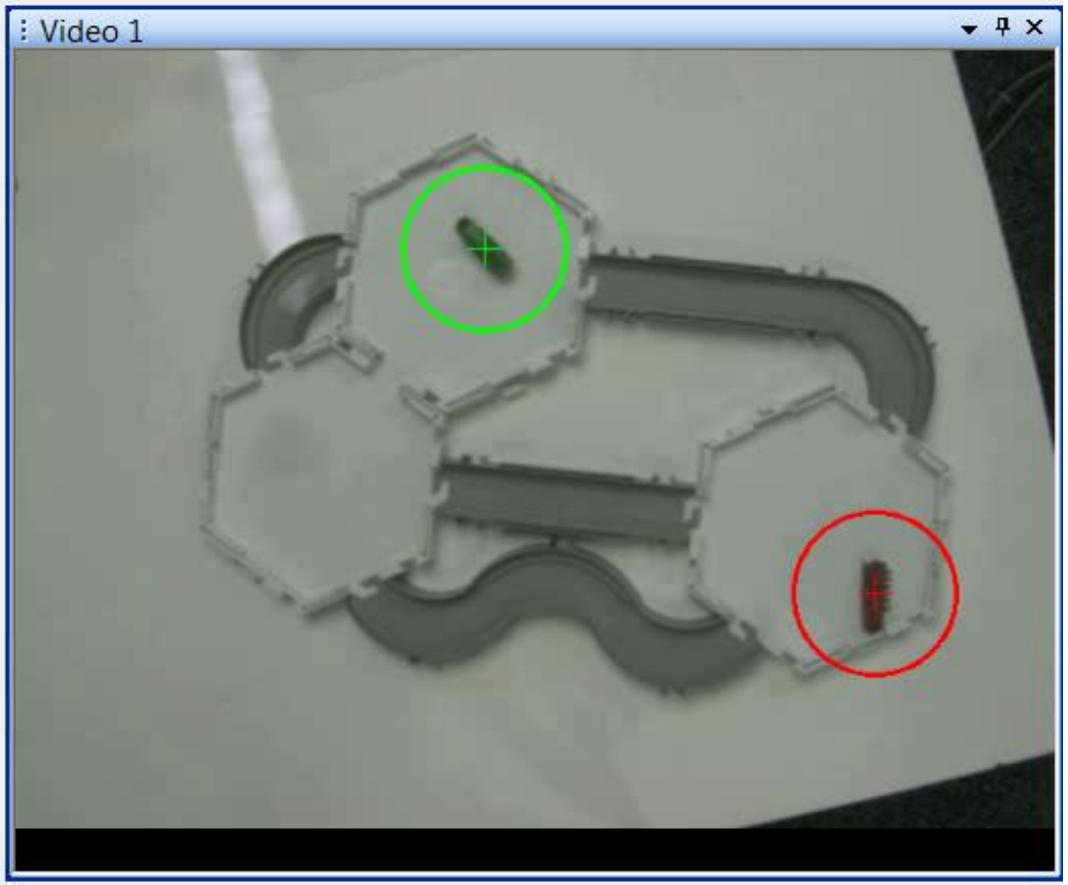
Dynamic Zones for Social event
社交行為-動態區域分析

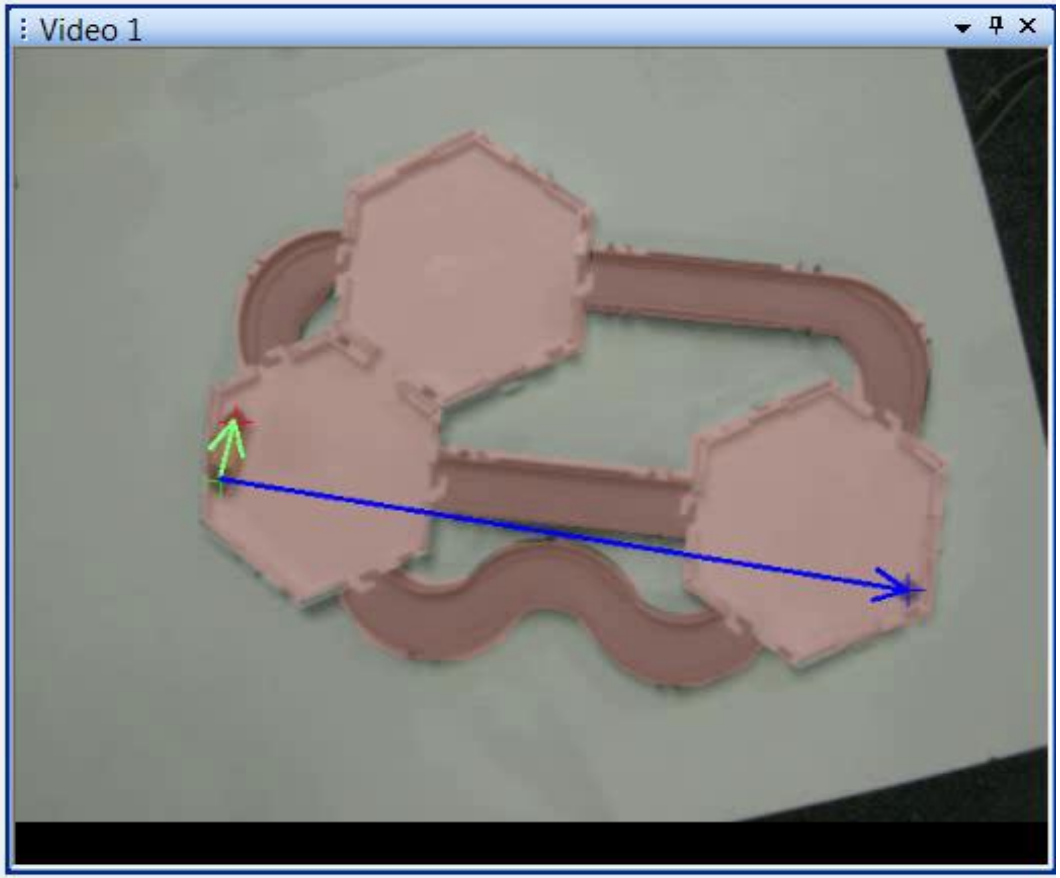
Areas 1

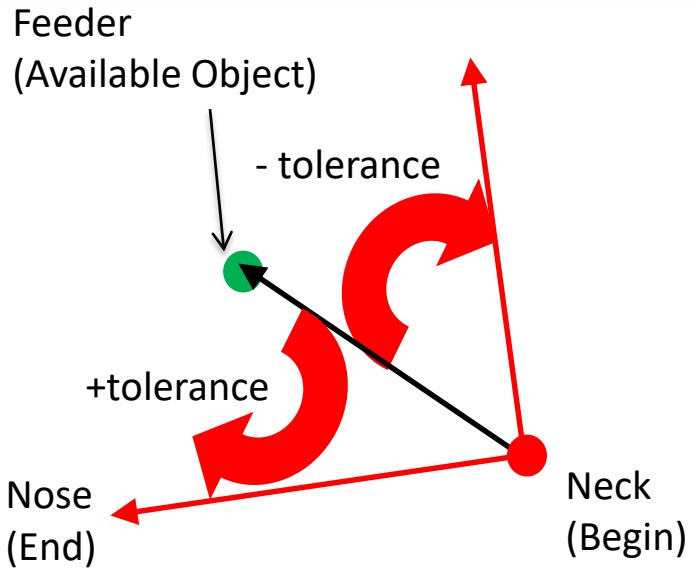
Arena	
Name	Arena
Outline	
Fill	
Shapes	0
Number of Zones	2
Zone ZD1.1	
Name	Zone 1.1
Outline	
Time Threshold (s)	0.1
Available Objects	Marker 1
Radius (pixel)	50
Zone ZD1.2	
Name	Zone 1.2
Outline	
Time Threshold (s)	0.1
Available Objects	Marker 2
Radius (pixel)	50



	Target		Object	Output	Count	Time, s		Track Length, pixel	
	Type	Name				Last	Cumulative	Last	Cumulative
	Zone ZD1.1	Zone 1.1	Mrk 2	N/A	4	0.6	1.8	127.5	322.0

Head Direction
頭部方向

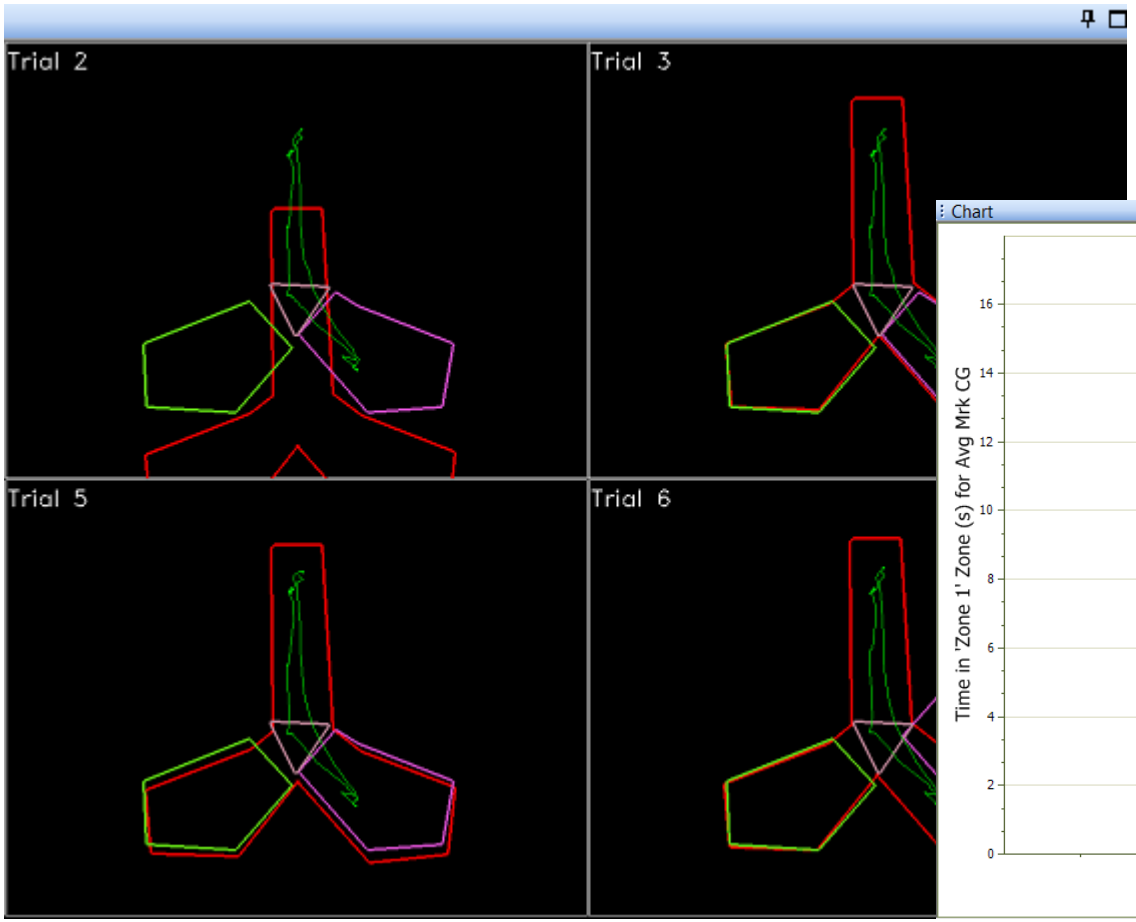


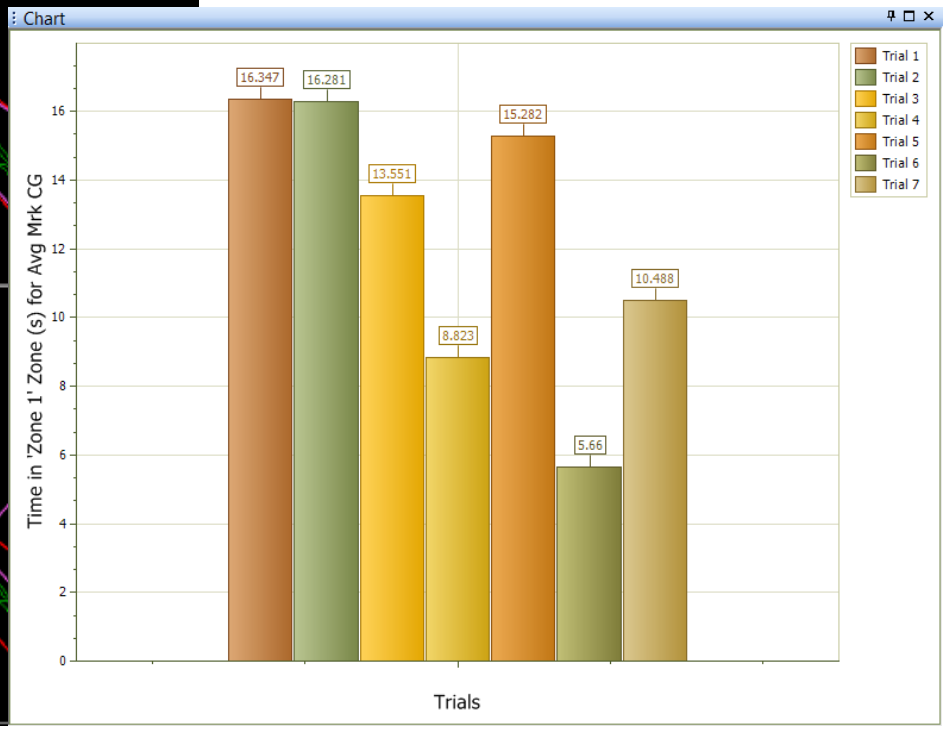


Event EV1.1

Name	Event 1.1
Color	
Target	Head Direction
± Tolerance	15
Begin	Marker 2
End	Marker 1
Time Threshold (s)	0.0
Object	Position of Object
Available Objects	Marker 3
Output Line #	N/A
Signal Type	Pulse
Pulse Duration (s)	1.0

數據分析





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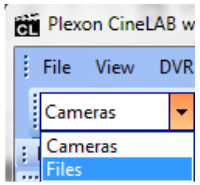
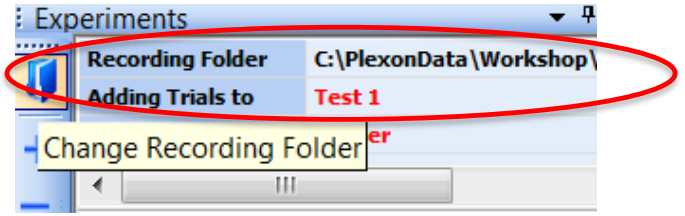
分析數據:選擇影片檔案

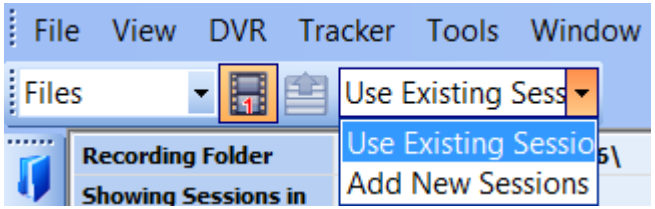
- 選擇Files模式



- 選擇要分析的檔案Select the **Rec Folder Icon** to view.

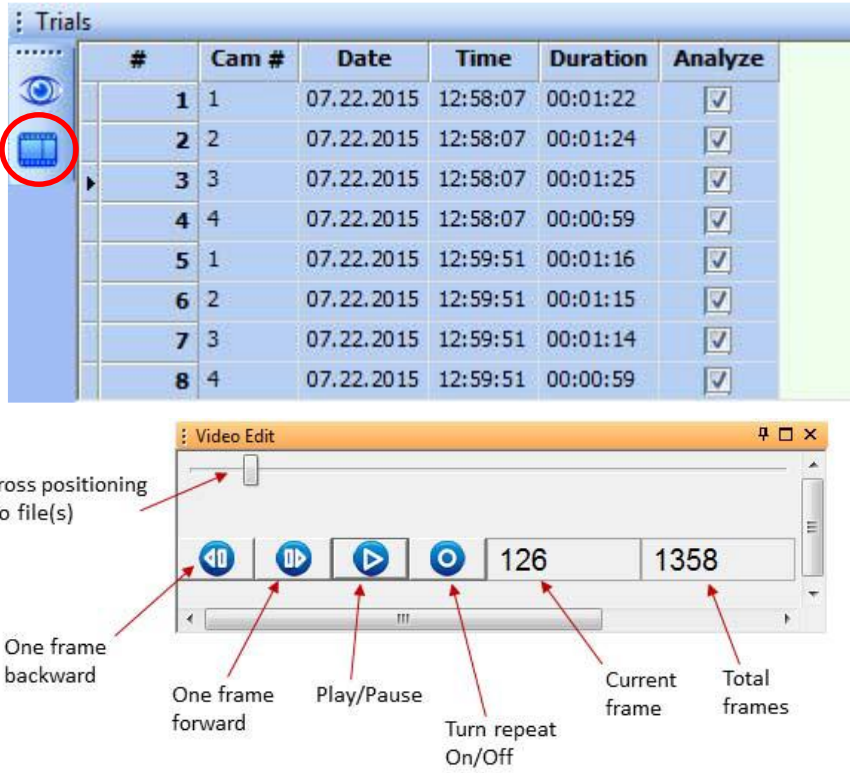


- 確認選擇 “Use Existing Sessions”



分析數據:選擇影片檔案

- 從清單選擇要分析的實驗
- **打開左邊“Open track video ”**可以看影片內容
- Video Edit視窗可以瀏覽影片片段內容



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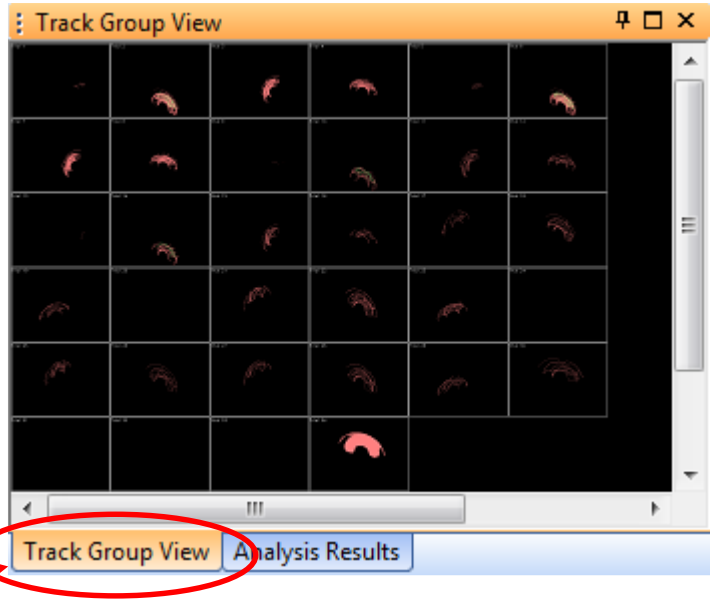
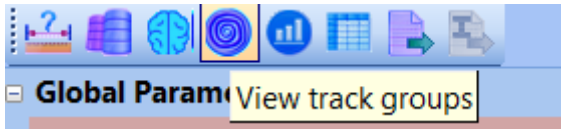
群組觀察軌跡
Track Viewing

- The **Track** is the detected pathway of travel of your subject while in the experimenter-defined arena
- You can view either an *individual* track or *track groupings*
  - Individual tracks can be viewed for the selected session by clicking on the eye icon in the trials panel
  - Group tracks can be viewed on the **Track Group View** tab on the far right panel of the user interface or by pressing the **Track Group View Button** on the Analysis Toolbar :





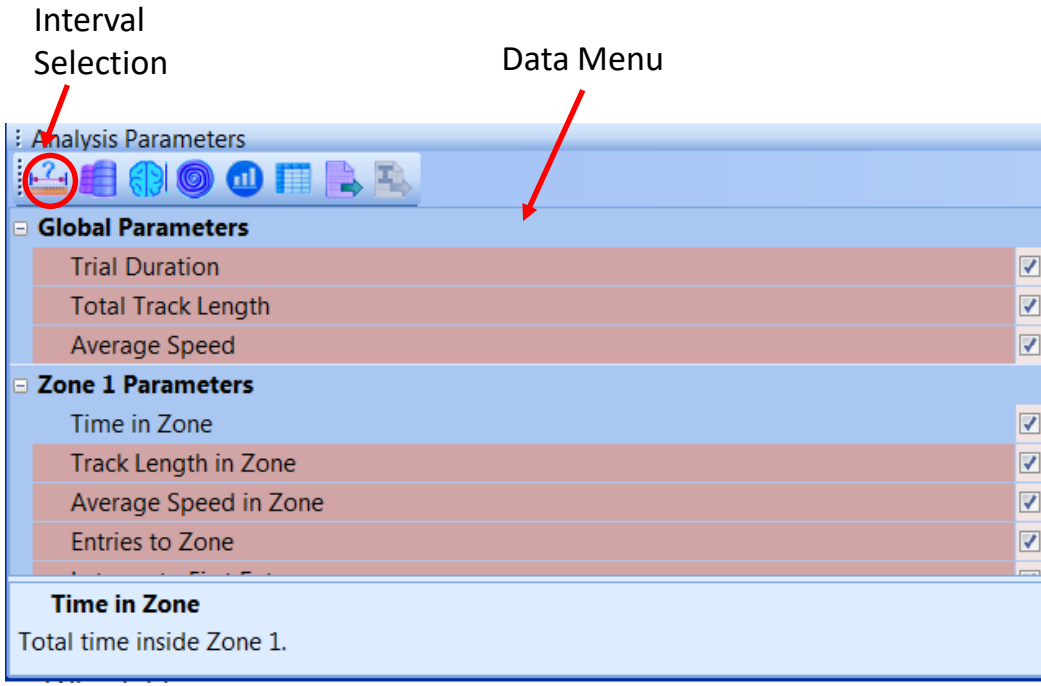
可分析基礎數據

- **Grouping**:
  - No Grouping (default)
  - Grouping based on user-defined variables
    - Select from Sessions Variables
- **Basic Output**:
  - Total Track Length
  - Average Speed
- **Zone Specific**:
  - Zone Track Length
  - Average Speed in Zone
  - Frequency of Zone entry occurrence
  - Latency of Zone entry (the first occurrence)
  - Time spent in Zone

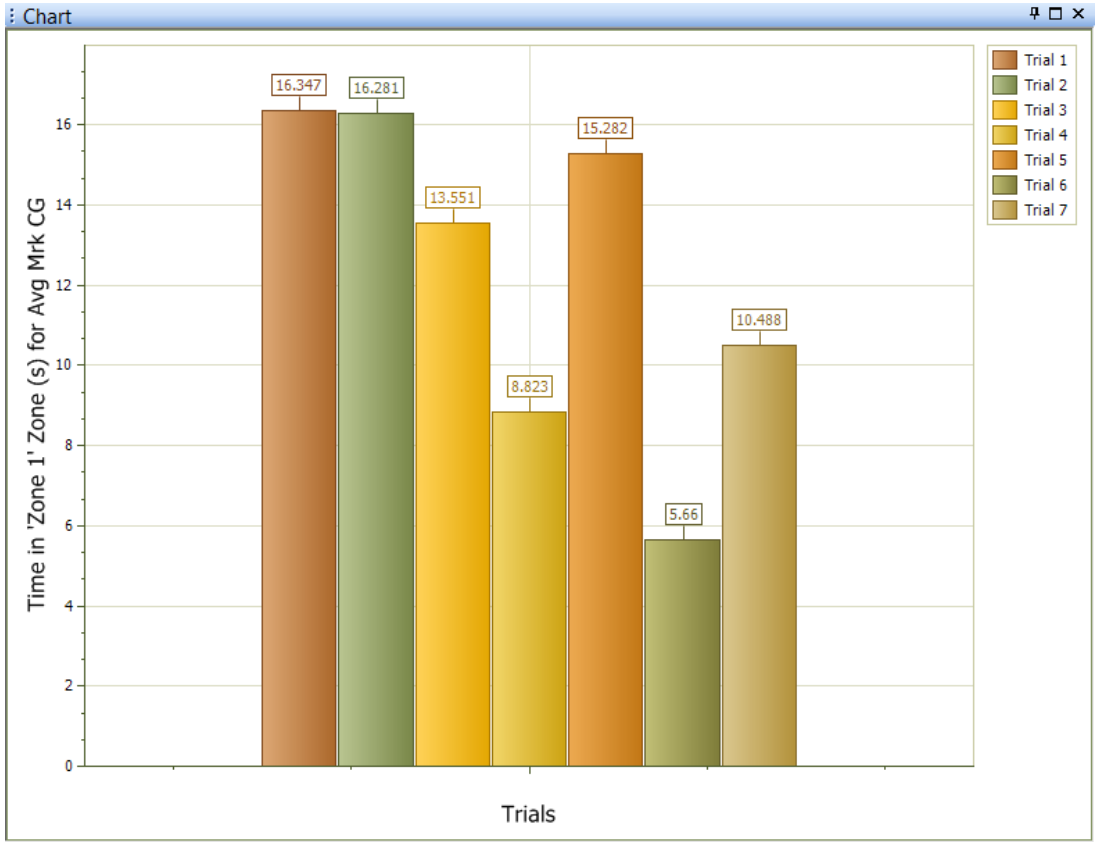
分析選項

Now that you know how to group your data, let's look at some more results automatically computed by the program.

- Select the parameter you are interested in viewing from the Data Menu
- Select the portion of the trial you are interested in viewing with the Interval Selection Icon:
  - You can skip this step if you wish to analyze the entire trial
- Pan through the data outputs by clicking through the parameter names
  - In the example to the right, Time in Zone graphical output would be displayed because it is highlighted in blue in the screenshot
- View the Graphical Outputs



柱狀圖數據呈現



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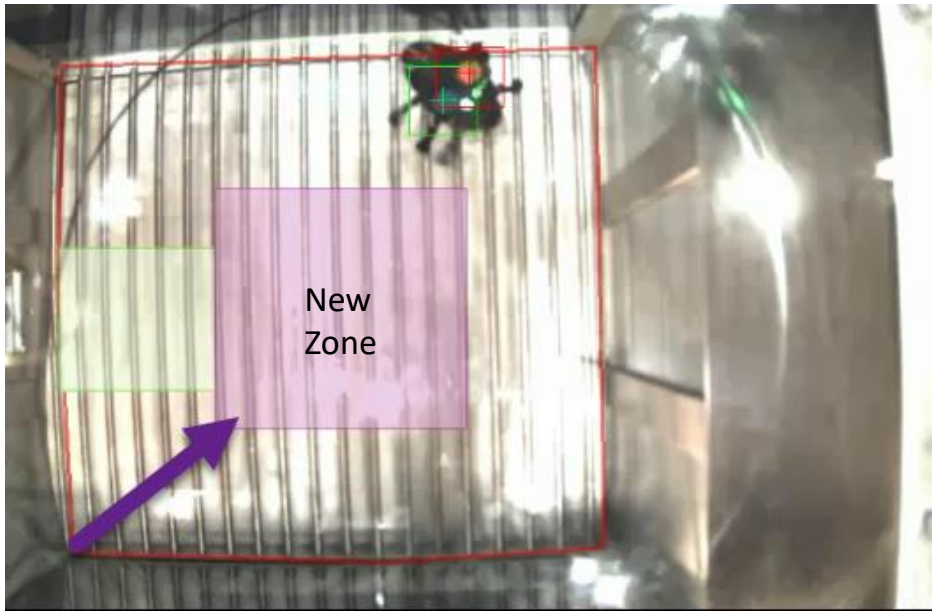
Analyzing Your Data: Post-Hoc Zone Alterations

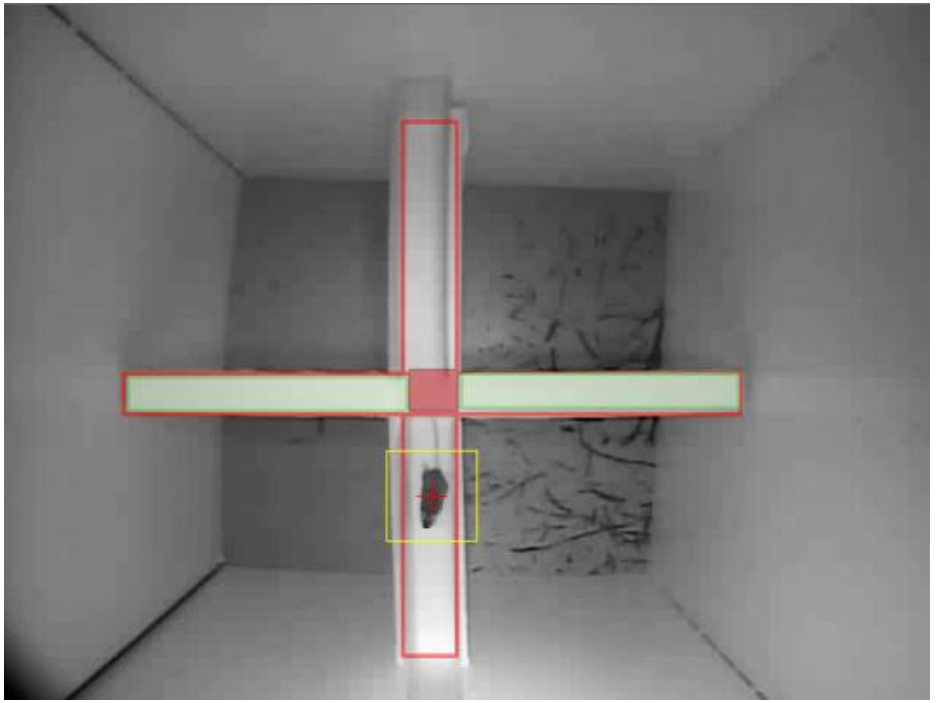
- Select Experiment 3
- Scenario: I want to add zones to my experiment.
- Solution: Add zones directly on the video playback screen!
- Click the Brain Icon 
- See your changes!



Video Playback

將設定套用在不同實驗
Overlays

- Use: Applying experimental settings from a different experiment to the current experiment.
  - View how different settings may affect result calculations
  - Compare/contrast different settings to decide what works best for additional experiments using a similar recording environment



- **<u>Only</u> available in Analysis Mode** – you **cannot** record additional trials in the same experiment with new settings

- Frame rate and tracking mode must match

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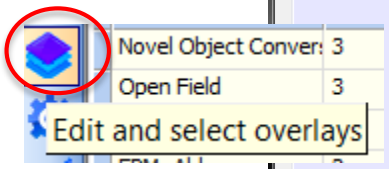
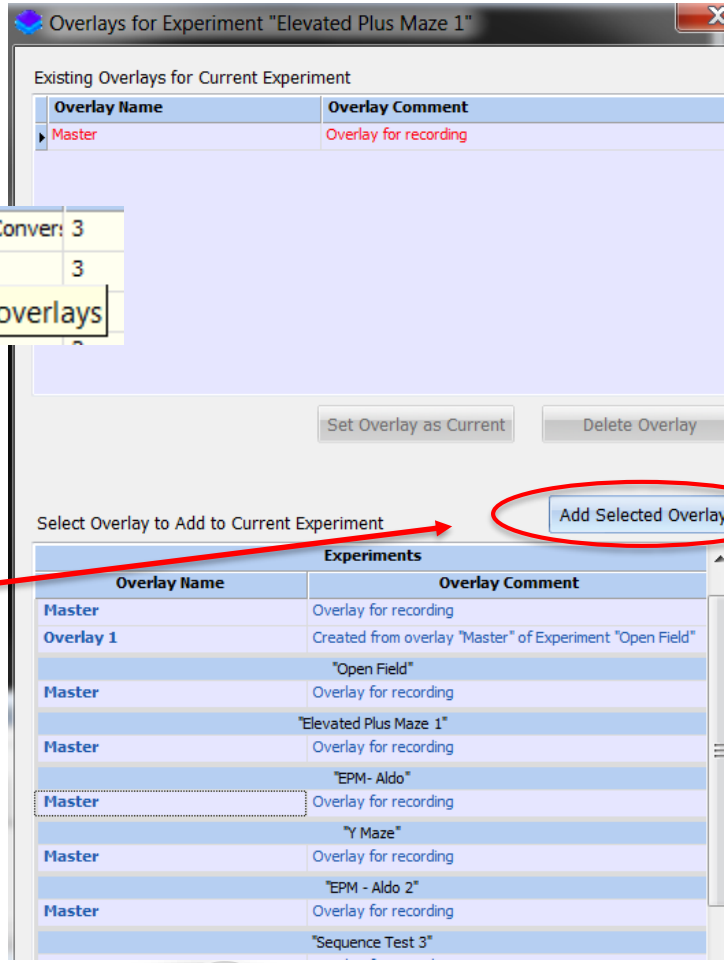
將設定套用在不同實驗Overlays

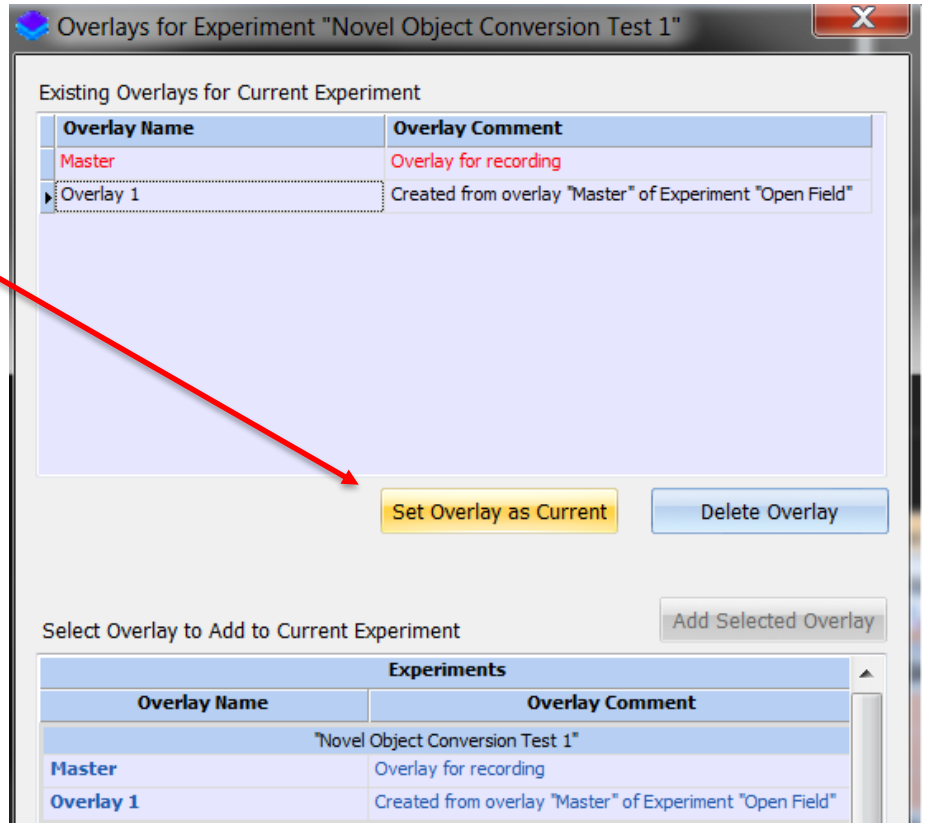
- Ensure you are in analysis mode
- Click the overlays icon
- Select overlay from experiment settings of a different experiment and “Add Selected Overlay”





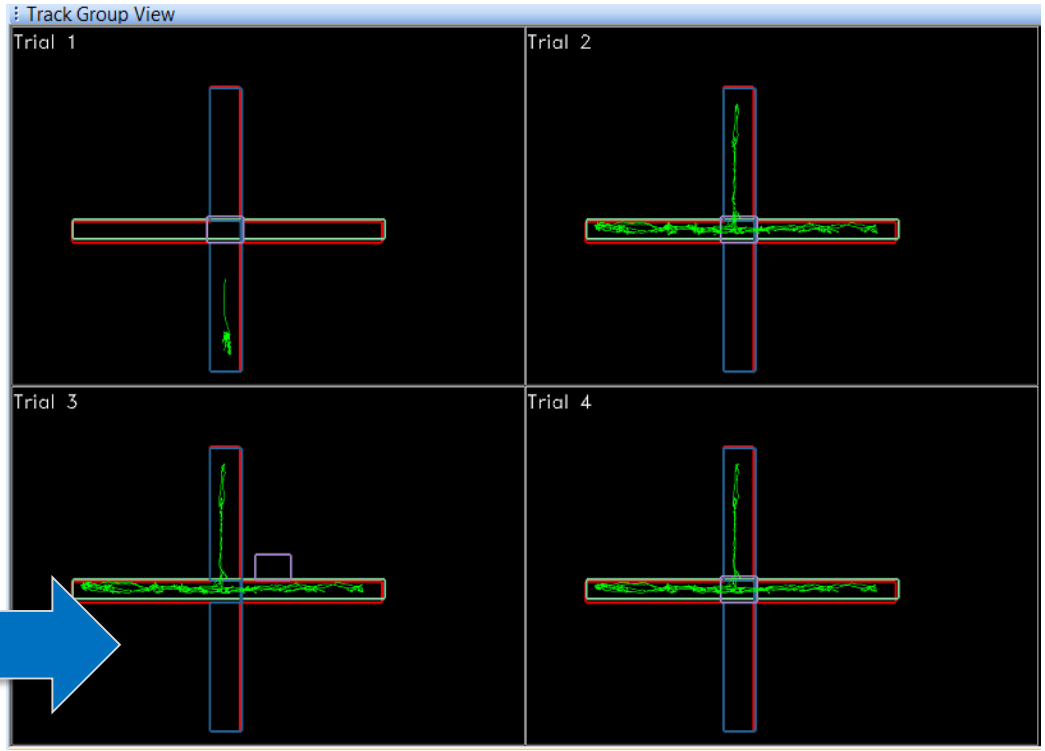
將設定套用在不同實驗Overlays

- Select new overlay and “Set Overlay as Current”
- See results change in Analysis Parameters Tab
- Exercise: Try using overlays with “T Maze Test 1” and “T Maze Test 2”
  - Apply T Maze Test 1 “Master” overlay to T Maze Test 2
  - Apply T Maze Test 2 “Master” Overlay to T Maze Test 1



改變不同設定參數

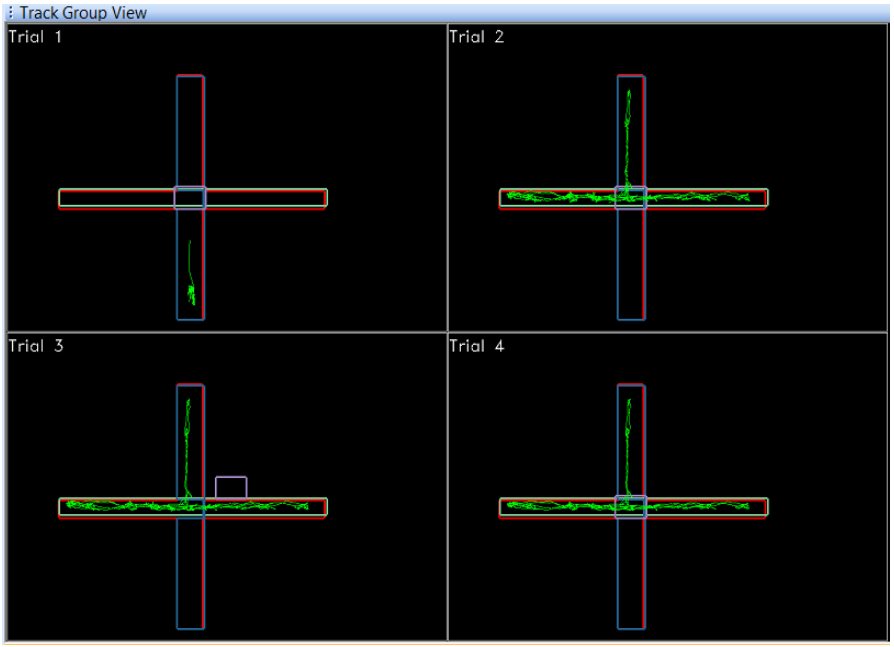
- Open Any Experiment in Files Mode
- Click on a Trial
  - Click the Open Track Video Icon 
- Modify the position of the Arena and Zones
- See your results change!



Additional Trials With Flexible Scenes

- **Note:** If you modify the arena and zone settings for a particular trial, all subsequent trials will have those **new** settings applied.

- Example: the new settings for trial 3 in this experiment would be applied to trials 5, 6, 7…
  - Manipulations to the arena and zone settings for these new trials can be applied after recording



數據輸出

- Click on the Export Results Icon in the Analysis Toolbar
- A popup window will ask you to save your file in a folder (make sure it's one that is easy to remember for importing your files into another program).
  - **Note**: Your file will be saved as a comma delimited (.csv) file
- Open your file in another program. Be sure to select "**All Files**" to view your file when opening it in another program (e.g., Excel)