

# Coordinating Tracking Data and Place Cell Activity with the CinePlex<sup>®</sup> System

Technical Note  
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## Introduction

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This document presents a sample experiment that makes use of the Plexon<sup>®</sup> CinePlex<sup>®</sup> tracking and marker capabilities to aid in the evaluation of hippocampus place cell activity. The goal of this experiment is to track an animal's movement in a maze, and then compare the animal's location in the maze (as a function of time) with the activity of the place cells in the animal's hippocampus. The Plexon OmniPlex<sup>®</sup> System is used to record the necessary neural data.

### **CinePlex Applications to Use**

In this experiment, we will track the x,y position of the animal in the experimental arena using the CinePlex Studio recording and tracking functions, while simultaneously recording neural activity of the place cells with the OmniPlex System. Then we will use the CinePlex Editor application to insert markers into the video stream to highlight the times during which substantial place cell activity is exhibited in the neural data.

The timing for the integrated OmniPlex/CinePlex System is controlled by a single clock, so the neural recording and the video stream are time-locked (synchronized) throughout the experiment.

This experiment requires the CinePlex Tracking license (CPT-V3) along with an OmniPlex license.

### **Integrated System**

The configurations presented in this document are:

- Applicable to a CinePlex System integrated with an Plexon OmniPlex<sup>®</sup> D Neural Data Acquisition System
- Based on Version 3.7 of CinePlex Studio and Version 3.6 of CinePlex Editor software

It is assumed that the CinePlex and OmniPlex Systems, including the PC and software, have been factory prepared and tested by Plexon prior to shipment.

### **Technical Support**

Plexon technical support is available by telephone at (+1) 214-369-4957 or by email at [support@plexon.com](mailto:support@plexon.com). Please contact us if you would like additional information or assistance with your experiments.

## Additional Resources

For detailed feature descriptions, licensing options, hardware requirements, start-up procedures and advanced configuration options, see the CinePlex and OmniPlex user guides on the Plexon website, [www.plexon.com](http://www.plexon.com).

### (Optional) Tracking the Head Direction

If you want to see where the animal's attention was during the experiment, consider attaching an LED headstage or purchasing LED equipped headstages with the Plexon system. The CinePlex System can record LED positions for each video frame. See the CinePlex System User Guide for detailed procedures.

## Startup and Operational Testing

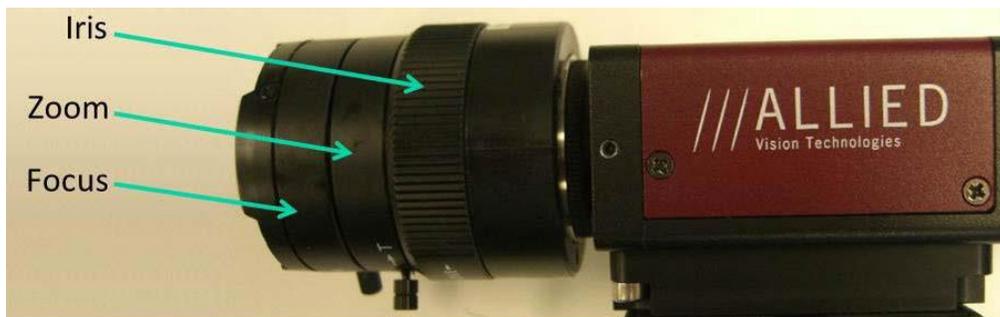
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Follow the general system startup procedure in the CinePlex System and OmniPlex System user guides to connect cables, prepare the cameras, and get data flowing in the CinePlex and OmniPlex Systems.

### Camera(s)

**CAUTION:** Never plug or unplug a camera FireWire cable with the PC power ON. Permanent damage to the camera could result.

One or more camera(s) were supplied with the original shipment of the CinePlex System. One camera is sufficient for this experiment (a two-dimensional maze) if it has a clear view of the entire experimental arena. It should typically be mounted above and orthogonal to the arena. In general, it is best to position the camera as far from the experiment as possible, then zoom as much as possible in order to fill the field of view with the area of interest without distortion.



Use the physical settings on the camera lens to obtain the best possible image. Do this before configuring any camera settings in the CinePlex software.

Note: The settings that are available on a particular lens depend on the lens make and model. Not all settings are available on all models.

More than one camera can be used if it is necessary to ensure you always have a clear view of the animal, but in this example we use one camera mounted directly above the center of the arena.

It is very important to mount the camera securely. Any movement of the camera could invalidate the spatial calibration and tracking data.

### **Lighting of the Experimental Arena**

Lighting for this experiment is very important, because the video images must be clear enough for the tracking algorithm to function efficiently. Lighting should be sufficient to view animal activity that is expected to occur during the experiment. Keep in mind that some cameras can react to lighting variations or flashes of light. Therefore, some preliminary video recordings should be done to ensure that tracking data is being faithfully recorded under all expected lighting conditions. If the experiment is to be performed in very dim lighting, consider using an infrared camera and illumination.

### **Physical Constraints**

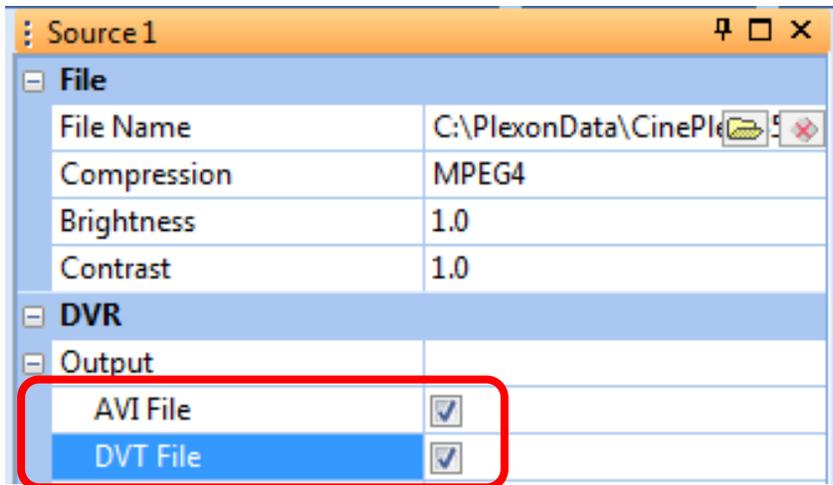
For experiments in which an animal will be moving through a large maze, consider other physical constraints in your experiments, such as a need for a longer headstage cable or clips to hold a headstage securely to the animal.

## **Tips for Better Tracking and Data Management**

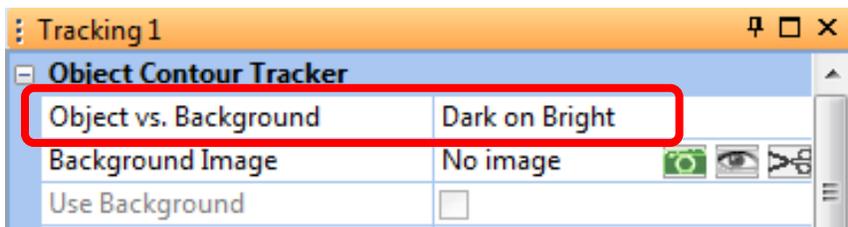
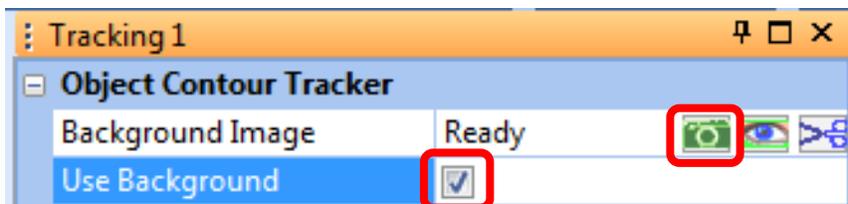
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**AVI File** – It is strongly recommended that you select the Audio Video Interleaved (AVI) checkbox to record the original video with embedded data. This file can be very useful later if you want to retrack the video with revised settings.

**DVT File** – You should also select the digital video tracking (DVT) checkbox, which configures the system to create a DVT file during the recording session. This file contains all the positional data (x,y coordinates) in a format that can easily be opened in a text application, spreadsheet, or many external programs. (See the image, below.)

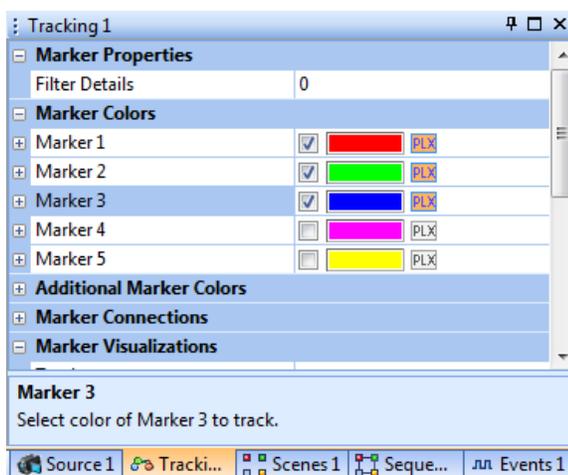
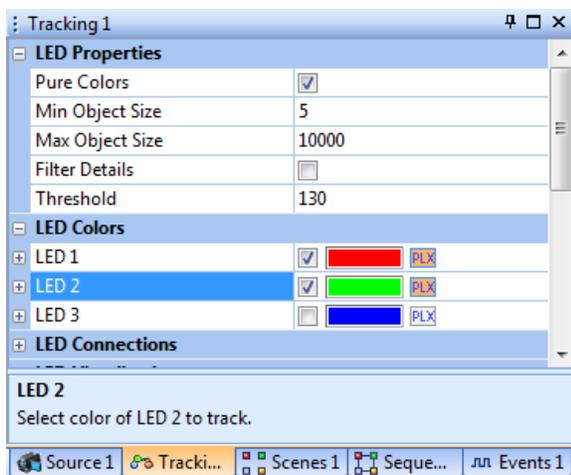
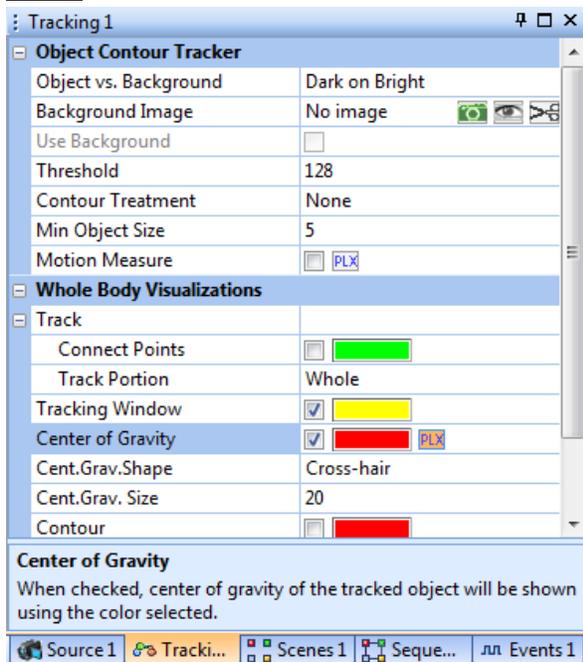


**Background subtraction** – If you are using Object Contour (whole body) tracking, it may be useful to subtract the arena background. Selecting the **Use Background** checkbox configures the system to subtract the background image from the video, and typically improves tracking accuracy. However, you might find it sufficient to select **Dark on Light** or **Light on Dark** in the **Object vs. Background** row.



**PLX buttons** – You must select *at least one* **PLX** button to cause the CinePlex System to send coordinate data to the OmniPlex System. The diagrams below show examples of **PLX** buttons for different tracking modes – Object Contour, LEDs and Color Markers.

**Note:** When a **PLX** button is selected, it turns orange; when it is not selected, it is clear.



## Recording Procedure

Set up the camera, start the system and calibrate the arena by means of the procedures described in the CinePlex Studio User Guide. Then record the neural and video data together as described in the user guide.

**Note:** When CinePlex Studio is in the Armed state (  ), the cassette icon in the status bar is yellow (  ). When recording starts, the icon turns green (  ).

During the recording process, verify that the system is successfully tracking the animal's location in the arena.

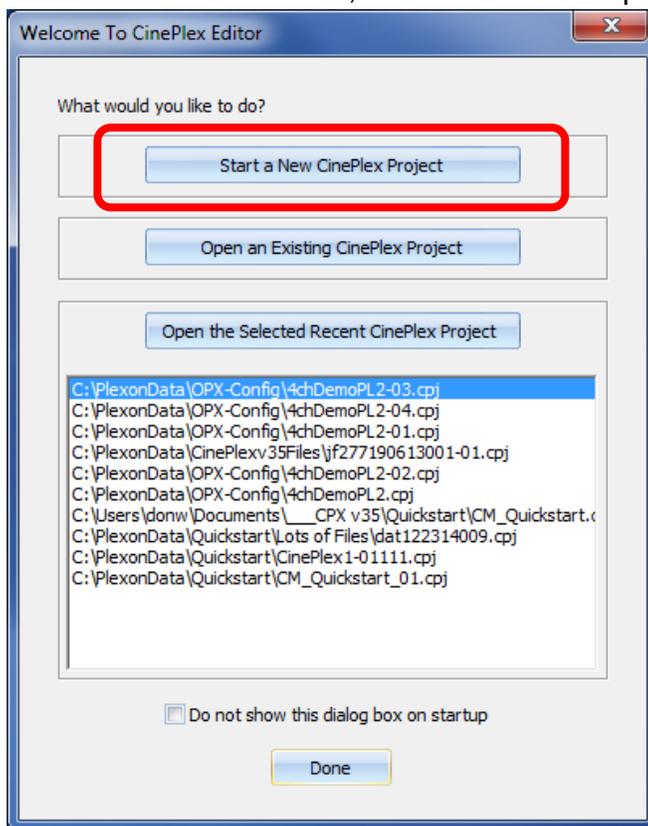
If you want to save all the settings in this Studio file, select **Save Settings** from the File dropdown menu or click the save icon in the toolbar (  ). The system will prompt you to enter a new name for this settings file, although you can enter the same (original) name of the file if you want to overwrite it.

## Viewing Neural and Video Files Simultaneously

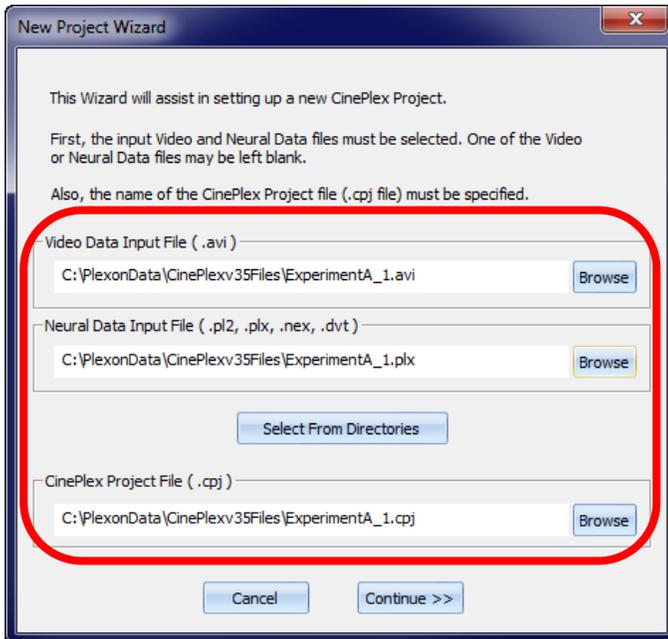
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To view the neural and video files together, use CinePlex Editor. Follow the procedures described in the CinePlex Editor User Guide. The highlights are presented below.

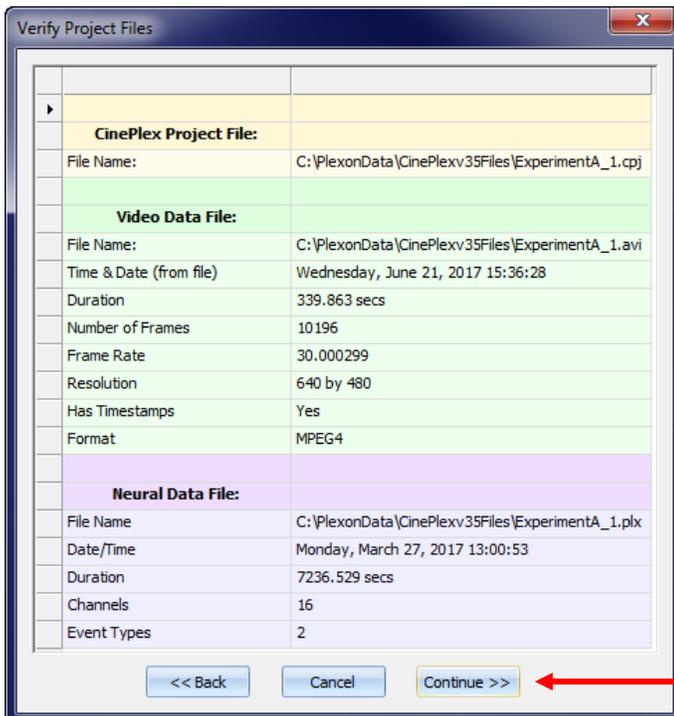
1. Double-click the CinePlex Editor icon (  ). Wait for the CinePlex Editor application main window to appear on the monitor.
2. On the Welcome screen, start a new Editor project by clicking on the **Start** button.



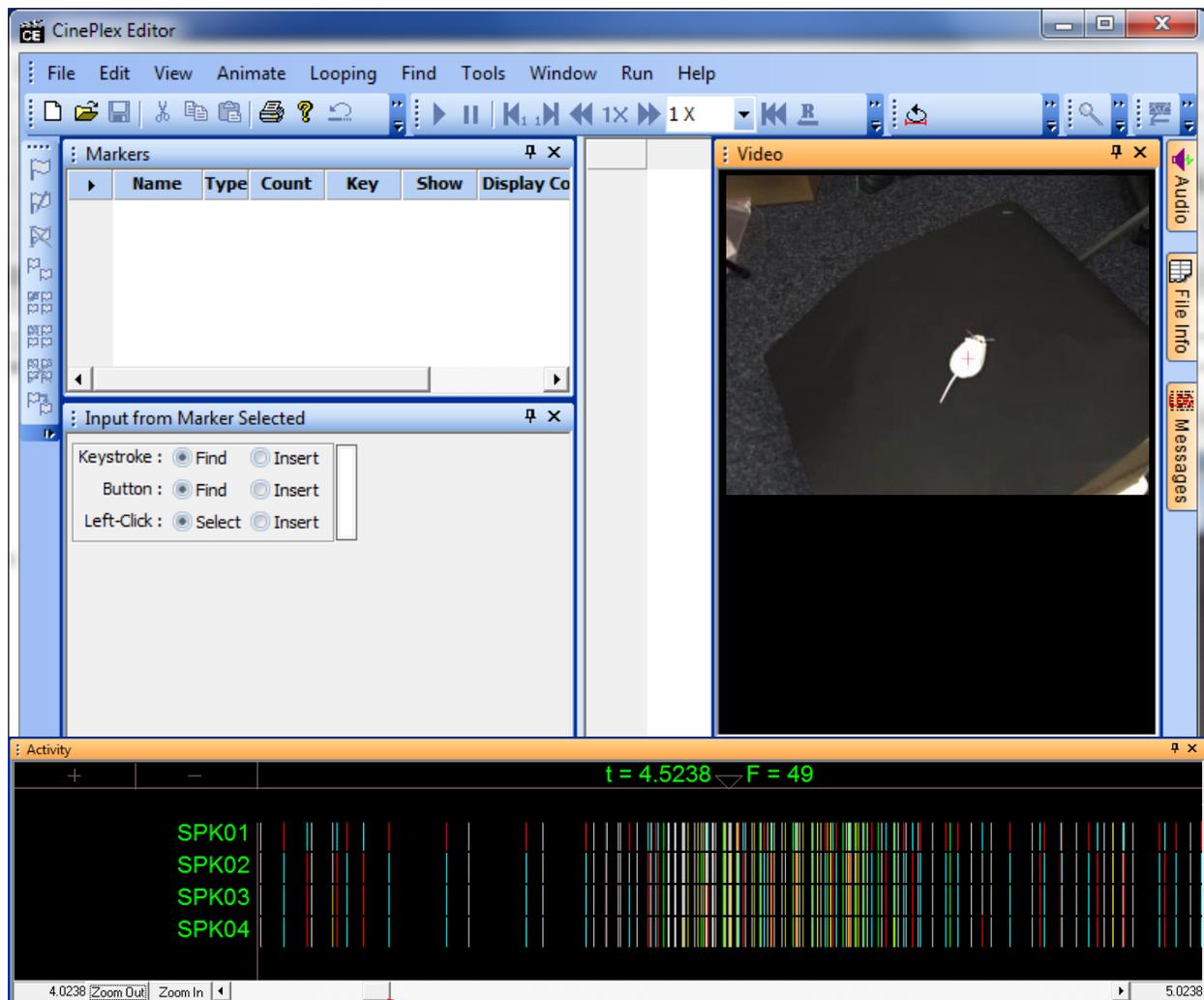
3. Browse to select the AVI file and the neural data file that were recorded during a specific experiment. In the example below, the files are from the folder “CinePlexv35Files.” The “\_1” at the end of the AVI file name indicate that the original video was taken with Camera 1. Use an appropriate name for the project (cpj) file. Then click the **Continue>>** button.



4. In the Verify Project Files dialog box, verify that the information is correct. Then click the **Continue>>** button.



CinePlex Editor creates the CPJ file and opens the CinePlex Editor main view.



**Slider**

5. View the animal's activity frame by frame by moving the slider left and right. The video images and neural activity indicators are time-locked (synchronized), because both recordings were created under the control of a single clock.

## Adding a Marker to Identify Important Points in the Data

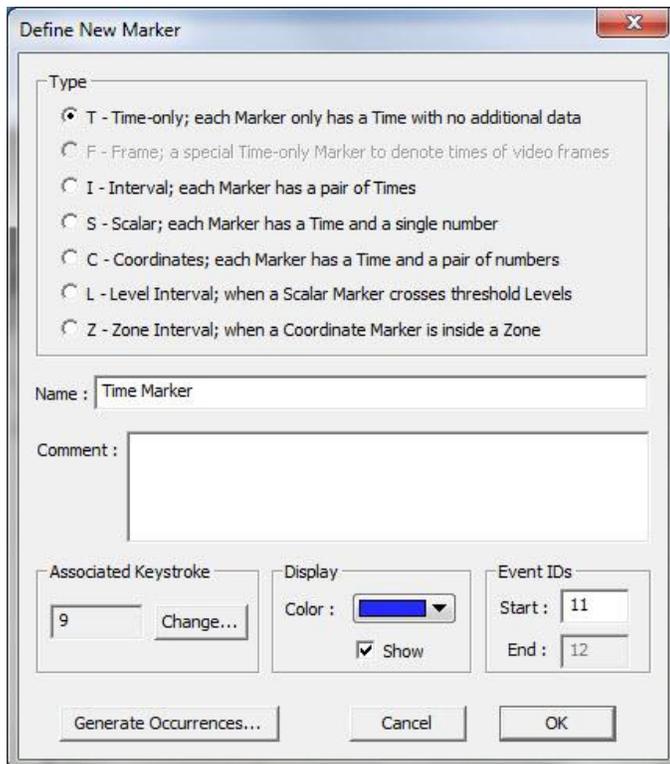
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In this example, you will want to add markers to the video stream to identify time intervals during which there is substantial place cell neural activity. You do this by [1] defining a marker to bracket the intervals of place cell activity, then [2] entering marker occurrences whenever place cell activity is seen in the neural data stream.

### Defining the Marker “Place Cell”

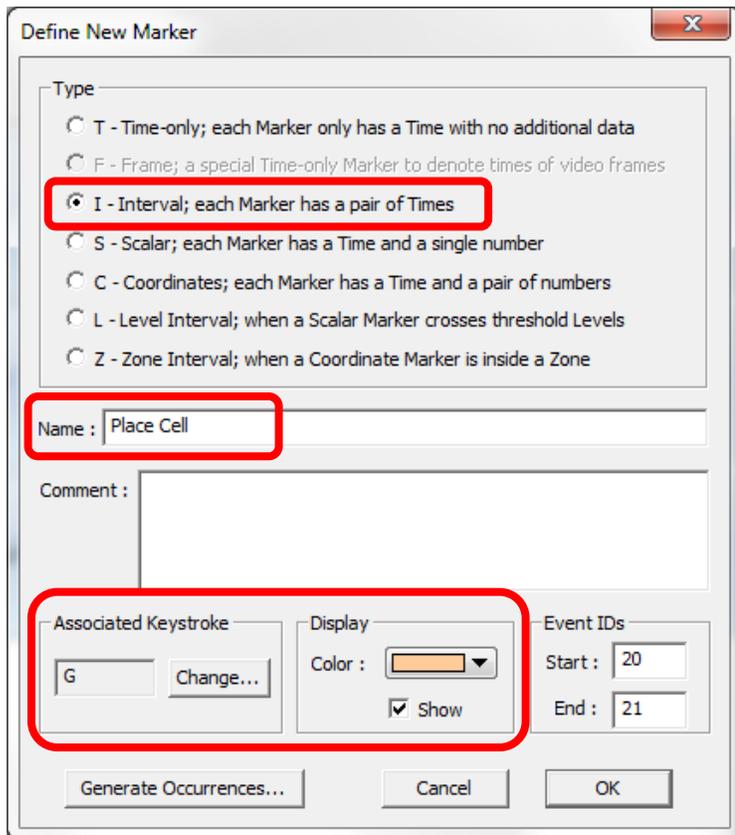
1. Click **Play** (▶) to start the video and neural data, then click **Pause** (⏸). (Although pausing is not required, the video can be distracting when you are performing other operations in CinePlex Editor.)
2. Define a new marker to denote the time intervals of place cell activity. This can be done in three ways:
  - a. Click the **New Marker** button ,
  - b. or, from the Edit menu, click **Define New Marker**,
  - c. or, from the right-click menu in the Markers window, select **Define New Marker**.

The Define New Marker dialog box appears.



Note: The F (Frame) marker is a special time-only marker applied automatically by CinePlex Studio to associate a time with each video frame. This marker always appears grayed out in the Define New Marker dialog box because it is system-generated.

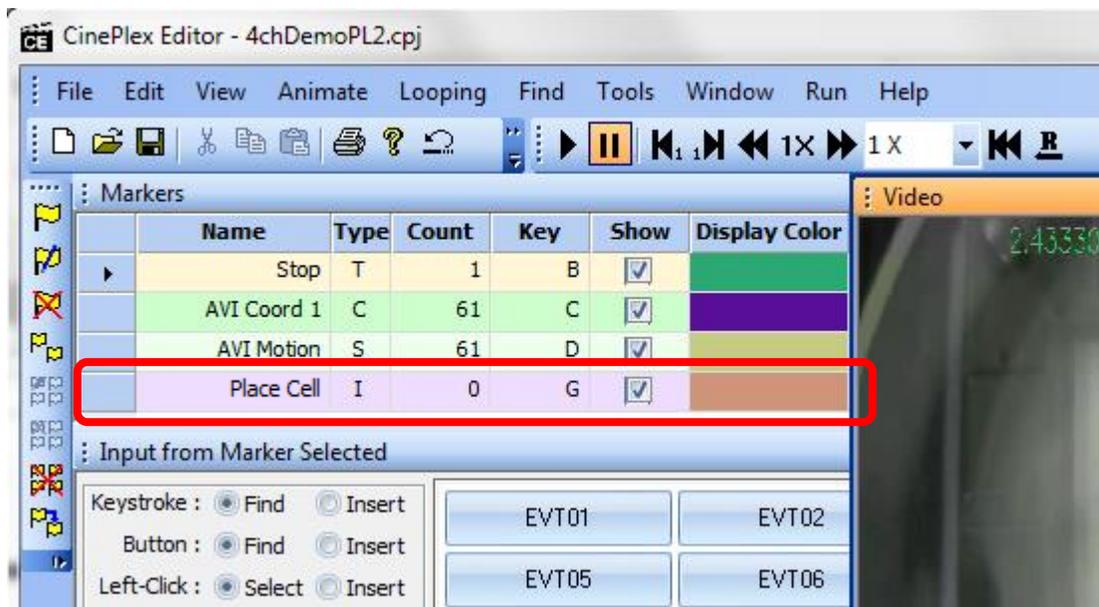
3. In the **Type** area, click “I” to define an interval marker.
4. In the **Name** box, in the place of the default name Interval type “Place Cell.” Retain the default settings for the other items.



Notice in the example above that the **Associated Keystroke** (which will be used for Marker Occurrences) is “G” and the display **Color** is beige. You can change these values if desired.

5. Click **OK**.

The Place Cell entry appears in the Markers window. However, there are no occurrences of this marker, so the value in the Count column is 0.



## Adding Marker Occurrences To Intervals with Place Cell Activity

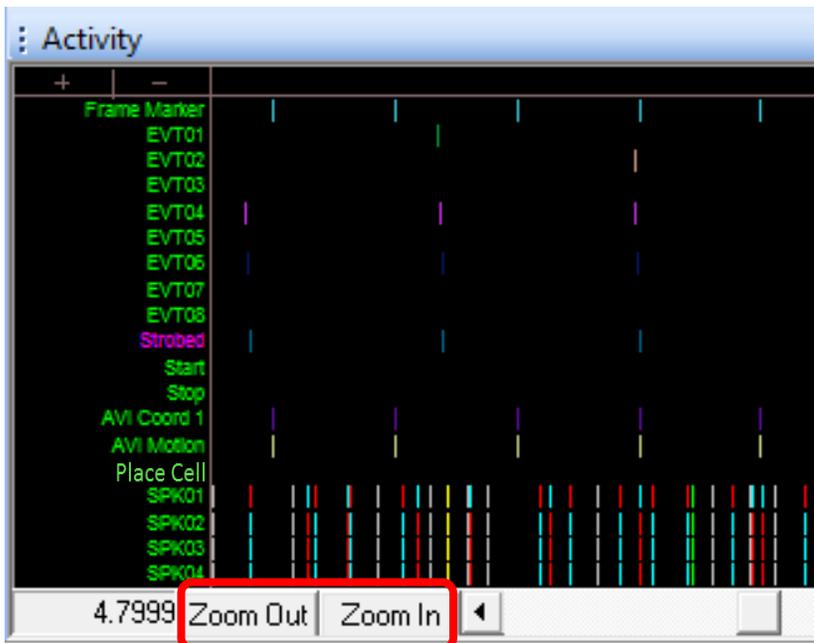
Initially, we *rough in* all the marker occurrences during one continuous pass through the video, then we go back and adjust each marker occurrence to be as accurate as possible. Then this information is saved to a NEX file, from which we can easily load it into the NeuroExplorer® application for analysis.

### Denoting place cell activity in the video (*roughing in* marker occurrences)

1. Click **Play** (▶). Adjust the Faster and Slower settings (◀◀ 1X ▶▶ 1/4 X) to a comfortable speed to view the video, which balances the desire to get through the video quickly against the risk of missing enhanced place cell activity should it occur too quickly to spot.

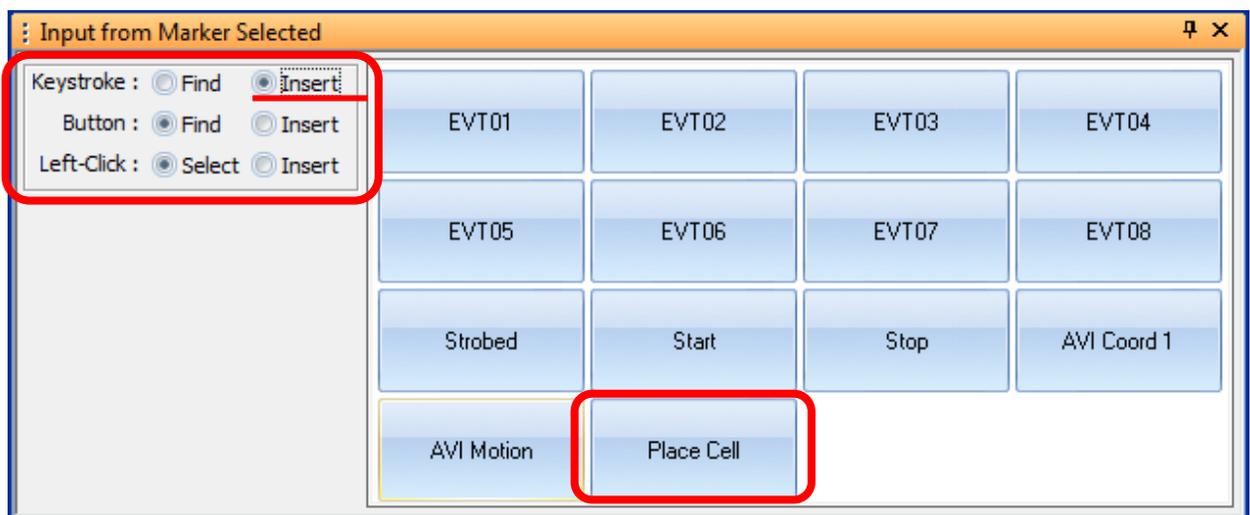


- Use the **Zoom In** and **Zoom Out** buttons at the bottom of the Activity window to adjust the time range such that, should the place cell activity be spotted in the neural data, a key on the keyboard can be hit before the time of the first place cell activity scrolls out of the Activity window.

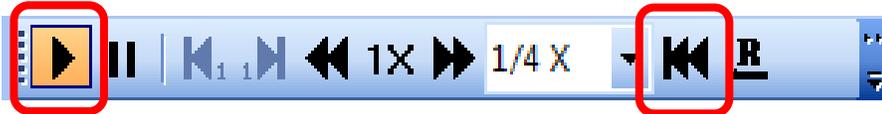


- In the Input window, at Keystroke, click **Insert**. (You could just as easily use the Button or Left-click input method here.) CinePlex Editor is now armed to enter new occurrences of the new marker when you press that marker's associated key.

Notice that “Place Cell” is displayed as one of the available Markers.



- Click **Rewind**. Click **Play**. Position a finger over the associated keystroke as you watch for the “Place Cell” event.



- Watch the video closely as it plays. When you see the place cell activity *starting* in the neural data, quickly press the associated key (“G” in this example).

When you see the place cell activity stopping in the neural data, press the key (“G”) again to complete the interval. These two keystrokes (starting and stopping) define this particular marker occurrence as a “Place Cell” interval. The interval appears in the Activity window.

You can press the key as many times as necessary during the playing of the video to identify all the place cell activity intervals. Accuracy is not important at this point; fine-tuning will come later. Go through the entire video.

The screenshot shows the Plexon software interface. On the left, there is a 'Markers' table with the following data:

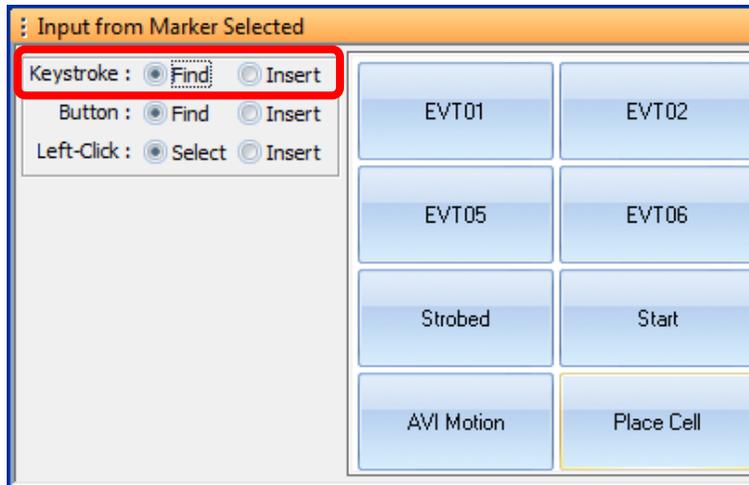
Name	Type	Count	Key	Show	Display Color
Start	T	1	A	<input checked="" type="checkbox"/>	
Stop	T	1	B	<input checked="" type="checkbox"/>	
AVI Coord 1	C	61	C	<input checked="" type="checkbox"/>	
AVI Motion	S	61	D	<input checked="" type="checkbox"/>	
Place Cell	I	10	G	<input checked="" type="checkbox"/>	

Below the table is a 'Keystroke' section with options for Find and Insert, and a 'Button' section with options for Find and Insert. There are also buttons for EVT01 through EVT08, Start, Stop, AVI Coord 1, AVI Motion, and Place Cell.

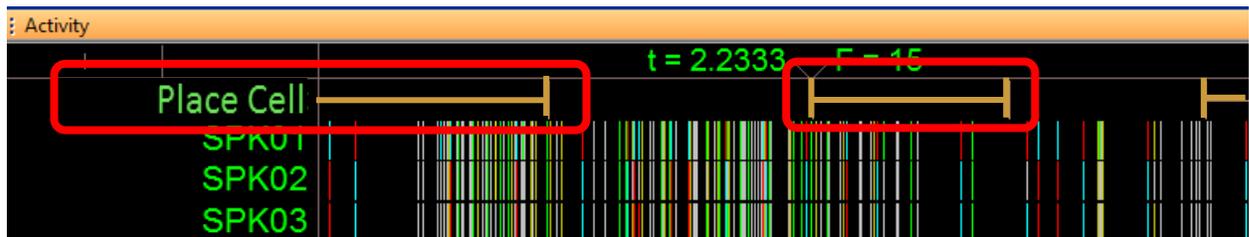
The main window displays a video stream of a mouse. A red crosshair is overlaid on the mouse. A red arrow points to the 'Key' column in the Markers table, and another red arrow points to the 'Place Cell' interval in the Activity window. The Activity window shows a timeline with a 'Place Cell' interval highlighted in pink. Below the Activity window, there are four channels labeled SPK01, SPK02, SPK03, and SPK04, showing neural data spikes.

## Making fine adjustments to marker occurrences

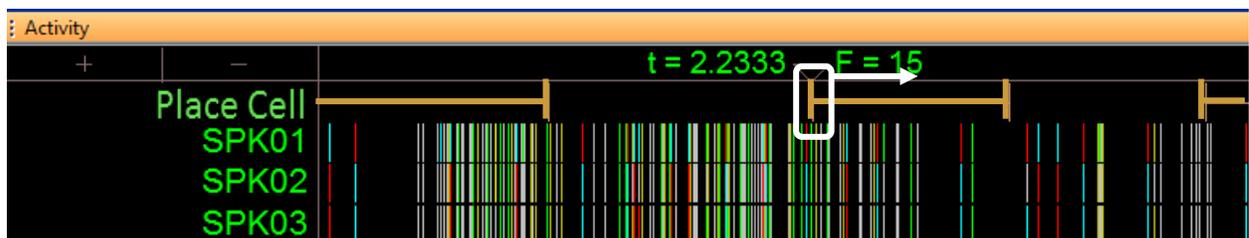
- Now we will go back through and adjust the time of the “Place Cell” marker occurrences to be more accurate. We will use the **Find** function to quickly locate our marker occurrences. In the Input window, after Keystroke, click **Find**.



- Rewind the video file, then press the assigned key for the “Place Cell” marker. This causes the system to jump to the start of the first marker occurrence in the Activity Window. Notice that the complete interval for each occurrence is displayed (start to finish). Use the left and right arrow keys on the keyboard to look at the neural data to decide where the place cell activity actually occurs.



- In the Activity window, drag the marker occurrences to the correct time. To do so, press and hold the Shift key, then click near the tick mark for the marker occurrence. Drag the tick mark to the desired time and release the mouse button. You can move the Start and Stop tick marks for each interval in this manner.



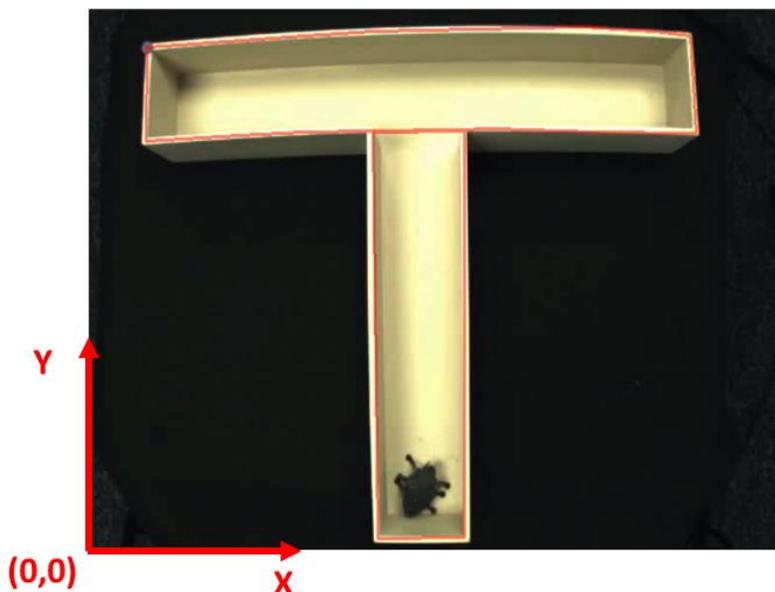
9. Review the recorded tracking data and see if any of the recorded x,y coordinates suddenly went to (0,0) for one or more frames. This can happen, for example, if an LED light was occluded by a cable or object in the arena. If you see several such (0,0) coordinates in the data, consider correcting them manually in your file with a simple arithmetic interpolation. If there are many such (0,0) coordinates, you should modify the values of the Tracking parameters in the user interface then retrack the AVI file. Refer to “Retrack AVI Procedure” in the CinePlex Studio User Guide for the necessary procedure.
10. If you want to save all the settings in this Editor project file, select **Save Project As...** from the File dropdown menu or click the save icon in the toolbar (  ). The system will prompt you to enter a new name for this settings file, although you can enter the same (original) name of the file if you want to overwrite it.

## Data Analysis

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Now that you have marked the time intervals during which place cell neural activity occurred, you can look back through the video, focusing on those intervals of interest, and see what the animal was doing during those specific intervals.

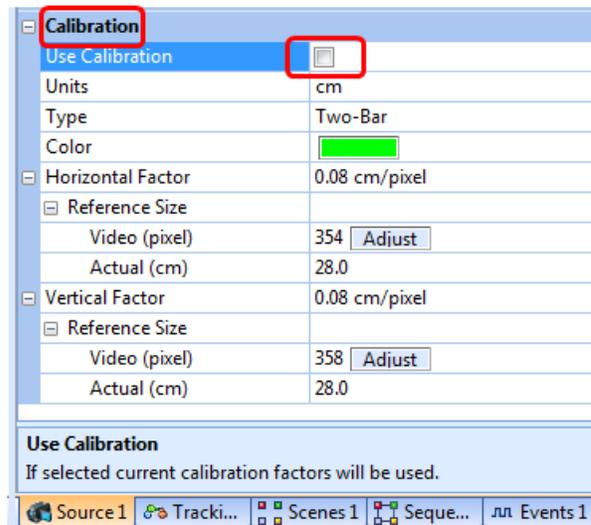
The DVT file that the system created during the experiment contains time-stamped x,y coordinates that you can use in further analyses. The coordinate system has its origin at the lower left corner of the video frame, as shown in this diagram.



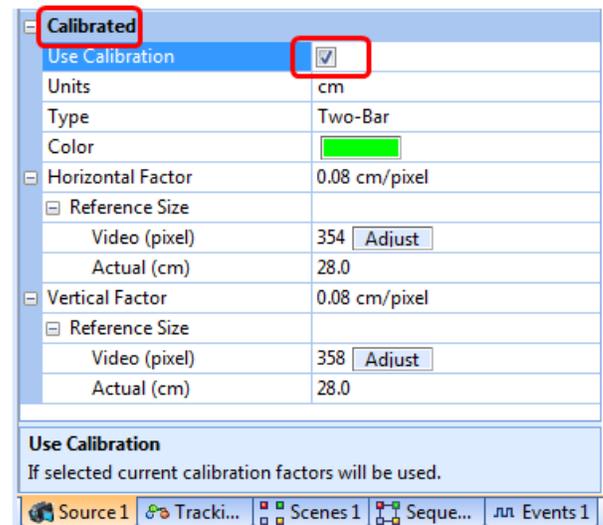
Be aware that the DVT coordinates (and the coordinates in the PLX and PL2 files) are always expressed in the 0-1023 format, not in pixels. There is an algebraic relationship between the DVT coordinate system and real pixels. See “Extractor Procedures” in the CinePlex Studio User Guide for further information about DVT coordinates and pixels in uncalibrated arenas.

Note: Remember that the coordinates will be extracted from the AVI file in pixels if the **Use Calibration** checkbox was not checked during recording. See the examples below.

**Use Calibration unchecked**  
when recording started -  
Extracted coordinates will be in pixels



**Use Calibration checked**  
when recording started -  
Extracted coordinates will be in cm



## Saving the Data

To save the data: On the File menu, click Export to NEX, and if desired, select the appropriate **What to Export** checkboxes. Click **Export!** to write the file.

Note: Exporting markers is supported for PLX files but not PL2 files.

Perform additional analyses and data management procedures in the NeuroExplorer application.

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