VIDEO TRACKING INTERFACE

SOF-840

USER'S MANUAL

DOC-086
Rev. 2.6

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notes

Table of Contents

CHAPTER 1 | INTRODUCTION

The Video Tracking Interface can be used to record locomotor activity in either Open Field arenas or home cage environments.

Open-Field Activity (OFA) monitoring is a sensitive method of measuring both gross and fine locomotor activity in small animals. In general, computerized OFA characterizes multiple end points of motor behavior, and as such, has proven to be a powerful assessment tool with many applications in behavioral pharmacology, toxicology, and genetics. For example, dopamine agonist studies can use distance traveled in centimeters as a measure of gross motor activity that is amenable to quantitative analysis. Opiate tolerance studies can examine overall distance traveled.

The Video Tracking Interface (VTI) system is an alternative to infrared (I/R) beam tracking systems traditionally used for monitoring Open-Field Activity (OFA) in rats and mice. With the Video Tracking Interface system, video cameras mounted in the cubicles perform video tracking of standard X and Y locomotion of subjects and, in conjunction with the Activity Monitor (SOF-811) software included with the system, defines an X and Y coordinate "map" of subject location and activity.

The cubicles used for VTI have slide-out floors that can accommodate a variety of housing cage platforms to permit monitoring of test subjects for longer periods of time – hours to days – in standard home cages, giving test subjects access to proper litter-bedding, food and water. Furthermore, VTI can collect tracking data from up to 2 cameras (one per cubicle) and each camera is capable of tracking up to 2 rat or 4 mouse test subjects (one subject per cage), providing the investigator with the potential to collect OFA data for up to 4 rats or 8 mice, per testing period.

General Computer Environment

The minimum recommended system is as follows:

- A 2.3 GHz or faster Dual Core computer with at least one free PCI slot for a single camera. Each additional camera requires additional processing power.
- Windows 2000, XP or Vista
- 1GB of RAM
- 1024 x 768 or larger screen resolution
- 500 Megabytes of free disk space (more space if videos are saved)
- CD-Rom drive and/or DVD recordable drive for archiving video files
- Mouse
- Network - In order to collect the data from a remote system, the remote system must be connected to the tracking computer via a Windows network.

There are several chamber platforms available:

- NIR-VID–515, One Rat Open Field
  Dimensions: 17.0"W x 17.0"D x 12.0"H (43.2 x 43.2 x 30.5 cm)
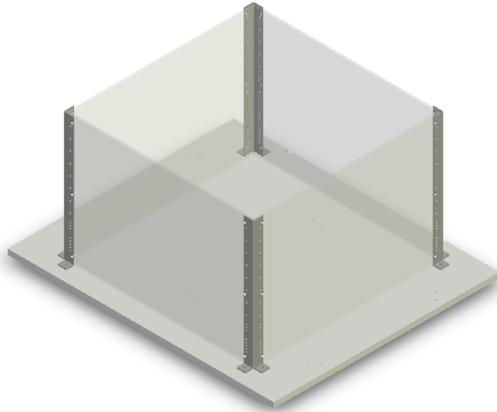
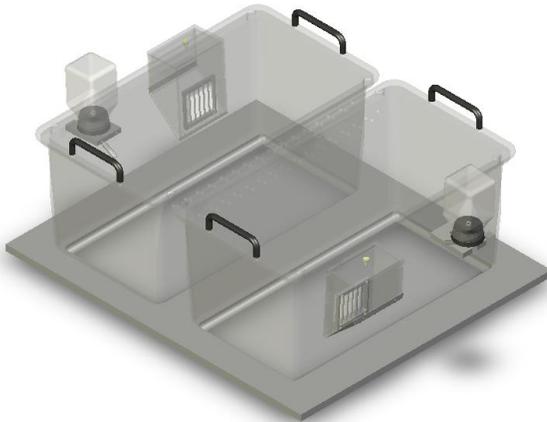- NIR-VID-510, Two Mouse Open Fields
  Dimensions: 11.0"W x 11.0"D x 8.0"H (28.0 x 28.0 x 20.3 cm)
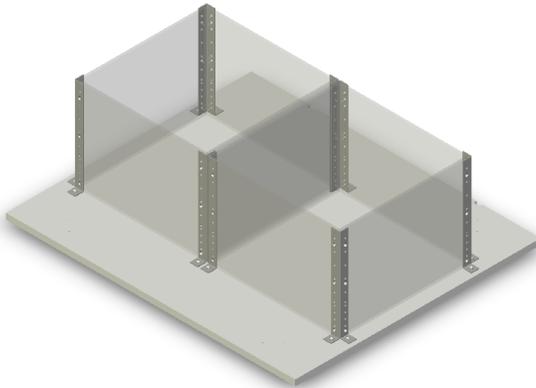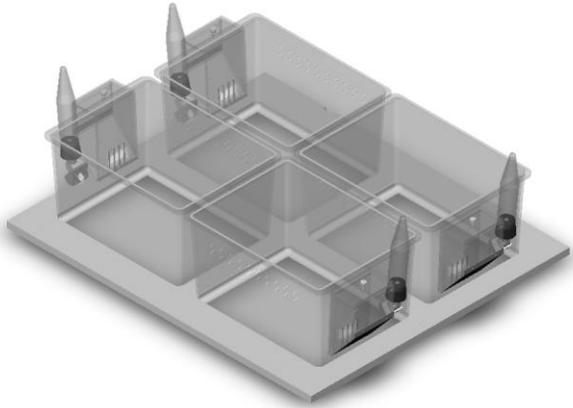

- NIR-VID-415, Two Rat Home Cages
  Dimensions: 18.0"W x 10.0"D x 8.5"H (45.7 x 25.4 x 21.6 cm)

- **NIR-VID-410, Four Mouse Home Cages**

Dimensions: 10.0"W x 8.0"D x 6.0"H (25.4 x 20.3 x 15.2 cm)

Mazes

Elevated mazes are often used to detect anxiety, and the Video Tracking Interface can now be used to track animals on plus mazes and zero mazes. The Video Tracking Interface (VTI) supports mouse and rat plus and zero mazes.

The maze part numbers are as follows:

- **VPMM-LM, NIR Mouse Plus Maze**
  Dimensions: 56"H x 41" W x 30"D (142.2 x 104.1 x 76.2 cm);
  Required Floor Space: 4' x 7' (1.3 m x 2.3 m)
- **VPMR-LR, NIR Rat Plus Maze**
  Dimensions: 69"H x 56" W x 45" D (175.3 x 142.2 x 114.3 cm);
  Required Floor Space: 5' x 9' (1.6m x 3m)
- **VZM-MS, Mouse Zero Maze**
  Dimensions: 18" diameter x 87" H (45.7 x 221.0 cm);
  Required Floor Space: 24" x 32" (61 x 81.3 cm)
- **VZM-RS, Rat Zero Maze**
  Dimensions: 27" diameter x 87" H (68.6 x 221.0 cm);
  Required Floor Space: 3' x 4' (91.4 x 121.9 cm)

CHAPTER 2 | SOFTWARE

Video Tracking Interface Menu Items

When the Video Tracking Interface (VTI) software is opened, the screen shown in Figure 2-1 will appear:

Figure 2-1 - VTI Screen
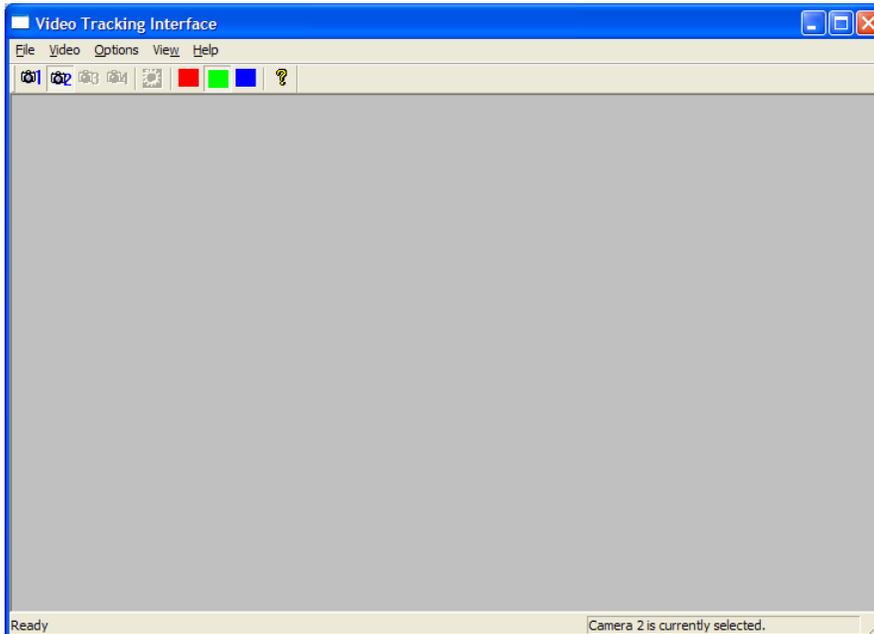
File Menu Options

Figure 2-2 - File Menu

The following options are available under the "File" menu:

- **Preferences:** Sets the camera names, turns video saving on and off, and sets the directory that video will be saved in.

- **Exit:** Closes the Video Tracking Interface (VTI) program

Video Menu Options

Figure 2-3 - Video Menu
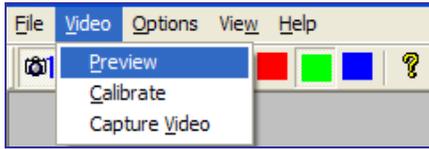
The following options are available under the "Video" menu:

- **Preview:** Displays the live image from the camera currently selected. Also displays the chamber tracking boundaries for each mouse/rat chamber and the current tracking location of the mouse/rat.

- **Calibrate:** Depending upon which type of device is being used with VTI (basic open field chambers, home cage platforms, or mazes) the "Calibrate" option will open one of following windows, shown in Figure 2-4 below. This utility allows calibration of the tracking system with respect to the type of device being used.

Figure 2-4 - Calibrate Screens
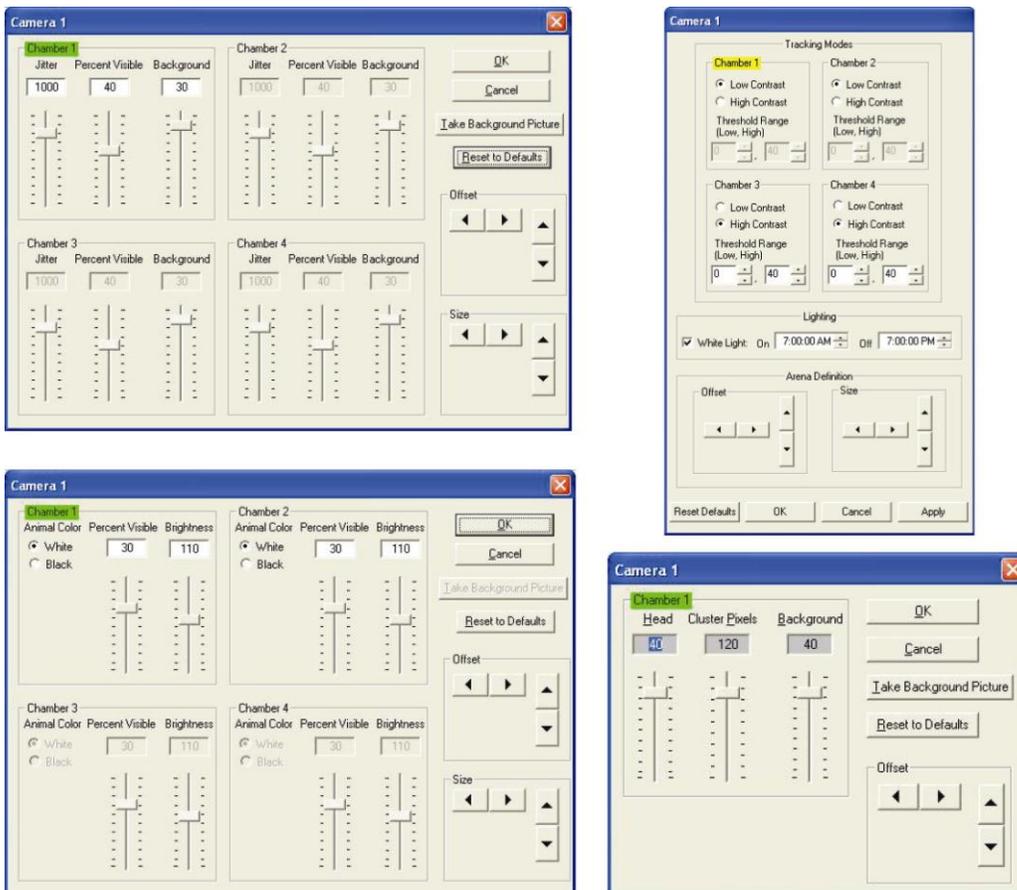
- **Capture Video:** Starts video capture and tracking for all cameras to be operated by the system. This option displays the camera images and sends tracking data to the data collection program, typically Activity Monitor or Video Maze.
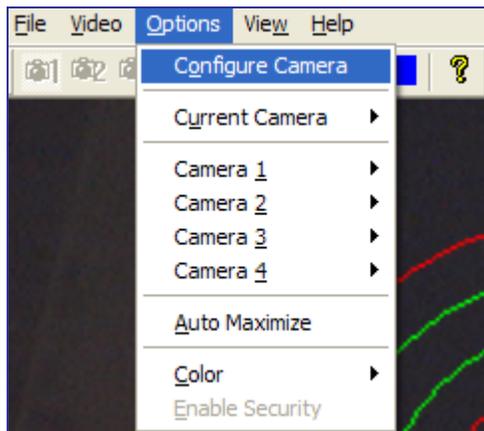
Options Menu Options

Figure 2-5 - Options Menu

The following options are available under the "Options" menu:

- **Configure Camera:** Adjusts the camera settings, including Brightness, Gain, Auto Exposure, etc. (Must be in Preview mode to adjust camera settings).

- **Current Camera:** Selects the camera from which live images are displayed in "Preview" mode, "Calibrate" mode and "Capture Video" mode. Choices are Camera 1 – 4, depending upon the number of cameras hooked up to VTI.   "Current Camera" may also be selected/changed by clicking the appropriate buttons on the Toolbar (described later)

- **Camera 1:** Sets the chamber type that Camera 1 is monitoring. The options include:
  - NIR Tracking
    - One Rat Open Field (NIR-VID-515)
    - Two Mouse Open Fields (NIR-VID-510)
    - Two Rat Home Cages (NIR-VID-415)
    - Four Mouse Home Cages (NIR-VID-410)
  - Color Tracking
    - One Rat Open Field (VID-515)
    - Two Mouse Open Fields (VID-510)
    - Two Rat Home Cages (VID-415)
    - Four Mouse Home Cages (VID-410)
  - VPMM-LM (NIR Mouse Plus Maze)
  - VPMR-LR (NIR Rat Plus Maze)
  - VZM-MS (Mouse Zero Maze)
  - VZM-RS (Rat Zero Maze)

- **Camera 2:** Sets the chamber type that Camera 2 is monitoring.

- **Camera 3:** Sets the chamber type that Camera 3 is monitoring.

- **Camera 4:** Sets the chamber type that Camera 4 is monitoring.

- **Auto Maximize:** When enabled, the main program screen will automatically maximize at appropriate times.

- **Color:** Changes the color of the chamber tracking boundaries, which are displayed in "Preview" mode and in "Calibrate". The choices are red, green and blue and can also be selected by clicking the appropriate buttons on the Toolbar (described later)

- **Enable Security:** Because (X, Y) coordinate data is being sent via the network from Video Tracking Interface (VTI) to the data collection software, this feature allows only authorized users to view the VTI data, keeping networked computers secure. Please contact the network administrator if there are any problems using this feature. Please note that if VTI and the data collection software are installed on the same computer, this option will have no effect.

View Menu Options

The following options are available under the "View" menu:

- **Toolbar:** Allows Toolbar, at the top of the screen under the menu options, to be turned on or off.

- **Status Bar:** Allows Status Bar, at the bottom of the screen, to be turned on or off. The Status Bar displays the current status of the VTI program, including the number of frames processed during video tracking.

Help

The following option is available under the "Help" menu:

- **Enter Password:** Allows entering passwords for different video tracking configurations.

- **About Video Tracking Interface…** Displays the current version of the Video Tracking Interface software.

Toolbar Options

Figure 2-6 - Toolbar

The Video Tracking Interface toolbar (shown above in Figure 2-6, also shown in Figure 2-1) contains several buttons that correspond these commonly used options:

[camera 1 icon]   - Sets camera 1 as the current camera.

[camera 2 icon]   - Sets camera 2 as the current camera.

[camera 3 icon]   - Sets camera 3 as the current camera.

[camera 4 icon]   - Sets camera 4 as the current camera.

[configure icon]   - Configures the camera (brightness, gain, exposure, etc.).

[red icon]   - Sets the chamber border color to red.

[green icon]   - Sets the chamber border color to green.

[blue icon]   - Sets the chamber border color to blue.

[help icon]   - Displays the current version of Video Tracking Interface.

Status Bar Options

Figure 2-7 - Status Bar

The Video Tracking Interface status bar (shown above in Figure 2-7, and also in Figure 2-1) displays status and information messages about the program. The left side of the status bar displays information about each option in the program. The right side of the status bar displays information about the current status of the program. When in "Preview" or "Capture Video" mode, the right side will display the number of frames (images from the camera) lost per total number of frames sent to the data collection software and the percentage of lost frames. It is important to check this information when previewing or capturing an experiment, because if the computer is too slow or there are too many programs running in the background, the Video Tracking Interface system will lose frames. Ideally this number should be 0, however a percentage of frames lost less than 0.1 percent is usually acceptable.

CHAPTER 3 | SETTING PREFERENCES

In Video Tracking, click **File | Preferences**. This will open the Preferences dialog.

Figure 3-1 - Preferences Dialog
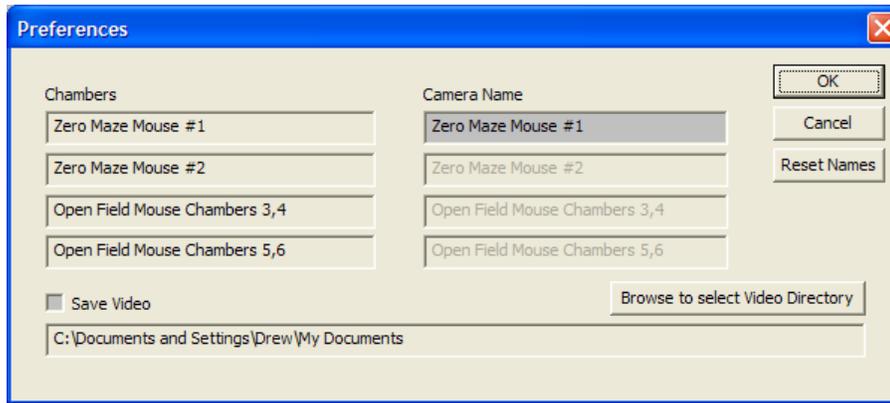
Each camera is assigned a default name. The chamber type and chamber number being monitored by the camera determines this default name. For example, in Figure 3-1 chamber type selected for Camera 1 is a Zero Maze, and it is assigned chamber 1. Camera 2 is also setup as a Zero Maze, and is assigned chamber 2. Camera 3 is setup for open field mouse chambers, and is assigned chamber numbers 3 and 4. The camera name can be changed by clicking on the default camera name and entering the desired name. However, it is important to note that the camera name is used when saving video, and therefore the camera name should describe the video it is capturing.

If Save Video is checked, VTI will save video when capturing data to a Windows Media Video file (.wmv) located in the Video Directory. By default, the Video Directory is set to the "My Documents" folder, but the directory can easily be changed by clicking on "Browse to select Video Directory".

When all settings are correct, click **OK**.

Configuring the Camera

In Video Tracking, click **Video | Preview**. This will display the video from the current camera. Select **Options | Configure Camera** to adjust the camera parameters. Depending on the model of camera(s) being used with the system, some or all of the following options may be available:

- Brightness – adjusts the black level of the camera.

- Auto Exposure – allows the camera to control exposure time.

- Sharpness – affects the clarity of detail in an image.

- White Balance – calibrates a camera's color response to take into account different "color temperatures" of light (i.e., fluorescent light is greenish; sunlight, more blue; incandescent light, yellowish). This calibration allows the camera to define what the color white is under any of these various lighting conditions.

- Saturation - the saturation of a color is the degree to which the color departs from neutral gray of the same brightness. An attribute of perceived color, or the percentage of hue in a color. Saturated colors are called vivid, strong, or deep.

- Shutter – determines how long the camera sensor is exposed during each frame capture.

- Gain – a multiplier for the camera sensor signal output.

Calibrating the Tracking System

Depending upon whether basic activity chambers, home cage platforms, or mazes are used for activity monitoring, one of the following Calibration windows will be used to calibrate the tracking system.

Figure 3-2 - Calibration Window for Open Field Chambers (VID-510 / VID-515)
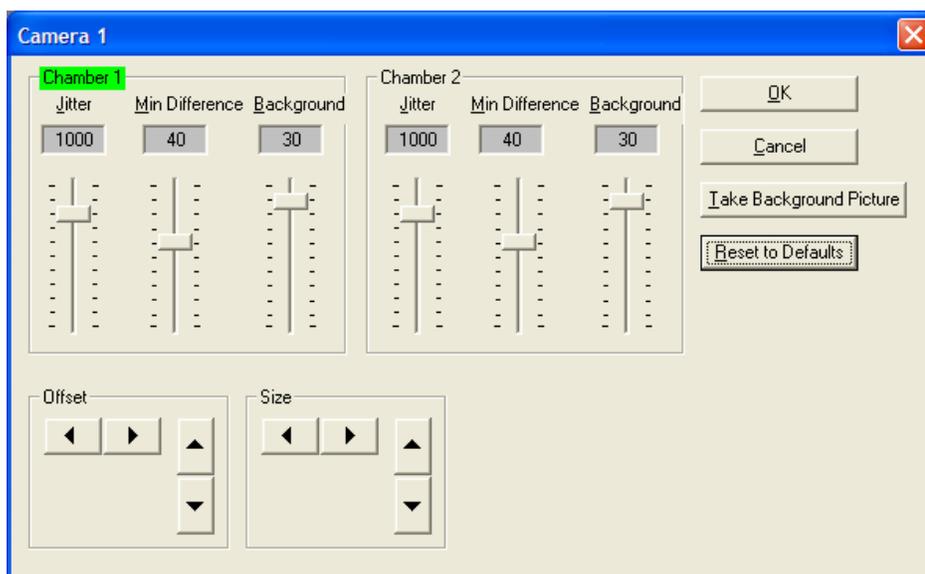
Figure 3-3 - Calibration Window for NIR Open Field Chambers (NIR-VID-510 / NIR-VID-515)
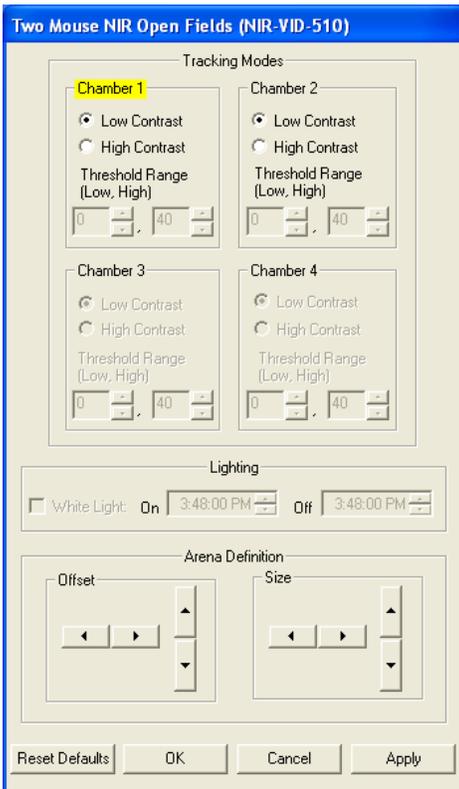

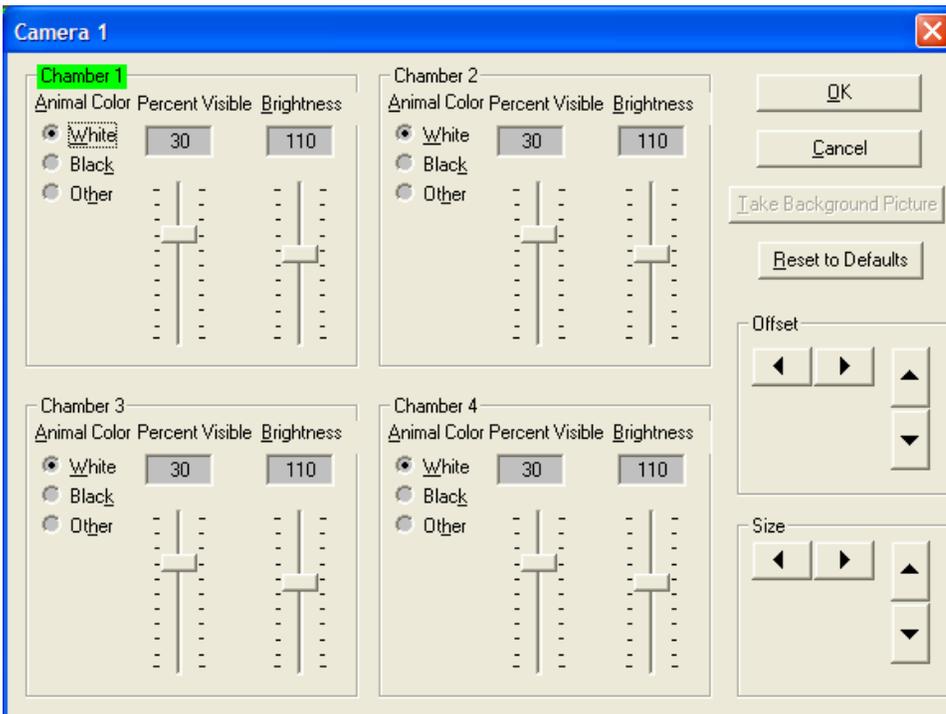*Figure 3-4 - Calibration Window for Home Cage Chambers (VID-410 / VID-415)*

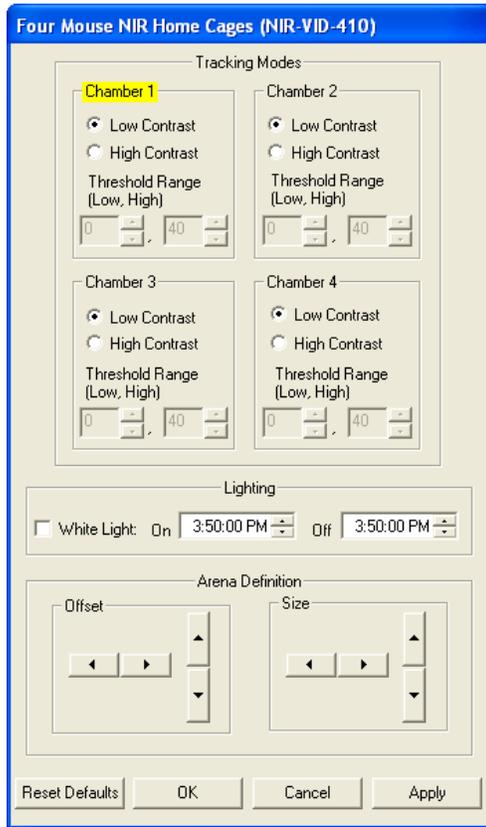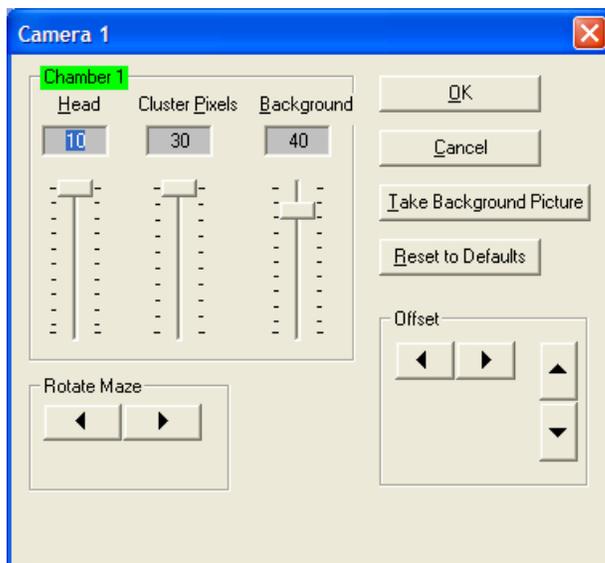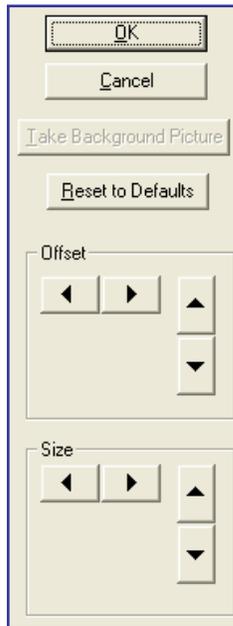*Figure 3-5 - Calibration Window for NIR Home Cage Chambers (NIR-VID-410 / NIR-VID-415)*

Figure 3-6 - Calibration Window for Mazes (VPM-MS, VPM-RS, VZM-MS, VZM-RS)

The panel shown in Figure 3-7 appears within each Calibration window:

Figure 3-7 - Calibration Controls

The function of each of the four buttons at the top the panel shown in Figure 3-7 is self-explanatory and will be explained further in the calibration instructions at the end of this chapter.

Below these four buttons are two sets of arrow buttons, one for setting the orientation ("Offset") and one for setting the size ("Size") of the chamber tracking boundaries.

The "Offset" arrow buttons (right, left, up, down) indicate which direction to move the chamber-tracking boundary.

The right "Size" arrow button increases the width of the chamber-tracking boundary and the left arrow button decreases it, while the up arrow button increases the height of the chamber-tracking boundary and the down arrow button decreases it.

To adjust the size of the mazes, click and drag the maze lines when in calibrate mode.

On the windows depicted above in Figure 3-2 and Figure 3-4, notice there multiple panels for calibrating the tracking parameters for up to four activity monitoring chambers/cages simultaneously. Each panel is labeled ("Chamber 1…Chamber 4") and has slider bars for setting tracking parameters. For each parameter the scale of the slider bars ranges from a minimum value (top of scale) to a maximum value (bottom of scale).

Calibration Instructions

Select **Options⏐Camera 1….Camera 4** to designate the type of chamber(s) used by each camera in the system. The choices include:

o NIR Tracking
   ▪ One Rat Open Field (NIR-VID-515)
   ▪ Two Mouse Open Fields (NIR-VID-510)
   ▪ Two Rat Home Cages (NIR-VID-415)
   ▪ Four Mouse Home Cages (NIR-VID-410)
o Color Tracking
   ▪ One Rat Open Field (VID-515)
   ▪ Two Mouse Open Fields (VID-510)
   ▪ Two Rat Home Cages (VID-415)
   ▪ Four Mouse Home Cages (VID-410)
o VPMM-LM (NIR Mouse Plus Maze)
o VPMR-LR (NIR Rat Plus Maze)
o VZM-MS (Mouse Zero Maze)
o VZM-RS (Rat Zero Maze)

Using Multiple Cameras

For multiple camera systems, each camera must be calibrated individually. Begin by selecting the desired camera from the **Options** pulldown menu, and then select the system being used (listed above). The calibration procedure depends on the system being used. Detailed instructions for each system follow.
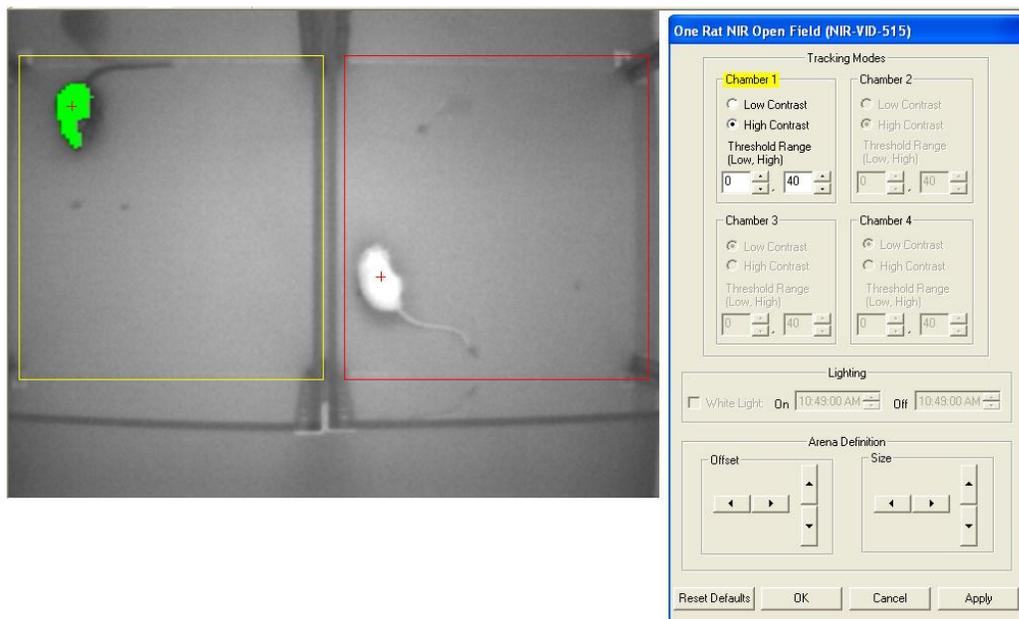
Repeat this process with each additional camera that is connected to the computer. Once calibration is complete for each camera, select **Capture Video** from the **Video** pulldown menu and video from each camera will be displayed on the screen.

NIR Tracking

Calibrating NIR Open Field Activity Chambers (NIR-VID-510 / NIR-VID-515)

1. Refer to Figure 3-3 while completing these calibration instructions. Select the camera connected to the open field chamber if there is more than one system.

2. Place the animals in the chambers.

3. Turn on the NIR light and close the cubicle doors.

4. Adjust the offset and size of each on-screen chamber so that the video tracking boundaries match the chamber boundaries. To switch to a different chamber, click inside that portion of the dialog, as shown in Figure 3-8. The active chamber will be highlighted in yellow.

Figure 3-8 - NIR Open Field Calibration

5. Select the desired tracking method for each chamber, Low or High Contrast. Select Low Contrast when using dark colored mice on a dark colored background or light colored mice

on a light colored background. Select High Contrast when using light colored mice on a dark colored background, or vice versa.

6. If High Contrast Mode is selected, use the Threshold Range controls to select the grayscale range that the animal occupies, lower values for darker mice and higher values for lighter mice. As these values are adjusted portions of the chamber screen will light up in green, the software will track these regions. To optimize tracking, minimize portions of the chamber that are highlighted green and maximize the green highlighting of the animal.

Calibrating NIR Home Cages (NIR-VID-410 / NIR-VID-415)

7. Refer to Figure 3-5 while completing these calibration instructions. Select the camera connected to the home cage if there is more than one system.

8. Ensure that the chambers are oriented so that the ends with the water tubes and food hoppers attached are facing the cubicle walls. Place the animals in the chambers and attach the lid spacers on the rear edge of Chambers 1 and 2. These spacers will reduce glare from the lights.

9. Turn on the NIR light and close the cubicle doors.

10. Adjust the offset and size of each on-screen chamber so that the video tracking boundaries match the chamber boundaries. To switch to a different chamber, click inside that portion of the dialog, as shown in Figure 3-9. The active chamber will be highlighted in yellow.

Figure 3-9 - NIR Home Cage Calibration

11. Select the desired tracking method for each chamber, Low or High Contrast. Select Low Contrast when using dark colore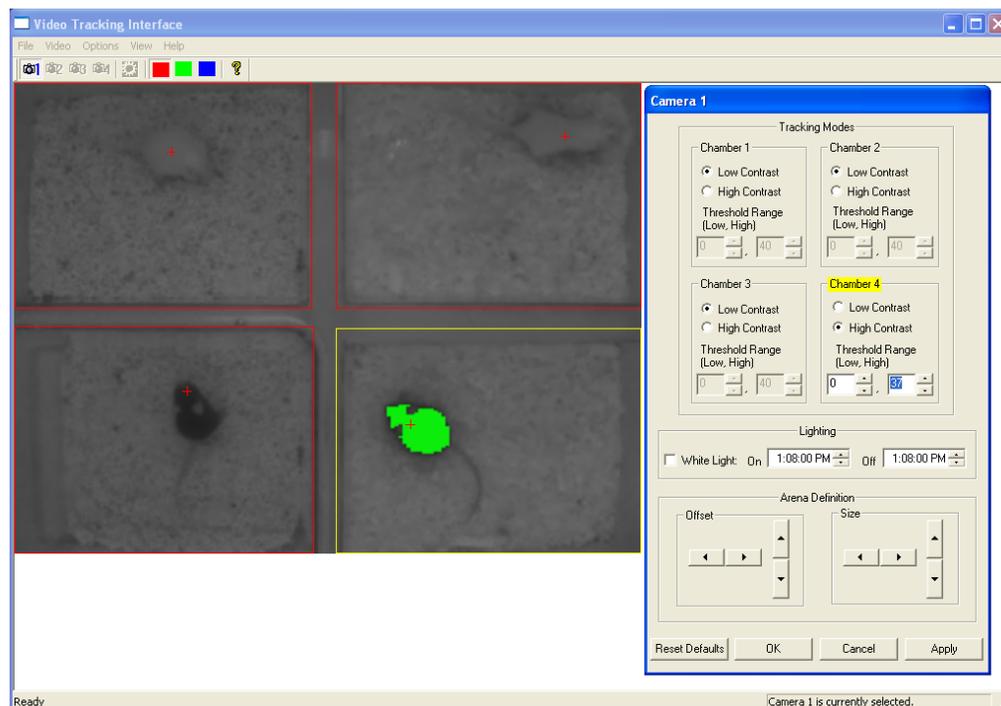d mice on a dark colored background or light colored mice on a light colored background. Select High Contrast when using light colored mice on a dark colored background, or vice versa.

12. If High Contrast Mode is selected, use the Threshold Range controls to select the grayscale range that the animal occupies, lower values for darker mice and higher values for lighter mice. As these values are adjusted portions of the chamber screen will light up in green, the software will track these regions. To optimize tracking, minimize portions of the chamber that are highlighted green and maximize the green highlighting of the animal.

13. If desired, use the Lighting controls on the Calibration dialog to set the day/night cycle for the white light. To enable this feature check the box next to the timer settings (labeled "White Light") and set the timers to the appropriate times.   To control the white light manually, uncheck the box and the timer will not run. Video Tracking Interface uses the Windows system clock, so be sure the computer's clock is synchronized with the time in the facility. When using the timer, the White Light toggle switch on the NIR-100 should be set to REMOTE and the NIR Light toggle switch should be set to ON.

Color Tracking

Calibrating Open Field Activity Chambers (VID-515 / VID-510)

1. Set the current camera to Camera 1.

2. Remove all animals from the chambers, and insert the chambers into the Video Tracking cubicles. Turn the cubicle light on and close the cubicle doors. Note that with a Near Infrared system, the lighting needs to be the same for both calibration and capturing of video. Turning the visible or NIR lights on and off while capturing video will corrupt the tracking data.

3. Select Video | Calibrate on the VTI menu. The screen shown in Figure 3-2 should appear. Click the button "Take Background Picture".

4. Adjust the offset and size of each chamber so that the Video Tracking boundaries match the chamber boundaries. To switch to a different chamber, click on that chambers text (e.g. Chamber 2). When this is done the VTI screen should look similar to Figure 3-10.

Figure 3-10 - Video Tracking Interface Screen

5. Open the cubicle doors and insert the animals into the chambers.

6. Close the cubicle doors.

7. The VTI software should now be tracking the animals correctly with the default Jitter, Min Difference, and Background settings.

8. Slight adjustments may need to be made to accurately track animals. Figure 3-11 shows both black and white mice being tracked with a Jitter setting of 100, a Min Difference setting of 40, and a Background setting of 40. the Jitter was changed to allow smoother tracking of the animals position.

9. When calibration is complete, click OK and repeat the calibration steps for each camera.

Figure 3-11 - Adjusted Settings
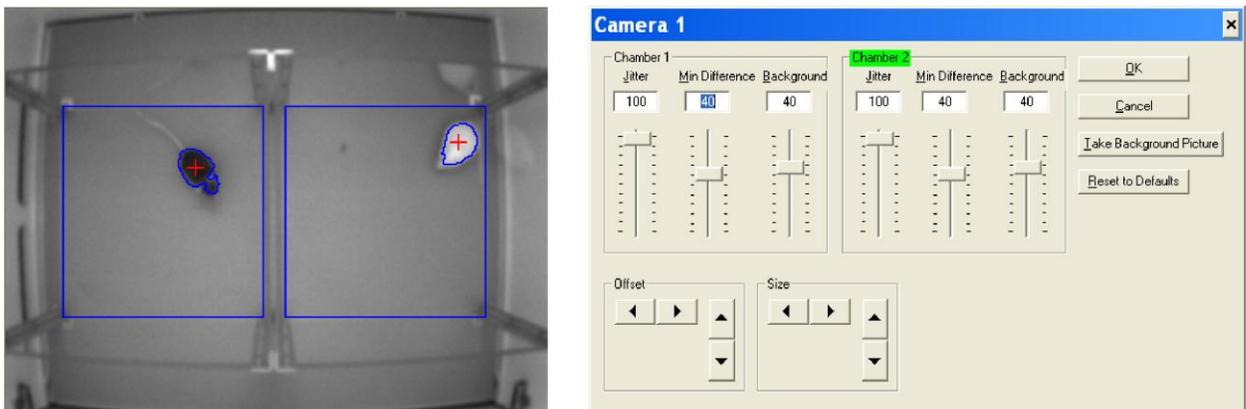
Jitter – determines how often the center of the animal is moved. If jitter is set very low the animals position will be very accurate, however the animals position will move slightly every frame, even though the animal is not moving. If the jitter is set very high the animal's position will only move when the animal has moved a significant distance. It is recommended to set this value to 1000, which offers a good balance of obtaining an accurate center of the animal without excessive movement.

Background - determines how much the current image pixels must differ from the background image pixels in order to be considered a valid pixel.

Min Difference – the minimum number of pixels that must be valid in order for the image to be analyzed.

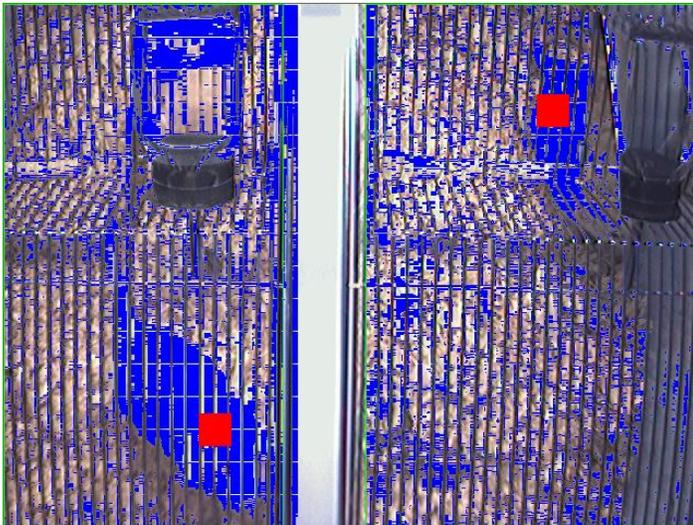**Calibrating Home Cages (VID-415 / VID-410)**

1. Set the current camera to Camera 1. Refer to Figure 3-12 for the VID-415 and Figure 3-13 for the VID-410 while completing these calibration instructions.

2. Insert the animals into the home cages, and then insert the home cages into the video Tracking cubicles. Ensure that the home cages are oriented correctly, as in Figure 3-13. Turn on the cubicle light and close the cubicle doors.

3. Adjust the offset and size of each chamber so that the video tracking boundaries match the chamber boundaries. To switch to a different chamber, click on that chambers text (e.g. Chamber 2).

4. Select the appropriate animal color for the subject (note that only white or black animals are supported when using the home cages. The activity chambers support all colors of animals.)

5. The blue areas on the camera image indicate which pixels the VTI software interprets as valid. Ideally the only blue in the image should be on the animal and the water bottle. The blue areas will change depending on the animal color and brightness selected. If the animal is white, any pixels that are brighter than the currently specified brightness will be considered valid. If the animal is black, any pixels that are darker than the currently specified brightness will be considered valid. The red square is the actual location the VTI software is tracking. The red square will be placed on the last location of movement that contains a valid square. Typically for white animals the default values of 30 for the percent visible and 110 for the brightness will work well, and for a black animal a value of 75 for the brightness may work. Adjust the brightness value as necessary until the software tracks the animal.

6. When calibration is complete, click **OK** and repeat the calibration steps for each camera.

Brightness – determines how dark/bright a pixel must be to be considered valid.

Percent Visible – the image is broken into small blocks, or grids, which are then analyzed by the software. Each block must have at least the percent visible number of pixels in order to be considered valid. For example, if percent visible is set to 40, then each block must have at least 40% of the blocks pixels considered valid in order for the entire block to be considered valid. The home cage algorithm works by taking the valid blocks, and then looking for movement in the valid blocks. A valid block with movement is determined to be the animals' location.

Home Cage Orientation – VID-410 – When using the VID-410 chambers, changing the orientation of the home cages may improve the accuracy of the video tracking, especially with black mice. Feel free to experiment with different orientations and calibration settings to obtain the best tracking accuracy.

Figure 3-12 - VID-415 Calibration

NOTE: White bedding will not work with the VTI system. For best results use wood chip bedding, preferably bedding with a strong red or yellow color.

Figure 3-13 - VID-410 Calibration
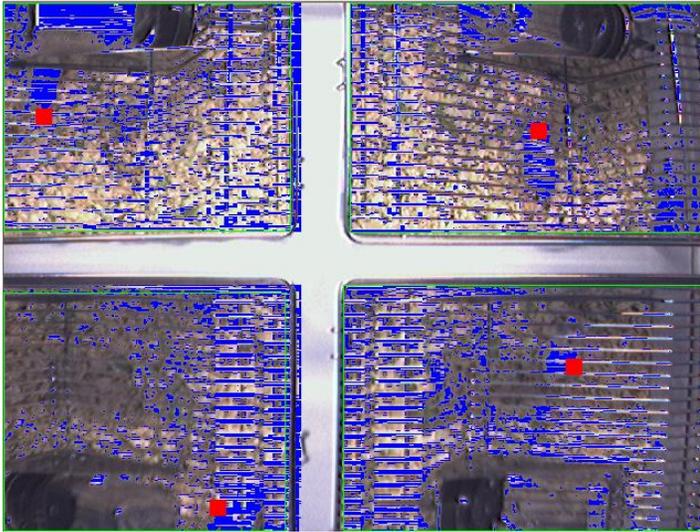
NOTE: White bedding will not work with the VTI system. For best results use wood chip bedding, preferably bedding with a strong red or yellow color.

Calibrating NIR Video Plus Maze (VPMM-LM & VPMR-LR)

1. Set the current camera to Camera 1. Refer to Figure 3-14 while completing these calibration instructions.

2. Ensure that the Plus Maze is evenly lit, with no shadows and a consistent background.

3. If necessary, rotate the camera lens to focus the camera. Adjust the maze lines (the green lines shown in Figure 3-14) so that the lines match the outline of the maze, as shown. The lines can be moved using the offset buttons shown in Figure 3-7, or by clicking and dragging the lines.

4. Before placing any animals on the maze, click "Take Background Picture".

5. The tracking algorithm works by calculating the difference between the current image and this Background Picture, therefore the background must stay constant during the trial for the tracking algorithm to work correctly. It is especially important that the lighting remain constant, therefore it is recommend that the maze not be lit by sunlight.

6. There are three properties that can be adjusted that affect the tracking. (Refer to Figure 3-6) The first property is the "Background". "Background" refers to the difference between the background image and the current image. Typically a Background value of 40 works

well for white or black animals. For brown animals the "Background" value may need to be reduced. The second property is the "Cluster Pixels". "Cluster Pixels" refers to the number of contiguous pixels that exceed the difference (the background setting) between the current image and the background image. The third property is labeled "Head". "Head" is the minimum number of pixels that must be valid in order for a head dip to occur, and is also the minimum number of pixels that must be valid for an exploration to occur.

7. As the tracking algorithm works by comparing the contrast of each pixel, it is important that there be a significant contrast difference between the floor and the animal. The contrast between the floor and the animal is used to detect head dips.

8. When calibration is complete, click OK and repeat the calibration steps for each camera.

Background – the difference between the background image and the current image. An image is composed of pixels (320 pixels wide by 240 pixels tall in this case), and each pixel from the current image is compared to the corresponding pixel in the Background Picture. If the difference between the current pixel and the Background pixel is greater than the background difference, the pixel is considered valid.

Cluster Pixels – the number of contiguous pixels that must exceed the difference (the background setting) between the current image and the background image in order for the animal to be detected.

Head – the minimum number of pixels that must be valid in order for a head dip to occur, also the minimum number of pixels that must be valid for an exploration to occur.

Figure 3-14 - Video Plus Maze (VPM-MS & VPM-RS)
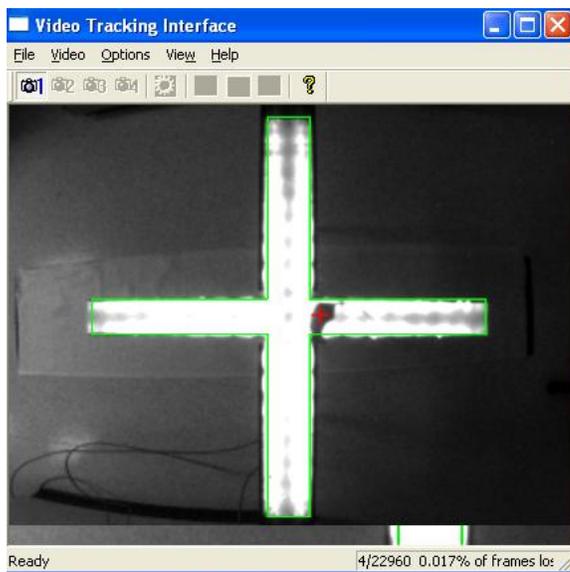
### Calibrating Video Zero Maze (VZM-MS & VZM-RS)

1. Set the current camera to Camera 1. Refer to Figure 3-15 while completing these calibration instructions.

2. Ensure that the Zero Maze is evenly lit, with no shadows and a consistent background. MED Associates has included built-in lights, which greatly assist in achieving proper lighting.

3. If necessary, rotate the camera lens to focus the camera. Rotate the camera so that the closed sections are in the upper-right and lower-left part of the image, as shown in Figure 3-15. If the camera can't be rotated, rotate the maze outline by clicking the "Rotate Maze" buttons on the calibration screen. Adjust the maze lines (the green lines shown in Figure 3-15) so that the lines match the outline of the maze, as shown. The lines can be moved using the offset buttons shown in Figure 3-7, or by clicking and dragging the lines.

4. Before placing any animals on the maze, click "Take Background Picture".

5. The tracking algorithm works by calculating the difference between the current image and this Background Picture, therefore the background must stay constant during the trial for the tracking algorithm to work correctly. It is especially important that the lighting remain constant, therefore it is recommend that the maze not be lit by sunlight.

6. There are three properties that can be adjusted that affect the tracking. (Refer to Figure 3-7) The first property is the "Background". "Background" refers to the difference between the background image and the current image. Typically a Background value of 40 works well for white or black animals. For brown animals the "Background" value may need to be reduced. The second property is the "Cluster Pixels". "Cluster Pixels" refers to the number of contiguous pixels that exceed the difference (the background setting) between the current image and the background image. The third property is labeled "Head". "Head" is the minimum number of pixels that must be valid in order for a head dip to occur, and is also the minimum number of pixels that must be valid for an exploration to occur.

7. As the tracking algorithm works by comparing the contrast of each pixel, certain shades of brown are very close to the blue background. If the system is having difficulty in tracking an animal, the blue background on the maze runway can be removed to reveal a white background. Also consider removing the blue plastic mats from under the maze in these cases.

8. When calibration is complete, click **OK** and repeat the calibration steps for each camera.

Background – the difference between the background image and the current image. An image is composed of pixels (320 pixels wide by 240 pixels tall in this case), and each pixel from the current image is compared to the corresponding pixel in the Background Picture. If the difference between the current pixel and the Background pixel is greater than the background difference, the pixel is considered valid.

Cluster Pixels – the number of contiguous pixels that must exceed the difference (the background setting) between the current image and the background image in order for the animal to be detected.

Head – the minimum number of pixels that must be valid in order for a head dip to occur, also the minimum number of pixels that must be valid for an exploration to occur.

*Figure 3-15 - Video Zero Maze (VZM-MS & VZM-RS)*
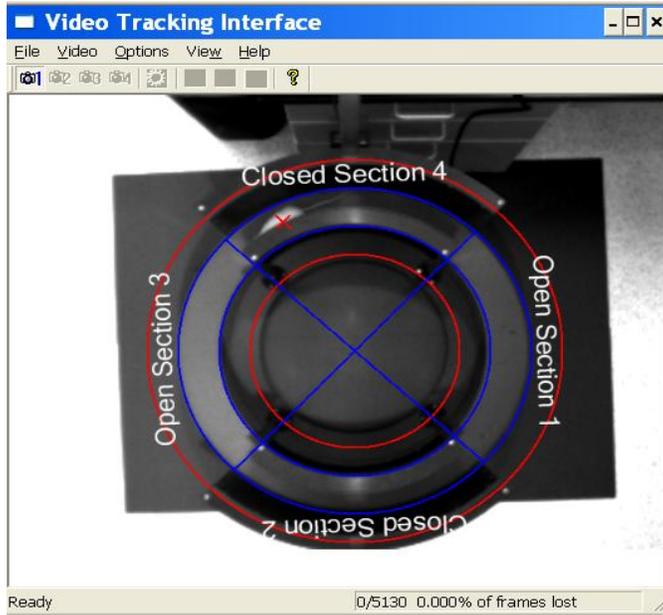
CHAPTER 4 | RUNNING AN EXPERIMENT

NOTE: Calibration of the tracking system must be completed before proceeding (for directions see **Error! Reference source not found.**).

1. If Mazes are being used, please refer to the Video Maze manual for information on how to begin an experiment.

2. Otherwise open Activity Monitor and select **File⎮Preferences** to open the following Preferences screen in Activity Monitor, depicted in Figure 4-1:

Figure 4-1 – Activity Monitor Preferences Screen
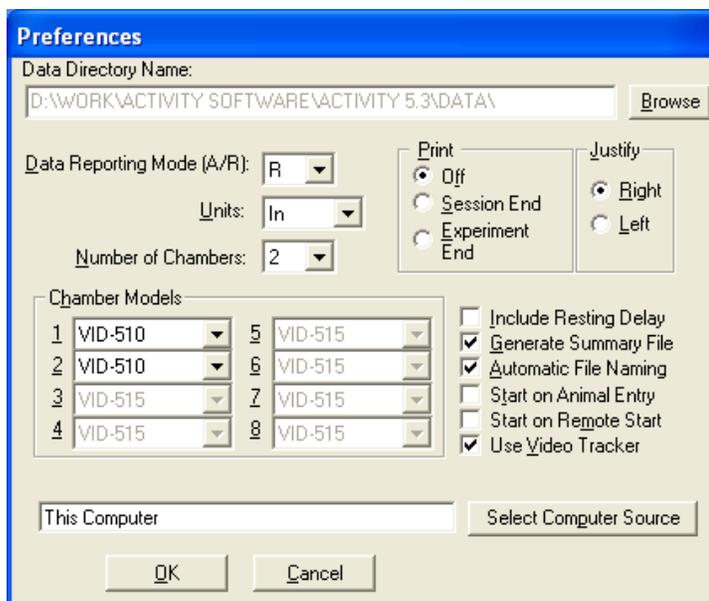
3. Enable the **Use Video Tracker** check box as shown above.

4. The **Select Computer Source** option selects the computer that the Video Tracking Interface (VTI) program is running on. Click on the "Select Computer Source" button to browse for the appropriate computer. If Activity Monitor is running on the same computer as VTI then click on "My Network Places" and click **OK** to set the computer to as its own source, as shown in Figure 4-1.

5. Select the Number of Chambers to the number of animals that VTI is tracking. For example:

    VID-515 / NIR-VID-515 acquires data from one animal per camera, so for a two-camera setup the number of chambers is two.

    VID-510 / NIR-VID-510 acquires data from two animals per camera, so for a two-camera setup the number of chambers is four.

    VID-415 / NIR-VID-415 acquires data from two animals per camera, so for a two-camera setup the number of chambers is four.

    VID-410/NIR-VID-410 acquires data from four animals per camera, so for a two-camera setup the number of chambers is eight.

6. Select the Chamber Model for each chamber to the appropriate type.

7. Click OK.

8. Refer to the Activity Monitor manual, Setting up an Ambulatory Experiment, starting with the **Run│Open Experiment** step.

9. Configure the Activity Monitor Open Experiment form with the appropriate settings, but do not start the boxes (typically the boxes are started when the OK button is pressed).

10. Insert the animals into the Video Tracking chambers, and in the Video Tracking program select **Video | Capture Video** to start video tracking.

11. Now in Activity Monitor hit the **OK** button in the Run Experiment form to start the Activity Monitor chambers.

12. Note that after an experiment is completed in the Video Tracking program selecting **Video | Stop Capture** stops the capturing of data. It is important that after the experiment has ended, video capturing be stopped, the chambers cleaned and new animals be put in before video capturing is restarted.

APPENDIX A | INSTALLING VIDEO TRACKING INTERFACE DRIVERS AND SOFTWARE

The Video Tracking Interface package for the Open Field and Home Cage systems work in conjunction with the Activity Monitor software and the maze systems work in conjunction with the Video Maze software. The Video Tracking Interface and the included supporting software (Activity Monitor or Video Maze) can be installed on the same computer, or two separate computers that are connected via a Windows network.

The appearance of the installation screens will vary slightly for each system, but the procedure is the same.

Insert the Video Tracking Interface CD, and the installation process should start automatically. If it does not then open "My Computer", open the CD drive "Video Tracking" and double-click on "autorun.exe". A screen resembling the one shown in Figure 4-2 will appear. Click **Install the … software** and a screen resembling the one shown in Figure 4-3 will appear.

Figure 4-2 - Example Main Screen
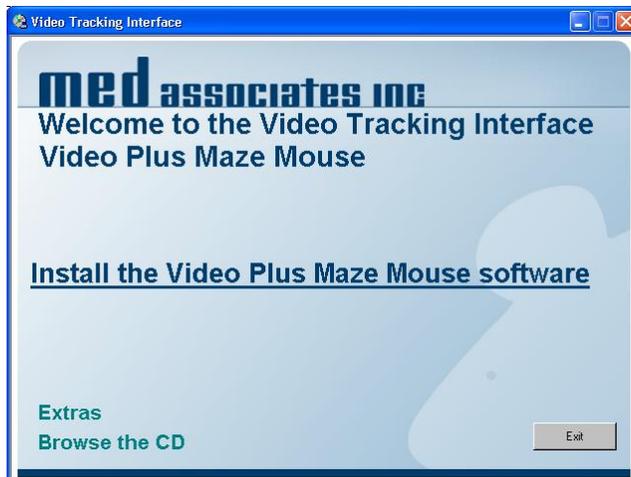
Click **Install** to begin installation of the Fire Wire Camera Driver, the Video Maze or Activity Monitor software and the Video Tracking Interface. As these installations are completed, their status will be indicated on the screen. A successful installation will be indicated with a green check mark and an unsuccessful installation will be indicated by a red X, refer to Figure 4-4. If any portion of the installation is unsuccessful, please contact MED Associates Customer Support.

Figure 4-3 – Example Installation Screen

Figure 4-4 – Example of Installation Status Indicators

Once the installation has been completed successfully, a screen resembling the one shown in Figure 4-5 will appear.

Click **Finish** to exit this screen. It is now necessary to complete the secondary portion of the Fire Wire Camera driver installation prior to using the camera. Refer to Appendix B for detailed instructions.

Figure 4-5 - Installation Complete

APPENDIX B |FIREWIRE CAMERA DRIVER INSTALLATION
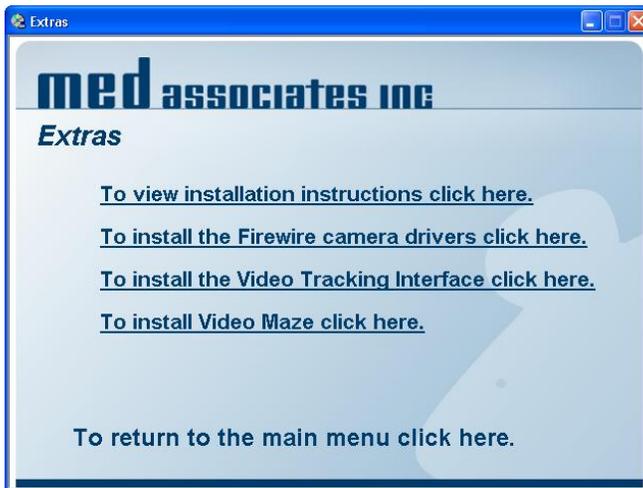
Installing the Firewire Camera Driver

Insert the Video Tracking Interface CD, and the installation process should start automatically. If it does not then open "My Computer", open the CD drive "Video Tracking" and double-click on "autorun.exe". A screen resembling the one shown in Figure 4-6 will appear. Click **Extras** and the screen shown in Figure 4-7 will appear.
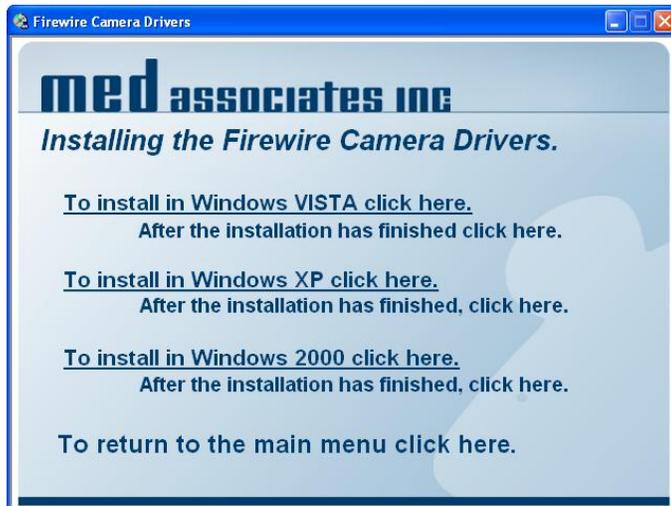
Figure 4-6 - Example Main Screen
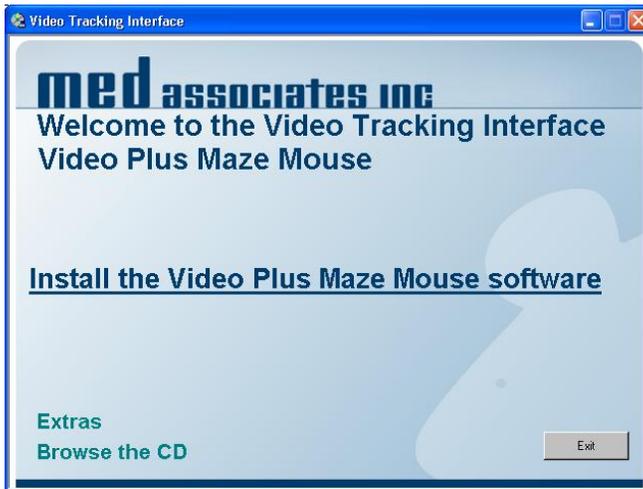
Click **To install the Firewire camera drivers click here** and the screen shown in Figure 4-8 will appear.

Figure 4-7 - Example Extras Screen

Click on the operating system being used and follow the subsequent steps to complete the Fire Wire Camera Driver installation.

Figure 4-8 – Example Firewire Camera Drivers Screen

Installing the Firewire PCI Card

Always turn off the power before working on the computer. Neglecting this precaution may cause serious damage. Install the Firewire card in any available PCI slot, following the directions provided with the computer for installing a PCI card.

## Installing the Firewire Cameras

Using the included Firewire cable, plug the first camera into any Firewire port on the Firewire PCI card. Then plug the second camera into another Firewire port on the Firewire PCI card. Note that only two cameras can be plugged into a Firewire card.

APPENDIX C | TROUBLESHOOTING

"Black Screen"

If the Camera Window displays a completely black image, there are several possible causes. The issue could be caused by a problem with the camera or connection, or the problem may be within the camera's settings. Refer to the following lists and ensure that none of these problems are the cause.

Figure 4-9 - "Black Screen"
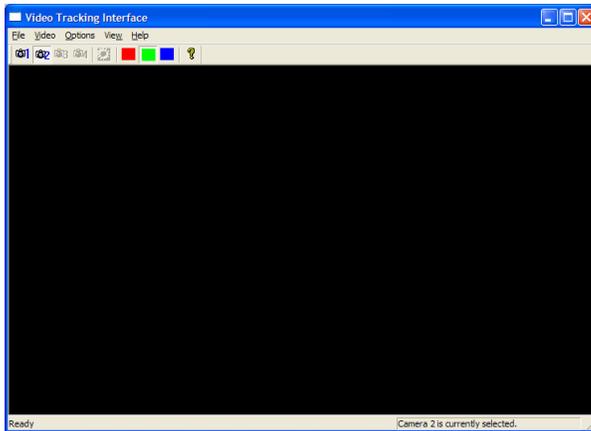
Equipment Issues

- Camera lens cap is on.
- Camera lens not properly attached to camera body.
- Camera lens aperture rotated to closed position.
- Loose Firewire cable connection at camera or computer.
- Light in Sound Attenuated Cubicle (or other closed environment) is out.
- Computer requires reboot.

Camera Settings

- Brightness level not set to automatic, and level is too low.
- Auto Exposure level not set to automatic, and level is too low.
- Gain level not set to automatic, and level is too low.
- Shutter level not set to automatic, and level is too low.

Resolution

If the equipment issues have been eliminated, set all the camera features to "Automatic". If Automatic settings are not available, set Brightness, Auto Exposure, Gain, and Shutter to the highest values possible. In most cases it is recommended that the automatic camera controls be disabled and that the camera be controls be set manually. This allows the user more control and ensures that the camera settings are uniform for multiple cameras setups. It also ensures the automatic camera adjustments will not interfere with animal tracking.

APPENDIX D | CONTACT INFORMATION

Please contact MED Associates, Inc. for information regarding any of our products.

Visit our website at www.med-associates.com for contact information.

For technical questions, email support@med-associates.com.