med associates inc

Instrumentation and software for research

USB VIDEO FEAR CONDITIONING

SOF-843 VIDEO FREEZE®

USER'S MANUAL

DOC-321
Rev 1.2

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notes

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CHAPTER 1 |INTRODUCTION

The **USB Video Fear Conditioning System** (VFC) and the **Video Freeze® Software** system allows the researcher to easily construct customized experimental protocols and obtain a quantitative measurement of conditioned immobility, or "freezing." Video Freeze® Software is an important advancement in behavioral testing technology because it provides a reliable, automated means of monitoring the effects of fear conditioning in rodent species.

The "Protocol Setup" section in this manual (see Chapter 6) outlines the steps involved in designing the stimulus conditions for the experiment, and the section titled "Starting an Experimental Session" describes the options pertaining to dependent measures.

The VFC system is entirely automated and records video on a PC hard drive. Digital video cameras are used to simultaneously capture data from up to four fear-conditioning chambers (mice or rats). The Video Freeze® run-time display provides information on current CS-US (Conditioned Stimulus-Unconditioned Stimulus) durations and inter-trial intervals, as well as displaying the video input while a session is being conducted.

All data is stored to the hard drive, and can be analyzed using the Video Freeze® Data-Analysis Utility (refer to Chapter 11 "Analyzing "*.raw" Data in Video Freeze®"). Use this data-analysis utility to simultaneously view the video and quantitative results. The data-analysis user interface allows researchers to focus on specific intervals within any given trial for detailed examination of the results.

Med Associates is dedicated to providing the scientific community with reliable and innovative behavioral-testing tools. Please contact technical support at support@med-associates.com with questions regarding the USB Video Fear Conditioning System.

CHAPTER 2 | HARDWARE GUIDE

Figure 2-1 – NIR-022SD Sound-Attenuating Cubicle with VFC-008 Fear Conditioning Chamber, VID-CAM-MONO-5 USB Video Camera, ENV414S Aversive Stimulator, and NIR-100 Light Controller

Figure 2-2 – VFC-008 Fear Conditioning Chamber with VFC-716 SmartCtrl Panel

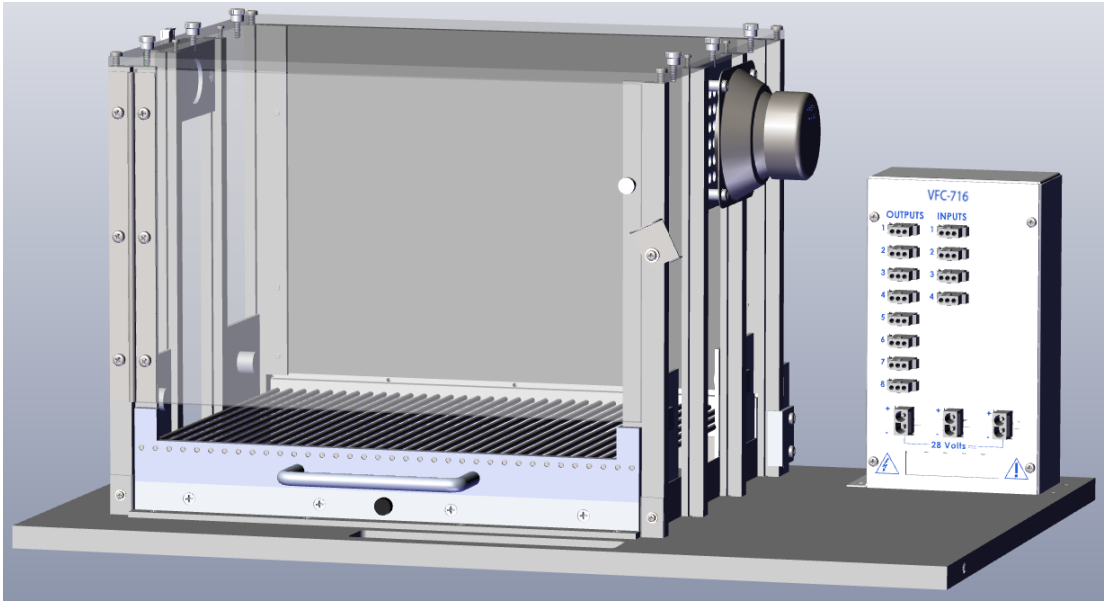


Figure 2-3 - VFC-716 SmartCtrl Panel Default Connections

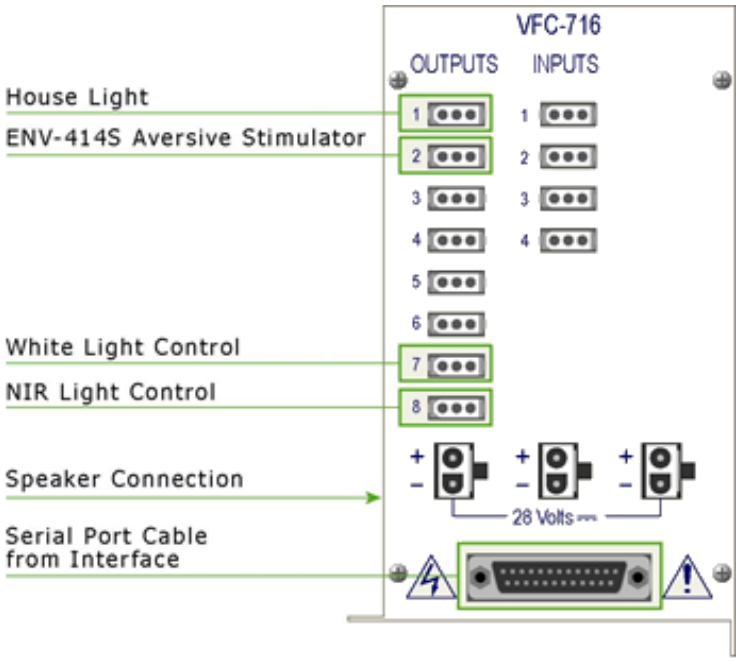


Figure 2-4 - SG-6080D Cabinet with DIG-700G Decode Card and VFC-100 Modules

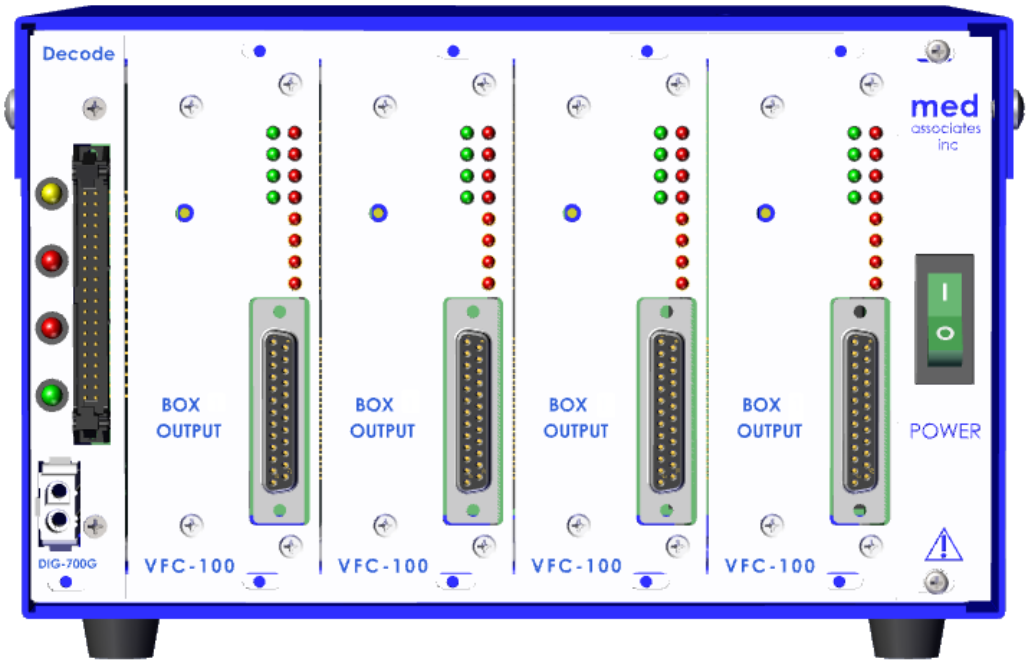


Figure 2-5 - DIG-704PCI-2 Interface Card (Installed in Computer)

Figure 2-6 - ENV-414S Stand-Alone Aversive Stimulator/Scrambler

Figure 2-7 - ENV-005-QD Quick Disconnect Harness (US Pat. No. 6412441 B1, Canadian Pat. No. 2,368,344, UK Pat. No. 1226750, Australian Patent 772111, France 1,226,750, Germany 602 05 143.6, Italy 1,226,750, the Netherlands 1,226,750, European Pat. No. 1226750, Other Foreign Patents Pending)

Figure 2-8 - VID-CAM-MONO-5 USB 3.0 Monochrome Video Camera

Figure 2-9 - NIR-100 Light Controller Front Panel and Rear Panel

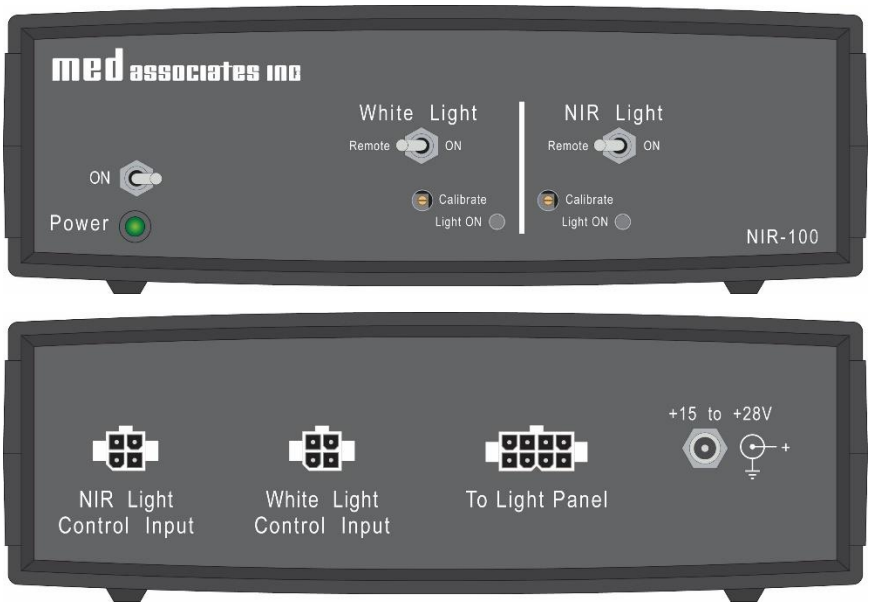


Figure 2-10 - NIR-100R Light Panel (Mounted on top of the Cubicle)

Table 2.1 – Hardware Guide

Part Number	Description	Quantity	Location
VFC-022MD	Sound-Attenuating Cubicle (SAC)	Up to 4 per Computer	Houses Chamber
VFC-008	Conditioning Chamber	One per SAC	Inside the Cubicle
SG-6080D	Interface Cabinet with 28 VDC	One for every four Chambers	Outside the Cubicle
DIG-700G	Interface Decode Card	One per Interface Cabinet	SG-6080D Interface Cabinet
VFC-100	Stimulus Output Cards	One per Chamber	SG-6080D Interface Cabinet
DIG-704PCI-2	PCI Interface Card	One for every four Chambers	Computer
ENV-414S	Aversive Stimulator	One per Chamber	Outside the Cubicle
ENV-005-QD	Quick Disconnect Harness	One per Chamber	Back of Chamber
VFC-716	SmartCtrl Panel	One per Chamber	Inside the Cubicle
ENV-229M	Stimulus Light with Diffuser	One per Chamber	Inside the Chamber
VID-CAM-MONO-5	USB3 Video Camera	One per Chamber	Left Cubicle Door
NIR-100	Light Controller	One per Chamber	Outside the Cubicle
NIR-100R	Light Panel	One per Chamber	On top of the Cubicle
ENV-025F	Fan	One per Chamber	Inside the Cubicle

Note, in addition to the hardware listed above, the VFC system also supports use of the Omni Control hardware which includes the DIG-705, IC-124, and OSC-112. Configuring a VFC system to support use of the Omni Control hardware is documented in Chapter 3 “Configuring the Software”.

Cable Guide

Figure 2-11 – DIG-700C Ribbon Cable

Figure 2-12 - SG-210CP 2-Pin Molex Cable

Figure 2-13 - SG-210CB-25 Serial Port Cable

Figure 2-14 - SG-210G-10 DB-9 Cable

Figure 2-15 - SG-216A-10 3-Pin Molex Cable

Figure 2-16 - CAB-USB3-A-MB-3M Cable

Figure 2-17 - NIR-101 Light Control Input Cable

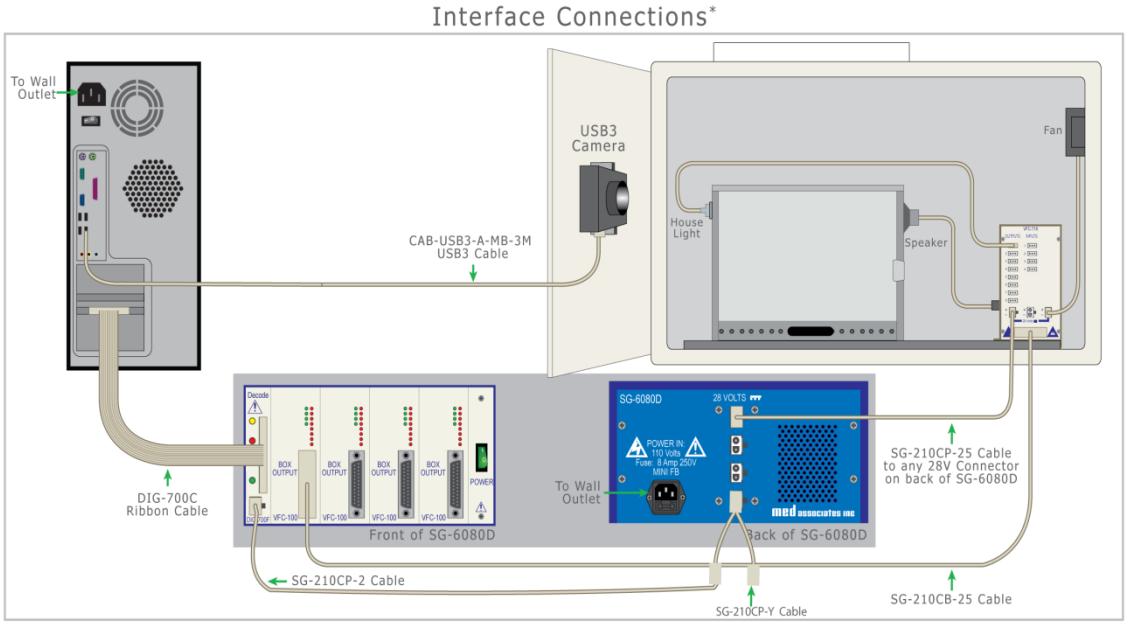
Figure 2-18 - NIR-102 Light Control Interface Cable

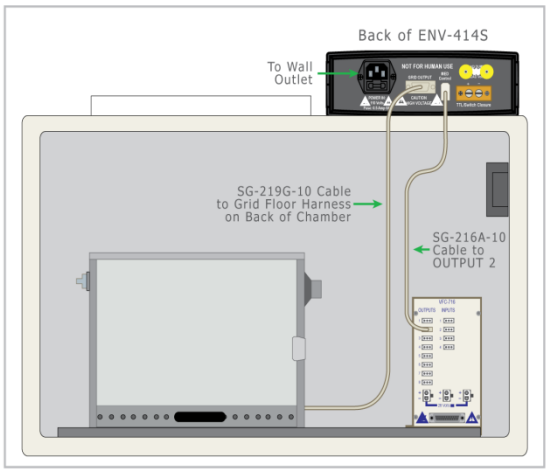
Figure 2.20 – SG-210CP-Y 2-Pin Molex Y Cable

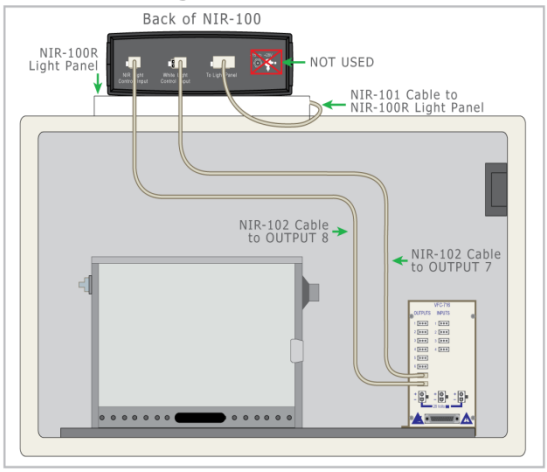
CHAPTER 3 | WIRING GUIDE

Prior to making any connections, be sure that all equipment is turned off. Failure to do so may result in equipment damage.

Quick Reference







* All cables running into the Sound Attenuating Cubicle (SAC) Except CAB-USB3-A-MB-3M should be run through the grommet plate on the right side of the SAC. USB Cable to camera runs through port in SAC on left wall.

Step-by-Step Instructions

1. Using the DIG-700C Ribbon Cable, connect the DIG-700G Interface Decode Card located in the SG-6080D cabinet, to the DIG-704PCI-2 card located on the back of the computer.

NOTE: Press cable connector firmly and evenly into DIG-704 until locking tabs engage, a strong tight connection is also required at the DIG-700G end

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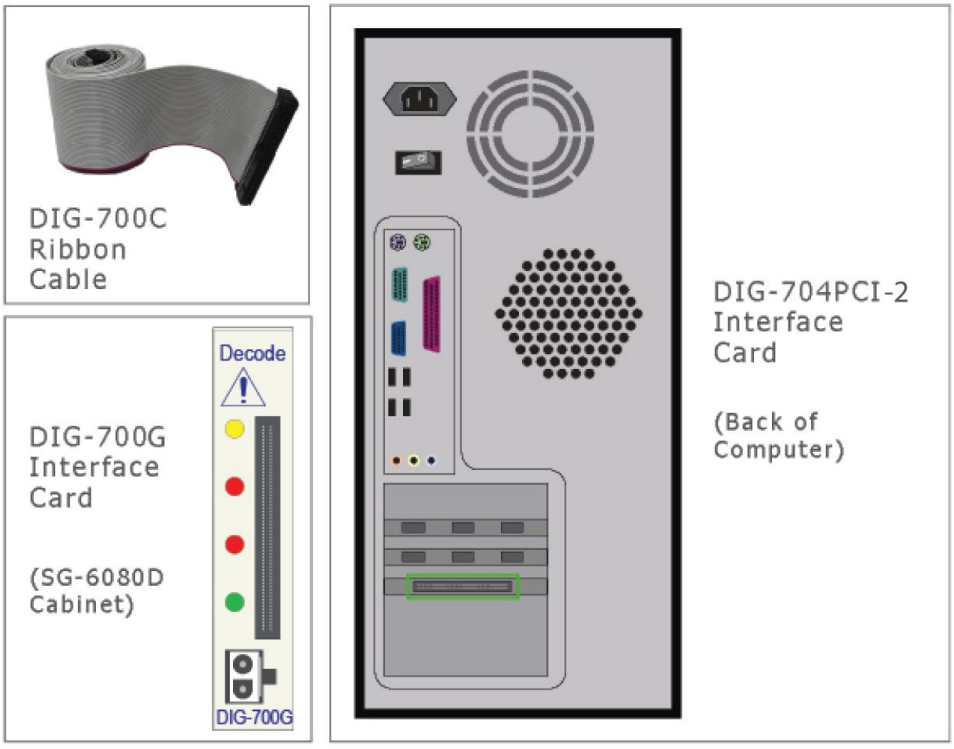
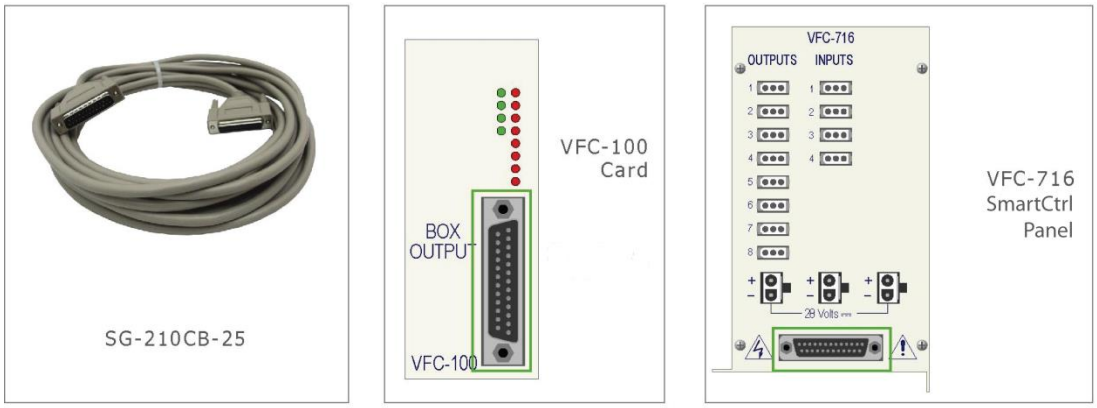


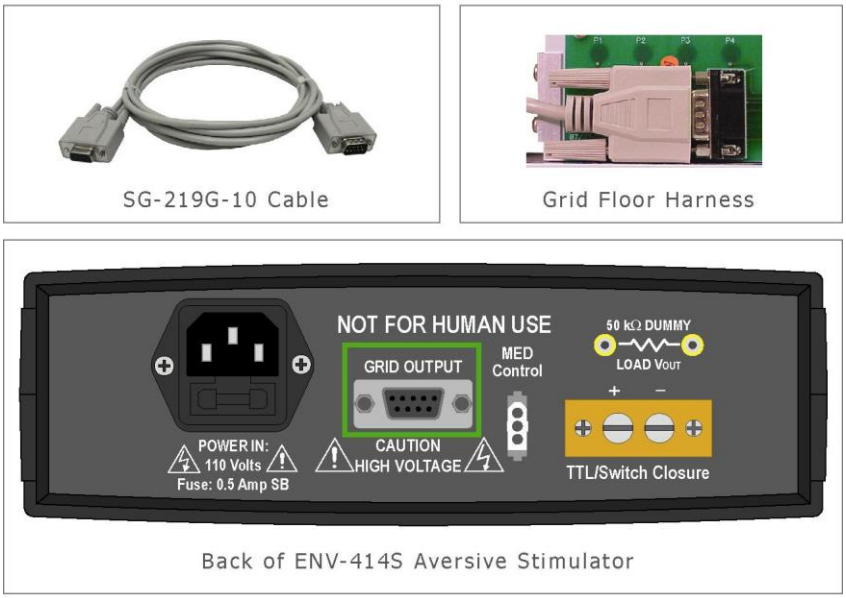
2. Using an SG210-CP-2 2-pin Molex cable, connect the 28V port on the DIG-700G Interface Decode Card located in the SG-6080D cabinet, to any available 28V port on the rear of the SG-6080D cabinet.

3. Using an SG-210-CB-25 25’ serial port cable, connect each VFC-100 stimulus output card located in the SG-6080D cabinet to the corresponding VFC-716 SmartCtrl Panel. For example, if there are multiple chambers, the VFC-100 card labeled “1” should be connected to the VFC-716 in Chamber 1, and so on.



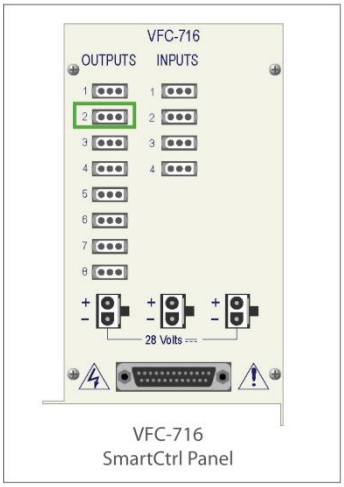
* All cables running into the Sound-Attenuating Cubicle (SAC) should be run through the grommet plate on the right side of the SAC.

4. Using the SG-219G-10 DB-9 cable, connect the Grid Output connector on the back of the ENV-414S Aversive Stimulator to the ENV-005-QD grid floor on the rear of the VFC-008 Conditioning Chamber (inside the Sound-Attenuating Cubicle).



* All cables running into the Sound-Attenuating Cubicle (SAC) should be run through the grommet plate on the right side of the SAC.

5. Using an SG-216A-10 3-pin Molex cable, connect the **MED Control** connection on the back of the ENV-414S Aversive Stimulator to the **OUTPUT 2** connection on the VFC-716 SmartCtrl Panel.



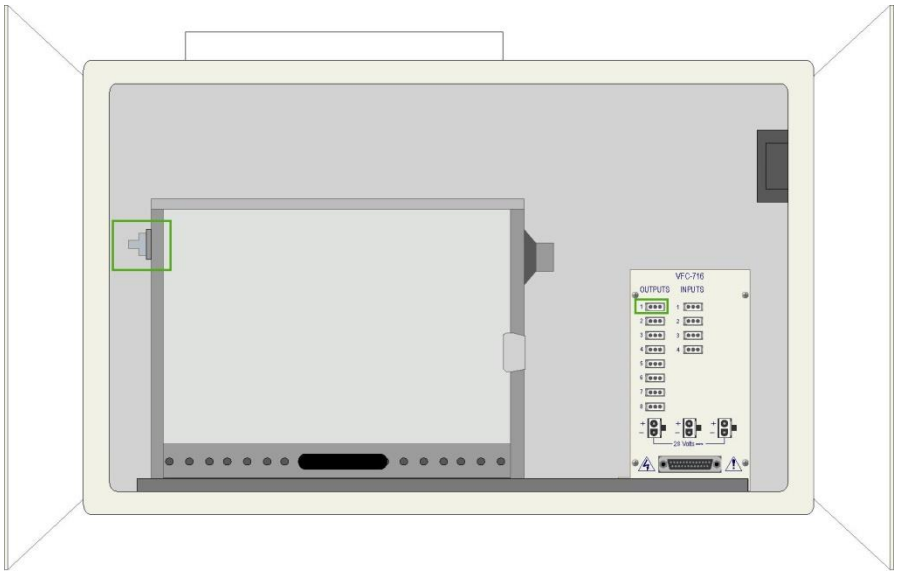
VFC-716
SmartCtrl Panel

SG-216A-10 Cable

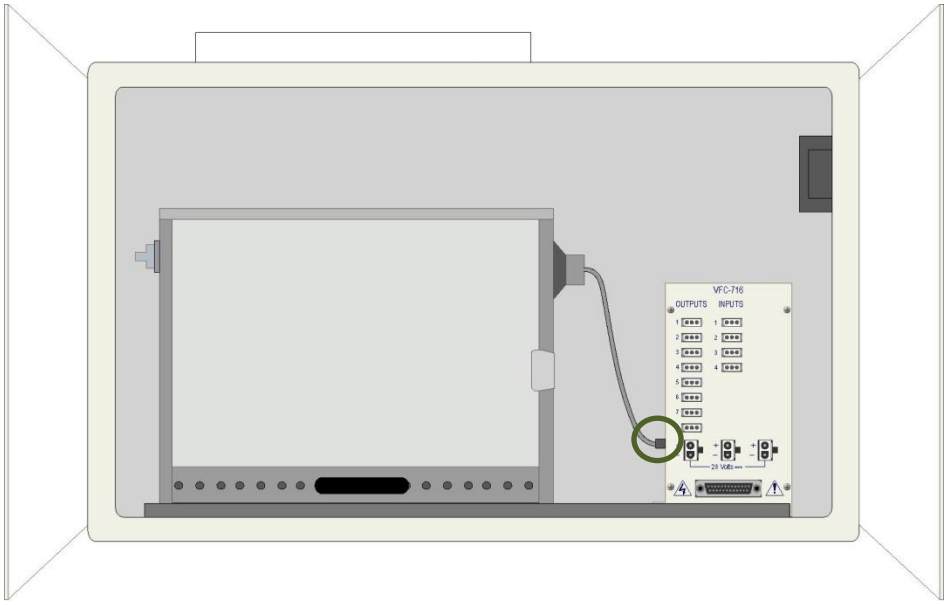
Back of ENV-414S Aversive Stimulator

* All cables running into the Sound-Attenuating Cubicle (SAC) should be run through the grommet plate on the right side of the SAC.

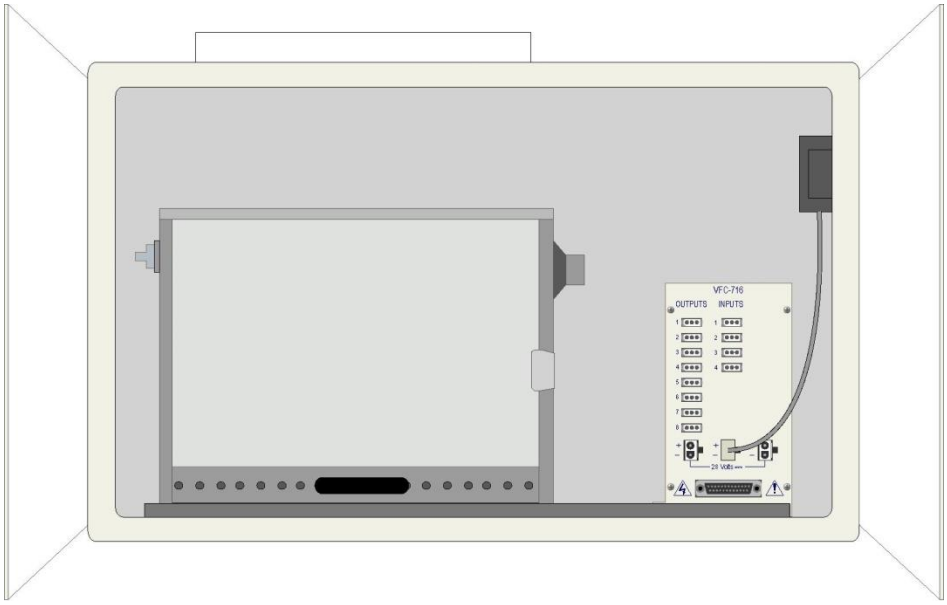
6. Connect the ENV-229M Stimulus Light with Diffuser (on the left side of the Conditioning Chamber) to the **OUTPUT 1** connection on the VFC-716 SmartCtrl Panel.



7. Connect the speaker to the input on the side of the VFC-716 SmartCtrl Panel.

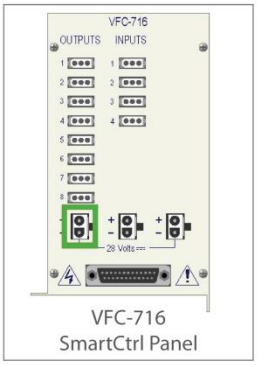


8. Connect the fan inside the Sound-Attenuating Cubicle to one of the three 28V connections on the VFC-716 SmartCtrl Panel.

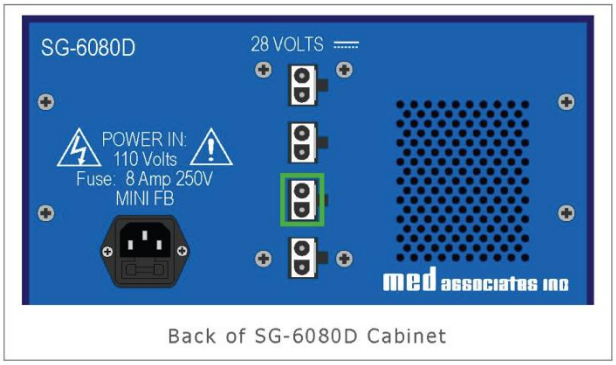


9. Using an SG-210CP-25 2-pin Molex cable, connect any available 28V connector on the VFC-716 SmartCtrl Panel in each Chamber to a 28V connector on the rear of the SG-6080D cabinet, using the SG-210PC-Y cable to connect a forth chamber if needed for a 4th chamber. See chapter 3 Interface connections.


SG-210CP-25



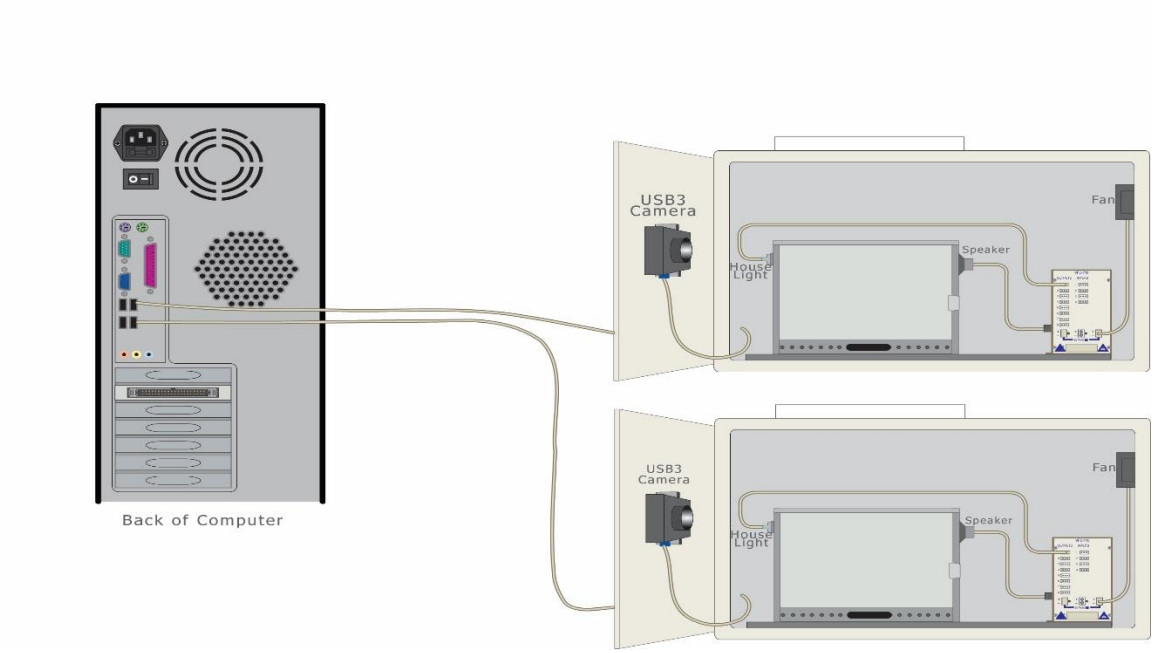
VFC-716
SmartCtrl Panel



Back of SG-6080D Cabinet

* All cables running into the Sound-Attenuating Cubicle (SAC) should be run through the grommet plate on the right side of the SAC.

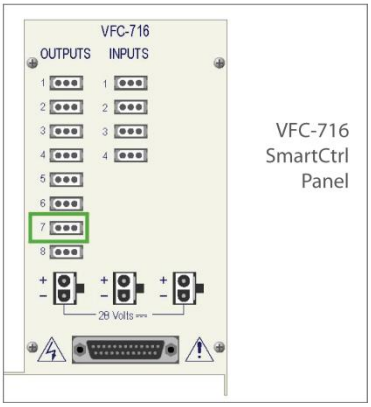
10. Using the USB cables (CAB-USB3-A-MB-3M), connect the USB3 Cameras to the USB3 ports on the back of the computer, indicated by blue internal tabs (instead of black for USB 2.0 which is not compatible). The screw-in locking connectors on the USB cables should be used where available, and sharp bends in the routing of the USB cable should be avoided. If additional length in USB3 cable is needed to reach the Computer, an optional USB3 hub (USB-VID-HUB-USB3) can be used. **Additional information relating to routing of the USB camera cables and recommended configuration of the SAC can be found in "Appendix E | USB Topology".**



Back of Computer

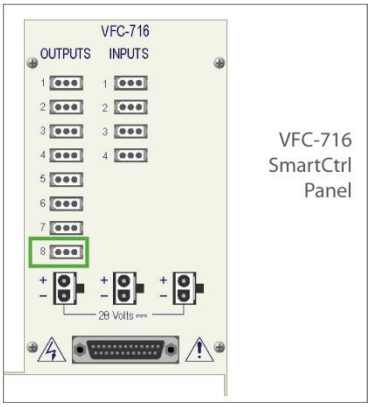
11. Using a NIR-102 cable, connect the White Light Control Input on the rear of the NIR-100 Light Controller to the OUTPUT 7 connector on the VFC-716.

* All cables running into the Sound-Attenuating Cubicle (SAC) should be run through the grommet plate on the right side of the SAC.

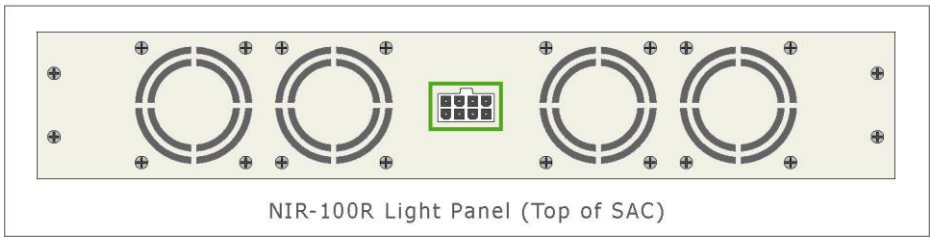


12. Using another NIR-102 cable, connect the NIR Light Control Input on the rear of the NIR-100 Light Controller to the OUTPUT 8 connector on the VFC-716.

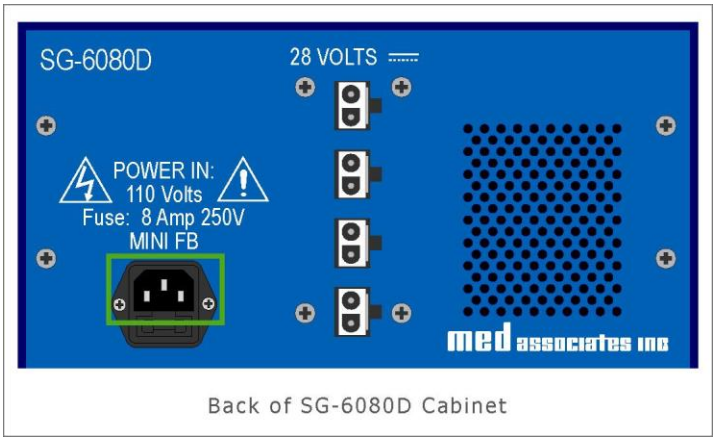
* All cables running into the Sound-Attenuating Cubicle (SAC) should be run through the grommet plate on the right side of the SAC.



13. Using the NIR-101 cable, connect the To Light Panel connector on the rear of the NIR-100 Light Controller to the only connector on the NIR-100R Light Panel.



14. Using the included power cord, plug the SG-6080D cabinet into a standard wall outlet.

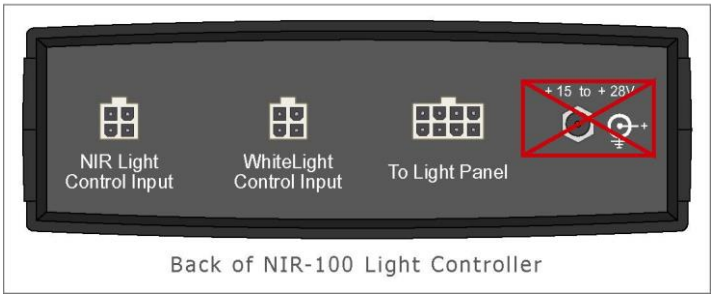


Back of SG-6080D Cabinet

15. Using the included power cord, plug the ENV-414S Aversive Stimulator into a standard wall outlet.

Back of ENV-414S

NOTE: It is not necessary to apply power to the NIR-100 Light Controller via the +15 to +28V DC connector (for standalone use only). Power is supplied via the Light Control Input connectors.



Back of NIR-100 Light Controller

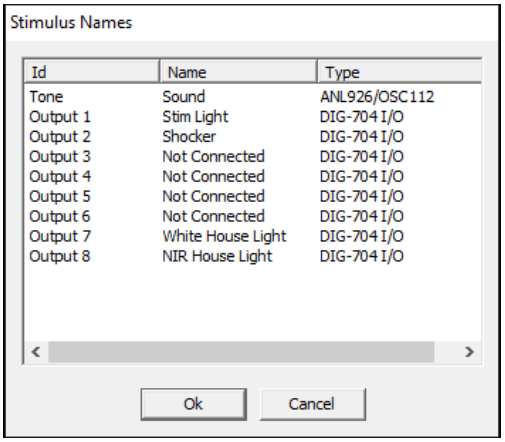
Configuring the Software

Once the hardware and drivers are correctly installed, configure the software following the steps below.

Launch the video Freeze software by double clicking the desktop icon.

From the **File** menu, select **Stimuli Definition**. The following will appear:

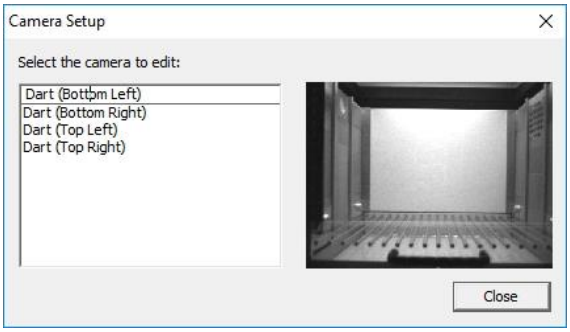
Figure 3.1 - Stimulus Names



The **Stimulus Names** screen (Figure 3.1) is shown with the default titles. The **Name** column is the only column that may be changed; the **Id** and **Type** columns may not be changed. To change the Name of a stimulus, click on the current name and enter the new name. When all the names are correct, select **Ok**.

Now select **File** | **Camera Identities.** The following screen will appear:

Figure 3.2 - Camera Setup

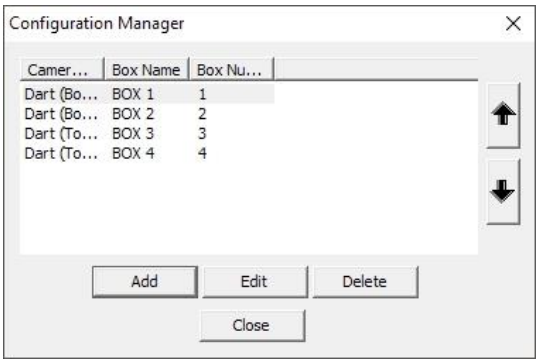


If a camera is missing from this list, recheck the USB cable connection from the camera to the USB3 port on the PC.

The **Camera Setup** screen is used to name the cameras and identify which camera is in which chamber. The camera name can be changed by clicking on the current camera name on the left side of the screen and entering a new name. The image on the right side of the screen corresponds to the selected camera. When the camera names have been updated, select **Exit**.

Select **File** | **Chamber Configuration.** The following screen will appear:

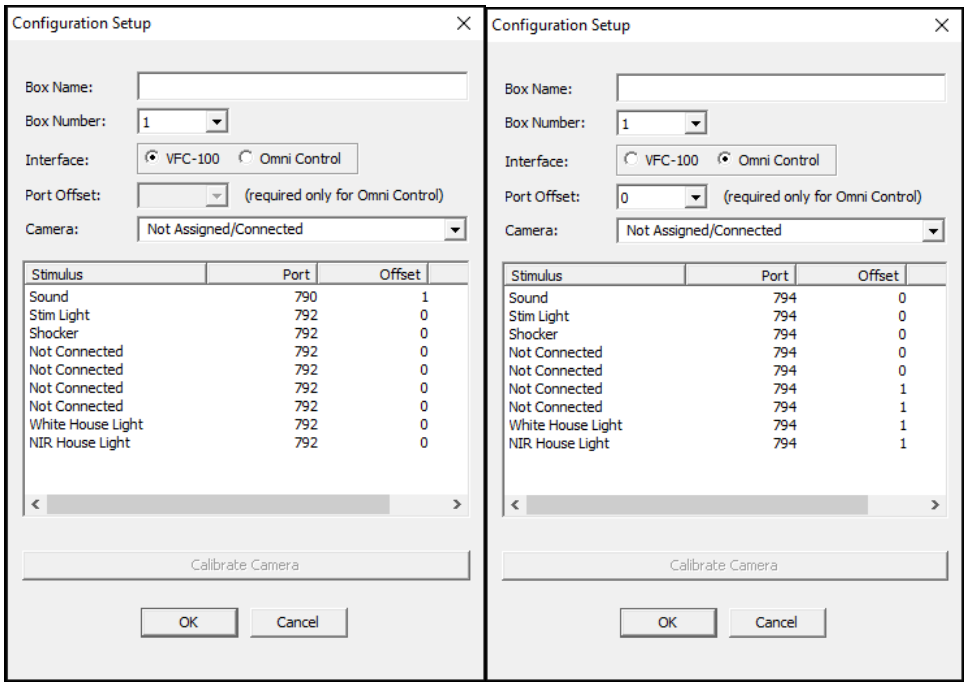
Figure 3.3 - Configuration Manager



The first time the Video Freeze software is run, the chambers may need to be added by clicking the ADD button, this step is performed as part of the system configuration by Med Associates, but after software re-install the chambers must be added.

The **Camera Ids** display order on the **Configuration Manager** screen can be changed by left-clicking on the camera to move, then clicking on the up or down arrow on the right side of the display (Figure 3.3). Camera Ids may be removed from this list by selecting them and selecting **Delete**. To add a new configuration to the list, click **Add**. The screen shown in Figure 3.4 will appear. To exit this screen, click **Close**.

Figure 3.4 – Adding a new Configuration



If chambers are added to a system, for example expanding from a 2-chamber system to a 4-chamber system, new configurations must be added via the configuration manager dialog add button.

Create a new configuration by entering the desired box name into the **Box Name** field. **Box Name** refers to an individual experimental setup, and includes the Sound Attenuating Cubicle (SAC), video camera, conditioning chamber, and all stimuli associated with that chamber. The hardware settings, including **Box Number, Interface, Port Offset,** and **Camera** must be set for each Box in the system.

The **Box Number** is associated with the desired hardware interface to be used: VFC-100 card or Omni Control device (OSC-112).

If using a **VFC-100** interface, determine which Box that each VFC-100 is connected to and refer Table 3.1 below to determine the appropriate **Box Number** for the default configurations.

If using an **Omni Control** (OSC-112) interface, determine which Box that each OSC-112 is connected to and refer Table 3.2 below to determine the appropriate **Box Number** for the default configurations. Note, that for Omni Control definitions, the **Port Offset** must also be defined by the user. The drop-down list for this parameter allows the user to select port: [0, 4, 8, or 12].

Table 3.1 – Default Stimulus Configuration Settings for a VFC-100 interface

	Box 1	Box 2	Box 3	Box 4
Audio				
Port	790	790	790	790
Offset	1	2	3	4
I/O				
Port	792	792	792	792
Offset	0	2	4	6
Bit for Output #1 (e.g. Stimulus Light)	1	1	1	1
Bit for Output #2 (e.g. Shocker)	2	2	2	2

Table 3.2 – Default Stimulus Configuration Settings for an Omni Control (OSC-112) interface

	Box 1	Box 2	Box 3	Box 4
Audio				
Port	794	794	794	794
Offset	0	4	8	12
I/O				
Port	794	794	794	794
Offset	0	4	8	12
Bit for Output #1 (e.g. Stimulus Light)	1	1	1	1
Bit for Output #2 (e.g. Shocker)	2	2	2	2

Next, select a camera from the **Camera** pull down menu.

Once all desired selections have been made, select **Ok**, and the Configuration Manager dialog shown in Figure 3.3 will appear again.

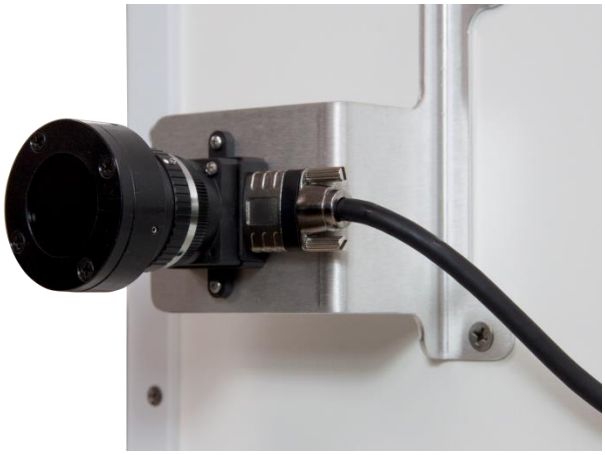
To calibrate a camera, select the camera to calibrate from the **Configuration Manager** screen (Figure 3.3) by either double clicking on the Id of the desired camera, or by highlighting the desired camera and clicking **Edit**. Click the **Calibrate Camera** button on the Configuration Setup dialog to display the Calibrate Camera dialog.

Refer to Chapter 4 for details on calibrating a camera.

CHAPTER 4 | CAMERA CALIBRATION

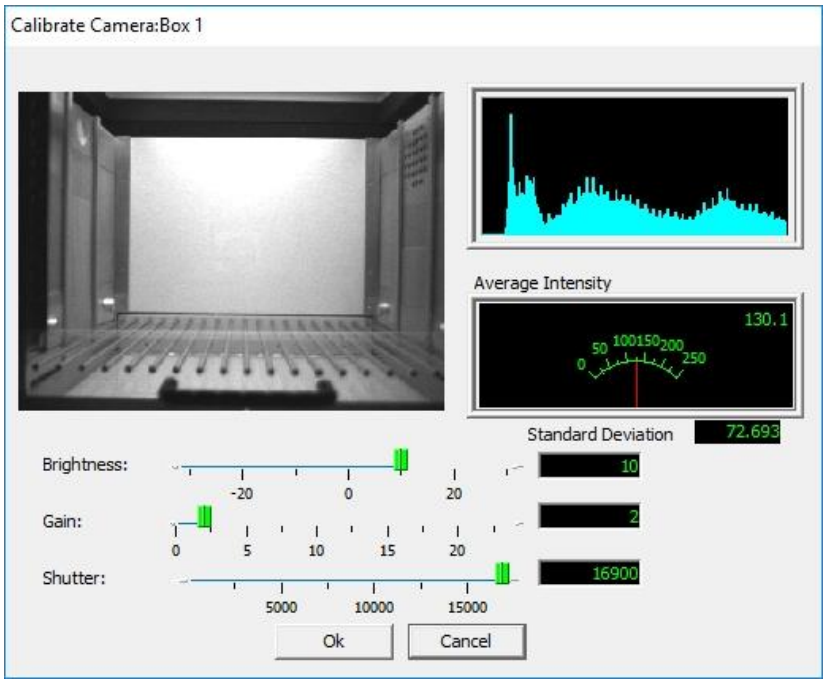
Calibration Procedure

Figure 4.1 – VID-CAM-MONO-5



Ensure that the Chamber is pushed all the way to the back of the Cubicle and the NIR light source is turned on in each box. The cameras in the MED-VFC system detect only NIR light; therefore there will be no image on the screen if the NIR light source is off.

Figure 4.2 - Calibrate Camera

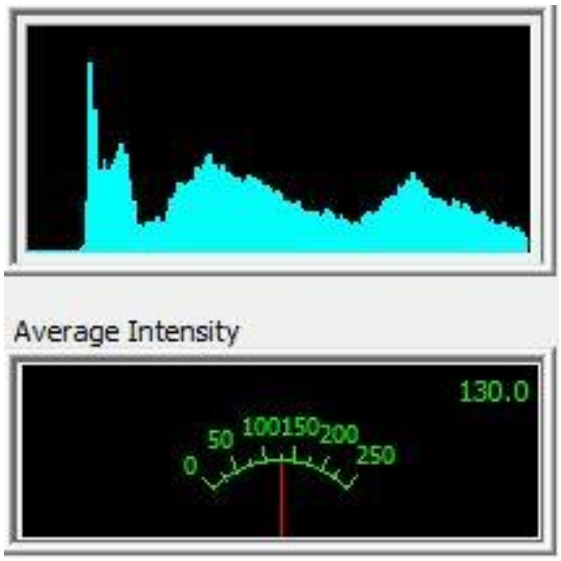


Default values are set for **Brightness** (0) **Gain** (0) and **Shutter** (10,000). The image will initially be viewable and may need to be fine-tuned.

MED Associates recommends an Average Intensity of approximately 130, this should be achieved by adjusting the **Brightness** and **Shutter** first and finally by increasing **Gain** (see Figure 4.2)**.** For examples of poor calibration see Figure 4.4.

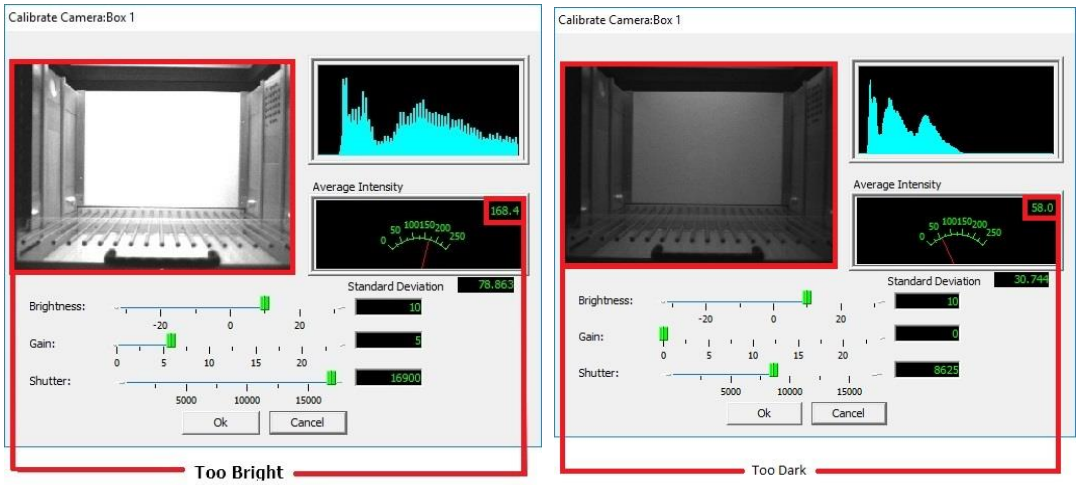
NOTE: The Brightness, Gain and Shutter may need adjustment and Average Intensity may need to be increased if a Contextual Insert is used. Adjust with a Contextual Insert in the chamber until a satisfactory image is attained.

Figure 4.3 – Average Intensity of Properly Calibrated Camera



The Video Display window should now show a clear, bright image of the empty Fear Conditioning Chamber. If the image is unclear the focus on the lens may need to be adjusted.

Figure 4.4 – Examples of Poorly Calibrated Cameras



Adjusting Camera Focus

MED Associates adjusts the focus and aperture settings on the camera prior to shipping Video Fear Conditioning systems. However, if focus adjustments are made, the camera settings (**Brightness, Gain, and Shutter**) will need to be adjusted using procedure below.

To adjust the camera focus, use the included .035" hex key to loosen the Focus Setscrew, shown in Figure 4.5. Rotate the Filter Housing until proper image clarity is achieved then tighten the Focus Setscrew.

The lens aperture should be locked in the fully open position (using the setscrew on the aperture ring; Figure 4.5). The white dot on the aperture ring should be lined up with setting **1.4** on the Filter Housing (Figure 4.6).

NOTE: The camera lens has adjustments for aperture and focus, both settings have been optimized by Med Associates during system building and subsequently locked via the set screws on the lens

Figure 4.5 - Focus and Aperture Rings on Video Lens

Figure 4.6 - Aperture Setting

Chapter 5 | Protocol Setup

Independent Variables

The Video Freeze® Software allows researchers to define stimulus intensities and durations, inter-trial intervals, inter-stimulus intervals, session durations, and number of trials per session. These variables are defined when constructing a protocol.

Dependent Variables

Freezing Behavior (defined as no movement other than breathing) is the primary dependent variable of the system. Freezing is defined using Motion Threshold and Minimum Freeze Duration.

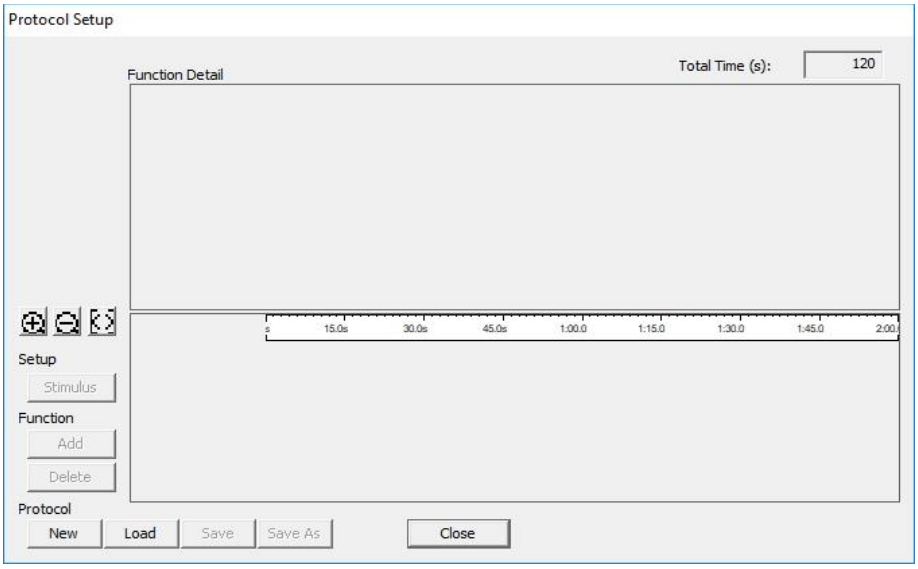
Protocol Setup Utility

Customized experimental protocols can be efficiently designed and implemented using the Protocol Setup utility of Video Freeze® software. This utility creates a "***.pro**" file that is later executed when conducting an experimental session.

Defining the Stimulus Conditions

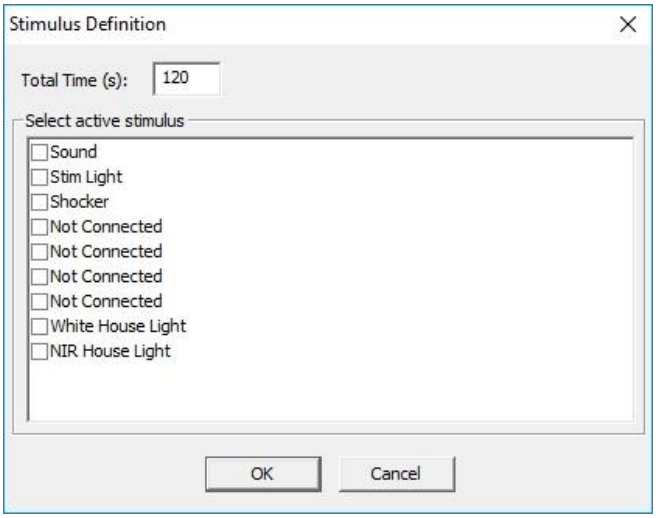
Select **File** | **Protocol Setup** and the following screen will appear.

Figure 5.1 - Opening the Protocol Setup Window



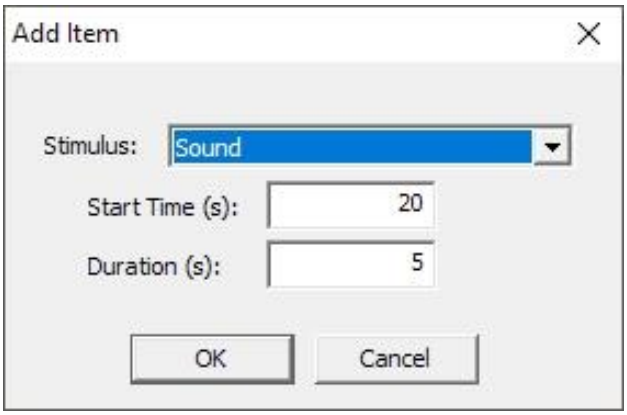
To define the stimulus conditions, click **New**. The Stimulus Definition screen will appear (Figure 5.2). Set **Total Time**, which is the total duration of the protocol in seconds, and select the Stimuli to be used in the protocol by checking the corresponding boxes. Note that only the Stimuli defined in **Stimuli Definition** appear on this list. When the desired stimuli have been selected, click **OK** to continue. These selections may be changed later by clicking **Stimulus** on the Protocol Setup screen.

Figure 5.2 - Stimulus Selection with User Defined Stimuli Names



To begin adding stimuli to the protocol, click **Add** and the Add Item screen will appear (Figure 5.3). This function is used to define the stimulus durations and intensities, and determines the onset and offset times for each stimulus. Use the pull-down menu to select the desired stimulus, and then enter the Start Time in seconds and the Duration in seconds. Click **OK** to add this item to the protocol. A representation of this item will appear on the Protocol Setup screen. An example is shown below.

Figure 5.3 - Add Item



Click **Add** again to add another stimulus to the protocol, or right-click on the stimulus representation shown on the Protocol Setup screen and click **Copy** (see Figure 5.4). Then, right-click near the desired start time for the item and select **Paste**. A representation of the new stimulus will appear.

Click on a stimulus representation (the red or green rectangle) to modify the properties of the stimulus. Properties may be modified either by changing the values in the "Function Detail" area of the screen, or by pressing and holding the "Ctrl" key while simultaneously clicking and dragging the ends of the stimulus representation. Any changes made to the properties using the mouse will be displayed in the "Function Detail" area of the screen. In Figure 5.4, the first representation of the sound stimulus has been selected.

The "Function Detail" area of the screen is also where other Stimuli parameters are defined, as shown in Figure 5.4. The Volume (dB), Frequency (Hz), Rise Time (ms), and Channel [1:2] can be changed, as well as whether the audible stimulus is a Tone or Noise. Note, that the Channel parameter is only needed when defining a sound stimulus for the Omni Control (OSC-112) interface.

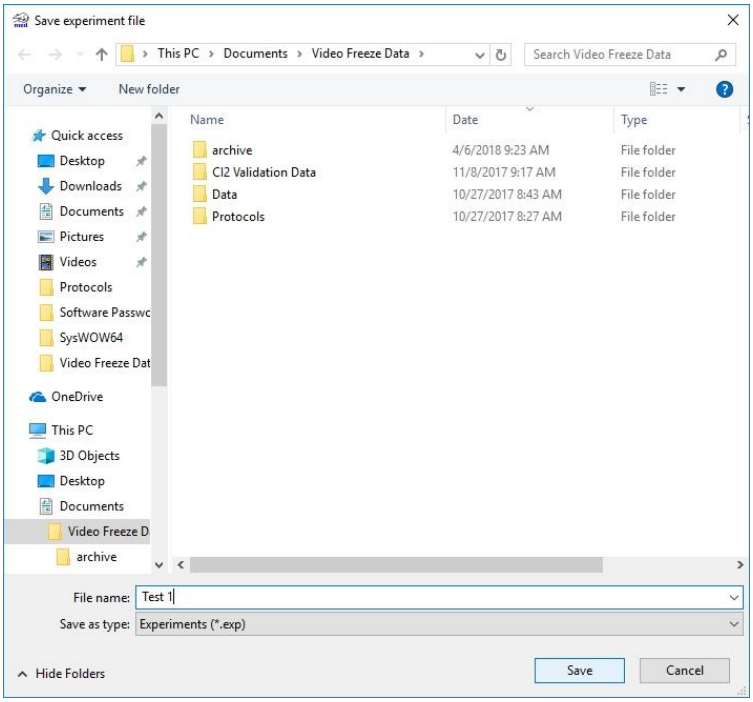
In the example shown in Figure 5.4, Stimulus 1 (sound) begins 10 seconds after the experiment starts and turns off 30 seconds later. Stimulus 2 (shocker) begins 30 seconds after the experiment starts and the duration is 10 seconds. All stimuli are independently defined.

Figure 5.4 - Editing Protocol Setup

Saving a Protocol

Once all of the desired stimuli have been added to the protocol, select **Save As** to save the protocol and all stimulus properties. Save the protocol to the desired directory, it may be useful to create a folder titled "Video Freeze Protocols." The program will automatically save the file with a "*.pro" extension.

*Figure 5.5 – Saving a *.pro File to a Specified Directory*



Modifying an Existing Protocol

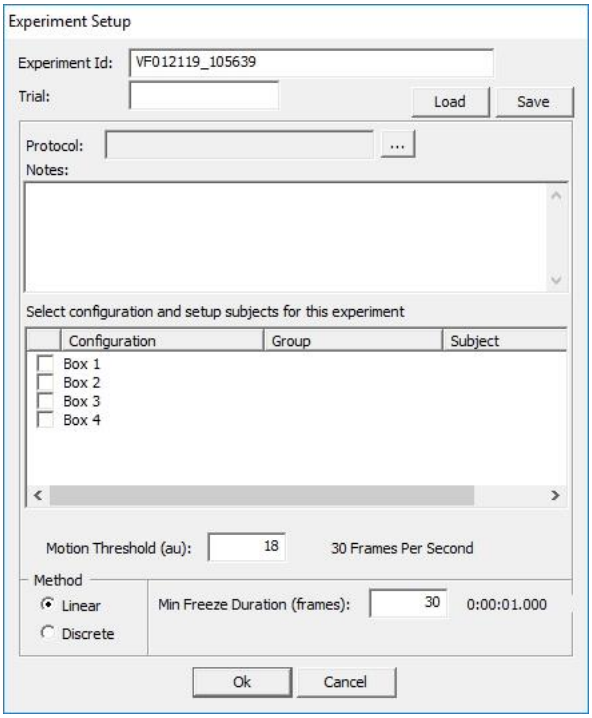
To modify an existing protocol, go to **File | Protocol Setup**, and then select **Load** and choose the desired protocol. Click on a stimulus representation to modify the properties of the stimulus. Properties may be modified either by changing the values in the "Function Detail" area of the screen, or by pressing and holding the "Ctrl" key while simultaneously clicking and dragging the ends of the stimulus representation. Any changes made to the properties using the mouse will be displayed in the "Function Detail" area of the screen. In Figure 5.4, the first representation of Stimulus 1 has been selected. Click on a stimulus representation and click **Delete** to remove it from the protocol.

CHAPTER 6 | STARTING AN EXPERIMENTAL SESSION

From the main Video Freeze menu, choose **Experiment** | **Start** and the Experiment Setup screen, shown below, will appear.

NOTE: Video Freeze will detect when a USB camera is disconnected while within the <Experiment> screen by displaying an error dialog box on the screen. The disconnected camera will still be shown as part of the experiment, but the image displayed will be a static image of the last good frame captured. If the camera is reconnected while the experiment is still active, Video Freeze will detect that the camera was reconnected, but the already defined/running experiment will not dynamically start capturing images from that re-connected camera. The user is required to <Stop> the experiment and <Restart> another experiment after the camera has been re-connected.

Figure 6.1 - Experiment Setup



Experimental Setup

The Experiment Setup screen includes the following fields/menus:

Experiment Id: If **Auto Experiment ID Generation** is selected on the **Options** menu, a filename will automatically appear in the **Experiment** window. This filename will include the date and time of the experiment, and follows the form: "VFMMDDYY_HHMMSS" This information can be altered to suit the particular experiment.

Trial: Enter trial/session information in the **Trial** field.

Load and Save: Select **Save** to save the experimental configuration (*.exp) for later use, and choose **Load** to use a previously saved experiment file.

Protocol: Next to the **Protocol** title, click the box labeled "**…**" to open the protocol (*.pro) file saved earlier. (See Saving a Protocol Page 29).

Notes: Enter any relevant information here, such as instructions on how to complete the experiment.

Motion Threshold: See Threshold Values below.

Sample Rate: The sample rate refers to the number of video frames scanned per second. This value is fixed at 30 fps.

Method: See Chapter 7.

Min Freeze Duration: The number of contiguous frames of movement less than the motion threshold that defines a Freeze event

Ok: Starts the experiment.

Cancel: Cancels and exits.

Threshold Values

The subject's behavior is quantified as an *index of motion*. All movement within the conditioning chamber is registered by the software as a change in video pixel composition over time. Therefore, robust and fast movements will be recorded as large relative changes in video-pixel composition, and small, refined, and slow-movements will be registered as relatively smaller changes in pixel composition. The index of motion has a range of 0 – 76,800 and during an experiment, movement is represented as a graph (motion index vs. time) in a non-cumulative manner.

Motion Threshold refers to the limit above which all behavior will register as movement (Not freezing) in the index of motion. When movement falls below this threshold, this behavior will be counted as freezing, depending upon the method of observation chosen. A Motion Threshold of 10-20 arbitrary units is recommended.

NOTE: The Motion Threshold can be modified after the experiment is complete during data analysis. The **Method of Observation** and **minimum freeze duration** can also be changed in data analysis.

Minimum Freeze Duration (number of frames) defines whether or not a Freeze Episode is recorded when a subject freezes during the session and the motion index falls below the Motion Threshold. The subject might momentarily stop moving, but this immobility may not actually represent a freeze. The Minimum Freeze Duration prevents these brief immobility events from being recognized as freeze episodes.

If the Minimum Freeze Duration is 30 frames, then the subject must remain immobile for one second to register as a freeze, as the video acquisition rate is 30 frames per second.

CHAPTER 7 | METHODS OF OBSERVATION

Linear Method (Default)

MED Associates recommends using the Linear Method as it takes advantage of all data collected by the software

The dependent measures resulting from the linear method are:

1. **Percent Freeze**: time immobile / total session time. The run-time window (see the section on "Recording the Session") of Video Freeze also provides:

   Instantaneous Percent Freeze: time spent immobile / current session time

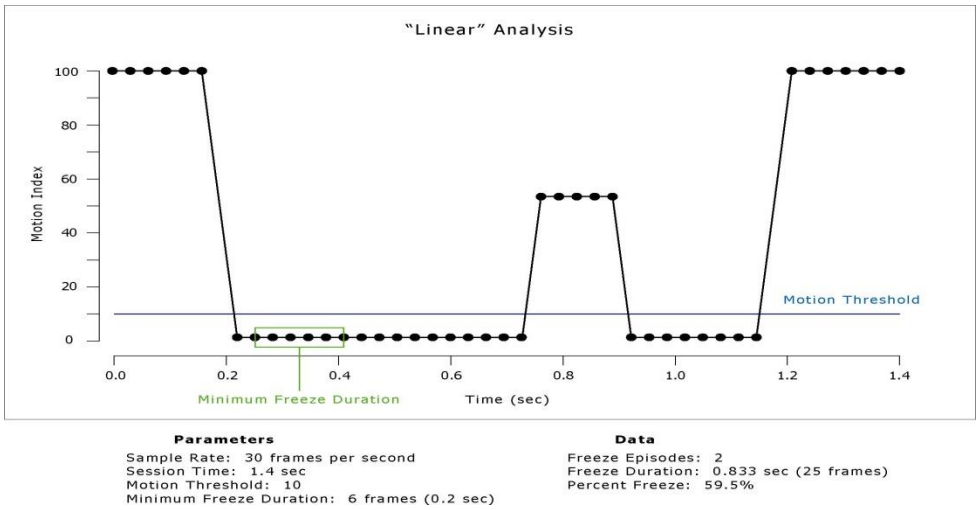
   Cumulative Percent Freeze: time spent immobile / total session duration. This percent freeze score will increase as the session progresses because time immobile will increase relative to total session time. Cumulative Percent Freeze will approach Instantaneous Percent Freeze as the session progresses.

2. **Freeze Episodes**: number of freezing events, defined by Motion Threshold and Minimum Freeze Duration

3. **Freeze Duration**: total amount of time the subject spends immobile.

In Figure 7.1 each filled-in circle represents a video frame (30 fps). The subject freezes at 0.2 seconds (because movement is below the Motion Threshold), and remains freezing until ~0.75 seconds. Using the linear method, the first Freeze Episode is recorded at 0.4 seconds because the Minimum Freeze Duration (6 frames) has expired. Movement is recorded at 0.75 seconds, and the subject is immobile again at ~0.95 seconds. Once the Minimum Freeze Duration has expired (at ~1.15 seconds), the second Freeze Episode is recorded. Note that the Motion Threshold must be exceeded before a second Freeze Episode can be recorded; therefore, when using the linear method, Freeze Episodes must be separated by periods of movement.

Figure 7.1 - Description of the Linear Method



Discrete Method

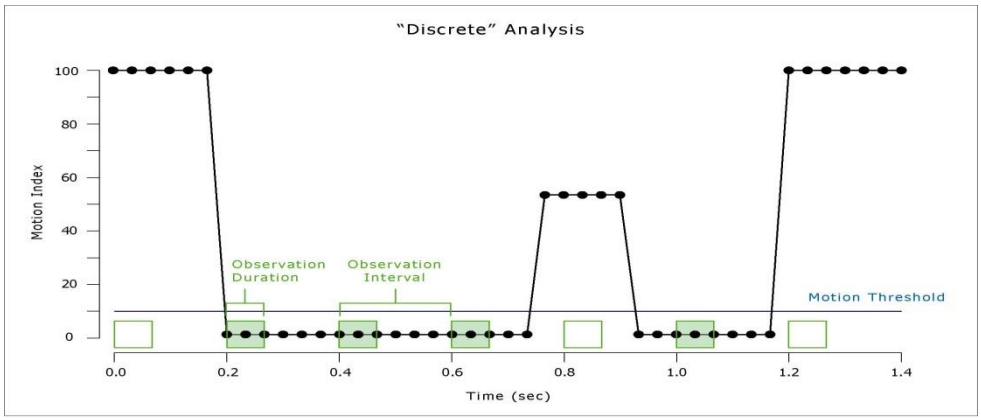
The discrete method is designed to mimic manual methods of monitoring freezing behavior. The standard manual method of tracking freezing involves visually scanning each fear-conditioning chamber at regular **Observation Intervals** (e.g. Every 4 seconds), and observing behavior for a specified duration of time (**Observation Duration**, e.g. 1 second). If the subject in the chamber is freezing for the entire Observation Duration (1-second), a **Freeze Count** is recorded for that event. If the subject is mobile during any portion of that observation period, then no Freeze Count is tallied. Therefore, the number of "counts" recorded by the end of the experimental session serves at the quantitative measure of freezing. Therefore, the discrete method yields:

1. **Freeze Count**: number of freezing observations
2. **Percent Freeze**: number of freezing observations / total number of possible observations

Observation Interval and Duration are used to determine whether a Freeze Count is registered for a freezing event. In the Figure 7.2, the green squares represent "observations." A Freeze Count is recorded only when there is no motion during the observation duration. In Figure 7.2 the highlighted green squares are Freeze Counts; therefore four Freeze Counts are recorded for the session. Note that the frequency of Freeze Counts is greater than the number of Freezing Episodes recorded using the linear method. This is because several Freeze Counts can occur without a record of movement using the discrete method.

Observation Interval and Duration must be set to greater than 1 fps, and the Observation Duration must be less than the Observation Interval. The Observation Interval defines the time period between the beginning of one observation and beginning of the next. Therefore, with a Sample Rate of 30 fps, and an Observation Interval of 15, there will be two observations per second.

Figure 7.2 - Description of the Discrete Method



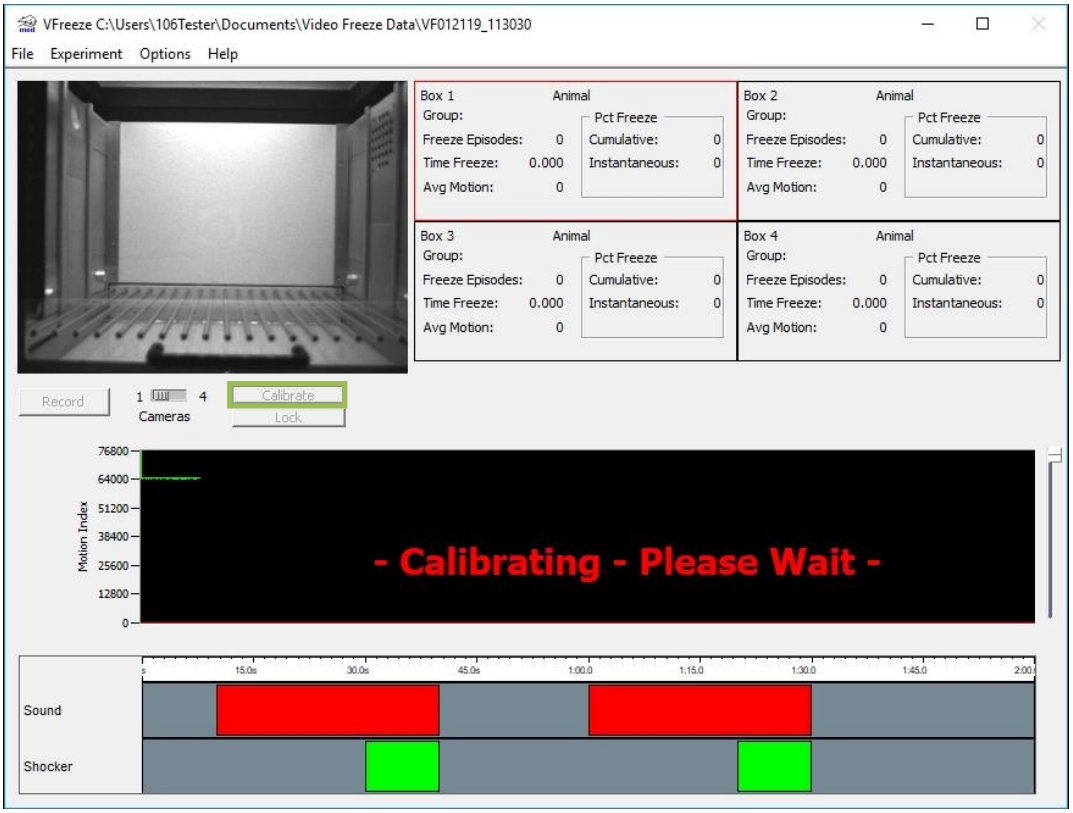
Parameters
Sample Rate: 30 frames per second
Session Time: 1.4 sec
Motion Threshold: 10
Observation Interval: 6 frames (0.2 sec)
Observation Duration: 3 frames (0.1 sec)

Data
Freeze Counts: 4
Total Counts: 7
Percent Freeze: 57.1%

CHAPTER 8 | VIDEO CAPTURING SYSTEM CALIBRATION

Prior to loading the subjects into the chambers, the video-capturing system must be calibrated. Ensure that the Chamber is pushed all the way to the back of the Cubicle, the NIR light source is turned on in each box and all doors are closed. Click the **Calibrate** button on the acquisition screen, indicated by the green outline in Figure 8.1.

Figure 8.1 - The Run Time Screen during Calibration



After the process is completed the green motion index line should be at zero, indicating all chambers are calibrated. The slider bar at the right side of the Motion Index graph can be adjusted to view the motion values more closely.

When ready to begin the Experiment, click the **Lock** button. The **Record** button will now become available.

CHAPTER 9 | RECORDING THE SESSION

To begin an experiment, load all the animals into the chambers, close the doors, and click the **Record** button. The Motion Index graph will display movement and the stimuli will be presented in an automated fashion. The session is complete when the Motion Index recorder reaches the end of the timeline. At the completion of the session, the video will stop recording.

To view the video and motion-index data from a particular chamber, click on the data window of the desired chamber (for example Chamber 1 in Figure 9.1) with the 1-4 slider set to the "1" position. The motion index graph displays the movement from the box with the data window outlined in red. Mouse click the data window to display the Motion Index graph for that particular box. The red outline will indicate the box whose data is displayed in the Motion Index graph. To view the video from all four chambers simultaneously, click the Cameras 1 - 4 slider control located under the video display. A single click toggles between 1 and 4 video displays.

Figure 9.1 - The Video Freeze Run Time Screen

NOTE: The video cameras are extremely sensitive to movement. Therefore, any vibration in the laboratory environment may register as movement in the Motion Index.

CHAPTER 10 | DATA ANALYSIS

Types of Data Files

When an experiment is conducted, the Video Freeze® Software simultaneously creates three different types of data files. One is a video file ("***.wmv"**) that can be viewed by Windows Media Player for a single chamber. For multiple chambers go to file> extract video stream, then select boxes 1-4 or individual boxes, this will create 1 **.wmv** file per box, which may then be played using Window's Media Player (see Figure 10.1). Another is a summary file ("***.txt"**) that can be exported to any spreadsheet program such as Excel® or notepad (see the section on Exporting Data to a Spreadsheet). The third type is a "***.raw"** data file that can only be viewed using Video Freeze®. This "*.raw" file contains the motion-index data (see Figure 10.3). See the next section to learn how to use Video Freeze® to re-analyze the "*.raw" data file.

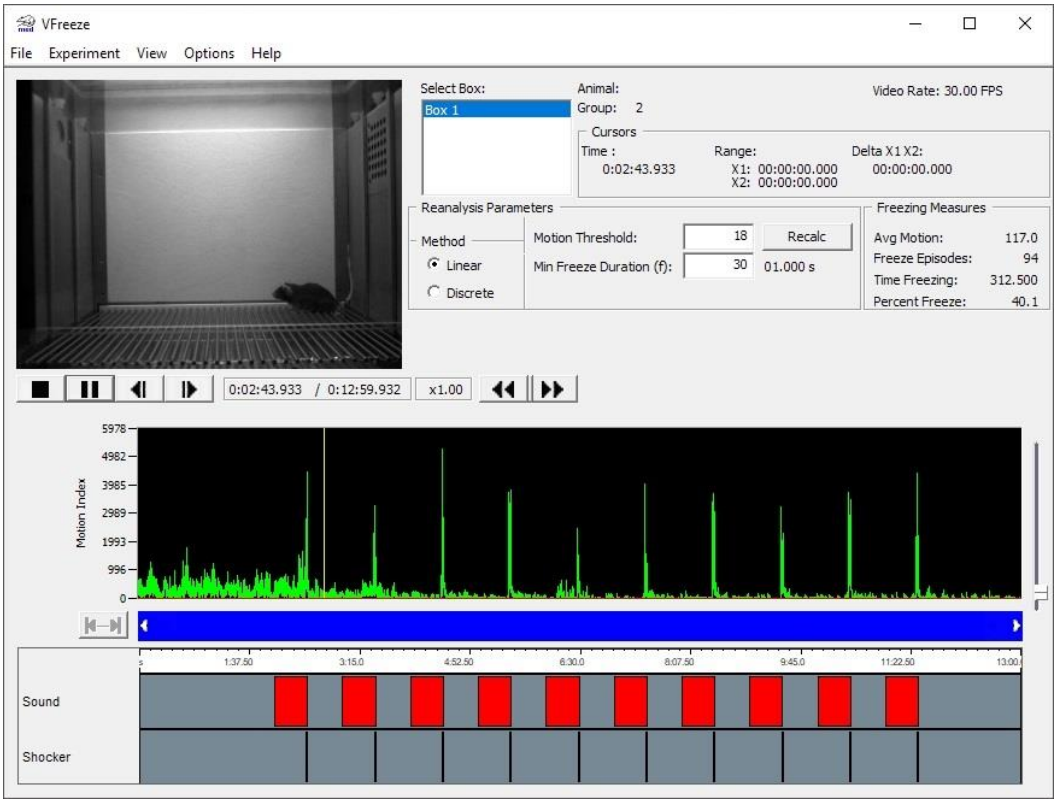
*Figure 10.1 - Windows Media Player File (*wmv)*

Figure 10.2 - Summary File (.txt)*

Analyzing "*.raw" data in Video Freeze®

Select **File | Open** and locate the "*.raw" data file of interest. The Video Freeze® Data Analysis window provides the same information as the run-time window (video, motion index, subject identifying information, freeze count, etc.).

Figure 10.3 - The ".raw" Data Analysis Window*



Pick which chamber to view from the "Select Box" list control

Use the stop/play/Frame step  play speed  tabs to play the video. If modifying the Method, Motion Threshold or Minimum Freeze Duration, click on the **Recalc** button to calculate Freezing Measures.

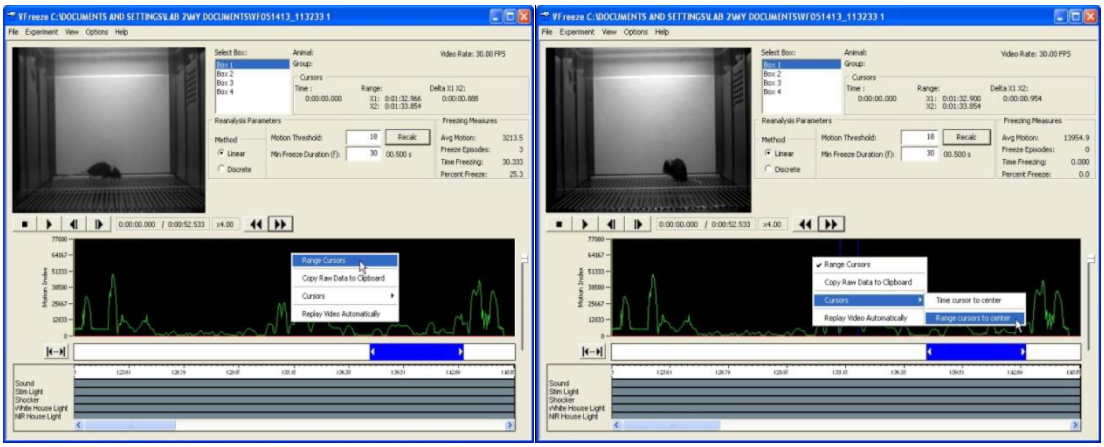
NOTE: These settings apply to all videos in the experimental session.

To focus on a specific period of time during the session, click on the blue timeline bar located below the motion index record. Adjusting the blue bar by dragging the cursor causes the motion-index to expand. The collapsed timeline bar represents the proportion of time (out of the total session time) represented in the immediately available motion index display. Click the Full scale button  directly to the left of the blue timeline bar to return to a view of the entire session.

Figure 10.4 – Adjusting the Blue Timeline

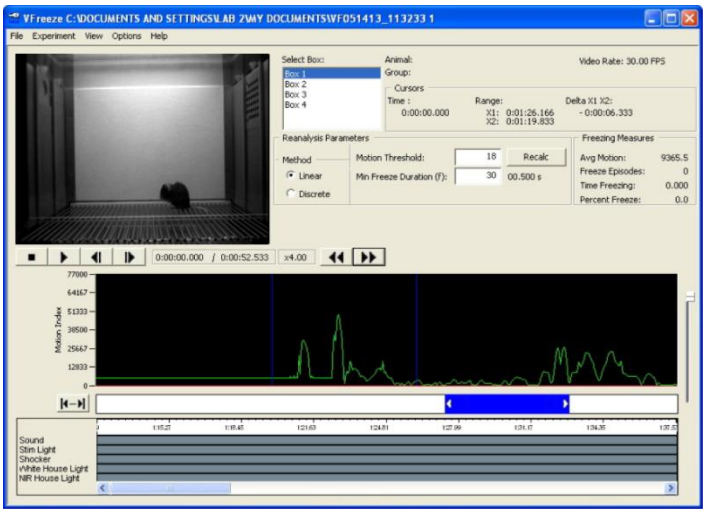
To obtain dependent measures from a *specified interval* of time within the session, use the **Range Cursors**. Select the Range Cursors with a right click on the motion index graph (see Figure 10.5), and then choose the **Cursors | Range Cursors to Center** option. Adjust the positioning and length of the specified interval by moving the vertical blue lines with a left-click and drag of the mouse. Drag the blue lines to the desired position in the Motion Index Graph, and then press **Recalc**. Adjusting the position of the Range Cursors will alter the data listed under the "Cursors" display on the data analysis screen.

Figure 10.5 - Selecting the Range Cursors



The figure below displays the Range cursors. Note that the Freezing Measures reflect only the data that exist between the two vertical lines on the Motion Index Graph.

Figure 10.6 - Positioning the Range Cursors



NOTE: If the range cursors are selected, only the data between the two cursors will be represented in the data file for export to Excel (see the next section for instructions on exporting).

Exporting Data to a Spreadsheet

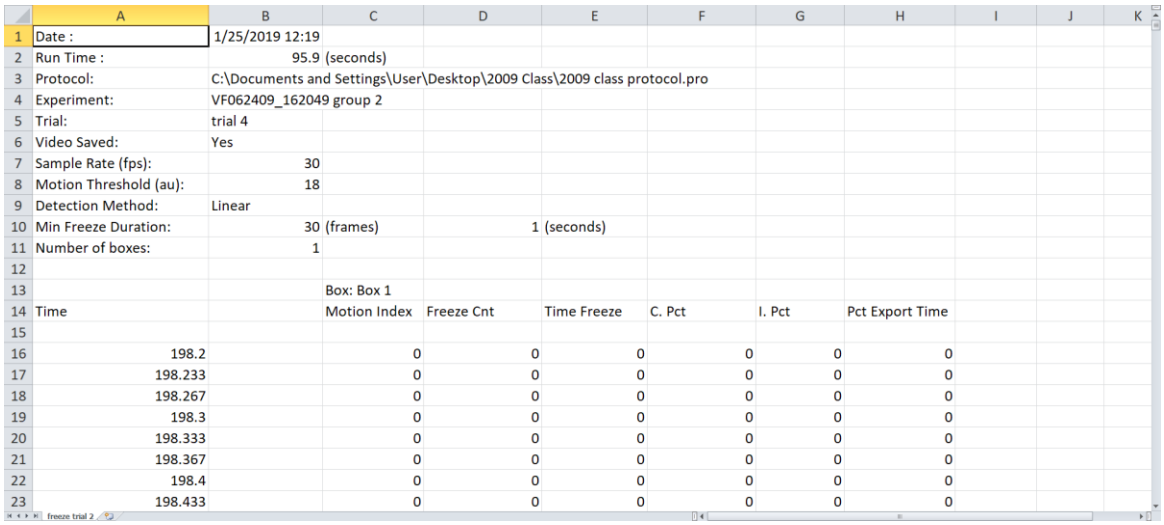
Exporting the data file to a desired location on the desktop computer by selecting **File | Export**. Creates a ".csv" file, that in turn can be opened by Microsoft excel, Open Office, Notepad or other spreadsheet software.

Once the file has been exported, go to that file location and double click on that named file and open it in selected spreadsheet software.

Exporting Video

Exporting a video file (“***.wmv**”) that can be viewed by Windows Media Player for a single chamber or multiple chambers, go to **file**> **extract video stream**, then select boxes 1-4 or individual boxes, select the (**“*.raw”**) file, this will create 1 **.wmv** file per box, which may then be played using Window’s Media Player (see Figure 10.1).

Figure 10.7 - Imported Data File in Microsoft Excel

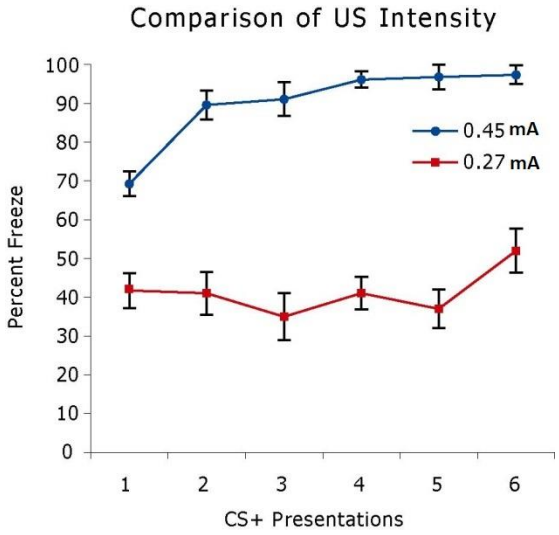


	A	B	C	D	E	F	G	H	I	J	K
1	Date :	1/25/2019 12:19									
2	Run Time :	95.9	(seconds)								
3	Protocol:	C:\Documents and Settings\User\Desktop\2009 Class\2009 class protocol.pro									
4	Experiment:	VF062409_162049 group 2									
5	Trial:	trial 4									
6	Video Saved:	Yes									
7	Sample Rate (fps):	30									
8	Motion Threshold (au):	18									
9	Detection Method:	Linear									
10	Min Freeze Duration:	30	(frames)		1	(seconds)					
11	Number of boxes:	1									
12											
13			Box: Box 1								
14	Time		Motion Index	Freeze Cnt	Time Freeze	C. Pct	I. Pct	Pct Export Time			
15											
16	198.2		0	0	0	0	0	0			
17	198.233		0	0	0	0	0	0			
18	198.267		0	0	0	0	0	0			
19	198.3		0	0	0	0	0	0			
20	198.333		0	0	0	0	0	0			
21	198.367		0	0	0	0	0	0			
22	198.4		0	0	0	0	0	0			
23	198.433		0	0	0	0	0	0			

CHAPTER 11 | COMPONENT ANALYSIS

One may choose to compare percent freeze across different events within a single experimental session. For instance, if several conditioned-stimulus/unconditioned-stimulus (CS-US) pairs are presented within a single session, then the amount of time the subject spends motionless during each presentation of each CS may provide meaningful information on the rate of conditioning.

Figure 11.1 - Data from Four CD-1 Mice During Fear Conditioning



The session represented in figure 11.1 was 30-min long with six presentations of a 30 second tone and 10 second aversive stimulus (stimuli co-terminated). Two subjects received 0.45mA aversive stimulus, and two received 0.27mA of aversive stimulus. Percent freeze during the first 20 seconds of each CS presentation is illustrated above.

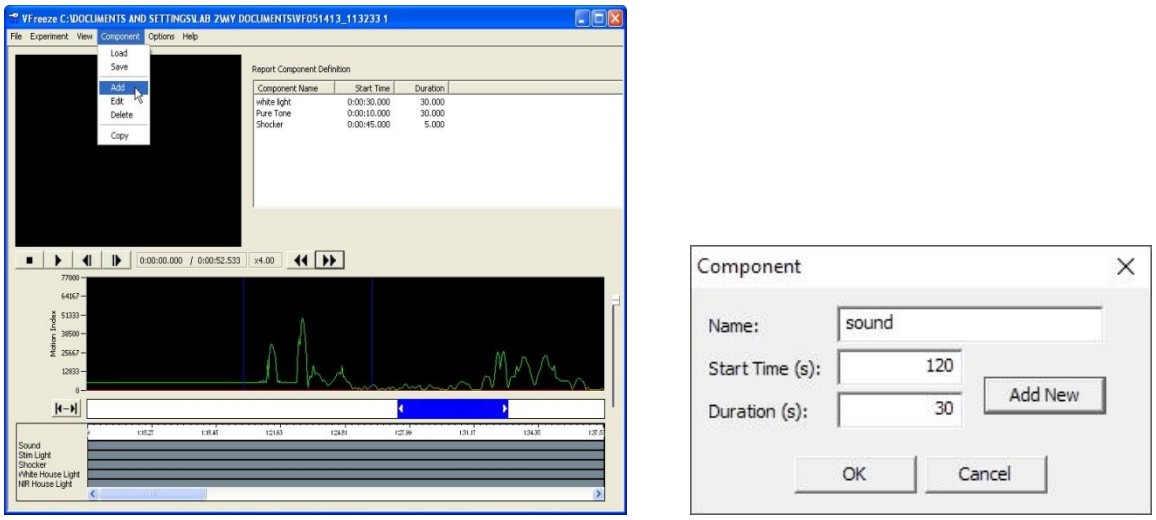
Component Summary Report

To begin, open a *.raw data file, and select **View | Component Setup**.

The **Start Time** and **Duration** of each event, or *component*, are user-defined. Therefore, one can compare percent freeze across any interval within a single experimental session.

To define each component, select **Component** | **Add** (see Figure 11.2). Name each component, and enter the **Start Time** (s) and the **Duration** (s). Percent freeze will be calculated for each component, and the Start Time and Duration of the component determine the parameters for the calculation.

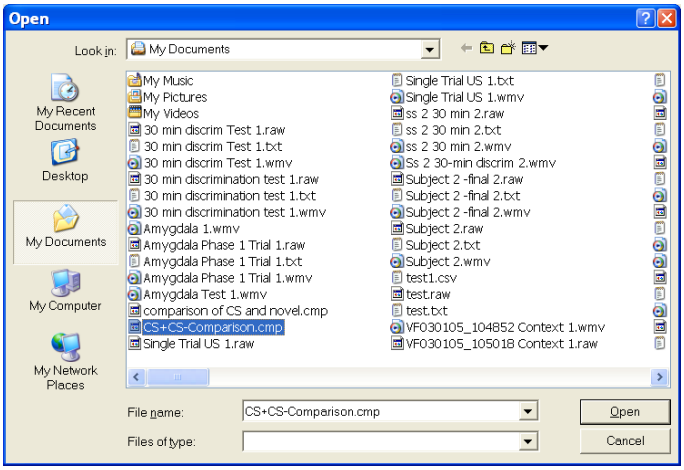
Figure 11.2 - Component Definition Display



Click **Add New** to add a component or **OK** when all the desired components for the analysis have been included. As new components are added to the analysis, each component will appear in the "Report Component Definition" display.

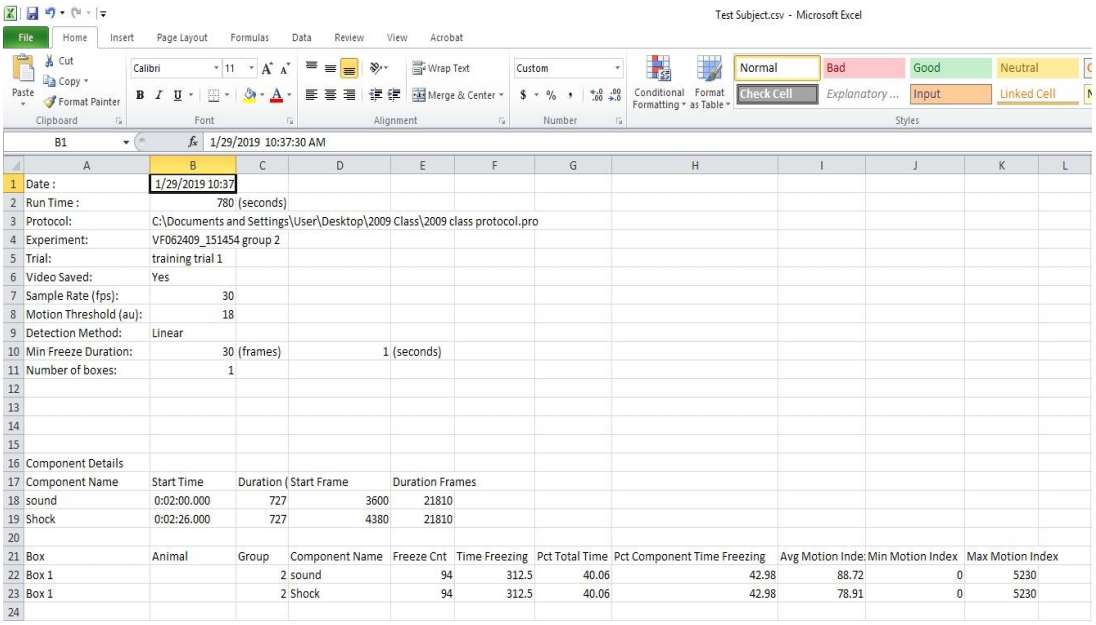
The component analysis ("*.cmp") is a file that can be saved independently of any "*.raw" data file. Choose **Component** | **Save**, and select the directory and filename for the component analysis, see below. This procedure has created a component analysis that can be applied to any "*.raw" data file.

Figure 11.3 - Saving a Component Analysis (.cmp file)*



To view a summary report of the component analysis, go to **File** | **Reports** | **Component Summary**, then type in a file name to save the report as a "*.csv". The "*.csv" file is a tab-delimited data file that can be read by any word-processing or spreadsheet program.

*Figure 11.4 - Component Analysis Results, *.csv file in Excel*



Test Subject.csv - Microsoft Excel

	A	B	C	D	E	F	G	H	I	J	K	L
1	Date :	1/29/2019 10:37										
2	Run Time :	780	(seconds)									
3	Protocol:	C:\Documents and Settings\User\Desktop\2009 Class\2009 class protocol.pro										
4	Experiment:	VF062409_151454 group 2										
5	Trial:	training trial 1										
6	Video Saved:	Yes										
7	Sample Rate (fps):	30										
8	Motion Threshold (au):	18										
9	Detection Method:	Linear										
10	Min Freeze Duration:	30	(frames)		1	(seconds)						
11	Number of boxes:	1										
12												
13												
14												
15												
16	Component Details											
17	Component Name	Start Time	Duration (	Start Frame	Duration Frames							
18	sound	0:02:00.000	727	3600	21810							
19	Shock	0:02:26.000	727	4380	21810							
20												
21	Box	Animal	Group	Component Name	Freeze Cnt	Time Freezing	Pct Total Time	Pct Component Time Freezing	Avg Motion Inde	Min Motion Index	Max Motion Index	
22	Box 1		2	sound	94	312.5	40.06	42.98	88.72	0	5230	
23	Box 1		2	Shock	94	312.5	40.06	42.98	78.91	0	5230	
24												

"*.csv" Dependent Variables

The "*.csv" file contains identifiers and component-analysis details, as well as four dependent variables, listed below.

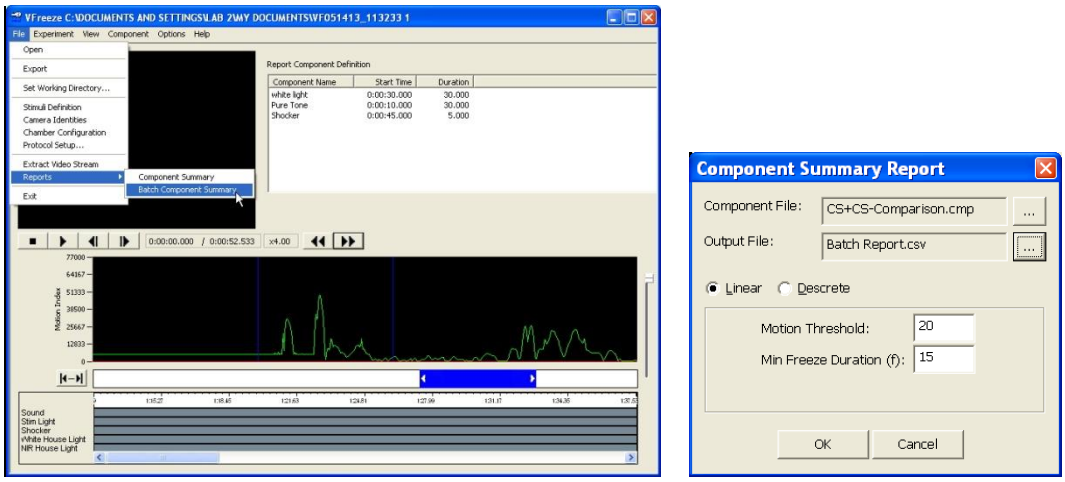
1. **Freeze Count or Freeze Episodes**: Number of freezing events, as defined by either the linear or discrete method of observation (respectively), during the component.
2. **Time Freeze**: Total number of seconds the subject spends motionless during the component interval.
3. **Percent Total Time Freeze**: The amount of time the subjects spends motionless during the session divided by the total amount of time for the session.
4. **Percent Component Time Freeze**: The amount of time the subject spends motionless during the component divided by the total amount of time within that component.
5. **Average Motion Index**: The average motion index during the component.
6. **Minimum Motion Index**: The minimum motion index during the component.
7. **Maximum Motion Index**: The maximum motion index during the component.

Batch Component Summary Report

To simultaneously apply a "*.cmp" file to multiple "*.raw" data files, go to **File | Reports | Batch Component Summary**. This option will allow the user to view and save a "*.csv" file that contains multiple experimental session data.

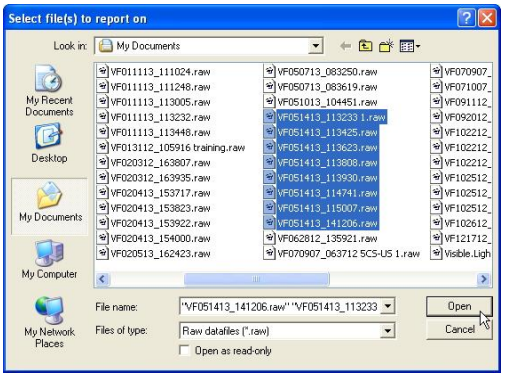
To generate a batch component summary report, click the first button marked "**…**" and select a "*.cmp" file. Then name the batch report using the second "**…**" button. Select a Method of Observation (Linear or Discrete) and define the threshold values. These setting will be applied to all of the *.raw data included in the batch summary report.

Figure 11.5 - Generating a Batch Summary Report Using Multiple ".raw" Data Files*



Select **OK**, and then choose the "*.raw" file or files to which the "*.cmp" analysis will be applied. To select multiple files at once, press the **Ctrl** key while using the left mouse button.

Figure 11.6 - Select the ".raw" File or Files*



Upon selecting the "*.raw" data files, the batch component analysis will be saved to the location specified for the output file. The batch component summary report will contain all of the dependent variables included in the component summary.

APPENDIX A | DRIVER AND SOFTWARE INSTALLATION

If the computer being used with the Video Fear Conditioning System was purchased as part of the system from MED Associates, the driver and software installation was completed at the factory. If the computer was not purchased from MED Associates, follow the instructions to install the hardware drivers and software programs.

Before beginning the installation, phone, fax or e-mail Med Associates with the registration information in order to receive the software installation password. This password will be necessary during the installation process.

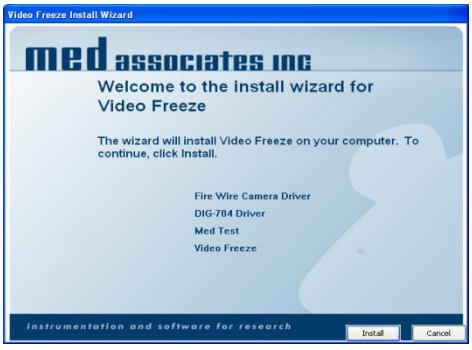
Insert the Video Freeze® CD into the CD-ROM drive and the screen shown in Figure A-1 will appear. Click **Install Video Freeze** and the screen shown in Figure A-2 will appear.

Figure A-1 - Installation Screen

Begin installing the drivers and software by clicking **Install**. Complete the steps to install the drivers and software, entering the desired User Name and Company as well as the password when prompted.

Successful installation of each item will be indicated by a green check mark, and a red X will indicate an unsuccessful installation.

Figure A-2 – Video Freeze Installation Checklist



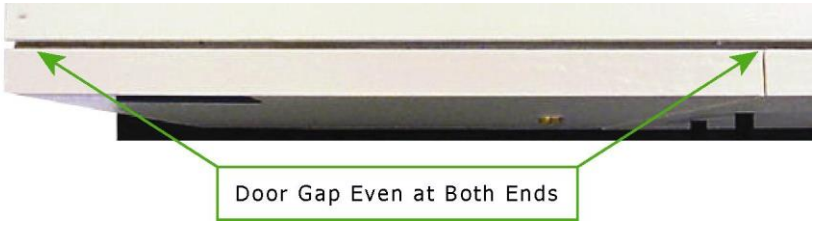
Driver and software installation has been successfully completed. Click **Finish** to close this screen.

APPENDIX B | DOOR HINGE ADJUSTMENT

The steps in this section should be followed to ensure that the Sound-Attenuating Cubicle (SAC) doors are properly adjusted.

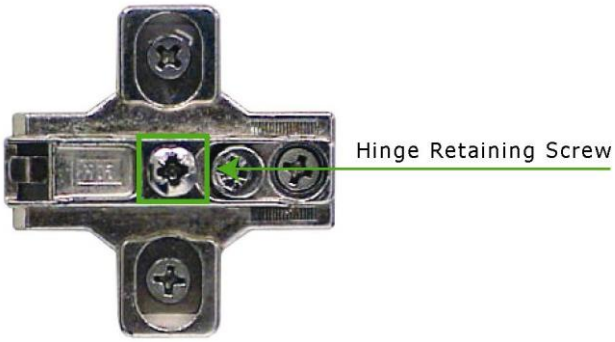
1. Close the left door and verify that it is properly sealed against the SAC. When the door is properly sealed the gap between the door and the SAC is equal at both ends, as shown in Figure B-1. If this gap is not even, proceed to Step 2.

Figure B-1 - Top View of SAC with Properly Sealed Door



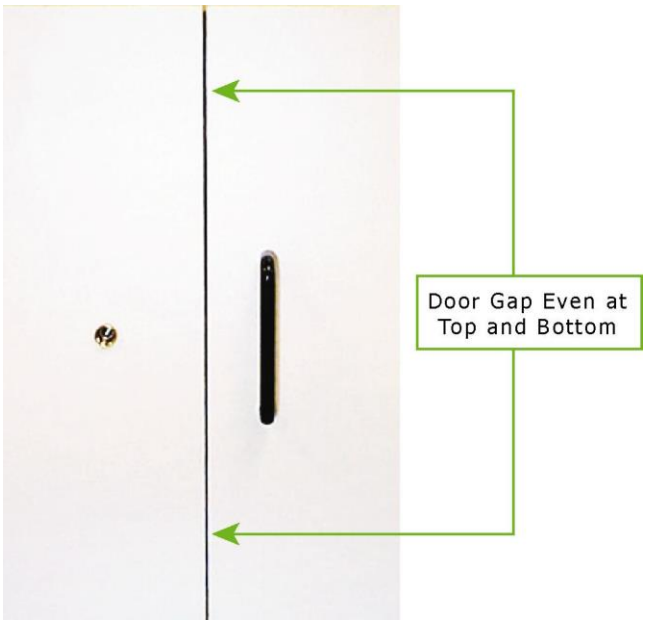
2. Loosen the hinge-retaining screw, shown in Figure B-2, and slide the hinge into the proper position. Once the door is sealed correctly, tighten the hinge-retaining screw. Repeat this step on the right door. It may take more than one attempt to achieve an acceptable seal.

Figure B-2 - Hinge Retaining Screw



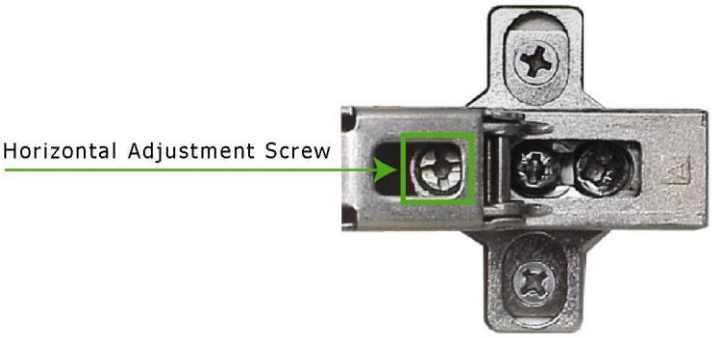
3. Close the left, then the right doors. The vertical seam between the two doors should be evenly spaced from top to bottom, as shown in Figure B-3. If they are not, proceed to Step 4.

Figure B-3 - Front View of SAC with Even Vertical Door Gap



4. Adjust the door position using the Horizontal Adjustment Screw. Turn the screw clockwise to move the door closer to the vertical center of the SAC, and counterclockwise to move it further. For example, if vertical gap between the doors is smaller at the top of the SAC than at the bottom, assess which door needs to be adjusted. If it is determined that the top of the right side door is too close to the center, then the Horizontal Adjustment Screw on the top hinge should be turned counterclockwise.

Figure B-4 - Horizontal Adjustment Screw



APPENDIX C | CAMERA FILTER CARE AND HANDLING

Proper care and handling of the camera filter are crucial to the proper operation of the system. Clean the filter gently only if necessary. Loose particles should be removed with a bulb puffer or with a filtered, pressurized air cleaner. If necessary, gently wipe the surface using anhydrous alcohol and lint-free lab towels. Use a new surface of the towel with each wipe. Avoid touching or wiping the filter with bare fingers.

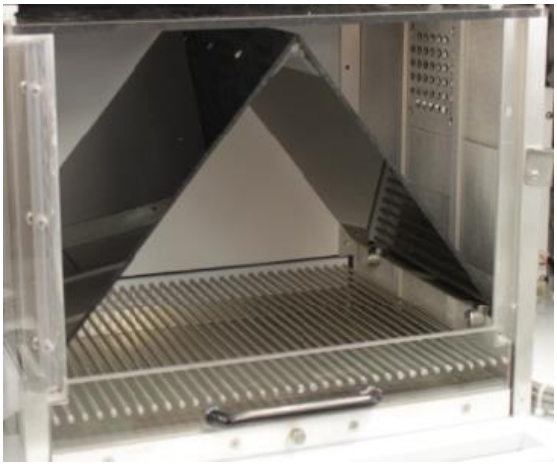
Figure C-1 – Camera lens Filters

APPENDIX D | INSTALLING CONTEXTUAL INSERTS

A-Frame Insert

Using the handle, pull the grid floor and waste pan forward until they are nearly removed from the chamber. Install two supports on one side of the chamber in the counter-bored notches of the support panels inside the chamber, secure them by placing the magnets on the outside of the chamber. Lift one side of the A-Frame so that it rests on the supports. While lifting the opposite side of the A-Frame add the last two supports and secure with magnets. Refer to Figure D-1.

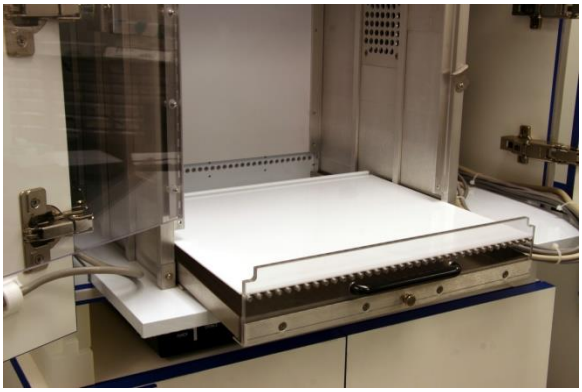
Figure D-1 - A-Frame Contextual Insert Installed



Smooth Floor Insert

Install the smooth floor contextual insert by sliding the chamber forward inside the SAC and opening the chamber door. Using the handle, pull the grid floor and waste pan forward until they are nearly removed from the chamber. Place the smooth floor insert over the grid floor. Refer to Figure D-2.

Figure D-2 - Smooth Floor Contextual Insert Installed



Curved Wall Insert

Begin installation of the curved wall contextual insert by sliding the chamber forward inside the SAC and opening the chamber door. Using the handle, pull the grid floor and waste pan forward until they are completely removed from the chamber. Place the insert inside of the chamber so that the ends are braced against the side wall supports of the chamber. Refer to Figure D-3. Be sure to install so that the orientation of the sound perforation and light cut-out are correct. Reinstall the grid floor and waste pan prior to placing animals in the chamber.

Figure D-3 - Rounded Back Wall Contextual Insert Installed

NOTE: When using Contextual Inserts, Camera Calibration and Average Intensity may need to be adjusted.

APPENDIX E | USB TOPOLOGY

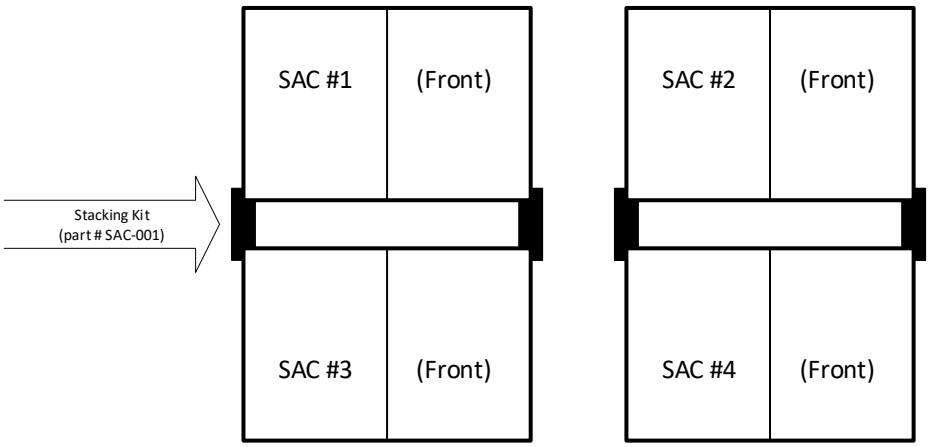
Recommended Configurations

The Video Fear Conditioning product (SOF-843) has been modified to support USB 3.0 cameras. SOF-843 versions 2.7.3 and earlier supported only IEEE-1394 (a.k.a. FireWire) cameras (VID-CAM-MONO-2A and VID-CAM-MONO-4). SOF-843 versions 3.0.0 and higher support both FireWire and USB 3.0 cameras (VOD-CAM-MONO-5). With the introduction of the USB 3.0 camera, the FireWire cameras, hubs, and cabling will be replaced with USB 3.0 cameras, cabling, and optional hubs. The USB 3.0 interface has different specifications regarding cable and hub requirements, and as a result, Med Associates has modified the Video Fear Conditioning bill-of-materials to accommodate these requirements. As a result of these requirements, the topology of the USB network will need to adhere to certain guidelines that conform to the specifications.

Listed below are the two recommended configurations that take into consideration the USB cable length restriction. These two options require stacking of the SACs. Three additional configurations are listed for those systems that require the Sound Attenuating Cubicles (SACs) to be positioned in a horizontal configuration (i.e. stacking of SACs is not an option).

The following diagram is a front view of (4) SACs that have been stacked using the Stacking Kit (SAC-001).

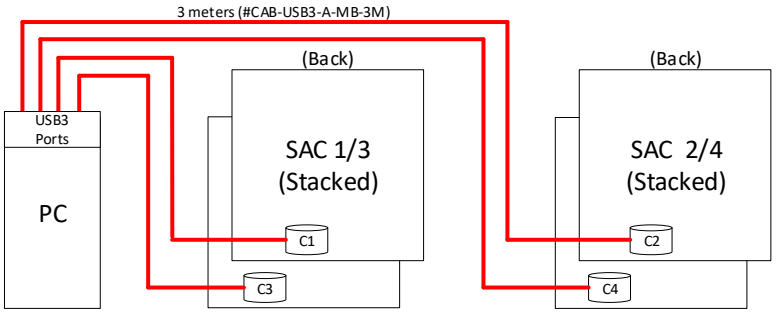
Figure E-1 –Stacked SACs (view from front)



NOTE: There is limited flexibility in the USB topology. Variations to the suggested topologies can be considered on a case-by-case basis when the recommended topologies do not fit a customer's system layout.

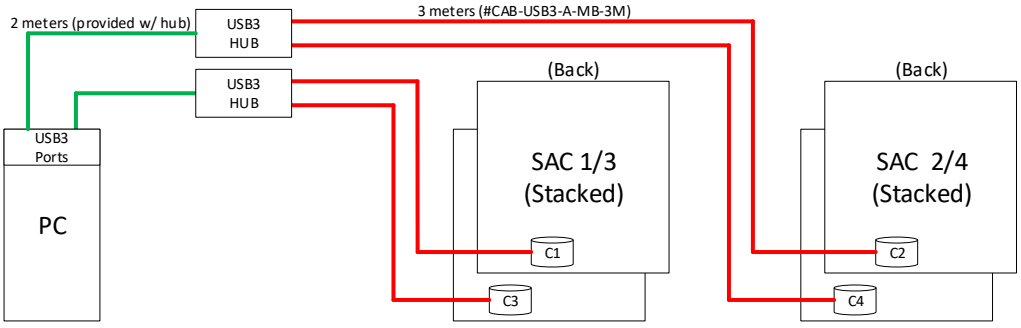
(Primary) Option #1: Connects (4) USB cameras directly into USB 3.0 ports on the back of the PC. The cameras are mounted in (4) SAC that are stacked two-high. Stacking of (2) SAC is critical to minimize the cable length required to span from the camera to the PC.

Figure E-2 –Primary Configuration (view from above)



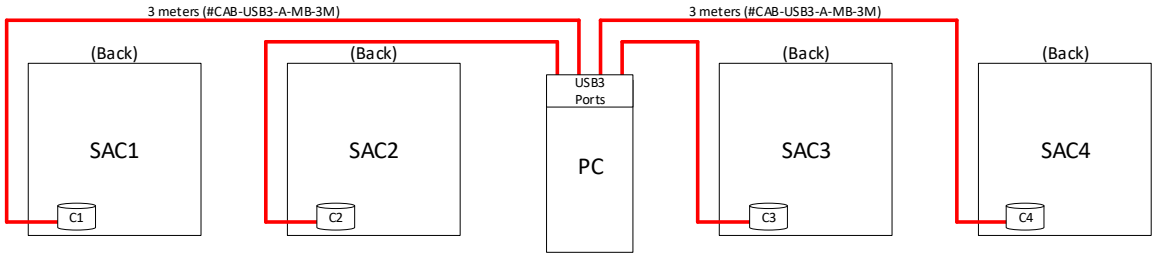
(Alternate) Option #2: This configuration can be utilized when the "Primary Option" is not permitted due to USB cable length restrictions (i.e. longer cable runs are required). This option connects (4) USB cameras to (2) USB 3.0 hubs (#USB-VID-HUB-USB3), and the (2) hubs are connected directly into USB 3.0 ports on the back of the PC. The system is designed to support no more than (2) USB cameras for USB hub. The cameras are mounted in (4) Sound Attenuating Cubicles (SAC) that are stacked two-high. Stacking of (2) SAC is critical to minimize the cable length required to span from the camera to the PC.

Figure E-3 –Alternate Configuration (view from above)



(Horizontal) Option #3: This configuration should only be utilized when the (4) SAC must be positioned in a horizontal configuration (i.e. stacking of SAC is not an option). This option connects (4) USB cameras directly into USB 3.0 ports on the back of the PC. Due to USB cable length restrictions, the PC must be positioned where all (4) USB cables extending from the (4) USB cameras can reach the back of the PC. To accomplish this, positioning the PC in the middle of the (4) SAC (two on each side) is the recommended position.

Figure E-4 –Horizontal Configuration (view from above)



(Alternate Horizontal) Options #4 and #5: This configuration should only be utilized when (4) SAC must be positioned in a horizontal configuration (i.e. stacking of SAC is not an option) and Option #3 is not permitted. This option connects (4) USB cameras to (2) USB 3.0 hubs (#USB-VID-HUB-USB3), and the (2) hubs are connected directly into USB 3.0 ports on the back of the PC. Due to USB cable length restrictions, the PC must be positioned where all (4) USB cables extending from the (4) USB cameras can reach the back of one of the USB hubs (maximum of two cameras per hub), and the (2) USB cables extending from each USB hub must reach the back of the PC. To accomplish this, two variations are diagrammed.

Figure E-5 –Alternate Horizontal Configuration (view from above)