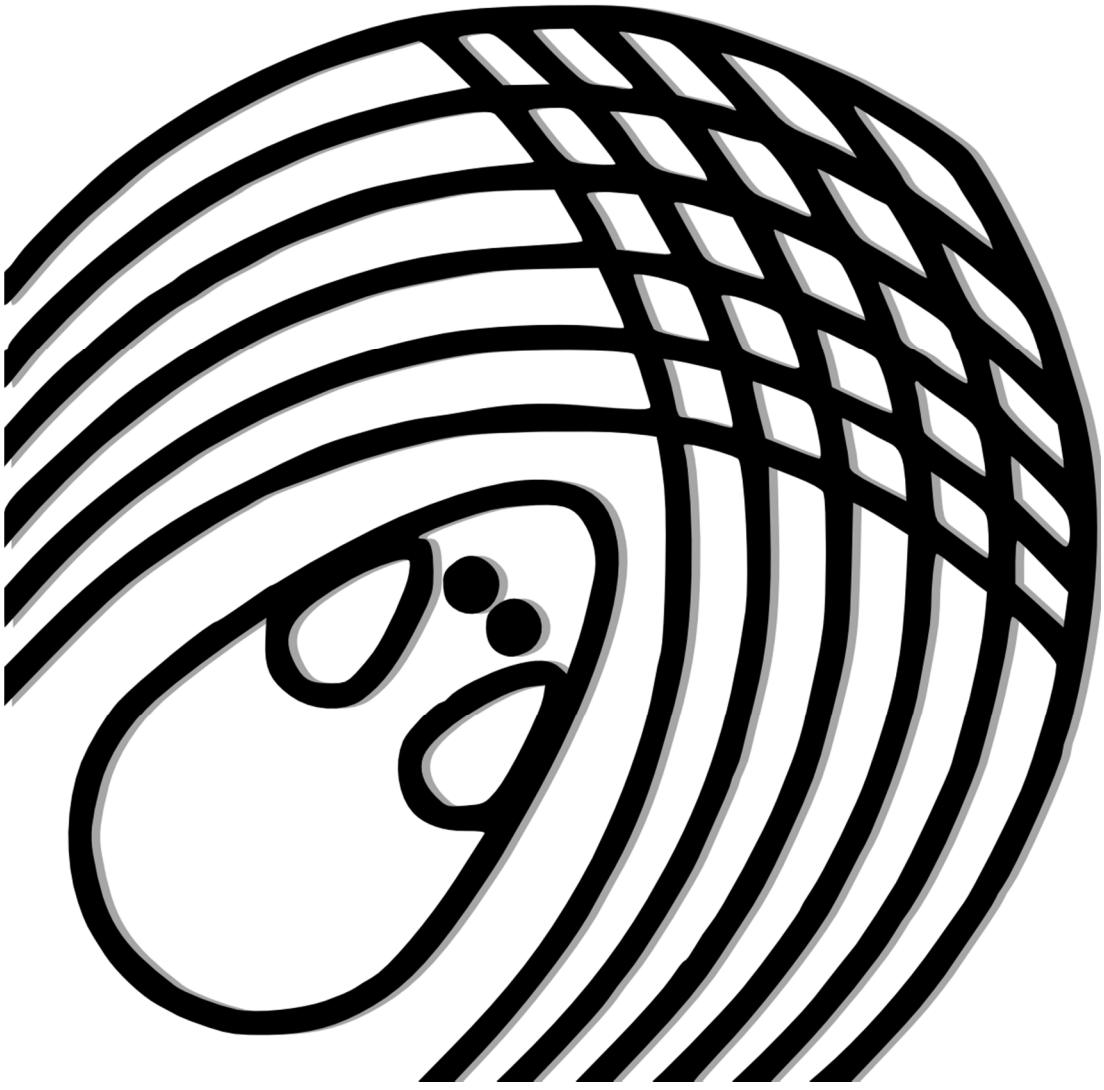
Fusion v5

SuperFlex Edition

Omnitech Electronics Inc.



Revised 5/16/2016

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1.0 Preparing the Hardware

A SuperFlex / Fusion system consists of any combination of hardware which interfaces with the Fusion Software. Please note: Depending on your configuration, not all software / hardware features contained in this manual may be applicable. Currently, the following components can be used with the Fusion system.

- Open Field (SuperFlex) – Locomotor Activity
- Open Field (SuperFlex CPP)– Conditioning Place Preference System
- Home Cage (SuperFlex HC) – Locomotor Activity
- Stimulus – Fear Conditioning
- Metabolic (PhysioScan) – O2/CO2/VO2/VCO2/RER
- Diet (DietMax) – Food/Liquid Consumption
- Circling Behavior (RotoMax) – Rotometer Activity
- Wheel – Running Activity
- Hole Poke – Baited Nose Poke
- Rota-Rod (AccuRotor)– Accelerating Rotating Rod
- ECC – Environmental Control Chamber
- Shuttle Box – Active/Passive Avoidance

1.1 Precautions

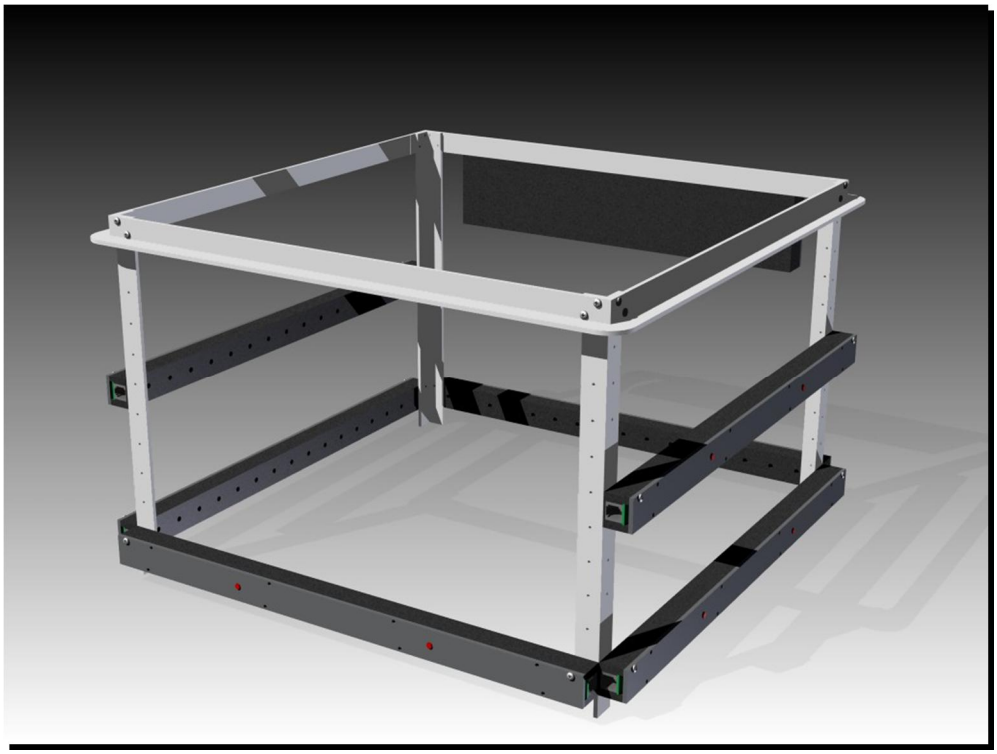
Always make sure that power is disconnected when connecting or reconfiguring system components. Some equipment can carry an electric charge despite being in an “off” state.

Fusion Hardware and associated computers should always be installed with EMI/RFI isolating power strips or on an uninterruptible power supply.

USB cables should not exceed 10 feet (3.3 m) in length and should be shielded and rated as 'USB 2.0 Hi-Speed'. 15 foot (4.5m) cables which meet the preceding conditions, under clean power, may work if cable length constraints are a significant issue in your laboratory space. Error-free operation cannot be guaranteed due to 15 feet (4.5m) being on the threshold of the USB 2.0 specification for maximum length. 'Dirty power' sources, USB cable length and USB cable quality directly correlate with communication quality.

1.2 Open Field - Locomotor Activity

Open Field is a system for storing, replaying, and analyzing open field locomotor activity data. Fusion Open Field uses a 16x16 grid of light beams to measure the locomotor activity of an animal.



1.2.1 Hardware Checklist

Before doing anything else, please check that the following components are accounted for (Each Unit):

- Fusion Node
- At least 2 beam sensor pairs
- Cage/Frame
- Power Adapter
- USB cable
- Interconnect cable (attached to each sensor)

1.2.2 Assembling the Hardware

The following steps explain how to properly assemble the Open Field Frame.

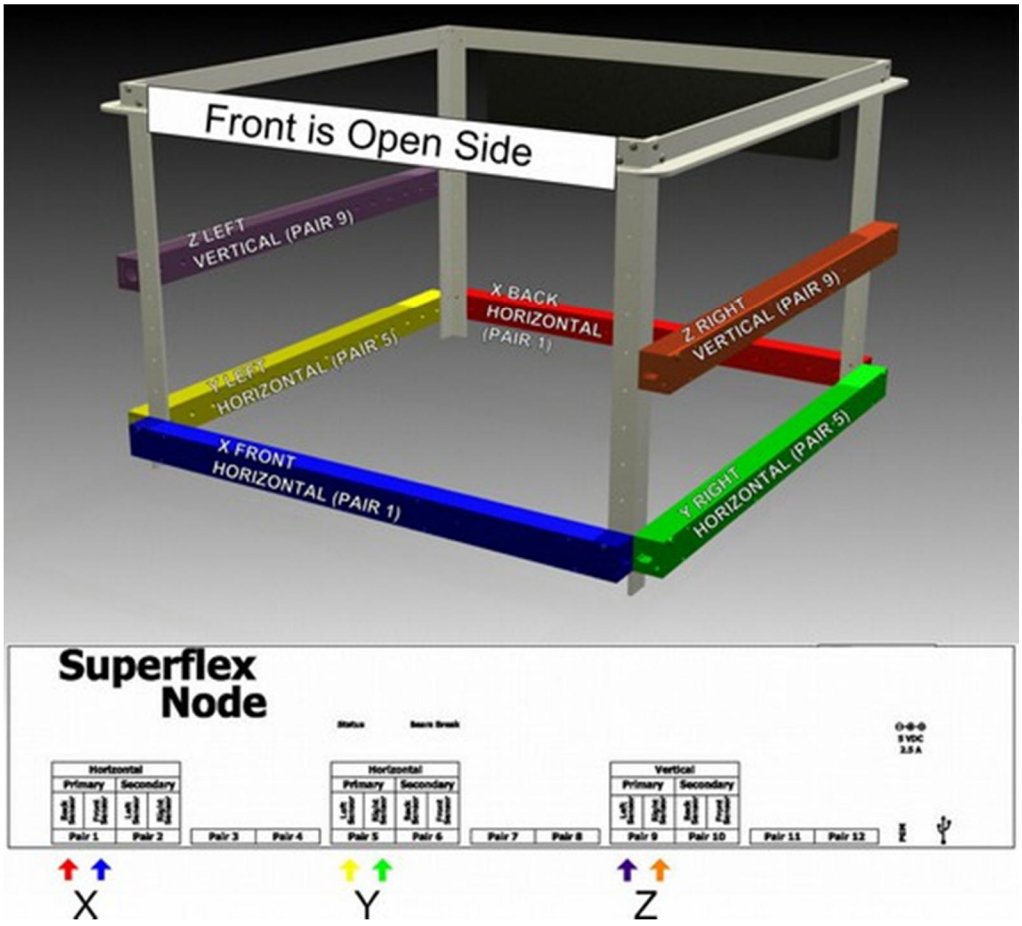
1.2.3 Cable Connection Guide

- Connect X Front Horizontal Sensor (with 2 Red Buttons) to **Pair 1 Front** Node Port
- Connect X Back Horizontal Sensor to **Pair 1 Back** Node Port
- Connect Y Left Horizontal Sensor to **Pair 5 Left** Node Port
- Connect Y Right Horizontal Sensor to **Pair 5 Right** Node Port

(This sensor features an attached cable which connects the X Front Sensor to the Node)

- Connect Z Left Vertical Sensor to **Pair 9 Left** Node Port
- Connect Z Right Vertical Sensor to **Pair 9 Right** Node Port
- Connect USB and POWER CABLE

ALL Power Cables should be connected to an EMI/RFI surge protector. An uninterruptable battery-backed-up power supply is highly recommended.



1.3 Open Field – Conditioned Place Preference

The Conditioned Place Preference (CPP) system allow researchers to perform classical conditioning experiments where environmental cues are repeatedly paired with a positive or negative stimulus. Over time, these pairings will cause the subject to associate the environmental cues with the positive or negative stimulus. CPP systems are typically used as a measure of drug abuse and addiction potential. Both 2-chamber and 3-chamber configurations are available for mice and rats with high contrast white/black stripes in horizontal and vertical orientation. Textured floor cues are likewise available.

1.3.1 Hardware Checklist

Before doing anything else, please check that the following components are present:

- Fusion Node
- At least 2 beam sensor pairs
- Cage/Frame
- Floor Cues
- Power Adapter
- USB cable
- Interconnect cable (attached to each sensor)
- Dividing panel height adjustment thumbscrews (#8-32 Plastic, Black)

1.3.2 Experimental Phases

Conditioned Place Preference studies typically have three phases:

Habituation Phase – This phase allows the animal to acclimate or habituate to the environment. The habituation phase can be recorded utilizing the Fusion Software to determine if the animal has an innate place preference by using the Zone Designer feature.

Conditioning Phase – This phase pairs the environmental cues and the unconditioned stimulus. The unconditioned stimulus is repeatedly presented and the animal is restricted to a single compartment with set environmental (wall and floor) cues.

Place Preference Testing Phase – This phase tests to determine if the conditioning was successful. The subject is presented with free access to all compartments and the time spent in each is recorded. This data can be compared against the initial Habituation Phase data to determine Place Preference Conditioning.

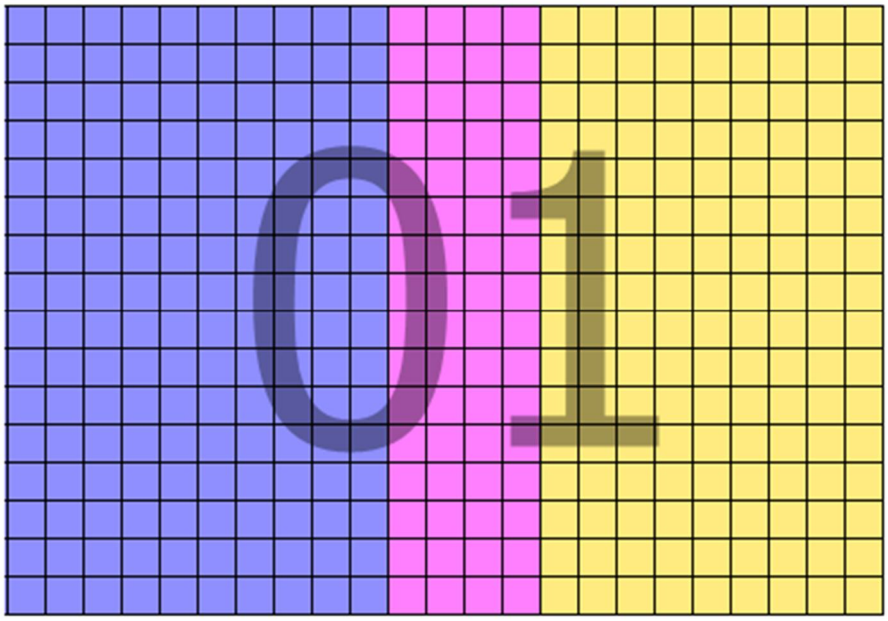
1.3.3 CPP Cages and Cues

The CPP frame can support either the industry standard 16” x 16” (40cm x 40cm) acrylic cage, or the three-chambered 24” x 12” (60cm x 30cm) cage.

Wall cues and floor cues may vary based on individual researcher preference. Typical cues involve textured floor cues and striped (horizontal and vertical) wall cues. Please contact Omnitech Electronics, Inc. for special requests.

1.3.4 CPP Zone Maps

For detailed information on the Zone Designer feature of the Fusion Software, please see the Zone Map section. A typical zone map for the CPP system may appear as depicted below. This zone map shows the three distinct zones (chambers) for zone-crossing purposes, allowing researchers to easily export place preference data.



1.3.5 Dividing Partition Height Adjustment

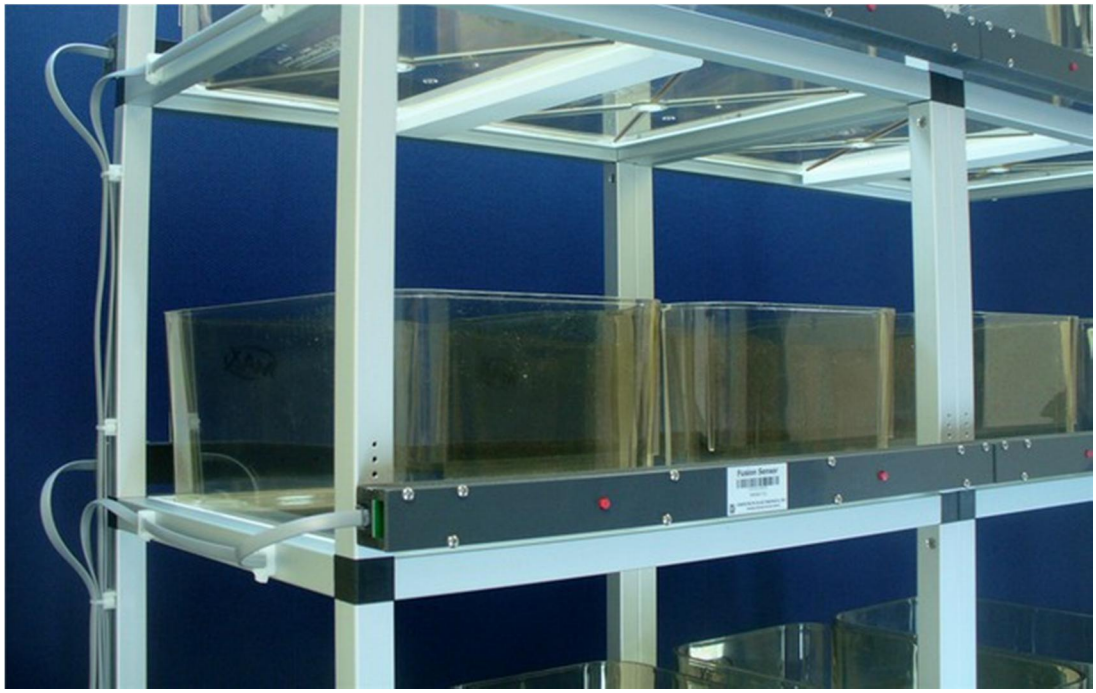
Adjust the center dividing panels by sliding the center panels up and inserting the height adjustment thumbscrews into one of the available adjustment holes. The panels should then be gently lowered so the thumbscrews rest on the top of the cage. This provides an easy means of adjustment and allows subjects access to all compartments.

1.3.6 CPP Cage Positioning

To align the acrylic CPP cage on the white base, place a CPP cage positioning spacer bar flat on the white base with one edge set inlaid against the metal frame. Then place the acrylic cage next to the spacer bar so that the edges are touching and gently slide the acrylic cage toward the CPP cage positioning spacer bar to ensure a snug fit. This aligns the CPP cage in the center of the white base.

1.4 Home Cage - Locomotor Activity

Home Cage is a system for storing, replaying, and analyzing home cage locomotor activity data. Fusion Home Cage uses a single axis of 16 light beams to measure the locomotor activity data of an animal. Home cage systems are available as individual units or as part of a space-saving rack.



1.4.1 Hardware Checklist

Please check that all hardware components are accounted for.

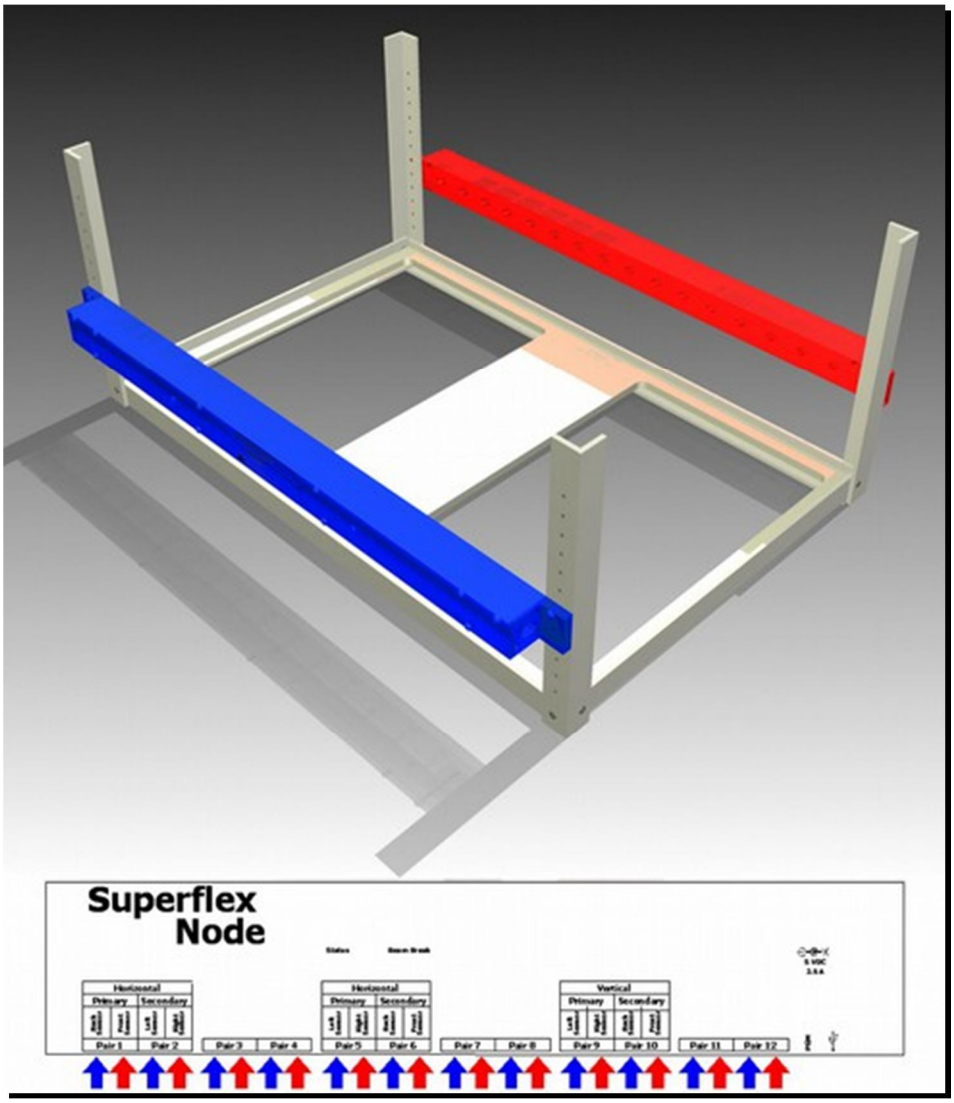
Components (Each Home Cage Cell):

- (1) pair of SuperFlex sensor bars (or two pairs if Vertical Sensors are included)
- Interconnect cables (attached to each sensor)
- Frame
- Home Cage (supplied by end user unless specifically purchased)

Components (Each Node):

- Fusion Node (can be used with up to 12 cages)
- AC Power Adapter
- USB cable

1.4.2 Connecting the Components



a) Connect the USB cable from the Node to the computer
b) Connect the power adapter to the power outlet
c) Connect the other end of the power adapter to the Node
d) Complete steps a-c for each node
e) Connect each cage sensor pair to one of the 12 channels (marked pair 1 through pair 12) on the Fusion Node (each sensor pair uses two connectors)
f) Complete step e for each cage
g) Launch Fusion software
h) A list of connected cages is displayed on the middle-left panel; Cage 1 is selected by default
i) Check cage orientation of each cage by moving your hand in the cage while watching the screen
j) If the orientation (left/right) is incorrect for any cage, the cables on that cage can be swapped to reverse the orientation.

1.4.3 Additional Configurations

Starting in Fusion 4.7n, an additional Home Cage configuration has been added that allows sensor add-ons as well as Locomotor sensor pairs to be used in experiments.

Supported add-ons include **Activity Wheels** and **Stimulus Hubs**. To incorporate the additional hardware, this configuration has a reduced maximum number of Home Cages. Eight Locomotor sensor pairs can be connected in Pairs 1 – 8 for eight cages. Pairs 9 – 12 are for the add-on sensors, with a maximum of eight add-ons, one for each cage. Connecting a Wheel or Hub into the first port of Pair 9 will join that Wheel or Hub to the Locomotor sensor pair connected in Pair 1. Similarly, connecting the Wheel or Hub into the second port of Pair 9 will join it to the Locomotor sensor pair in Pair 2, and connecting an add-on into the first port of Pair 10 will join it to the Locomotor sensor pair in Pair 3, etc.

1.5 Metabolic - O2/CO2/VO2/VCO2/RER

The PhysioScan Metabolic System which integrates with the Fusion Software is ideal for measuring oxygen consumption and carbon dioxide production in mice and rats using open circuit calorimetry providing in depth O2, CO2, VO2, VCO2, and RER data.

1.6 Diet - Food/Liquid Consumption

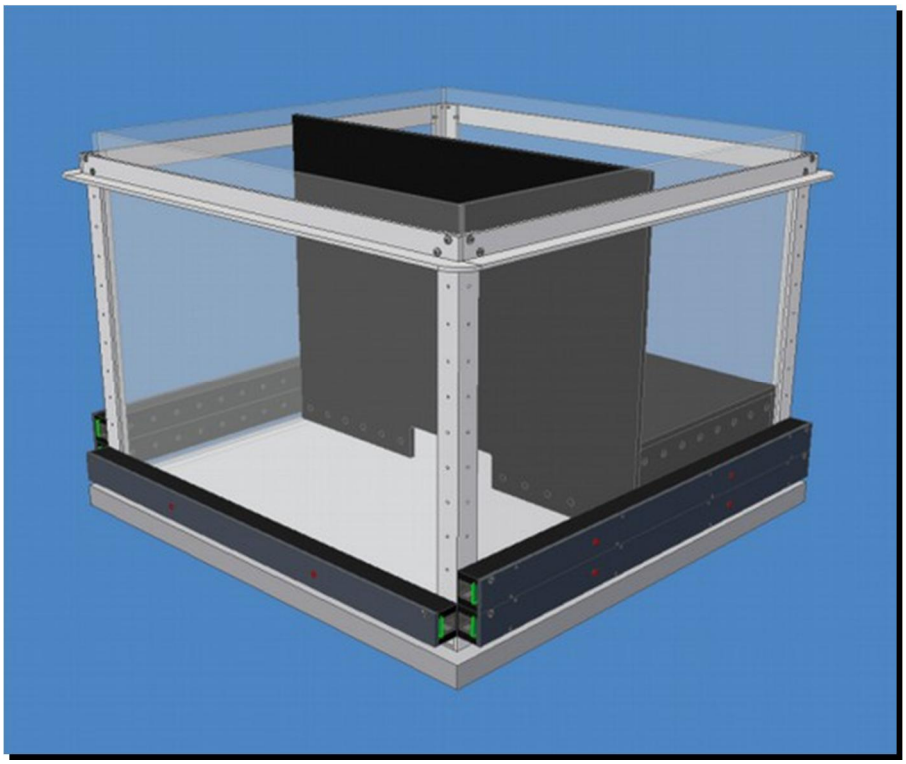
The DietMax Food/Liquid Consumption Monitoring System integrates with the Fusion Software and allows for precise food and liquid consumption monitoring in small animals over extended periods of time. Multiple stations can be used for preference studies.

1.7 Circling Behavior - Rotometer Activity

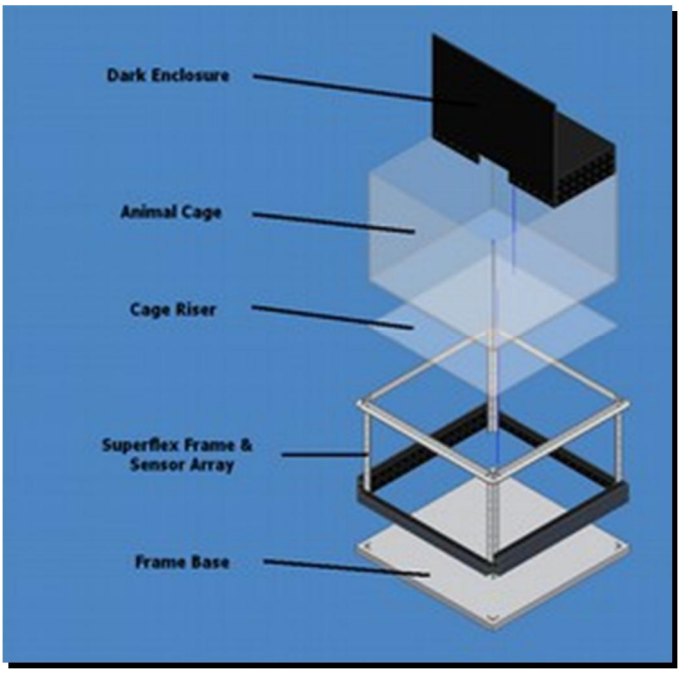
The Fusion based RotoMax Circling system is designed to study the circling behavior of animals. It excels in measuring tight clockwise, counter-clockwise, and net turns. When used in an open-field, the rotometer hardware integrates into the lid of the cage and plugs directly into the SuperFlex Node. Stand-alone bowl and cylinder systems are likewise available.

1.8 Dark Enclosure – Light/Dark Studies

The SuperFlex Open Field configuration can accommodate a variety of inserts to aid in various types of assays. The dark insert is transparent to the system's IR photobeams, however the insert is completely opaque to visible light. Mouse and rat sized enclosures are available in either 1/2 or 1/3 one-third open-field area dimensions.



1.8.1 Dark Enclosure Setup

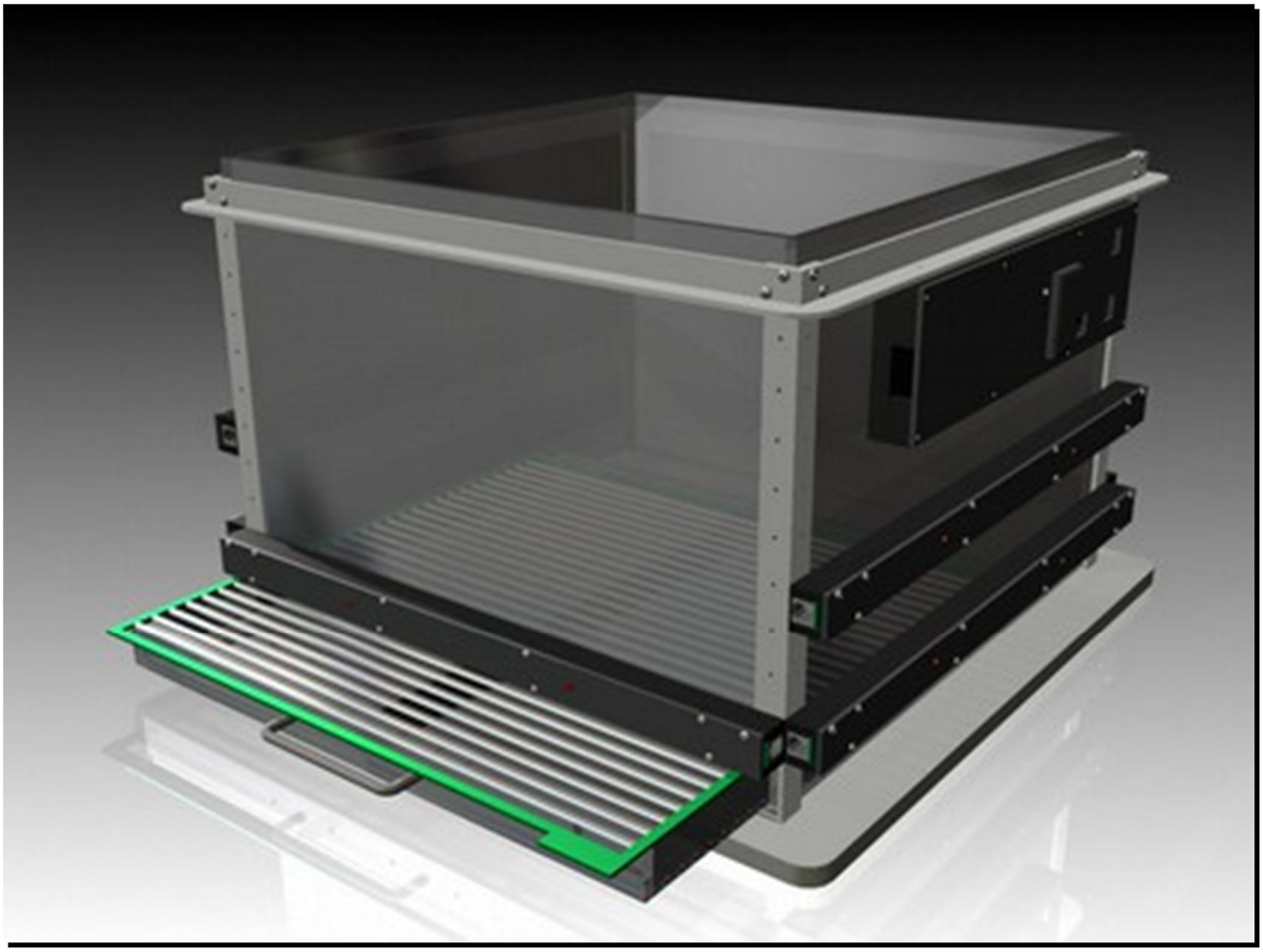


1.8.2 Dark Enclosure Zone Map

When the Fusion software is installed, a zone map is created in the “[Documents]/Fusion” directory called “Dark Enclosure.szm”. This zone map can be used to analyze dark enclosure experiments. Dark enclosures for light/dark studies are available from Omnitech Electronics, Inc.

1.9 Stimulus - Fear Conditioning

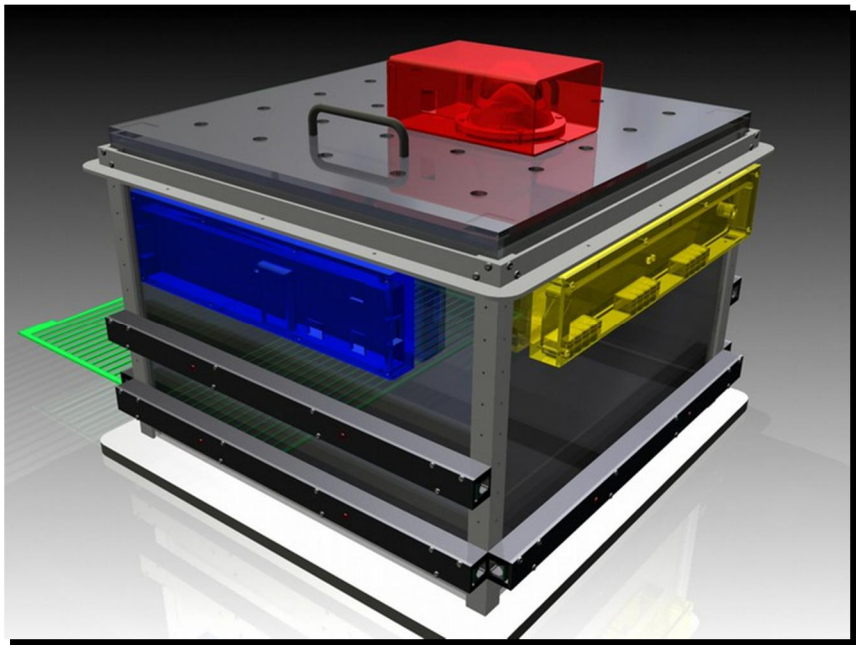
Stimulus is a system designed to gauge animal responses to anxiety inducing stimuli. The system is capable of sending three different kinds of stimuli; shock, light, and sound with a range of intensities. Moreover, because of the integration with the Fusion system, it is capable of tracking the animals' movement, reaction time and velocity as the stimuli are being activated.



1.9.1 Hardware Checklist

- Stimulus Hub
- Stimulus Lid (with Lamp and Speaker)
- Shock Tray
- Stimulus Hub Interconnect Cable (Connects to Fusion Node)
- Stimulus Lid Interconnect Cable
- Shock Interconnect Cable
- AC Power Cable

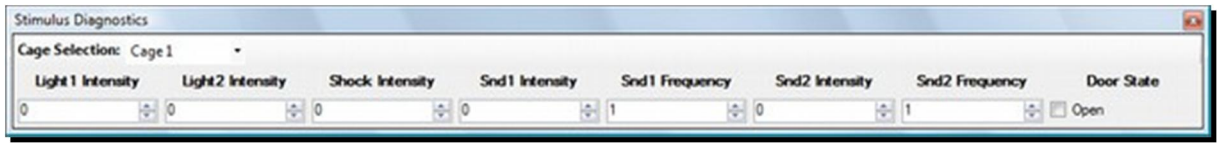
1.9.2 Connecting the Hardware



a) Connect the Fusion Node (on the port marked “Stimulus Hub”) to the Stimulus Hub (port marked “Fusion Node”).
b) Connect the Shock Grid to the Stimulus Hub (port marked “To Shock Grid”).
c) Connect the AC outlet to the Stimulus Hub (port marked “AC In”).

1.9.3 Stimulus Diagnostics

In order to use Stimulus Diagnostics, select “Stimulus Diagnostics” from the Tools pull-down menu. This may only be selected when an experiment is not in progress. All stimuli will be reset to their default values upon opening Stimulus Diagnostics.



a) Selecting a Cage

In order to select the cage on which to perform diagnostics, use the “Cage Selection” drop down list in the upper-left corner of the diagnostic window.

b) Testing Stimuli

In order to test a stimulus, change its value using the numeric boxes corresponding to that stimulus. All intensities are on a 0-255 scale, all frequencies are on a 1-20,000 Hz scale, and the door state uses a checkbox toggle (checked indicates “open” state).

c) Closing Stimulus Diagnostics

To close Stimulus Diagnostics, use the close button in the upper-right corner of the window. When Diagnostics is closed, the stimuli will reset to their default values.

1.10 Wheel – Running Activity

Activity Wheels measure the activity of an animal in an enriched environment. A wheel can be attached to the outside of a SuperFlex cage so it does not interfere with open field measurement. An accompanying sensor measures the number of rotations that the wheel travels. Close-off panels are available for non-wheel experiments.

1.10.1 Hardware Checklist

- Acrylic Wheel Cage
- Activity Wheel
- Wheel Sensor
- Wheel Cover Panel

1.10.2 Assembling the Hardware

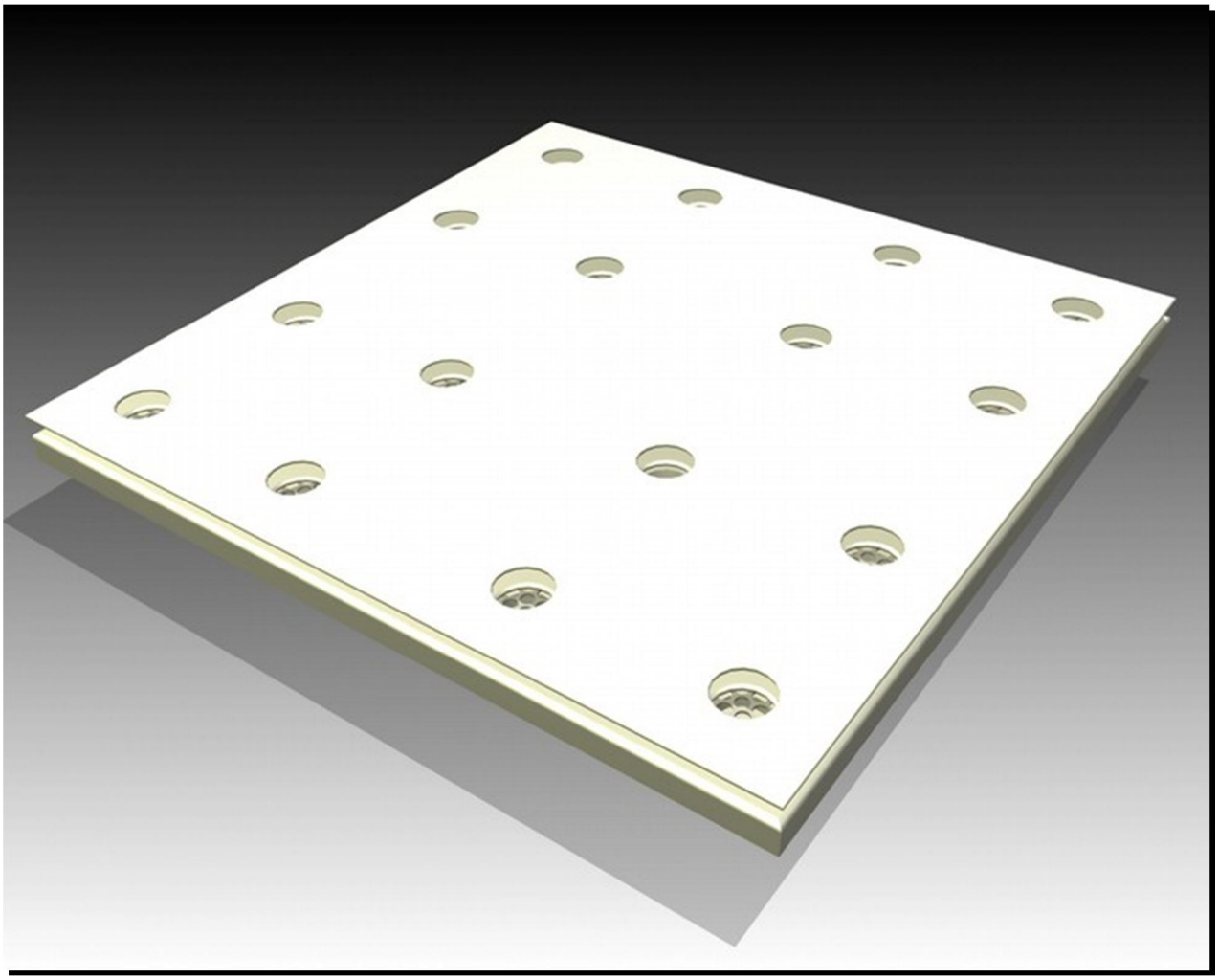
- Remove the solid acrylic cage from the Fusion open-field frame and replace it with the acrylic cage that has running wheel cut-outs in the frame, taking care to note the openings are in the front left and rear right quadrants.
- When utilizing the running wheel cover plates, be careful – the magnets are powerful. Allowing the magnets to snap together may shatter the magnets.
- Slide the plate across the face of the cage and carefully line up the magnets.
- Do not attempt to line up the magnets and lower the plate over the magnets.
- When attaching a running wheel, carefully slide the running wheel over the face of the cage to line up the magnets.
- Cover plates can be stored by facing two plates together and gently twisting until the magnets line up. To remove the plates from the storage state, twist the handles and pull gently apart.
- The cover plates can be used to condition laboratory animals to the running wheel by placing an animal in the running wheel and attaching the cover plate.

1.10.3 Connecting the Hardware

Connect each wheel sensor to the Fusion Node into the jacks marked “Wheel” using the interconnecting cable. In a “Wheel Only” hardware configuration, wheels can fully populate all Fusion Node ports.

1.11 Hole Poke - Baited Nose Poke

The Baited Nose Poke (a.k.a Hole Poke) Board is a classic assay designed for memory and learning studies which can be used with baited or un-baited cups. The targets in the cups can optionally be covered with a retaining screen to prevent removal of target object.

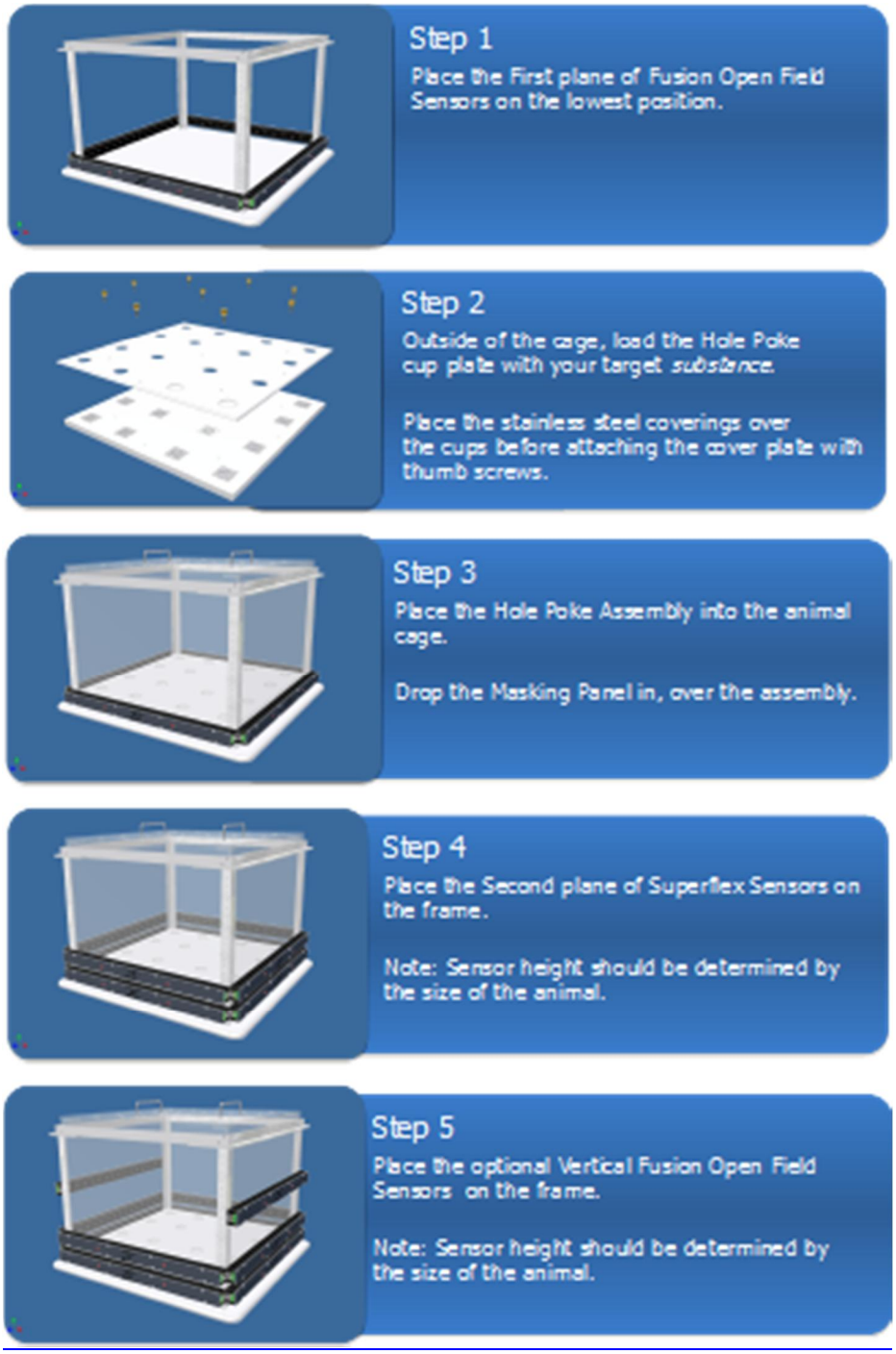


1.11.1 Hardware Checklist

Before doing anything else, please check that the following components are accounted for (Each Unit):

- (2) additional X-Y SuperFlex sensor pairs
- Hole Poke Base with integrated cups
- Hole Poke Screen Retaining Sheet
- Hole Poke Top Sheet
- (16) Baited Cup Screens

1.11.2 Assembling the Hardware



1.11.3 Connecting the Hardware

a) Connect each sensor bar to the corresponding port in the Fusion Node
b) Connect the USB cable from the Node to the computer
c) Connect the power cable from the outlet to the Node
d) Complete steps a-c for each cage

1.12 Environmental Control Chamber

Environmental Control/ Isolation Chambers are available for all Omnitech equipment for light and sound isolation. With SuperFlex systems, light cycles can be precisely controlled. Each chamber features power for the Fusion Node, a ventilation fan, and a software-controllable dome light.

1.12.1 Hardware Checklist

- Environmental Control Chamber
- Power Cable

1.12.2 Connecting the Hardware

Perform the following for each Environmental Control Chamber in the system.

- Connect the USB cable to the PC (or supplied USB hub).
- Run the other end of the USB cable through the rear-left tube vent down into the isolation chamber.
- Inside the chamber, connect the USB cable to the Fusion Node.
- Inside the chamber, connect the black power cable to the Fusion Node.
- Inside the chamber, connect the gray ECC interface cable to the port marked ECC (the sixth port) on the Fusion Node.
- Connect the power cable to the back of the Environmental Control Chamber to an outlet.

1.12.3 Changing the Dome Light Status

The on/off status of the dome light can be altered in one of two ways.

- The red trigger on the front of the ECC marked “Light Toggle” will change the state of the dome light (On->Off, Off->On).
- In the fusion software, under the “Tools” pull-down menu, select the “ECC Configuration” option. Each cage containing an ECC will be listed in the left column, and a corresponding checkbox in the right column will indicate its dome light status. Each checkbox can be clicked to toggle its status. This operation can be performed in the middle of an experiment.

1.12.4 Fan Problem Indications

It is critical for the health of any ECC occupants that the fan be on at all times. In the unlikely event that the fan is not working properly, the system will provide several warning signs.

- The red “Fan Trouble Indicator” on the front of the ECC will be illuminated.
- The corresponding subject status in the software will read “Check Fan”.
- In the Fusion Drawing (upper middle of the default readout), the fan icons will be illuminated red.

If the fan is not working, the ECC should not be used until the problem is solved.

2.0 Preparing the Fusion Software

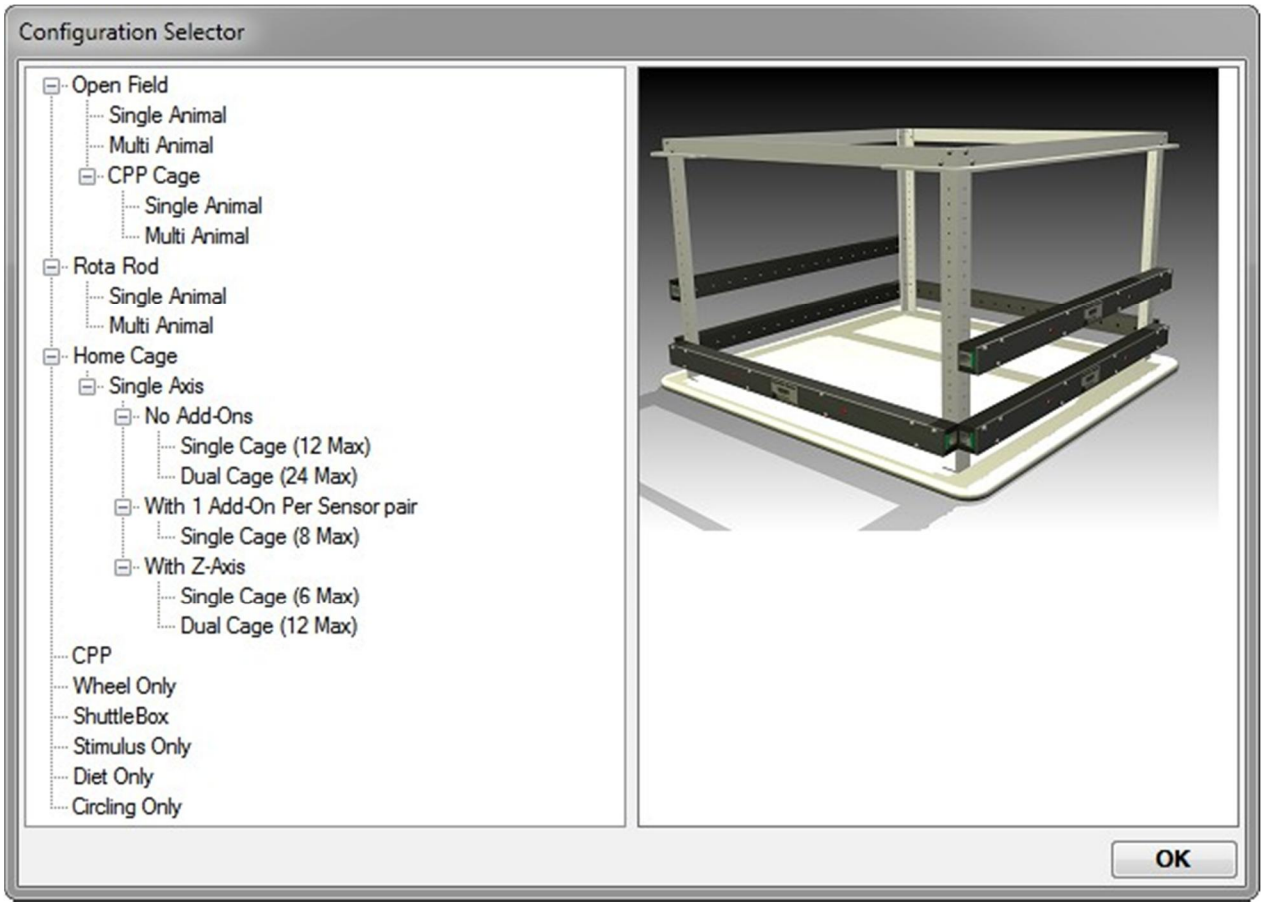
After the system has been assembled and connected, the software must be configured to work with the system.

2.1 System Requirements

- Windows XP/7/8/10
- Intel Core 2 Duo or AMD Athlon 64 X2 CPU Minimum
- At least 2 Gigabytes RAM
- Screen resolution of at least 1024x768
- Minimal background processes running concurrently

2.2 List of Hardware Configurations

When the Fusion system is first launched, a hardware configuration must be selected. This will determine what the port assignments are on each Fusion Node, and what features are available in the software. If an incorrect option is selected or if the hardware configuration changes, this dialog can be launched from the settings dialog.



Open Field. This is the primary Fusion mode. Beyond open field locomotor studies, it can incorporate all Fusion components into the system (Diet, Metabolic, Circling, Stimulus, Wheel). In this mode, each cage uses exactly one node. The open field configuration can be used in Single or Dual Animal mode (two animals per cage frame).

Home Cage. Designed for locomotor studies using a home cage. Home cage studies are generally single axis, but a vertical component can be installed above the primary axis. The home cage configuration can be used in Single or Dual Animal mode (two animals per cage frame).

Wheel Only. Uses only wheel hardware. The system can use up to 24 wheels per node in this configuration.

Diet Only. Uses only diet hardware. The system can use up to 24 scales per node in this configuration.

Stimulus Only. Uses only stimulus hardware. The system can use up to 24 stimulus units per node in this configuration.

Shuttle Box. A configuration specifically for Shuttle Box experiments. Each cage has one locomotor axis (x-axis) and a stimulus hub.

Circling Only. Uses only rotometer hardware. The system can use up to 24 rotors per node in this configuration.

Individual and Multi Rota-Rod. Uses Rota-Rod hardware. The system can support up to 4 Multi Rota-Rods or 16 Individual Rota-Rods.

2.3 Assigning Nodes

Fusion requires that nodes be assigned to the system in a particular order. This process is called “assignment”. When the hardware is connected and Fusion software is first launched, a dialog appears asking the user to perform an assignment. Each node can be assigned simply by pressing a trigger on any sensor connected to that node. As each node is assigned, its name will appear in the list of assigned nodes along with its rank. The order of the nodes (and therefore the cages) will be identical to the order in which they were assigned. In some cases, you may wish to reassign nodes once they have already been assigned – this can be done within the Settings dialog.

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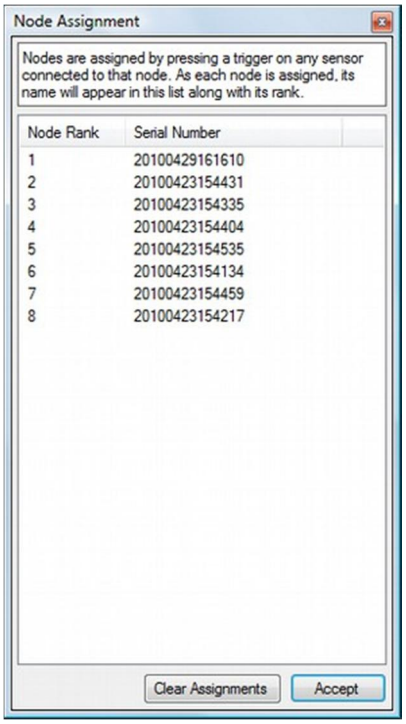
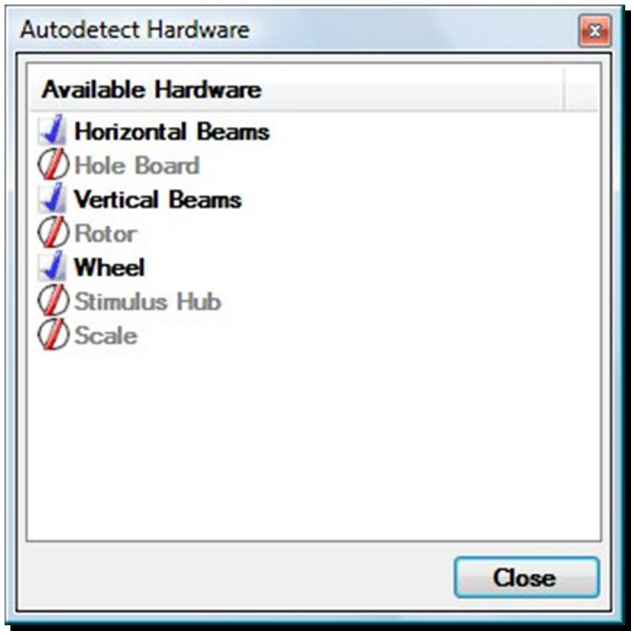


2.4 Detecting Hardware

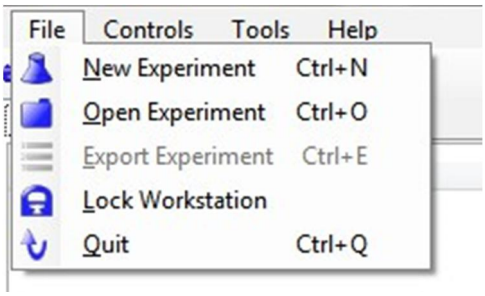
Fusion can automatically detect which hardware is connected to the system. The hardware detection dialog reports what Fusion has detected. This dialog does not require any human interaction.



A list of available hardware is shown in the dialog. The available hardware is determined by the hardware configuration. A blue checkmark will appear next to each hardware device that has been found, and a red slash next to each hardware device that has not been found. If Fusion has incorrectly detected the hardware, thoroughly check the connections going into the nodes. Fusion automatically detects which hardware is connected to the system based on which port devices are connected, so it is important that each component is connected to the correct port.

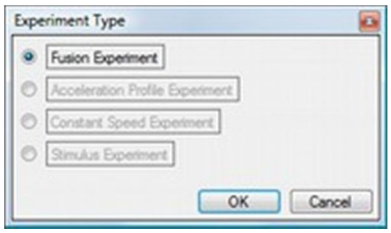
3.0 Creating a New Experiment

Start by selecting “New Experiment” from the main Fusion interface.



3.1 Experiment Type Selection

In some cases, more than one experiment type will be available. If this is the case, a dialog will present one of several experiment types to be chosen.



3.2 Fusion Experiment

All Fusion hardware uses this experiment type with the exception of the Rota-Rod. A Fusion experiment supports multiple subjects, phases, and batches. Some alternate types are:

Acceleration Profile Experiment (Rota-Rod Only)

In an Acceleration profile experiment, the user can create a precise speed map for the Rota-Rod to execute. In the drop-down box for this property, the user can choose from any previously saved acceleration profiles.

Constant Speed Experiment (Rota-Rod Only)

At the beginning of a Constant Speed experiment, the rod will begin rotating at a constant speed and remain at that speed for the duration of the experiment. The corresponding numeric box for this property chooses the constant speed at which the rod rotates. This number can range from -100 to 100 (0 is invalid). A negative number will cause the rod to rotate counterclockwise instead of the default clockwise rotation.

Stimulus Experiment (only with Stimulus hardware)

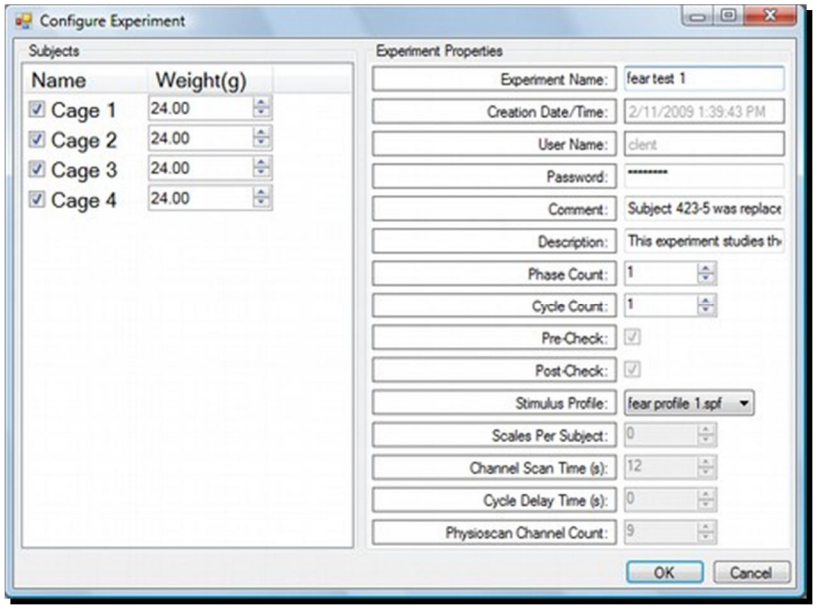
In a Stimulus Experiment, the user is able to select a “Stimulus Profile” with which to run the experiments. The experiment will automatically end when the Stimulus Profile has concluded. Initially, the listed duration for the experiment will be the maximum possible duration for the selected profile.

3.3 Cage Selection

Select cage(s) for the experiment by using the check boxes in the left column of the Experiment Properties dialog.

3.4 Experiment Properties

The experiment has several parameters that need to be set before continuing. Depending on what hardware is connected to the system, not all the experiment parameters listed here may be available. Once all of the information is entered, click OK to continue.



Name. An ID assigned by the Fusion Software to the corresponding cage or chamber.

Experiment Name. The name of the experiment. The name may have between 1 and 15 characters.

Creation Date/Time. This field will always be set to the time at which the experiment dialog was launched. This value cannot be changed manually.

User Name. The user name will always be the Windows user account name. This value cannot be changed manually.

Password. To encrypt[1] experiment information, type in a password. This field supports long lengths (up to 32767 characters).

Comment, Description. The comment and description fields are optional. These fields support long lengths (up to 32767 characters).

[1] Encrypting an Experiment File prevents unauthorized users from accessing the data in that experiment (either writing or reading). The password chosen will be required to open the experiment. Do not lose or forget the password.

Subject Default Sex. Assigns each generated subject a default sex that can be overridden on the next page by the user.

Subject Default Age Unit. Selects with what age unit (days / weeks / months / years) to regard the age numerical values.

Subject Default Age. Assigns each generated subject a default age that can be overridden on the next page by the user.

Phase Count (Fusion Experiment Only). Phase Count is the number of discrete experimental trials that are in a batch. This value may be 1-10. Recording must be manually restarted after each phase. When the "OK" button is selected, the user will be allowed to set the duration (in minutes) for each phase and provide a name for each phase.

Batch Count. Batch Count specifies how many times to record the phases specified by phase count. This is used to record how multiple subjects respond to the same set of stimuli.

Calculation: Experiment Duration= Average Phase Duration * Phase Count * Batch Count

Pre-Check, Post-Check. This is a Locomotor setting and can only be changed from the Locomotor Settings tab under the Settings option from the Tools menu.

Stimulus Profile (Stimulus Experiment Only). A stimulus profile can be run during the experiment if the Stimulus system is present. All Stimulus profiles in the current path will appear in this drop-down list. To select a profile from a different location on the computer, select "Browse" from the drop down list.

Scales Per Subject (Scale Hardware Only). The number of scales that are assigned to each subject. This is a Diet setting and cannot be changed here.

Channel Scan Time (Metabolic Hardware Only). This is a Metabolic setting and cannot be changed here.

Cycle Delay Time (Metabolic Hardware Only). This is a Metabolic setting and cannot be changed here.

Metabolic Channel Count (Metabolic Hardware Only). This is a Metabolic setting and cannot be changed here.

Trial Count (Rota-Rod Only). Rota-Rod experiments are divided into one or more "Trials". All trials in a Rota-Rod experiment are identical. If more than one Batch is selected, each batch will contain the number of trials specified by "Trial Count".

Acceleration Profile (Rota-Rod Only). In the "Acceleration Profile" experiment type, this selects the Acceleration profile to be executed on the Rota-Rod.

Constant Speed RPM (Rota-Rod Only). In the “Constant Speed” experiment type, this selects the rotational speed at which the Rota-Rod will spin for the duration of the trial.

Experiment Duration (m) (Rota-Rod Only). In the “Constant Speed” experiment type, this selects the total time (in minutes) for which the Rota-Rod will spin at the speed specified by “Constant Speed RPM” (above). If the animal does not fall for this time span, a “timeout” will occur.

3.5 Experiment Details

After the Experiment Properties dialog has been completed, the “Experiment Details” dialog will open.

Phase Names and Durations (Applicable Experiments Only)

Each phase in the experiment has its own duration and name. The phase names should be unique, whereas durations may be identical if desired.

Subject ID

Each ID may be edited for each batch. For instance, if there are 8 cages and 3 batches, the system will provide 3 * 8 = 24 Subject ID fields for that experiment. In order to change the ID, simply edit the text to the right of the corresponding ID / batch combination.

Subject Type

A meaningful subject descriptor.

Subject Sex

Unknown, Male, or Female.

Subject Age

A numerical value, interpreted by the Age Unit (selected in the Experiment Properties Window).

Subject Treatment

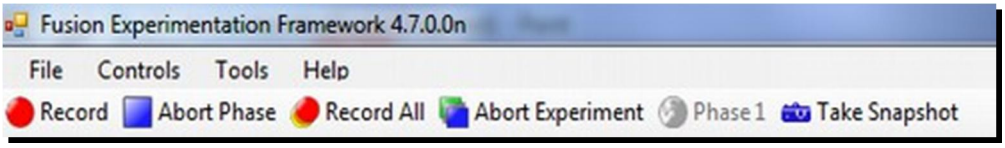
A meaningful object descriptor.

4.0 Running an Experiment

4.1 Starting an Experiment

Individual cages can be chosen on the left panel by clicking on the subject name. When a subject is selected, clicking the “Record” button will advance the state for that subject. Alternatively, use any trigger located on the sensors of the corresponding cage to advance the state. To simultaneously advance the states of all subjects involved in the experiment, use the “Record All” button. Many buttons in the toolbar have keyboard shortcuts which can be ascertained by using the “Controls” pull-down menu.

Note: Open Field / Home Cage / CPP / ShuttleFlex users: When Insertion Autostart is not enabled, subjects must be present within the cage before recording can begin.

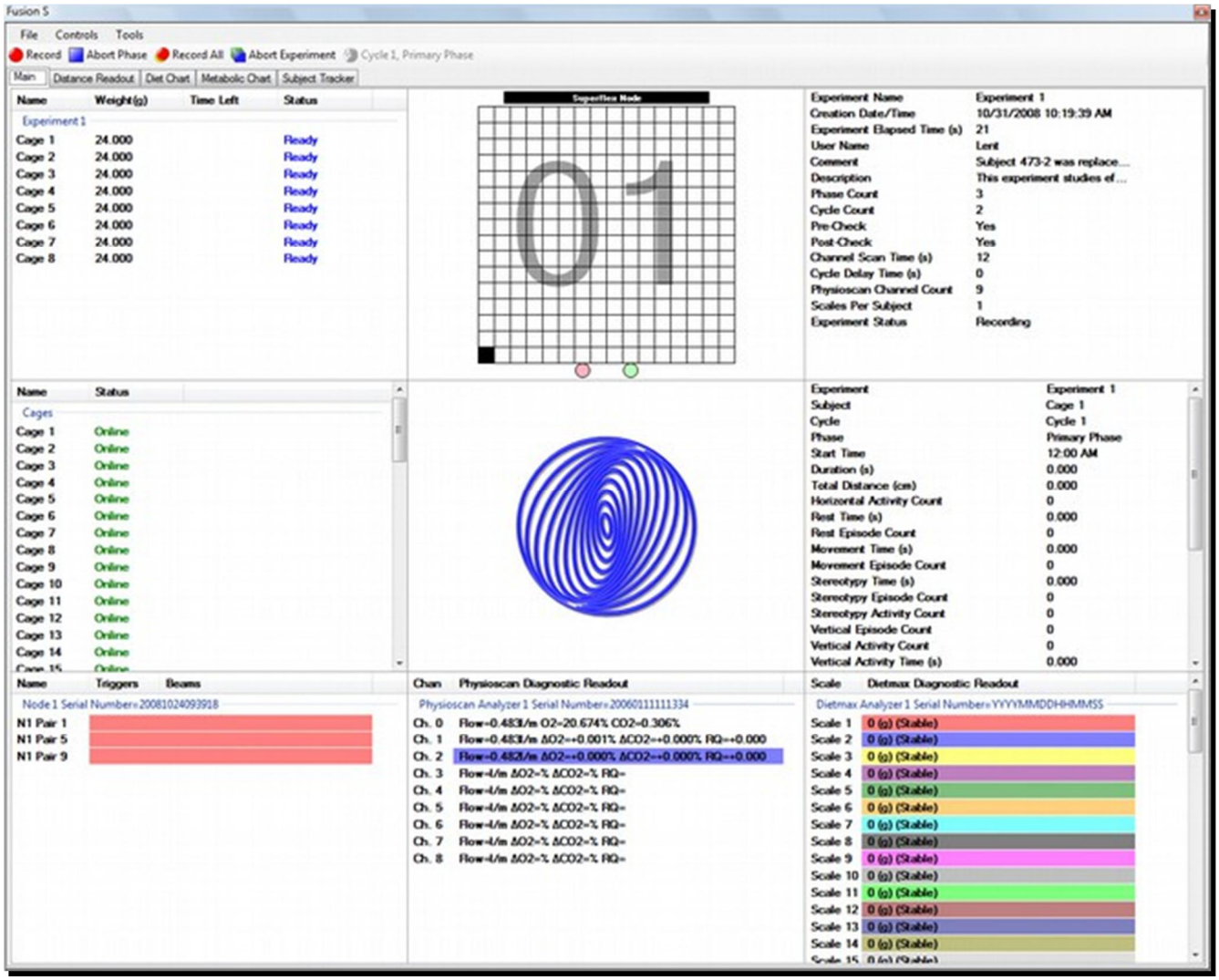


4.2 List of Recording States in the Fusion System

- ***Ready*** – In the “Ready” state, a subject is available to begin its phase or trial. Advancing the state when “Ready” is active will cause the subject to be recorded.
- ***Clear Rod*** (Rota-Rod only) – In the “Clear Rod” state, the rotating rod is stopped and the animal should not be on the rod or on the shock grid. Advancing the state while “Clear Rod” is active will cause the rod to start spinning at its initial speed and the system will advance to the “Insert Animal” state.
- ***Clear Cage*** (Locomotor only) – In the “Clear Cage” state, a Pre-Check is about to be performed, so the cage must be clear of all obstructions, including animals. Advancing the state while “Clear Cage” is active will cause the Pre-Check to be performed. If the Pre-Check is successful, the system will advance to the “Insert Animal” state.
- ***Insert Animal*** (Locomotor and Rota-Rod only) – In the “Insert Animal” state, the animal should be inserted into the cage or onto the rod. Advancing the state when “Insert Animal” is active will cause the subject to be recorded.
- ***Recording*** – In the “Recording” state, an experiment is in progress. A readout will indicate how much time remains in the current phase/trial, and all events while in the “Recording” state will be saved for later analysis. Advancing the state while “Recording” is active will do nothing. A phase/trial or experiment can be aborted while in progress.
- ***Completed*** – All Phases/Trials of all Batches have been run for a particular subject. No further experimentation can be conducted while in this state. Advancing the state while “Completed” is active will do nothing.

4.3 The Active Variable Display

When an experiment is created, Fusion will populate the variable display (middle-right) with all enabled display variables. These variables will display real-time variables for the currently selected subject, over the course of the current phase.



4.4 Stopping a Phase or a Trial

Without user intervention, Fusion records until the end of a phase or a trial and waits for the user. To terminate the phase for the current subject prematurely, click the “Abort Phase” (or “Abort Trial”) button. Please note that the aborted phase/trial cannot be modified or re-recorded later.

4.5 Aborting an Experiment

To terminate the entire experiment, use the “Abort Experiment” button. This button will terminate every phase of all subjects involved in an experiment. As with aborted phases/trials, aborted experiments cannot be either modified or re-recorded at a later time.

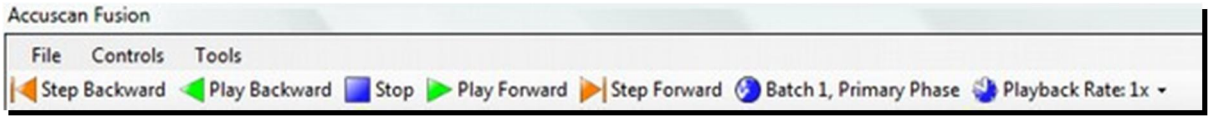
5.0 Playback of Experiments

5.1 Opening Experiment

To open a completed experiment, choose “Open Experiment” from the “File” menu. Even if you have just finished an experiment, you must still open it before you may playback or export that experiment.

5.2 Playback

The Fusion system allows the operator to replay the experiment forward and backward. To play forward, select “Play Forward”, and to play backward, select “Play Backward”.

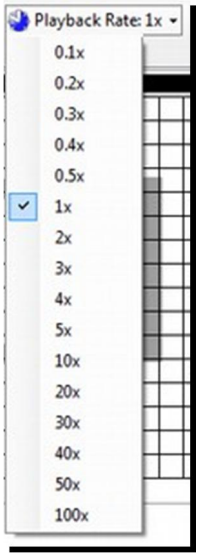


5.3 Stepping

During playback, the Fusion system can advance or retreat by a single event. To step, select either the “Step Forward” or “Step Backward” button. These buttons can also be held down to perform rapid stepping. Any event in the phase can be considered during the “step” operation, so if other data-collecting plug-ins are loaded, the result of a step might not be obvious (there may not be a change in the grid during every step).

5.4 Playback Rate

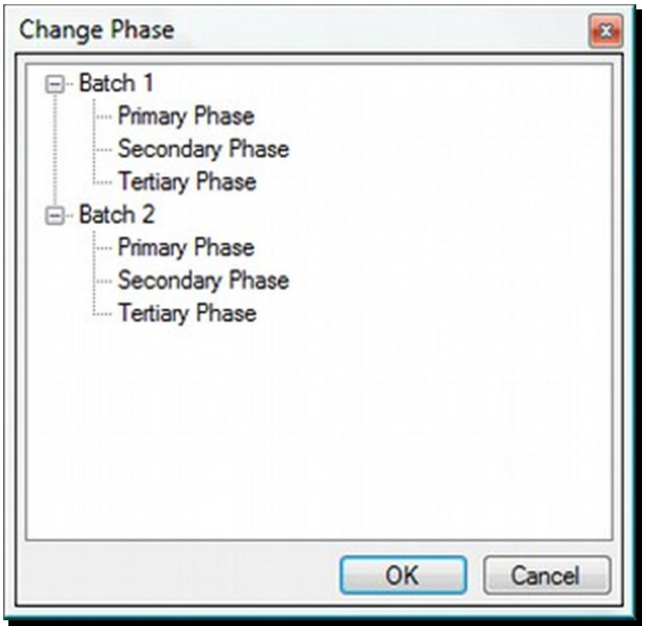
The speed of the playback may be selected from the “Playback Rate” drop down menu[2]. Note that the playback rate only affects continuous playback. “Step Forward” and “Step Backward” are unaffected by playback rate.



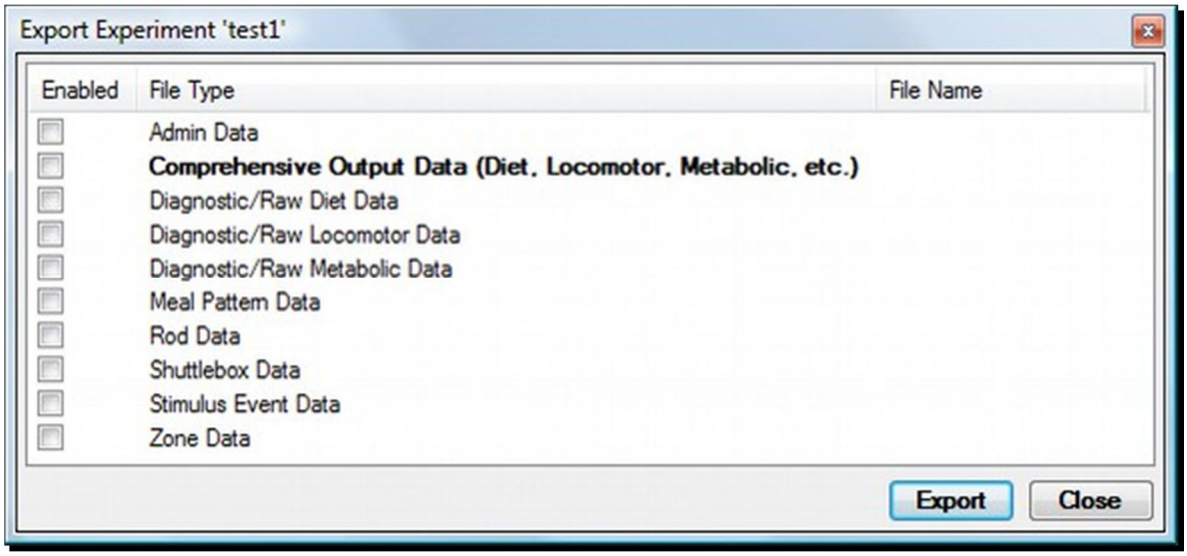
[2] Depending on the speed of the host computer, playback may not be able to accurately reach the faster playback rates.

5.5 Phase Selection

In order to change the currently selected phase, click on the name of the current batch and phase in the toolbar. This will bring up the Change Phase dialog containing a tree structure with the batches and phases of the experiment. When the desired phase is found, simply select it and click the “OK” button to change to that phase.



6.0 The Export Dialog



6.1 File Types and File Names

To export a file type, click the corresponding checkbox; this will open a file name chooser dialog. This allows you to name the file, and choose the file format. When the desired file types have been chosen, click the “Export” button to generate the files. Some file types are time consuming to export, so please be patient.

6.2 Comprehensive Output Data

Fusion’s primary export type is “Comprehensive Output Data”. Only those variables selected in the Export Variable settings will be exported.

6.3 Meal Pattern Data

Meal Pattern Data can be used to analyze meal patterns or diet preference. It separates each phase into separate meals (a span of contiguous consumptions). The parameters for what constitutes a meal can be changed in the diet settings.

6.4 Raw Beam Data, Raw Metabolic Data, Raw Scale Data

All three of these types export their respective values to the maximum time-resolution recorded by the system. They can be used to perform custom evaluations of the data using analysis programs.

6.5 Rod Data

Rod Data contains a list of Rota-Rod trials, the corresponding outcomes of those trials, and the time at which that outcome was achieved. Common possible outcomes include “Fall”, “No-Fall”, and “Abort” (user intervention).

6.6 Shuttle Box Data

Produces a breakdown of shuttle box activity with results divided by trial.

6.7 Stimulus Event Data

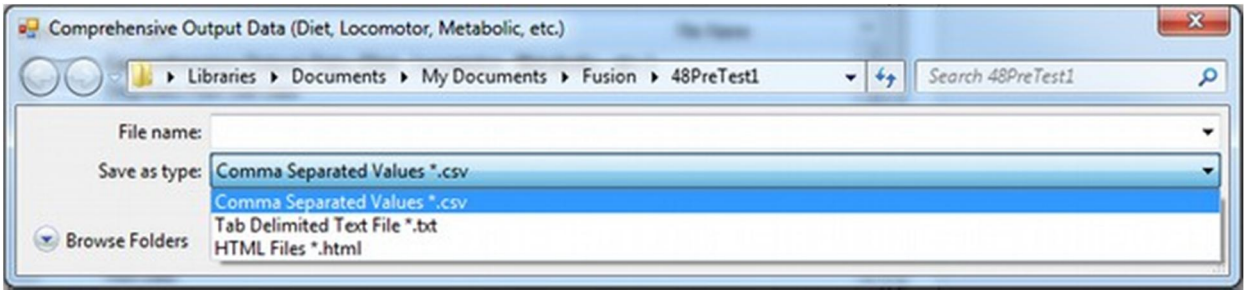
Produces an analysis similar to Comprehensive Output Data, but sampled according to the stimuli that were fired during the experiment. This data contains movement latency variables provided that the experiment contains locomotor activity.

6.8 Zone Data

If a zone is loaded, this file type contains zone analysis data. As with Comprehensive Output Data, only those variables selected in the Export Variable settings will be exported.

7.0 File Formats

Fusion can export in a variety of different file formats. In order to choose a file format, change the "Save as type" drop-down value in the file name dialog.



7.1 Comma Separated Values (.csv)

This is the default export format. Each row is separated by a line break, and each element in that row is separated by a comma. Most popular spreadsheet packages can open this file type (including Microsoft Excel and OpenOffice/LibreOffice Calc).

7.2 Tab Delimited Format (.txt)

Each row is separated by a line break, and each element in that row is separated by a tab. Most popular spreadsheet packages can open this file type (including Microsoft Excel and OpenOffice/LibreOffice Calc).

7.3 HTML (.html)

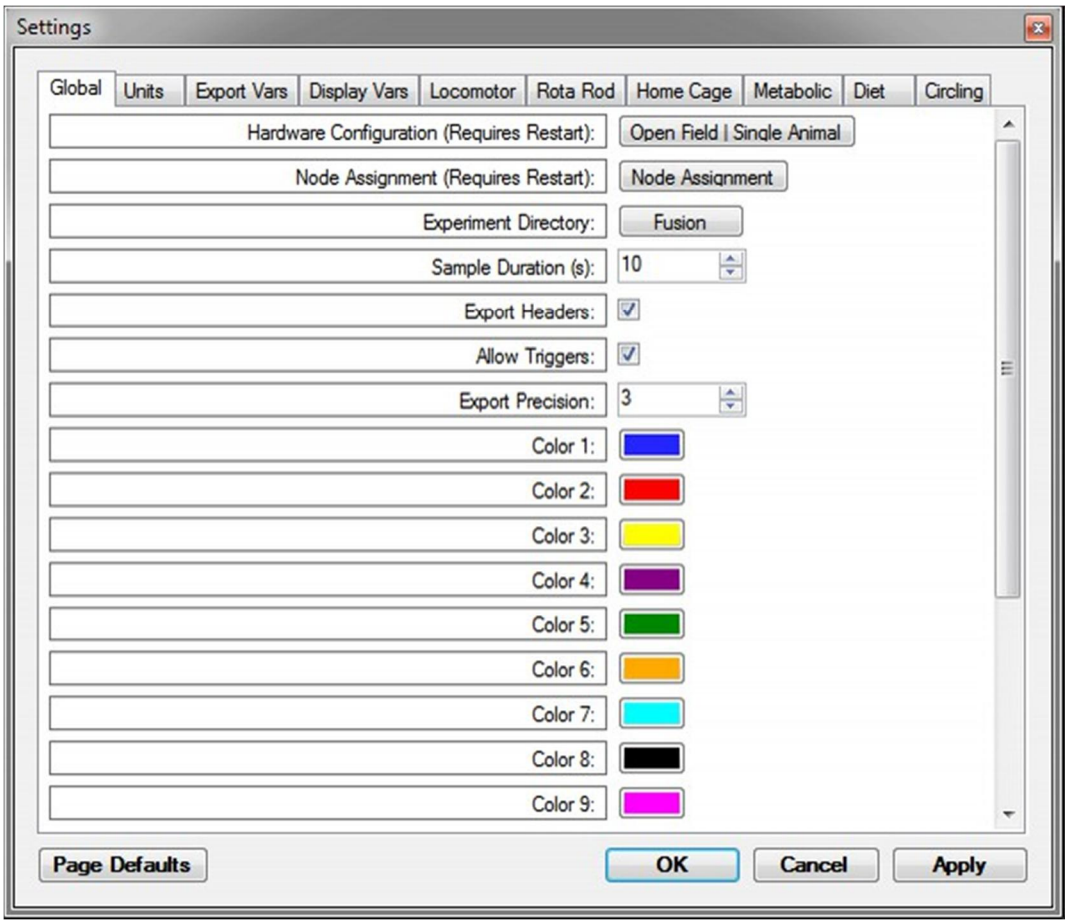
HTML files may be opened with web browsers. This format is ideal for publishing results to a website or quickly viewing results if no data formatting needs to be performed.

8.0 Experiment Settings

The Experiment Settings dialog contains all modifiable parameters of the Fusion system. In order to this dialog, select “Experiment Settings” from the Tools pull-down menu. This may only be selected when an experiment is not in progress.

8.1 Global Tab

These settings apply to the entire Fusion system.



8.1.1 Hardware Configuration

The Fusion system can integrate a wide variety of hardware. When Fusion is first launched, the user will be presented with a hardware configuration selection dialog (section 23). If the wrong hardware configuration was chosen or the hardware configuration changes, this button may be used to reselect the hardware configuration – changes will not take effect until the program is restarted.

8.1.2 Node Assignment

This button can be used to request a new Node Assignment with the reconfigurable SuperFlex Open Field system. Node assignment is discussed in detail in Section. Note: assignment may

not be performed when Fusion is running. However, assignments may be cleared, thus forcing Fusion to initiate an assignment when it is subsequently launched.

8.1.3 Experiment Directory

This property will change the directory that the program uses for new experiments. Be sure to choose a directory that the current user has permission to modify. For more information on the experiment directory, see section.

8.1.4 Font

This font will be used throughout the Fusion Application to display text. In many cases, it may be desirable to adjust the font when the resolution of the PC is changed in order to ensure readability. Beware; every piece of text has an allocated space within the Fusion system. Changing the font may cause the text to be undesirably cropped.

8.1.5 Samples and Sample Duration Configuration

The Fusion activity output file separates phases into smaller time bins known as “samples”. The “Sample Duration” setting can be used to configure the duration of these samples. The default Sample Duration is 10 minutes.

8.1.6 Allow Triggers

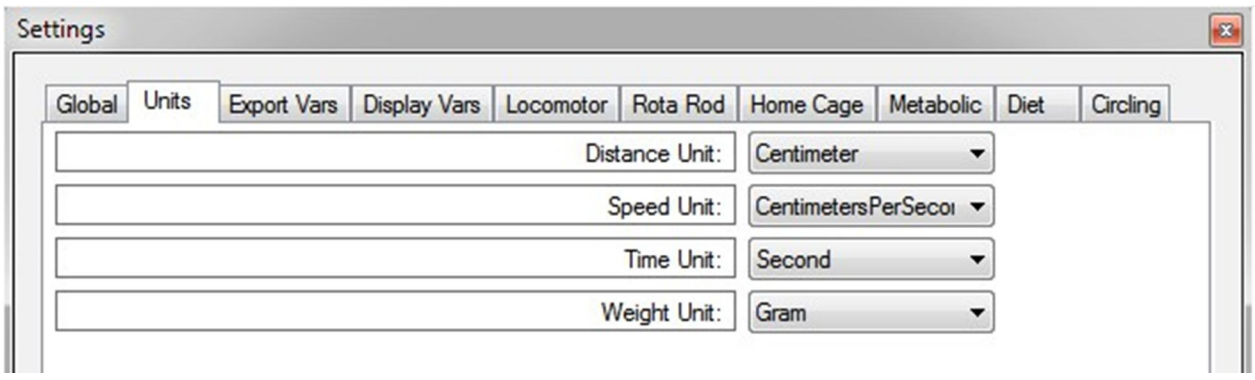
On each Fusion sensor, there are one or more triggers. Each trigger begins recording the current phase for the corresponding cage (if a phase is prepared for recording). In a “Two Animal Mode” locomotor system, each locomotor trigger corresponds to a particular subject. This property specifies whether to allow use of the triggers for this purpose.

8.1.7 Colors

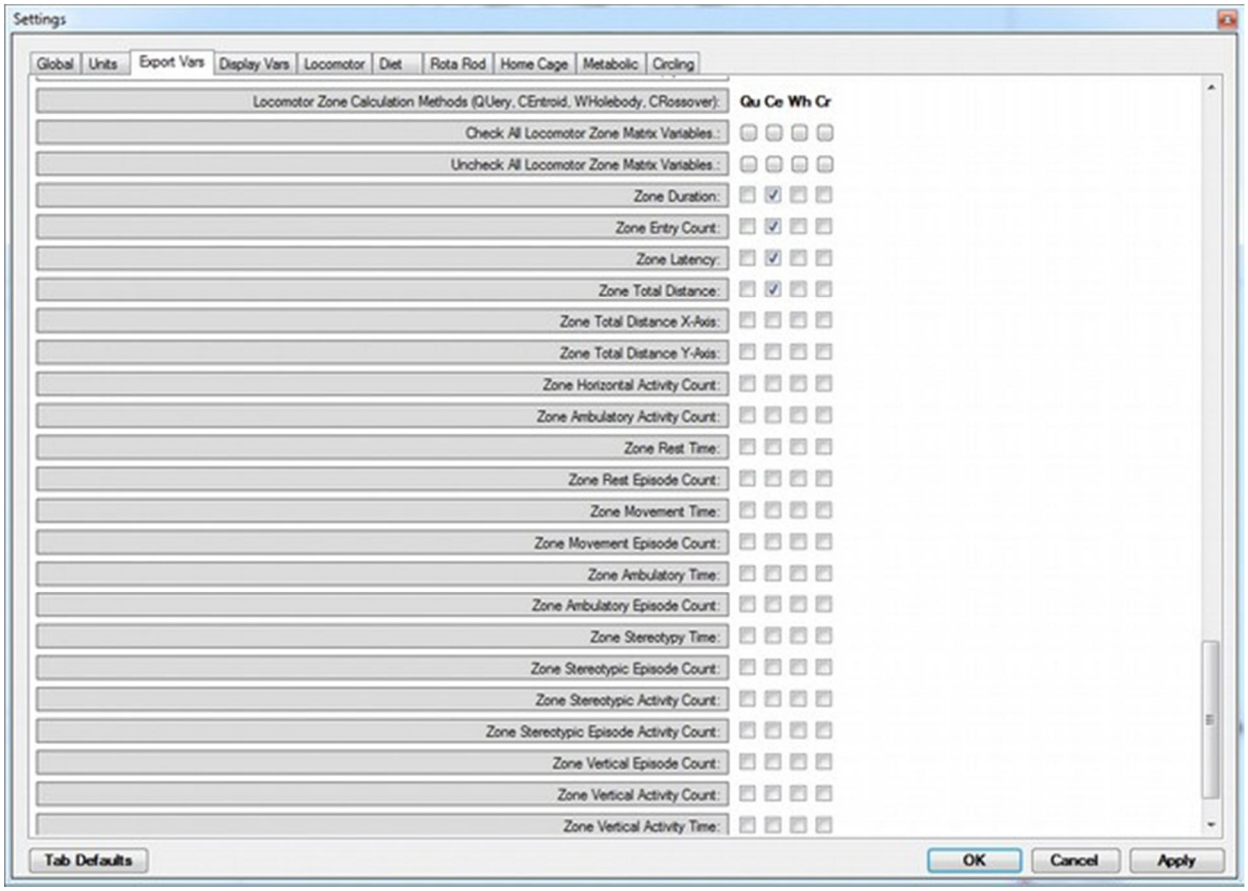
The colors chosen here will be used throughout Fusion Application. For instance, they will be used in the Fusion Zone system and the metabolic variable graph.

8.2 Units Tab

The units selected here will be used throughout Fusion (both in the application and in exported data files) when the program displays quantitative properties. The default units are Centimeters, Centimeters /Second, Seconds, and Grams.



8.3 Export Variables Tab



Each Fusion variable may be exported in the “Comprehensive Output Data” file type. The user has the opportunity to enable or disable each variable for the purposes of either export or display. To activate a variable, simply make sure that the variable’s checkbox is selected (single click will toggle the checkbox). If the variable is not desired, remove the check from the box.

Variables are available fourfold when performing locomotor zone exports. Four columns of check boxes are provided for each variable since Fusion is capable of defining Zone activity in four different ways: Query (Qu), Centroid (Ce), Whole Body (Wh), and Crossover (Cr).

In the **Query (Qu)** method, if any part of the subject is occupying a cell contained by a zone, the subject is considered to be “inside” that zone.

The **Centroid (Ce)** method considers the center of the subject to be the only location for the subject. Consider a subject occupying a square of cells with corners (2,2) and (4,4). The subject’s center is considered to be (3,3), and in the Centroid method of zone calculation, this is exclusive location of the subject. The subject would be considered “inside” a zone if (3,3) is selected by that zone.

In the **Whole Body (Wh)** method, the subject is only considered to be “inside” a zone if every cell occupied by that subject is contained in the zone.

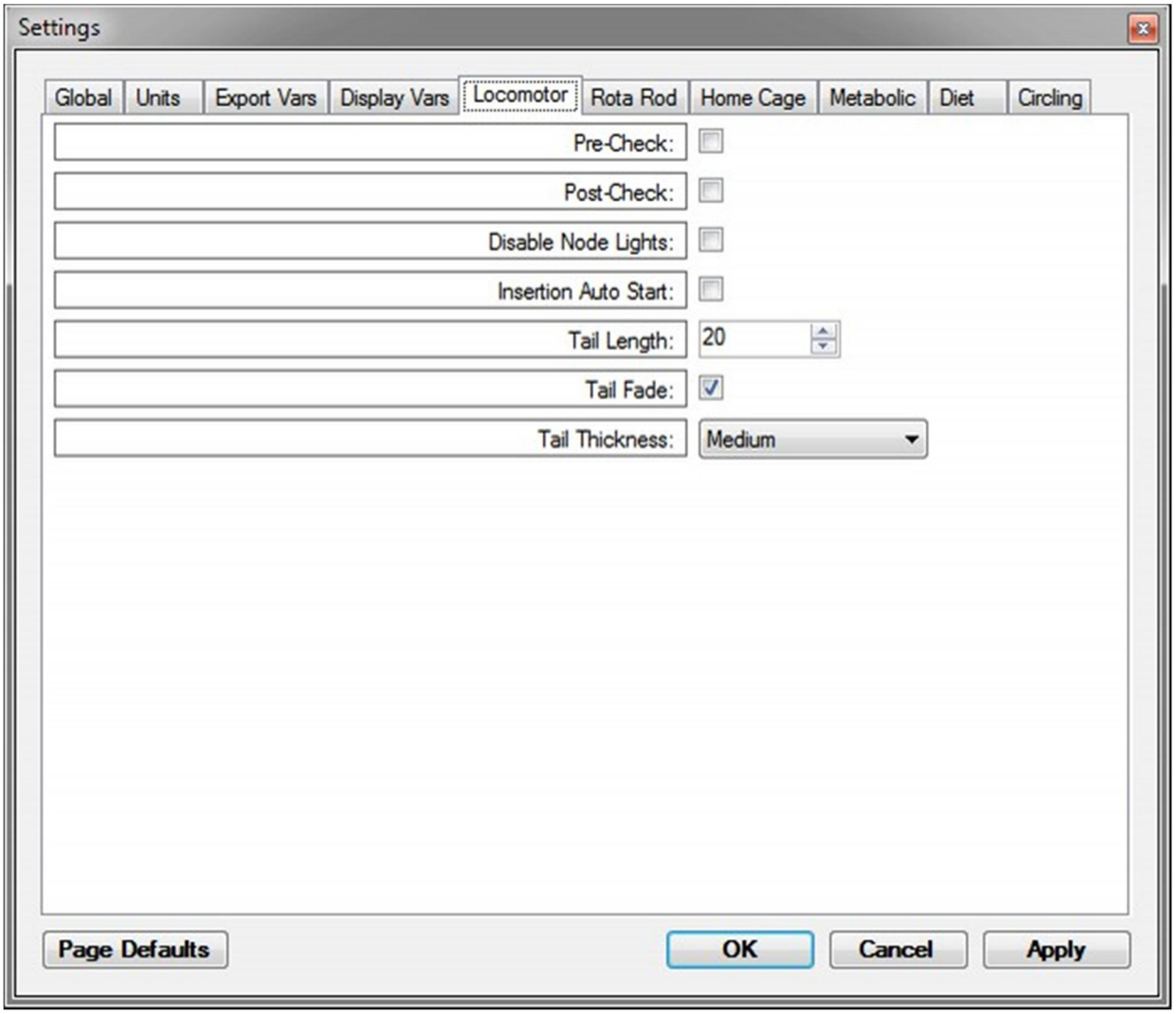
In the **Crossover (Cr)** method, which is similar to Whole Body, the subject is considered to be in the last confirmed zone until the animal's entire body crosses into a new zone.

8.4 Display Variables Tab

Most of the variables in the Fusion System can be displayed in real-time during an experiment. In order for a variable to be displayed in real-time, it must be enabled. This dialog is similar to the "Export Variables" dialog (Section 38), except that it determines what variables are displayed during the experiment, not what variables are exported after the experiment is complete.

8.5 Locomotor Tab

These settings apply to the locomotor component of the Fusion system.



8.5.1 Pre-Check & Post-Check

Before the experiment is started (pre-check) and after the experiment has ended (post-check) (i.e. before the animal is inserted in the cage and after the animal is removed from the cage), the Fusion software can check to make sure all beams are unbroken in relevant cages. This helps to ensure that the equipment is operating properly, and there are no undesirable obstructions within the cage. When these properties are checked, the system will not allow the experiment to progress when beams are inappropriately blocked.

8.5.2 Insertion Auto Start

Normally, while in the “Insert” state, the PC record button or hardware trigger must be used to start a phase. When “Insertion Auto Start” is enabled, the system will start automatically when the animal is inserted in “Insert” mode.

8.5.3 Tail Length

Status Page activity tail/path length to draw, based on the number of previous centroid points.

8.5.4 Tail Fade

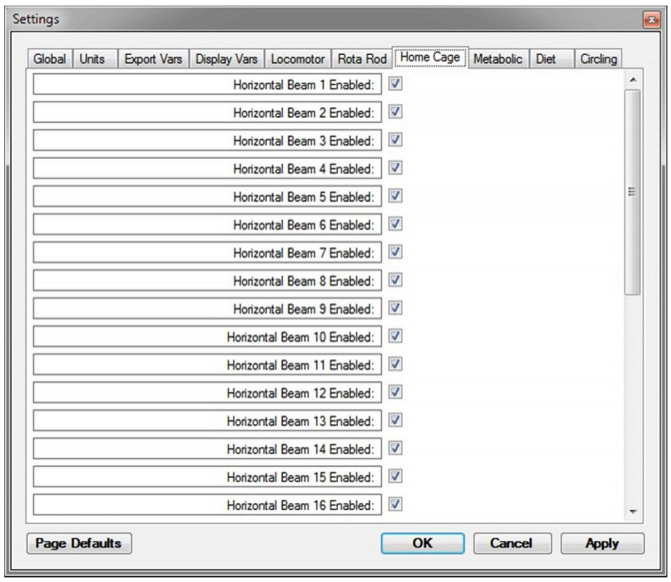
Status Page Enable/Disable activity tail fade over time.

8.5.5 Tail Thickness

Allows user to set the thickness of the activity tail on the status page.

8.6 Home Cage Tab

In the Home Cage configuration, specific beams can be universally enabled or disabled. This feature might be used if each cage has a planned obstruction which should not be identified by the software as an animal or if the cage is shorter than the sensor and the cage end walls are causing interference.



8.6.1 Enabled Horizontal Beams

Each beam in the horizontal (lower) sensors can be enabled or disabled in this section. A check represents an enabled state. By default, all horizontal beams are enabled.

8.6.2 Enabled Vertical Beams

Each beam in the vertical (upper sensors can be enabled or disabled in this section. A check represents an enabled state. Vertical beams appear after the horizontal beams in the Home Cage tab. By default, all vertical beams are enabled.

9.0 Variable List (availability dependent on installed hardware)

Ambulatory Activity Count. A count of sensor changes (beam breaks) due to ambulation.

Ambulatory Count. The total number of ambulatory episodes. Episodes are separated by rest periods of at least 1 second.

Ambulatory Episode Average Velocity. An average of the velocities of all ambulatory episodes.

Ambulatory Episode Median Velocity. The median of the velocities of all ambulatory episodes.

Ambulatory Episode Peak Average Velocity. The highest average velocity of all ambulatory episodes.

Ambulatory Time. The length of time that the subject spent in ambulatory activity. Ambulatory activity is defined as a period in which ambulation occurred.

Animal Weight. The weight of the animal (user supplied).

Arm Movement Count. The number of times that the wake arm was actuated.

Average Speed. The average speed the subject travels between the hole pokes (Calculated as: Distance Traveled To Hole / Time Subject Travels between holes)This is only available when additional XY sensor pairs are connected to monitor locomotor activity during nose-poke experiments. For the purpose of this variable, the location of the subject is defined as the centroid (center of mass) of the subject.

Batch. The name of the relevant batch.

Cage CO2. The concentration of carbon dioxide as a percentage for each test cage (subject).

Cage Flow Rate. The air flow rate for each test cage.

Cage O2. The concentration of oxygen as a percentage for each test cage (subject).

Cage. The name of the relevant cage.

Clockwise Turns. The number of complete clockwise turns traveled by the subject attached to a rotor.

CO2 Change. Cage CO2 - Reference CO2

Consumption. Time course of the amount of weight removed from the scale.

Counter-Clockwise Turns. The number of complete counter-clockwise turns traveled by the subject attached to a rotor.

Displacement from Previous Hole. The linear distance from the last hole poke to the current hole poke or from the first recorded position to the first hole poke.

Distance Traveled To Hole. The distance the subject travels after poking a hole until arriving and poking the next hole. This is only available when additional XY sensor pairs are connected to monitor locomotor activity during nose-poke experiments. For the purpose of this variable, the location of the subject is defined as the centroid (center of mass) of the subject.

Distance Traveled to Hole. The nonlinear distance traveled from the last hole poke to the current hole poke or from the first recorded position to the first hole poke. For the purpose of this variable, the location of the subject is defined as the centroid (center of mass) of the subject.

Duration. Represents time elapsed since the start of the experiment.

Experiment. The name of the relevant experiment (this value is user created at acquisition time).

Hole Name. The name of the hole poked by the subject.

Hole Type. The type of hole – user defined – as empty, non-target or target.

Horizontal Activity Count. A count of sensor changes (beam breaks).

Legacy Left-Front Time. The time spent in the left-front portion of the SuperFlex Open Field cage. The area is 4” x 4” (10.16cm x 10.16cm). This represents one of the four legacy corner variables.

Legacy Right-Front Time. The time spent in the right-front portion of the SuperFlex Open Field cage. The area is 4” x 4” (10.16cm x 10.16cm). This represents one of the four legacy corner variables.

Legacy Left-Rear Time. The time spent in the left-rear portion of the SuperFlex Open Field cage. The area is 4” x 4” (10.16cm x 10.16cm). This represents one of the four legacy corner variables.

Legacy Right-Rear Time. The time spent in the right-rear portion of the SuperFlex Open Field cage. The area is 4” x 4” (10.16cm x 10.16cm). This represents one of the four legacy corner variables.

Legacy Center Time. The time spent in the center portion of the SuperFlex Open Field cage. The area is 8” x 8” (20.32cm x 20.32cm) in the center of the cage.

Legacy Margin Time. The time spent in the margin portion of the SuperFlex Open Field cage. This is any area that is not defined by the Legacy Center area. The area is 4” x 16” (10.16cm x 40.64cm) along the left and right edges of the arena and 16” x 4” (40.64cm x 10.16cm) along the front and rear edges. This overlaps the corner times.

Legacy Center Distance. The distance traveled in the center portion of the SuperFlex Open Field cage. The area is 8” x 8” (20.32cm x 20.32cm) in the center of the cage.

Legacy Margin Distance. The distance traveled in the margin portion of the SuperFlex Open Field cage. This is any area that is not defined by the Legacy Center area. The area is 4” x 16” (10.16cm x 40.64cm) along the left and right edges of the arena and 16” x 4” (40.64cm x 10.16cm) along the front and rear edges.

Locomotor Clockwise Revolutions. Counts the number of clockwise revolutions that the subject travels in an open field.

Locomotor Counter-Clockwise Revolutions. Counts the number of counter-clockwise revolutions that the subject travels in an open field.

Movement Episode Count. The total number of locomotor episodes. Episodes are separated by rest periods of at least 1 second.

Movement Time. The length of time that the subject spent in activity. Activity is defined as a period in which ambulation or stereotypy occurred.

Net Turns. The number of complete clockwise turns minus the number of complete counter-clockwise turns traveled by the subject attached to a rotor.

O2 Change. Cage O2 - Reference O2

Phase. The name of the relevant phase (this value is user created at acquisition time).

Poke Duration . The duration the sensor beam stays broken during a nose poke.

Poke Sequence. The sequence number of the hole-poke by the subject.

Poke Start. The time at which the nose of the subject breaks the sensor beams - as a running total from experiment start time.

Production. Time course of the amount of weight added to the scale. This may occur if food is added to a depleted food reservoir.

Reference CO2. The concentration of CO2 as a percentage in Reference Channel 0 (typically room air).

Reference Flow Rate. The air flow rate being monitored in Reference Channel 0.

Reference O2. The concentration of oxygen as a percentage in Reference Channel 0 (typically room air).

Respiratory Exchange Ratio. The ratio between the amount of CO2 produced and O2 consumed (determined from comparing exhaled gasses to room air). VCO2/VO2

Rest Episode Count. The total number of resting periods. A resting period is defined as a period of inactivity greater than or equal to 1 second.

Rest Threshold. The time (in seconds) chosen by the user, to determine how long the subject must remain motionless for the program to register resting behavior. Valid entries range from 1 to 60 seconds. Caution: setting the Rest Threshold equal to or greater than the sample duration may result in the loss of Rest Threshold data.

Rest Time. The length of time that the subject spent at rest. A resting period is defined as a period of inactivity greater than or equal to 1 second.

Sample. The name of the relevant sample.

Start Time. This represents the time at which the experiment began (as expressed in the time zone in which the experiment was created).

Stereotypic Activity Count. The number of beam breaks due to stereotypic activity. If the animal breaks the same beam (or set of beams) repeatedly then the monitor considers that the animal is exhibiting stereotypy. This typically happens during grooming, head bobbing, etc.

Stereotypic Episode Activity Count. The number of beam breaks that occur during a period of stereotypic activity. If the animal breaks the same beam (or set of beams) repeatedly then the monitor considers that the animal is exhibiting stereotypy. This typically happens during grooming, head bobbing, etc.

Stereotypic Episode Count. This corresponds to the number of times that stereotypic behavior was observed in the animal. A break in stereotypy of 1 second or more is required to separate one stereotypic episode from the next. If the animal breaks the same beam (or set of beams) repeatedly then the monitor considers that the animal is exhibiting stereotypy. This typically happens during grooming, head bobbing, etc.

Stereotypic Time. The total amount of time that stereotypic behavior is exhibited. A break in stereotypy of 1 second or more is required to separate one stereotypic episode from the next. If the animal breaks the same beam (or set of beams) repeatedly then the monitor considers that the animal is exhibiting stereotypy. This typically happens during grooming, head bobbing, etc.

Subject. The name of the relevant Subject (this value is user created at acquisition time).

Total Displacement. The running total of the animal's "Displacement from Previous Hole" values. For the purpose of this variable, the location of the subject is defined as the centroid (center of mass) of the subject.

Total Distance Traveled. The total distance the subject travels during the experiment. This is only available when additional XY sensor pairs are connected to monitor locomotor activity during nose-poke experiments. For the purpose of this variable, the location of the subject is defined as the centroid (center of mass) of the subject.

Total Distance. The total distance that the subject has traveled. For the purpose of this variable, the location of the subject is defined as the centroid (center of mass) of the subject.

VCO2. Volume of carbon dioxide produced, normalized to body weight.

Vertical Activity Count. Cumulative vertical beam breaks.

Vertical Activity Time. When the animal rears up, this timer starts incrementing.

Vertical Episode Count. Each time the animal rears up, this is incremented by 1. The animal must go below the level of the vertical sensor for at least 1 second before the next rearing can be registered.

VO2. Volume of oxygen consumed, normalized to body weight.

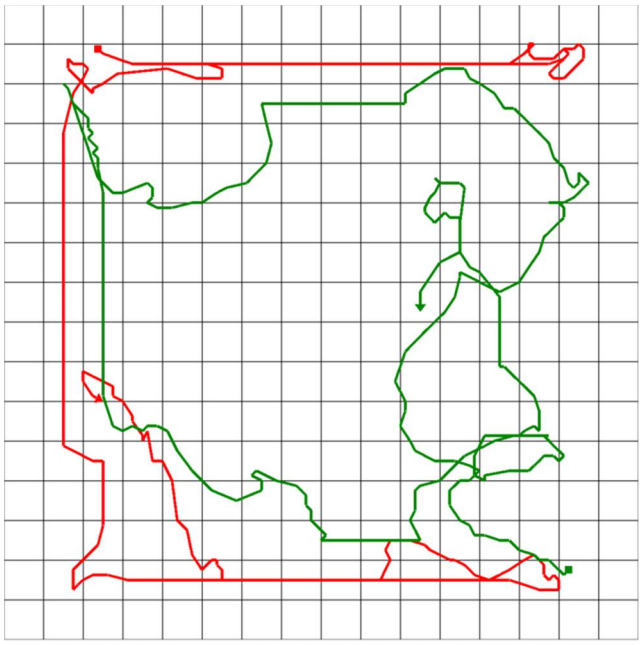
Wheel Rotations. The number of rotations traveled by the activity wheel.

10.0 Locomotor Activity Plotter

Fusion offers several ways of visually displaying activity data. To get started with the Locomotor Activity Plotter, select “Activity Plotter” from the Tools pull-down menu. This may only be selected when an experiment is loaded and is not in progress.

10.1 Path Plotting

10.1.1 Overview



The Path Plotter is accessible from the tools menu under 'Locomotor Activity Plotter'. The path plotter records and displays two dimensional movement paths as well as including vertical activity locations, of test subjects over specified time frames. The image below shows a single color, single trial plot. Single paths can also be colored via a gradient to show time progression, and up to 16 separate trial paths can be overlaid, in different colors, to create a single image for direct comparison. The path plotter does not produce valuable data in single axis configurations.

10.1.2 Instructions

To plot trials, choose the desired trials from the trials window by selecting the check boxes next to them. Checking a Batch check box or a Cage check box will select all of its child trials, thus it is not necessary for a trial's batch or cage to be checked for it to be plotted. Once all trials are selected, choose the desired Plot Mode. There are three options: Horizontal, Vertical, and Horizontal and Vertical, and they plot horizontal motion between centroid points as contiguous lines, centroid positions during vertical activity as colored dots, or an overlay of both. Horizontal plots are plotted in the order their trials were selected, so selecting Batch 1 Phase 1 and then Batch 1 Phase 2 will plot Phase 2 over Phase 1.

10.1.3 Trial Color Schemes

Trials are automatically displayed with the basic Fusion color scheme, which can be changed. Right-clicking or double-clicking on a Trial will display a color selection menu where you can choose to re-color trials as needed.

10.1.4 Time Frames

Optionally, you can input a specific Time Frame during the experiment for plotting. This option is found under the Configure menu. Entering all zeroes will plot the entire experiment for the

selected trials. When multiple trials of different lengths are selected, it is possible to plot all of one trial and most of another. It is never possible to plot a Time Frame that surpasses the end of the recorded experiment.

10.1.5 Line Gradients

Gradients are changes in color over time that can be applied when only a single trial is selected. If you have selected a gradient, the path will be one color for the first half of the plotted movement, and another for the second, or three colors, or a rainbow spectrum, or a grayscale fade. Otherwise, the path displayed will be based on the color of the trial.

10.1.6 Line and Dot Thickness

This option changes the thickness of the movement lines and size of the rearing dots.

10.1.7 Output Resolution

Files may be saved in two different resolutions: standard and high. Standard resolution creates an 800 x 800 pixel lossless Portable Network Graphic (PNG) plot image with experiment information at the bottom. High resolution behaves identically, but with an image size of 2400 x 2400 pixels.

10.1.8 Path Plotting Options

a) Plot Mode

Plot Mode selects which data to plot. The choices are horizontal, vertical, and both. Horizontal data is represented by a winding line representing the path of the animal's centroid. The line changes color according to a set gradient as time progresses. Vertical data is represented by red dots that indicate the position of the animal's centroid when vertical activity is detected. When both horizontal and vertical data are plotted, the horizontal data is plotted first, with the vertical dots drawn over the horizontal.

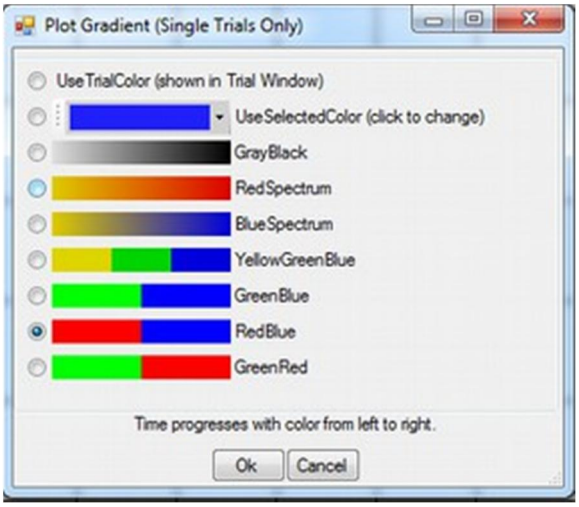
b) Plot Time Frame

Time frame is the user-defined window of time within the experiment that the Plotter uses to plot its data. Entering all zeroes will plot the entire experiment for the selected trials. When multiple trials of different lengths are selected, it is possible to plot all of one trial and most of another. It is never possible to plot a Time Frame that lasts longer or later than all selected trials.

c) Plot Gradient

The Gradient choice selects a series of color gradients for the animal's path. These are used to show the progression of time as the animal moves. Different types of gradient are offered: solid or gradual. Solid gradients are broken up into user-selectable single color, two or three colors, each representing one portion of the plotted time frame. Gradual gradients fade into each new color instead of abruptly transitioning. A greyscale gradual gradient is available as well. Note: the gradient chosen will always make a complete progression from its first color to its final color, even if the plot is not

for the entire experiment. Plotting an entire experiment with a two-color gradient will make each color represent one half of the total experiment time.



Multiple Trials are automatically displayed with the default Fusion color scheme. Colors can be changed by Right-clicking or double-clicking on a Trial which will display a color selection menu, where you can choose to re-color trials as needed.

Single Trial Paths, when the Overlap feature in the Configuration menu is enabled, will mark in black any segment of path where the subject doubled back over its previous path, or if it moved in laps that covered precisely the same lines.

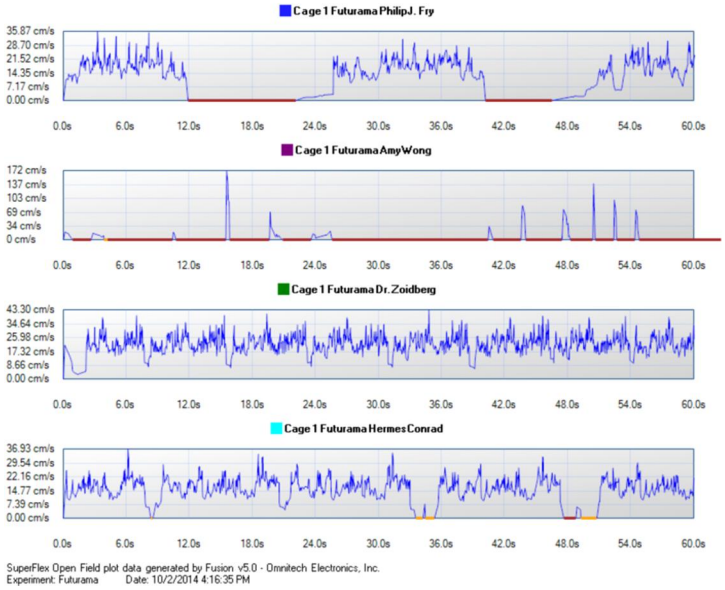
Multiple Trial Paths, when plotted simultaneously, can obscure each other by running over one another in the same direction. The Overlap feature in the Configure menu allows you to view overlap occurrences by marking them in black instead of the top trial's color. If the Overlap feature is disabled, Horizontal plots are plotted in the order their trials were selected, so selecting Batch 1 Phase 1 and then Batch 1 Phase 2 will plot Phase 2 over Phase 1.

d) Line and Dot size

This option adjusts the size of the lines and dots used to plot the data. Four choices are available: hairline, thin, medium, and thick.

10.2 Velocity Plotting

The Velocity Plotter allows you to see, at a glance, which animals show more activity over the experiment duration or via user-selectable time periods. Red lines represent user-definable rest periods during the trials. Other velocity variables that can be set are: Speed Units, Time Units, Plot Axis Override Value (allows the software or user to calculate the maximum Y axis value), Plot Maximum Axis (allows the user to manually set the maximum Y axis value), and Color schemes. The Velocity Plotter does not produce reliable data in single axis configurations unless the subject is located in a very narrow test chamber.



10.2.1 Overview

The Velocity Plotter is accessible from the tools menu under 'Locomotor Activity Plotter' and allows the creation of image files displaying velocities of different subjects over specified time frames.

10.2.2 Instructions

To plot trials, choose the desired trials from the trials window by selecting the check boxes next to them. Checking a Batch check box or a Cage check box will select all of its child trials, thus it is not necessary for a trial's batch or cage to be checked for it to be plotted. Once all trials are selected, click the 'Plot' button.

10.2.3 Time Frames

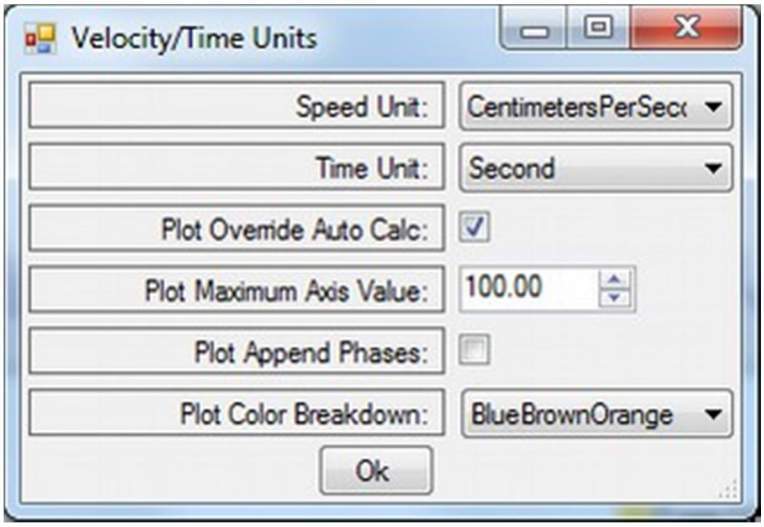
The velocity activity time frame can be adjusted by utilizing the “+” and “-” buttons on the top left of the graphing window. This will scale the time frame displayed in the graph(s). The adjacent master horizontal scroll bar changes the location of the selected time frame within the trial duration. For example, if the time frame was set to 30 seconds with the “+” and “-” buttons, and the trial length was 2 minutes, the scroll bar would set the location of the time frame (30 seconds), allowing the user the user to view any 30 second time frame within the trial.

10.2.4 Output Resolution

Files may be saved in two different resolutions: standard and high. Standard resolution creates an 800 pixel wide lossless Portable Network Graphic (PNG) plot image with experiment information at the bottom. High resolution behaves identically, but with an image 2400 pixels wide.

10.2.5 Velocity Settings

A variety of different settings exist to change the way the velocity graph is displayed. To change these settings access the Velocity/Time Unit setting window under the Configure Menu of the Activity Plotter.

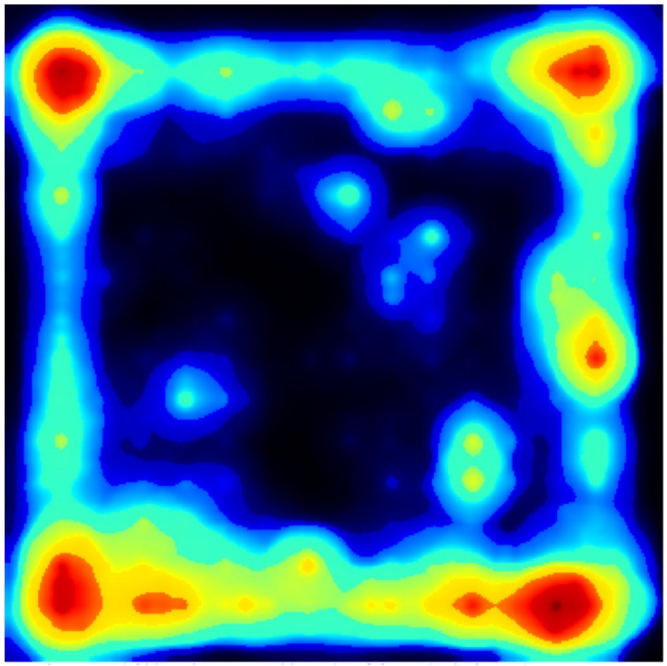


- **Speed Unit**: Unit of speed that is displayed on the Y axis of the graph.
- **Time Unit**: Unit of time that is displayed on the X axis of the graph.
- **Plot Override Auto Calc**: When checked, disables automatic vertical scaling of the Y axis based on the minimum and maximum values present in the plot data, and utilizes the *Plot Maximum Axis Value* to determine vertical scale.
- **Plot Maximum Axis Value**: Sets the maximum Y axis value of the Velocity graph. Will only take effect if *Plot Override Auto Calc* is checked.
- **Plot Append Phases**: When checked, in multiple phase and multiple batch experiments, velocity data from multiple phases of the same batch will be appended to the end of the previous phase for that batch. This generates one graph per batch. When unchecked, multiple graphs will be generated from multiple phases within each batch.
- **Plot Color Breakdown**: Plot Color Breakdown changes the color of the graph's data depending on what kind of data it is graphing. Selecting "None" will display all data as blue. "BlueBrownOrange" sets movement activity as blue, rest time as brown, and stereotypic activity as orange. "RedGreenPurple" sets movement activity as red, rest time as green, and stereotypic activity as purple.

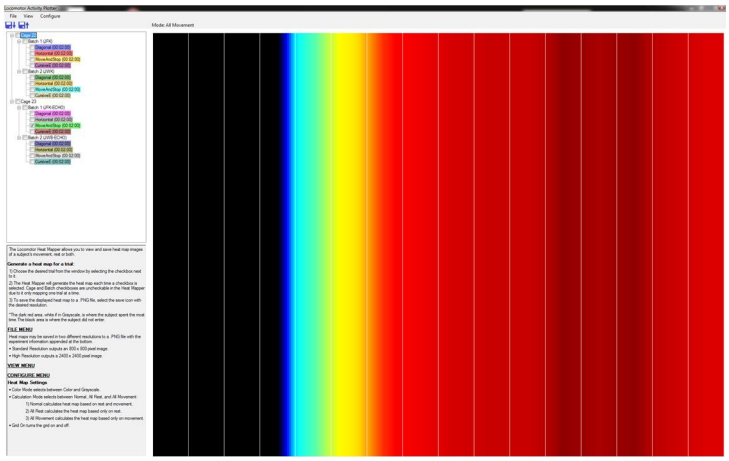
10.3 Place Preference Heat Maps

Heat Maps use color to visually indicate a place preference representation of time a subject has spent in a given area in relation to other areas. These heat maps can be exported in either high or low resolution images for analysis or for presentation. “Hotter” colors represent a larger time fraction spent in a location while “cooler” colors represent a smaller time fraction. If a subject spends time evenly in all portions of the cage, then red will be predominant as the majority of occupation time was equally distributed.

- Black = no or miniscule occupation time
- Blue / Green = minimal occupation time
- Yellow / Orange = moderate occupation time
- Red / Dark Red = peak occupation time



XY Axis (2-dimensional) Heat Map



X Axis (1-dimensional) Heat Map

10.3.1 Heat Map Configuration: Type

Fusion allows the user to choose the type of heat map they desire. Three types of Heat Map outputs are available and selectable under: **Configure → Heat Map Options**

1) All Rest: Rest Only (rest is user defined under **Tools → Experiment Settings → Locomotor Tab**)

2) All Movement: Activity locations exclusive of any rest activity

3) Combined Rest and Movement

10.3.2 Heat Map Configuration: Display Grid

The Display Grid allows the user to correlate beam locations to the Heat Map and to Zones mapped out in the Zone Designer. These white grid lines may be turned off under the ***Configure*** → ***Heat Map Options*** pull-down menu.

10.3.3 Heat Map Selection

To select and plot a Heat Map, simply select a trial in the Experiment View Window on the left side of the screen. Based on your setting in the Heat Map Configuration, Fusion will automatically generate a place preference heat map for that window.

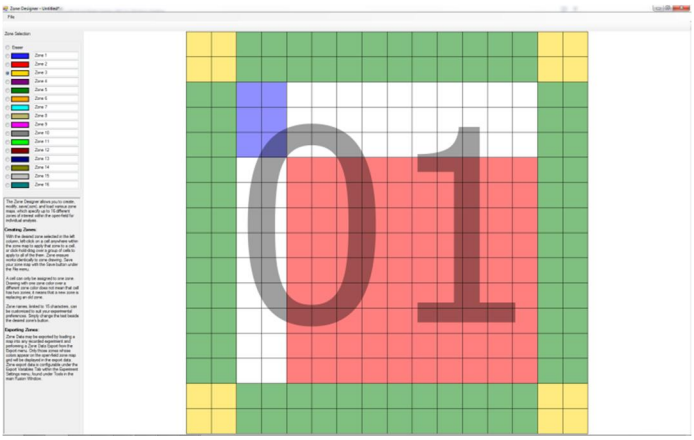
10.3.4 Saving a Heat Map

To export a Heat Map as either a high or low resolution graphic image, simply click on the representative disk icon in the upper left hand corner and follow the pop-up dialog. Low resolution exports are saved with a textual descriptive footer as an 800x800 pixel image, while high resolution exports are saved similarly, except at 2400x2400 pixels.

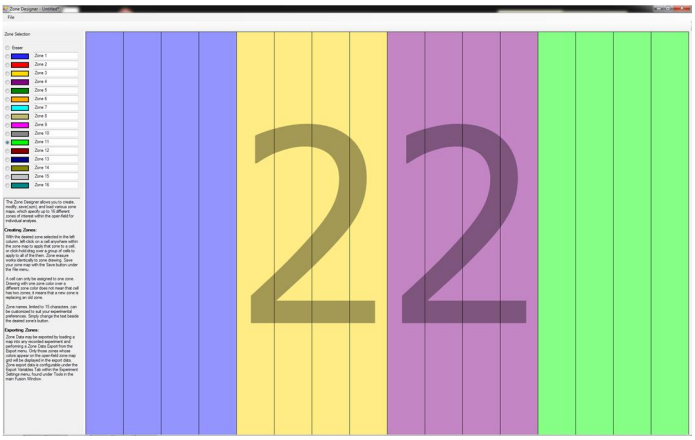
11.0 The Zone Designer

The Zone Designer gives the user access to a sophisticated system for activity analysis. The Zone Designer requires the Open Field or Home Cage System.

Zone Maps are always non-destructive – in other words, they will never affect a recording experiment, even if they are applied during that experiment. They will only affect the analysis of an already recorded experiment and can be changed and reapplied at any time.

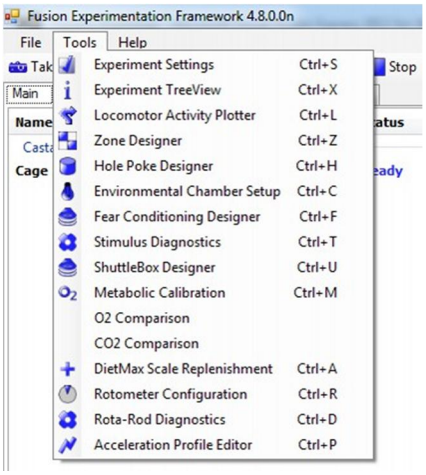


XY Axis (2-dimensional) Zone Map



X Axis (1-dimensional) Zone Map

11.1 Tutorial



11.1.1 Opening the Zone Designer

To get started with the Zone Designer, select “Zone Designer” from the Tools pull-down menu. This may only be selected when an experiment is not in progress. Once the Zone Designer is open, a blank zone is automatically loaded. The selected zone’s name and unique color are located in the upper-left corner.

11.1.2 Drawing on the Canvas

To draw the currently selected zone to the canvas, simply select the zone number / color in the color selector menu and then click/drag over the intended area.

11.1.3 Renaming Zones

To rename the zone, select the textbox next to the zone color in the upper-left corner of the screen and enter the desired name.

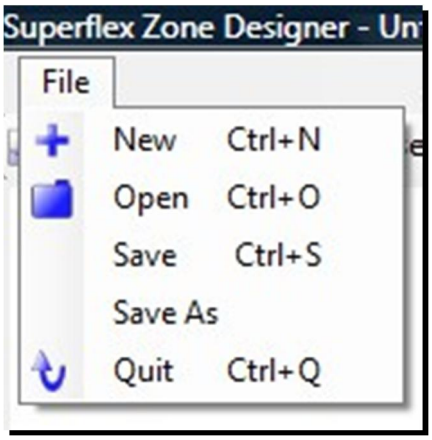
11.1.4 Selecting Zones

To select a different zone to apply to the canvas, select the Zone from the menu on the left side of the screen.

11.2 Managing Zone Maps

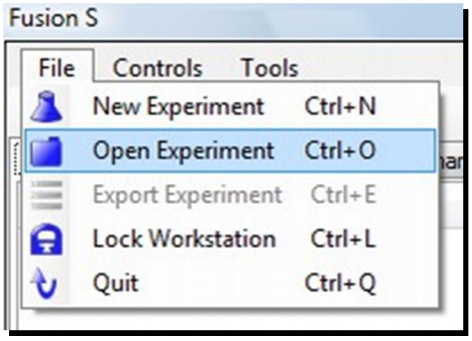
All Zone Map management operations can be accessed from the “File” drop down menu.

- **New** - Loads a new Zone Map with an empty canvas.
- **Open**-Opens a previously saved Zone Map.
- **Save** - Saves the current Zone Map using the current file name. If no file name has yet been chosen, a dialog will be opened permitting the user to choose one.
- **Save As** - Saves the current Zone Map using a new file name.

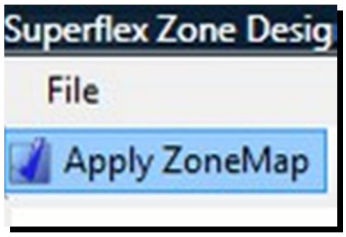


11.3 Applying a Zone Map

In order to use a zone map to obtain zone data, the zone map must be applied to a completed experiment. Start by opening an experiment in the main Fusion screen.

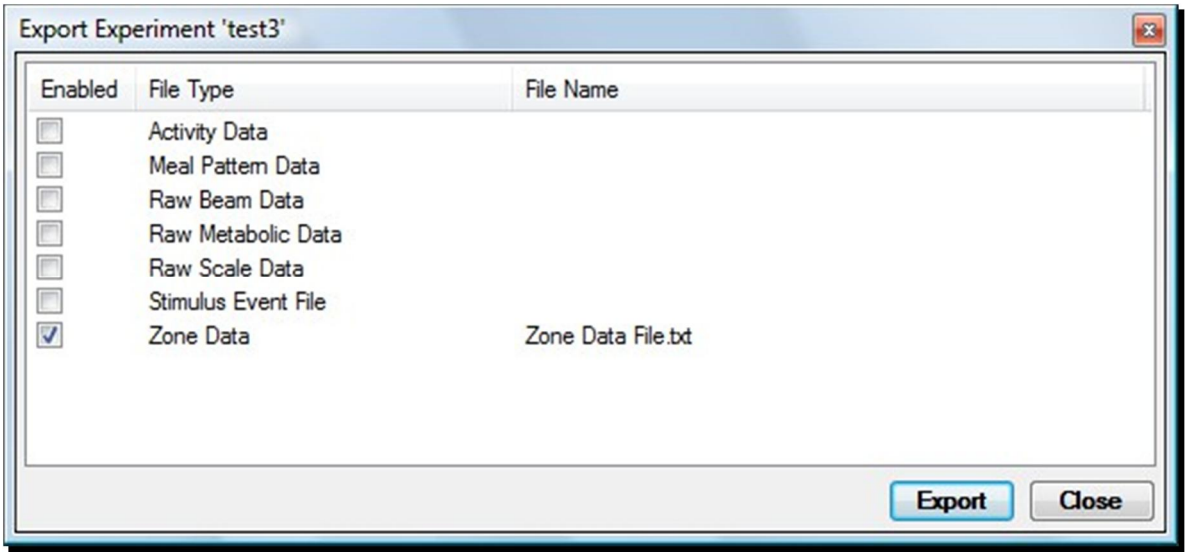


Next, enter the Zone Designer and open or create a Zone Map. Once you have loaded an experiment and prepared a Zone Map, the “Apply Zone Map” button will be available. Selecting this button will cause the Zone Map to appear in the main Fusion readout.



11.4 Exporting Zone Data

In the main Fusion interface, select “Export” from the File drop down menu.



Select “Zone Data” to choose the file name for the exported zone data, and then select “Export” to begin the export process. This can be quite time-consuming for large experiments and complicated Zone Maps, so please be patient.

11.5 Zone Variables

The Fusion Zone system includes several different variables for quantitative analysis of a zone map. Additionally, there are four different zone calculation methods. These can be configured for export under the **Export Variables** tab under **Tools** -> **Experiment Settings**.

11.5.1 Zone Variable Calculation Methods

For the purposes of variable calculation, Fusion Zone can determine whether a subject is “inside” a zone using one of three methods.

In the **Query** method, if any part of the subject is occupying a cell contained by a zone, the subject is considered to be “inside” that zone.

The **Centroid** method considers the center of the subject to be the only location for the subject. Consider a subject occupying a square of cells with corners (2,2) and (4,4). The subject's center is considered to be (3,3), and in the Centroid method of zone calculation, this is exclusive location of the subject. The subject would be considered "inside" a zone if (3,3) is selected by that zone.

In the **Whole Body** method, the subject is only considered to be "inside" a zone if every cell occupied by that subject is contained in the zone.

In the **Crossover** method, which is similar to Whole Body, the subject is considered to be in the last confirmed zone until the animal's entire body crosses into a new zone.

11.5.2 Zone Variable List

Entry Count. The number of times that the subject entered the zone.

Duration. The total amount of time that the subject spent in the zone.

Latency3. The time elapsed until the subject first entered the zone in a given phase.

Horizontal Activity Count. The number of times that a change in the sensors was detected within a zone.

Total Distance. The total distance traveled by the subject within the zone.

Rest Time. The total amount of time that the subject spent in rest within the zone. Rest is defined as a period of inactivity greater than 1 second.

Rest Episode Count. The total number of rest periods of the subject within the zone. Rest is defined as a period of inactivity greater than 1 second.

Movement Time. The length of time that the subject spent in activity within the zone. Activity is defined as a period in which activity occurred in intervals of less than 1 second.

Movement Episode Count. The total number of activity periods of the subject within the zone. Activity is defined as a period in which activity occurred in intervals of less than 1 second.

Stereotypy Episode Count. The number of episodes in which stereotypic behavior was observed in the subject while in the zone. A break in stereotypy of 1 second or more is required to separate one stereotypic episode from the next. If the animal breaks the same beam (or set of beams) repeatedly then the monitor considers that the animal is exhibiting stereotypy. This typically happens during grooming, head bobbing, etc.

[3] Latency values are identical for each sample in a given phase.

Stereotypic Beam Activity. The number of movements that occur during every period of stereotypic activity within the zone. If the animal breaks the same beam (or set of beams) repeatedly then the monitor considers that the animal is exhibiting stereotypy. This typically happens during grooming, head bobbing, etc.

Stereotypic Time. The total amount of time that stereotypic behavior is observed in the subject within the zone. A break in stereotypy of 1 second or more is required to separate one stereotypic episode from the next. If the animal breaks the same beam (or set of beams) repeatedly then the monitor considers that the animal is exhibiting stereotypy. This typically happens during grooming, head bobbing, etc.

12.0 The Hole Poke Designer

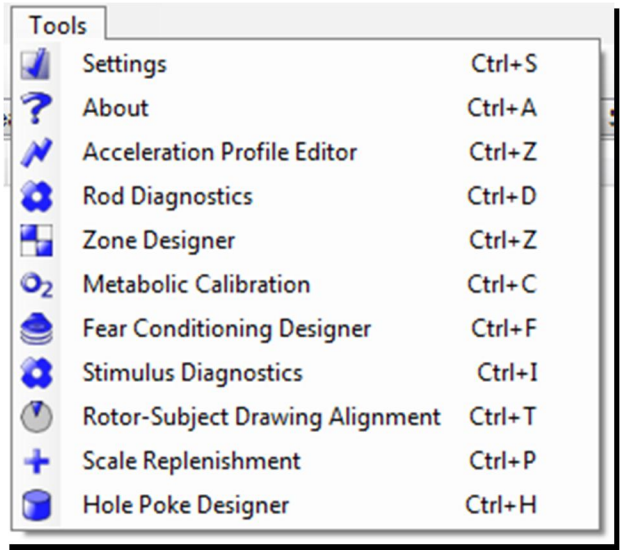
The Hole Poke designer is used to design Hole Poke maps for hole analysis. The Hole Poke Designer requires the Hole Poke hardware.

Hole Poke Maps are always non-destructive – in other words, they will never affect a recording experiment, even if they are applied during that experiment. They will only affect the analysis of an already recorded experiment.

12.1 Tutorial

12.1.1 Opening the Hole Poke Designer

To get started with the Hole Poke Designer, select “Hole Poke Designer” from the Tools pull-down menu. This may only be selected when an experiment is not in progress.



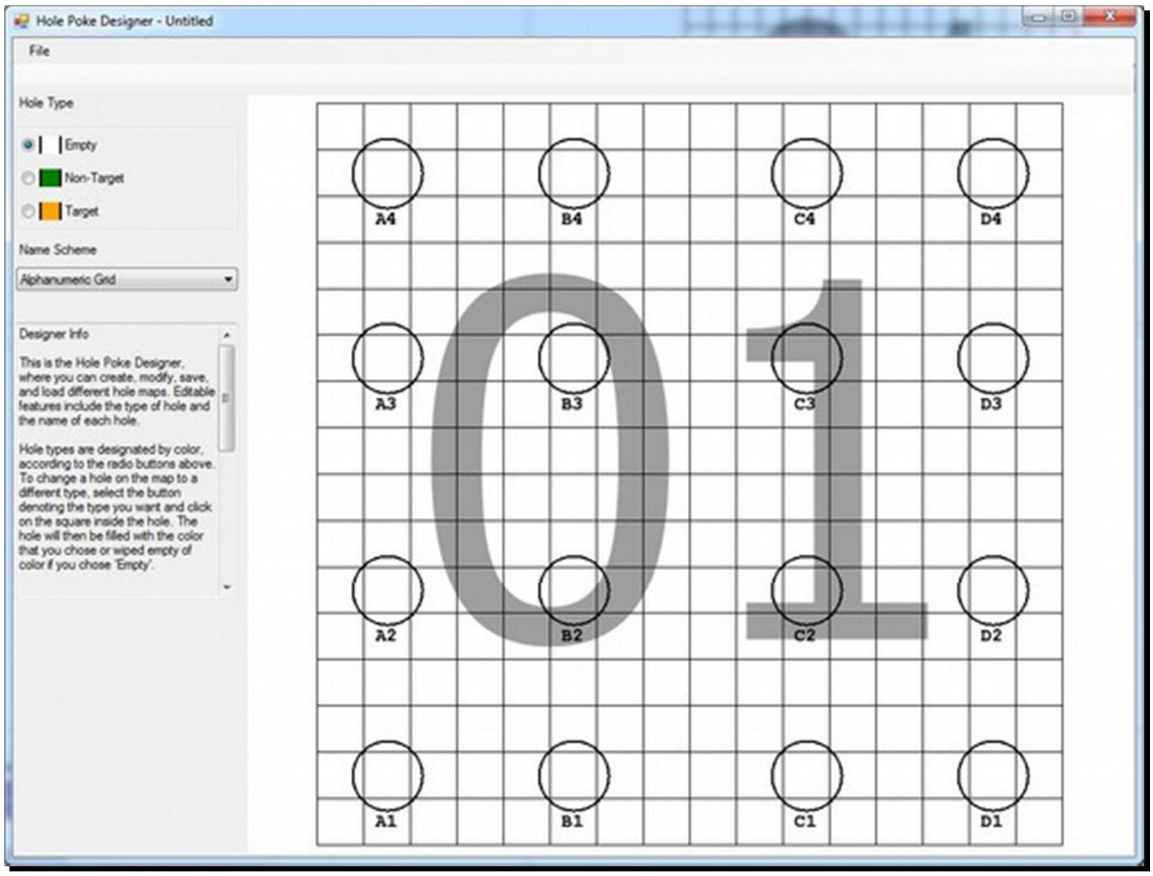
Once the Hole Poke Designer is open, a blank Hole Poke Map is automatically loaded. The selected hole’s state (empty, non-target, target) is located in the upper-left corner.

12.1.2 Applying a State to a Hole

Each hole can have one of three different states: Empty (White), Non-Target (Green), Target (Orange)

Animal exploration of an empty hole results in a “Mistrial”. Exploration of a Non-Target or Target hole results in a “Trial.” Exploration of Target hole also ends the Hole Poke experiment (Hole Poke variables such as Duration and Distance stop incrementing at this point).

In order to set a hole to a particular state, select that state from the corresponding radio button in the top left portion of the screen, and then click on a hole to apply that state.



12.1.3 Managing Hole Poke Maps

All Hole Poke Map management operations can be accessed from the “File” drop down menu.

- ***New***. Loads a new Hole Poke Map with an empty canvas.
- ***Open***. Activates an Open File dialog which allows the user to select a previously saved Hole Poke Map.
- ***Save***. Saves the current Hole Poke Map using the current file name. If no file name has yet been chosen, a dialog will be opened permitting the user to choose one.
- ***Save As***. Saves the current Hole Poke Map using a new file name.

12.1.4 Naming Schemes

Naming schemes are different options for labeling individual holes. One naming scheme may label each hole with a letter and number representing its position in a grid, while another might give each hole a single unique numerical designation. There are five standard naming schemes:

1) Alphanumeric Grid. Columns correspond to letters, rows to numbers. A1 starts in the bottom left.

2) Battleship Grid. Columns correspond to numbers, rows to letters, as in the game Battleship. A1 starts in the top left.

3) Cartesian Grid. A standard XY-style grid with coordinates separated by hyphens instead of commas to simplify data output with .csv files.

4) Discrete Numbering Bottom Grid. This scheme names each hole with a single number, starting in the bottom left and proceeding right until the end of the row, then moving up to the next row and continuing.

5) Discrete Numbering Top Grid. This scheme works just like Discrete Numbering Bottom Grid, but starts in the top left.

The default scheme for newly created hole maps is Alphanumeric Grid.

To apply a new scheme to a hole map, use the drop down menu on the lefthand side of the Hole Poke Designer, and select any of the standard schemes to apply. All holes will be instantly renamed according to the new scheme. Names are saved to files as well as hole types.

12.1.5 Custom Naming Schemes

Custom schemes are allowed as well as standard schemes. A custom scheme is created by clicking the cell below the desired hole to change its name. The text already below the hole will underline when the cursor is in the correct position. Upon clicking, a dialog box will appear, and the name can be entered there. Twelve characters maximum, letters, numbers, and hyphens only. No spaces. When a hole is renamed, the dropdown menu on the left-hand side will change to display "Custom," and the text "Custom" can be seen within the dropdown menu as well. This is not a selectable option. It is displayed to inform the user that the current name scheme is a nonstandard one. The scheme can be changed back to a standard scheme at any time by clicking one of the standard options under the dropdown menu. If the current scheme is "Custom," an extra prompt will appear cautioning the user that changing will lose all custom names.

12.1.6 Applying a Hole Poke Map

In order to use a Hole Poke Map to obtain hole poke data, the Hole Poke Map must be applied to a completed experiment. Start by opening an experiment in the main Fusion screen.

Next, enter the Hole Poke Designer and open or create a Hole Poke Map. Once you have loaded an experiment and prepared a Hole Poke Map, the “Apply Hole Poke Map” button will be available. Selecting this button will cause the Hole Poke Map to appear in the main Fusion readout.

12.2 Exporting Hole Poke Data

Once the Hole Poke Map is applied, the hole poke data is made available by selecting Hole Poke Data when creating the Fusion Comprehensive Output Data File. The comprehensive data export contains hole poke information such as duration and number of holes visited. A more detailed analysis, called Hole Poke Sequence Data, has now been added to the export menu. A Hole Poke Sequence is displayed for each cage used in the experiment. The sequence gives a list of each individual poke that occurs during an experiment, detailing poke number, poke start time, poke duration, hole poke displacement, hole poke displacement total, hole name, and hole type. If locomotor motion tracking axes are in use for the Hole Poke experiment as well, the export data will also display the distances traveled by the subject from the end of a previous poke to the beginning of the next, the cumulative distance traveled between pokes, and the average speeds during travel between pokes. Do not use Hole Poke with only one locomotor axis enabled (one sensor pair of motion tracking axes), as this configuration is unsupported.

12.3 Hole Poke Autostart Options

This addition to Hole Poke functionality allows the experiment to start recording data as soon as the software registers a beam break in the Hole layer has occurred. In other words, the experiment begins the first time the animal pokes its nose into any hole. First Poke Auto Start is found in the new experiment dialog, and will only be displayed if the software detects that Hole Poke sensors are connected. This setting overrides the setting Insertion Auto Start, found under the Locomotor tab of Settings. If Insertion Auto Start is enabled, but the user creates an experiment with First Poke Auto Start enabled, the experiment will not begin automatically recording if the animal is inserted into the cage. It will wait for the first poke.

13.0 Fusion Directories and Files

This section describes the locations and names of files and directories used by Fusion.

13.1 Directories

13.1.1 The Fusion Experiment Directory

All files saved by the Fusion system are stored in the Fusion experiment directory by default. The default for this directory is “[Documents]\Fusion” where [Documents] is the current user’s Documents directory. In Windows XP, this directory is “My Documents”. In Windows 7, this directory is “Documents”. This directory can be changed in the Settings dialog.

13.2 Files

13.2.1 Fusion Experiments (“.fxp” and “.atm”)

When an experiment is created in Fusion, a subdirectory is created below the Fusion experiment directory with the same name as the experiment. All files associated with that experiment will be located in that directory. The experiment contains two types of files – one header file (“.fxp” extension) and at least one data file (“.atm” extension).

13.2.2 Fear Conditioning Profiles (“.spf”)

Fear conditioning profiles use a “.spf” extension. These are saved by default in the Fusion experiment directory.

13.2.3 Zone Map Files (“.szm”)

Zone Map Files use a “.szm” extension. These are saved by default in the Fusion experiment directory.

13.2.4 Exported Analysis Files (“.txt”,”.csv”,”.html”)

When the Fusion system exports a data file, it uses that experiment’s directory by default (though a different directory can be chosen).

14.0 Appendix 1: VersaMax & DigiScan Legacy Software Comparability

Fusion's variable naming may differ from the variable naming in legacy software. For the purpose of comparability, this section specifies each legacy variable and its Fusion analog.

VersaMax Variables:	Fusion Equivalent Variables
HACTV (Horizontal Activity)	Horizontal Activity Count
TOTDIST (Total Distance)	Total Distance
MOVNO (Number of Movements)	Movement Episode Count
MOVTIME (Movement Time)	Movement Time
RESTIME (Rest Time)	Rest Time
VACTV (Vertical Activity)	Vertical Activity Count
VMOVNO (Number of Vertical Movements)	Vertical Episode Count
VTIME (Vertical Time)	Vertical Activity Time
STRCNT (Stereotypy Counts)	Stereotypic Episode Activity Count
STRNO (Number of Stereotypy)	Stereotypic Episode Count
STRTIME (Stereotypy Time)	Stereotypy Time
CWREV (Clockwise Revolutions)	Clockwise Revolutions
CCWREV (Counter-Clockwise Revolutions)	Counter-Clockwise Revolutions
MRGDIST (Margin Distance)	Margin Distance
MRGTIME (Margin Time)	Margin Time
CTRDIST (Center Distance)	Center Distance
CTRTIME (Center Time)	Center Time
LFTIME (Left-Front Time)	Left-Front Time
RFTIME (Right-Front Time)	Left-Rear Time
LRTIME (Left-Rear Time)	Right-Front Time
RRTIME (Right-Rear Time)	Right-Rear Time
WHEELROT (Wheel Rotations)	Wheel Rotations
RACTV (Rearing Activity)	– Not Implemented (See Rearing Note*)
RMOVNO (Number of Rearing Movements)	– Not Implemented (See Rearing Note*)
RTIME (Rearing Time)	– Not Implemented (See Rearing Note*) [4]

[4] ***Rearing Note:** VersaMax has two variable sets to track vertical beam breaks: Vertical and Rearing. Fusion has Vertical and Jumping. Vertical variables regard any beam breaks in the vertical sensor layer as the target activity, and record it. Rearing variables only regard vertical beam breaks as legitimate, recordable rearing activity if there is also a beam break in the horizontal sensor layer.

15.0 Contact and Warranty Information

15.1 Contact Information

Omnitech Electronics, Inc.

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15.2 Warranty

Omnitech Electronics, Inc. warrants its equipment for a period of 1 year from date of purchase to original purchaser including parts and labor. Extended warranties are available; please contact sales@omnitech-usa.com for further details.

Warranty is void for purchases not paid in full within allotted payment window, unless special arrangements have been made with Omnitech Electronics, Inc.

A Return Materials Authorization (RMA) form must be requested prior to returning any product for service. The form is available by contacting – support@omnitech-usa.com - Upon approval, the product should be packed well and insured for full value. All labor and parts will be covered under the conditions or the warranty as stated above. Non-warranty repairs will be invoiced on the basis of materials and labor required to execute the necessary repairs, plus freight charges. These charges will be subject to customer approval. PLEASE NOTE: Please keep all packing materials that came with this shipment.