

IonWizard 7.8

Acquisition

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1 What is Acquisition?

The lonWizard acquisition module provides a framework that supports data collection in lonWizard. There are four separate but related functions that are handled by the acquisition framework:

- 1. One-time configuration of the physical devices attached to the computer, their attachment to each other and the options that are installed. Some examples of physical devices are: computer interface cards, excitation light sources, system interfaces and PMT tubes.
- 2. Definition of tasks that can be performed. Tasks fall into two groups: recording tasks such as dual excitation fluorescence and video edge detection and output/control tasks such as analog output.
- 3. Creation of experiments, including selection of tasks and entering of specific duration and rate (epoch) information.
- 4. Execution of current experiment to produce a data set, including real-time control of some experiment parameters.

Acquisition Terms

The following terms will be used to describe hardware and software that are used in the acquisition module:

Interface Card A card that plugs into a slot inside the computer.

Computer PortOne of the "standard" ports that are usually built-in to the computer, such as

serial, parallel or USB ports.

Physical Device A generic term for a "box" that is connected to an interface card, a built-in

computer port, or another physical device by at least one cable.

Device IonWizard's representation of an interface card or a physical device. The

acquisition module uses devices to perform three main functions:

- Show how the interface cards and physical devices are connected to the

computer and, therefore, lonWizard.

- Specify the options that are installed in the specific physical device or the configuration options to use. For example, in a MuStep, you specify the

names of the filters that are installed.

- Access test functions for the interface cards or physical devices.

Hardware Tree A hierarchical list of the devices that that are used by the acquisition module.

Task Definition of how one or more devices are used to acquire or output data or to

control another device.

Task Type General categorization which defines the overall function of a task. The task

type is selected when a new task is created and it determines the values you must enter to create the task. lonWizard supports general purpose acquisition task types such as "Trace Recording" as well as special purpose task types

such as "Dual Excitation."

Epoch A specified duration of time during which a specified set of task parameters

will be used to "run" a task.

Experiment A list of one or more tasks and one or more epochs plus general options that

define how an experiment is run.



"Recording sources" are now called "Recording tasks 41" which are a subset of "experiment tasks."

2 Manual Convention

Let us begin with definitions of stylistic conventions used in this manual.

- <u>Underlined</u> text refers to the names of interface elements shown in the illustrations included in most sections.
- Italicized text refers to names given to specific parts of the IonWizard interface. These names can be either IonOptix names, for example *trace bar* or names of Windows controls, like *scroll bar* and are described in various sections of the manual.
- Bold text refers to mouse buttons or keystrokes that must be used in order to operate some function.
- The symbol § indicates the following name is a section in the manual.



A note icon indicates an important point that you should know.



An idea icon shows some ideas on how you can use a device or function.



A stop icon indicates a potential for personal injury, equipment damage or data loss.



The 4x icon will explain major differences from version 4 of IonWizard.

3 Acquisition Framework

As described already, the acquisition module provides a framework for data collection. This framework is expandable: specialized functions can be added to the core acquisition functionality as needed. The acquisition framework uses a layered architecture where each layer builds on elements created in lower layers. There are three layers: hardware, tasks and experiments. These layers are very important and we describe them below in a general overview. The remainder of this section documents the various tools used to control the framework.

Hardware

The hardware layer is a complete description of all the actual hardware present in your system. Create this layer by using the <u>Hardware Manager</u> dialog box to build a hardware tree. Hardware devices in this tree are accessed by tasks to perform experimental functions such as recording data.

The complete list of hardware devices available in a given situation is determined by the set of hardware component drivers installed on the computer system. This is the first level of expandability provided by the framework.

Tasks

Once the hardware tree has been established, you now create any tasks you will use in your experiments. These tasks range from simple trace recordings to complex dual excitation ratiometric fluorescence recordings. There is a single list of tasks on the system, build via the <u>Task Manager 13</u> dialog box. These tasks can be used in any combination desired in any number of experiments.

As with the hardware layer, the list of tasks available in a given situation is determined by the set of task plug-ins installed on the computer system. This is the second level of expandability provided by the framework. A complete list of available task plug-ins, and the tasks they provide is available in the Acquisition Tasks 40 section.

Experiments

When the task list has been established, you can now create experiments. Create any number experiments using the Experiment Manager dialog box. Then use the Parameters dialog box to select tasks, sampling rates and other features for each experiment. At any one time only one experiment is selected as current and available for execution.

3.1 Launching Acquisition

The acquisition module is launched by selecting <u>New</u> from the lonWizard <u>File</u> *menu*. The <u>Collect</u> *menu* appears when the module completes its initialization process and is ready for use.



You can only have one copy of the acquisition module open at a time.

Errors Launching Acquisition

It is possible that acquisition module will fail before presenting the <u>Collect menu</u>. For example, the framework will run checks on your saved hardware, task and experiment settings; if these tests fail, the module will present an *alert* message and then exit. In this case, you will never see the <u>Collect menu</u>.

To make these tests lonWizard may need to communicate with the acquisition hardware to learn its capabilities and/or current settings. As a result you should make sure all hardware is powered on prior to launching the acquisition module.



You should turn on all acquisition hardware BEFORE you start acquisition

It is also possible you will see *alert* messages after the <u>Collect</u> *menu* appears that indicate the system cannot proceed and needs repair. Further information is available in the <u>Experiment Repair</u> (29) section.

Tasks Manager...

3.2 Collect Menu

All lonWizard acquisition functions are accessed from the Collect menu which is divided into three sections:

The top section of the <u>Collect</u> *menu* provides access to the acquisition configuration tools:

Hardware... Opens the Hardware Manager *dialog* which

creates and manages the hardware tree by adding, configuring and testing devices.

Opens the Task Manager 13 dialog which

creates and manages the list of tasks that are available for use in experiments.

Experiments... Opens the Experiment Manager 18 dialog

which creates and deletes experiments and

selects the current experiment.

Parameters... Opens the Parameters 20 dialog to edit

parameters for the current experiment.

Trace Monitors... Opens the <u>Trace Monitors</u> 32 dialog to add/

edit trace monitors for the current

experiment.

Mark Text... Opens the Mark Text 34 dialog to edit pre-

defined mark text available to any

experiment.



Collect Menu - tools

The middle section of the <u>Collect</u> *menu* allows the user to show or hide <u>experiment tool bars</u> available in the current experiment:

Experiment Status If checked, display the Experiment Status

Tool Bar 35 .

Manual Control If checked, display the Manual Control Tool

Bar 36. Available depending on tasks and options selected in the current experiment

parameters.

Trace Monitors If checked, display the <u>Trace Monitors Tool</u>

Bar 331. Available when one or more trace monitors have been added to the current

experiment.

Vessel Measurement If checked, display the <u>Vessel Dimension</u>

Recording Task Tool Bar 76. Available when there is a Vessel Dimension Recording task

in the current experiment.



Collect menu - tool bars



Additional recording task tool bars will be individually displayed for each recording task in the current experiment that has a experiment toolbar. Refer to individual recording tasks [41] documentation for details on the tool bar functionality provided by each task..

The bottom section of the Collect menu provides tools for running the experiment 37:

Interactive If checked, any visible experiment tools bars

will update in real-time.

Start Starts the current experiment, disabled once

started

Stop Stops the current experiment, disabled

unless experiment is running

Pause a running experiment, disabled unless

experiment is running

Resume Resume a paused experiment, disabled

unless paused

Skip Trigger Skip wait for external trigger, disabled if not

waiting



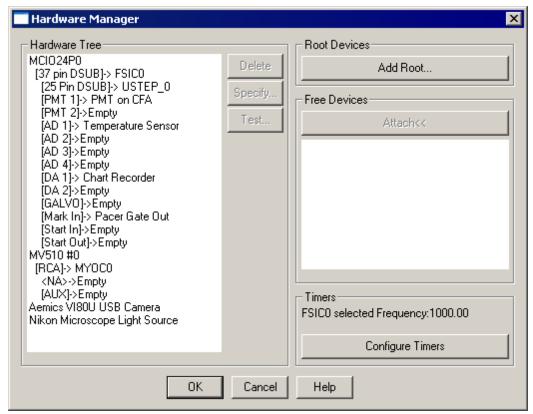
Collect menu - control

3.3 Hardware Manager Dialog

The <u>Hardware Manager 8</u> is used to define the hardware setup and provide hardware test functions. Setup of the Hardware Manager is the first step that should be done after software installation, as it is used by the software to determine available options for tasks and triggers. Generally, the <u>Hardware Manager</u> *dialog* will not need to be changed after the initial setup.



The Hardware Manager will generally only be edited during the initial software installation. While it is relatively easy to add devices, it can be difficult to remove devices from this tree. Once their device is added to the tree, certain physical devices, such as the camera, will need to be powered and connected for the software to start.



Hardware Manager

Selecting <u>Hardware...</u> from the <u>Collect</u> *menu* will display the <u>Hardware Manager</u> *dialog* which provides two main functions:

- 1. Manages the <u>Hardware Tree</u> using the <u>Add Root...</u> and <u>Attach<< buttons</u>.
- 2. Selects the primary system timer via the Configure Timers button in the Timers section.

Managing the Hardware Tree

The hardware tree shows how devices are connected in a hierarchal tree. At the left edge are the root devices which interface directly with normal Windows device drivers. Below each device is a list of connection points that it provides. When you highlight a connection point, you will see a list of supported devices that can be attached in the <u>Free Devices</u> section. When you attach a device, it will be listed next to the connection point following the arrow (->) and any connections that it provides will be listed below, indented an additional level. Eventually, you will attach all the devices that will provide input to or output data from lonWizard.



For details on specific connections or devices, please refer to the device's documentation in the <u>Acquisition Devices</u> 131 section.

Adding Root Devices

Root devices are specialized devices that connect directly to the computer and use a Windows device driver, such as interface cards and USB devices. In some cases, a root device may also be a "standin" for hardware that does not have any computer controllable parts, such as a single excitation light source. You must add the appropriate root device using the <u>Add Root...</u> button before you can add any non-root devices. See the <u>Add Root Device</u> 10 dialog for more information.

Attaching Devices

To attach a child device to a root device or other device with connection points, select the desired connection point in the <u>Hardware Tree</u> and then pick a device from the <u>Free Devices</u> section and click the <u>Attach<< button</u>. The device's <u>Specification</u> dialog will automatically open. Please see the device of interest in the Acquisition Devices section for details about the <u>Specification</u> dialog.

Deleting Devices

To delete a device and any attached child devices, select the device in the <u>Hardware Tree</u> then click the <u>Delete</u> button. You will be asked to confirm that you want to delete the device.



If you delete a device that is used in ANY task the Hardware Manager will warn you when you click OK. If you save your changes you will have to fix all broken tasks and affected experiments before you can run ANY experiments.

Specifying Device Options

Some devices have options that must be configured for the specific device. To specify the options, highlight the corresponding device in the <u>Hardware Tree</u> and then click the <u>Specify...</u> button. If the highlighted device does not have any options, the <u>Specify...</u> button will be disabled. The <u>Specification</u> dialog will also pop up automatically when adding a new device.



For details on the specify options for a specific device, refer to the "Specification Dialog" section for that device in the <u>Acquisition Devices</u> section.

Testing Devices

Some devices provide a test *dialog* that allows you to interact with the physical hardware directly from the <u>Hardware Manager</u>. To test the physical hardware, highlight the corresponding device in the <u>Hardware Tree</u> and then click the <u>Test...</u> *button*. If the highlighted device does not have a test function, the <u>Test...</u> *button* will be disabled.



For details on the test function for a specific device, please refer to the "Test Dialog" section for that device in the Acquisition Devices 131 section.

Configure System Timer

See the <u>Timer Configuration 10</u> dialog for instructions on selecting the system timer source.

3.3.1 Add Root Device Dialog

Clicking the <u>Add Root...</u> button in the <u>Hardware Manager</u> 8 dialog will display the <u>Add Root Device</u> dialog. It displays a list of all supported root devices in the <u>Type of Device</u> section. If you uncheck the <u>Show All Devices</u> checkbox, you will hide hardware devices whose required Windows device driver is not installed.



Add Root Device Dialog - Select device

After you have selected a device, you will see a list of available instances of the device. Select the instance that you want to use and then click the <u>Add button</u>. The device's <u>Specification dialog</u> will automatically open. Please see the device of interest in the <u>Acquisition Devices</u> 131 section for details about the <u>Specification dialog</u>.



If the instance list is empty, it means that you have already installed the maximum supported number of the device type in the hardware tree.



For details on specific devices, please refer to the "Connections" section of the <u>Acquisition Devices [13</u>] section.



Add Root Device Dialog - Select instance

3.3.2 Timer Configuration Dialog

The <u>Configure Timers</u> button of the <u>Hardware Manager</u> dialog will open the <u>Timer Configuration</u> dialog. The <u>Timer Configuration</u> dialog allows you to select the master interrupt source to use in the system and configure a specific pacing frequency. This pacing frequency determines that rate at which data is sampled.

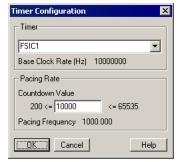
Selecting Timer

The <u>Timer</u> *drop-down menu* will show all devices in your current hardware tree that are capable of being the system timer source. Generally, you should select the devices with the fastest <u>Base</u> <u>Clock Rate</u> as the system timer, which is the default selected value. In some cases, notably the System Interfaces, you must select a specific device in order for the device to function properly. Please refer to the "Requirements" section of the specific device in the <u>******</u> 131 Acquisition Devices 131 section for any device specific restrictions that may exist.

Once you select a timer, the <u>Base Clock Rate</u> and <u>Pacing Rate</u> section of the dialog will change based on the characteristics of the selected device.



When you change between devices, the dialog will try to adjust the countdown value so that the resulting Pacing Frequency is the same. Be sure to check that the



Timer Configuration Dialog

Pacing Frequency is correct after changing the timer.

Setting Pacer Frequency

To select a specific pacing frequency, enter the correct <u>Countdown Value</u> to 'divide' the <u>Base Clock Rate</u> of the selected timer to get the desired <u>Pacing Frequency</u>.



Consult IonOptix before using pacing frequencies greater than 1000Hz.

3.4 Acquisition Filter Manager

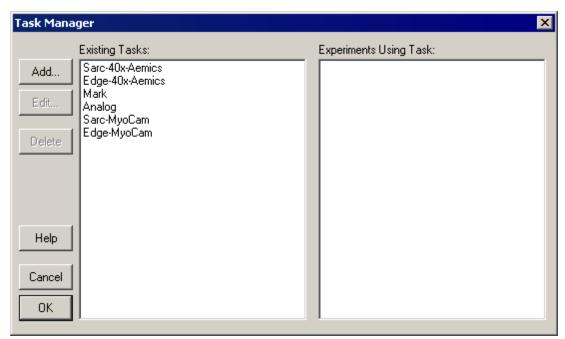


Acquisition Filter Manager Dialog

An acquisition filter is a piece of software that can be attached to an acquisition data stream that somehow changes that data. Each filter has an input, for example an image stream from a camera, and an output such as another image stream. The filter will process each piece of data that flows through it performing some modification, for example rotating the image or sharpening it. The output of the Filter can then be used in any regular acquisition task like SoftEdge or SarcLen.

The <u>Acquisition Filter Manager</u> dialog box shown in the image above lets you set up a filter and attach it to the desired data stream.

3.5 Task Manager Dialog



Task Manager Dialog

The <u>Task Manager</u> is used to define a collection of tasks that are available for use in one or more experiments. Tasks are created by selecting a desired task type (see § <u>Acquisition Tasks 40</u>) for a list of standard acquisition task types) and then selecting the specific devices to use and entering various labels and calibration values needed to run the selected task type.

To access the <u>Task Manager</u> dialog select "Task Manager..." in the <u>Collect</u> menu or click on the <u>Manage</u> button in the <u>Parameters</u> of dialog.

Here is a description of the parts of the Task Manager dialog.

Existing Tasks Shows the names of all currently defined tasks in the system. Click on a task to

select it (highlighted with the blue bar).

If the task name is listed in red there is an error that needs your attention, refer to

Task Manager Errors 15.

Experiments Lists experiments that are using the currently selected task.

Add... Creates a new task.

Edit... Edits parameters for the currently selected task.

Delete Deletes the current task after confirmation.

Cancel Undoes ALL changes and closes dialog. Clicking the "X" in the upper-right corner

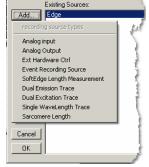
is the same as Cancel.

OK Saves ALL changes and closes dialog.

Creating a New Task

When you click the <u>Add...</u> button, a list of all available task types that are installed on your system will be displayed. When you select a task type, you will be shown the corresponding <u>...Task</u> dialog. The exact information that will need to be entered in the <u>...</u> <u>Task</u> dialog depends on the task type selected - refer to the specific task documentation for details.

While the exact task parameters list varies by task type, every task has a <u>name field</u>. The name is displayed in the <u>Task Manager dialog</u> and is used when selecting a task for use in an experiment. If you define multiple tasks of the same task type, you should attempt to name each task differently so you know which task is which.



Add Recording Source List



Some task types, such as Cell Length Recording task, are extra-cost options that may not be present on your system.



Details for the parameters are located in the 'Task Settings section of each task type of the <u>Acquisition Tasks</u> 40 section. Some task types are documented separately.

Editing an Existing Task

When you highlight an existing task and click the <u>Edit...</u> button, the appropriate <u>Task</u> dialog will be displayed. When you edit the parameters of an existing task, you will affect any new data collected using an experiment with this task. Previously saved data files are NOT affected by changes to a task.

It is likely that editing a task will mark the experiments that use the task as "needing review" in which case the framework will enter the repair state 29.



If you change the name of an existing task all saved user limits and templates will be reset.



Details for the parameters of each task type are located in 'Task Settings' section of the task type documentation. Some task types are documented separately.

Deleting a Task

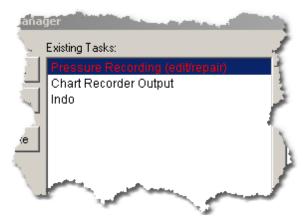
To remove a task from the system, highlight the desired task then click the <u>Delete</u> *button*. Before the task is deleted, you will be shown an <u>alert dialog</u> 16 that will describe the repercussions of this action. It is possible that deleting a task will break the experiment setup in which case the framework will enter the <u>repair state</u> 29.



If you delete a task that is used in an experiment you will have to edit/review the experiment

Repairing Tasks

As described in Framework Repair Process [29], it is possible to break tasks by editing the hardware tree. In that case the acquistion framework will enter the repair state and you will be foreced to resolve the task errors before proceeding. When you enter the Task Manager in this situation, all broken tasks will be highlighted in red text as shown in the figure below. The text in parenthesis after the task name indicates the actions needed to correct the task errors.

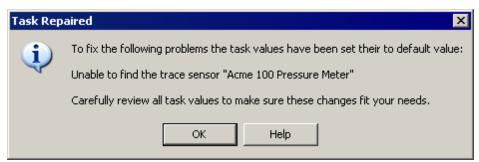


Once all the broken (red) tasks have been fixed, you will still need to review any experiment that used a repaired task before the framework re-enables data acquisition.

3.5.1 Alerts

The <u>Task Manager</u> can display a number of *alert dialog boxes*. These *alerts* are documented in the following sections.

3.5.1.1 Task Repaired



Task Repaired Message

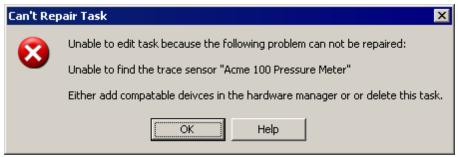
This *alert* indicates that the task you are about to edit has been repaired and you need to review the repair. Task repairs occur as a result of a change to hardware tree. The actual nature of any repair performed by a task is task specific, but it typically amounts to selecting a new hardware element of the required type from the existing set of elements.

For example, assume you have a task that uses a trace sensor named "Acme 100 Pressure Meter". You decide that you need a better pressure meter and purchase a shiny new Acme 5000 Pressure Meter. To use this new meter, you modify the hardware tree by deleting the old one and adding the new one. This action likely breaks the existing task that was recording pressure.

To correct this situation, the task will attempt to repair itself by selecting the first trace sensor it sees in the hardware tree (if a task cannot repair itself, then it must be deleted - see <u>Can't Repair Task</u> 16). If this sensor is your new pressure meter, then the task is repaired as you would expect and you can simply click Ok in the task dialog to accept the change.

If your system has more than one trace sensor however, you will want to review the selected one to make sure the repair operation selected the Acme 5000 and not, for example, your temperature sensor. If the repair was incorrect, you will need to select the correct sensor and then click <u>Ok</u> to save the changes.

3.5.1.2 Can't Repair Task



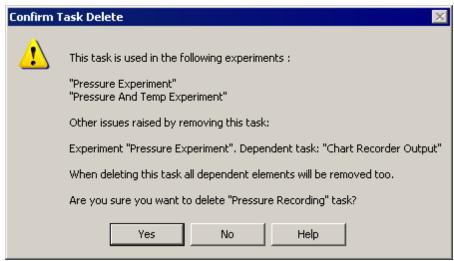
Cannot Repair Task Message

This *alert* indicates that the task you are trying to edit cannot be repaired because a resource type it requires no longer exists in hardware tree.

For example, assume you have a task that uses a trace sensor named "Acme 100 Pressure Meter". You decide you don't need pressure data any more and give your meter to your colleague across the hall. You then delete the meter from your hardware tree. If you system does not contain any other trace sensor, you get the above message when you try to edit the task. Typically in this case you would delete your pressure recording task.

However, if after seeing this message you realize you really do need to record pressure, then you need to get back your pressure meter and add it to the hardware tree again. This action will enable repair of the task.

3.5.1.3 Confirm Task Delete



Task Delete Confirmation Message

Deleting a task from the <u>Task Manager</u> dialog requires confirmation. The confirmation *alert dialog* lists the repercussions of removing the task. The possible repercussions are:

- 1. If the task is in use by any experiment, it will be deleted from that experiment.
- 2. Any secondary task that references the task being deleted will also be deleted.
- 3. If all tasks are removed from an experiment, the epochs in that experiment will also be deleted.
- 4. Any epoch that referenced the task will be deleted.

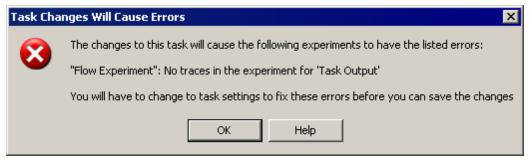
5. Any trace monitors that referenced the task will be deleted.

In the figure above, we are about to delete a task called "Pressure Recording". We see that two experiments were using that task. We see also that in "Pressure Experiment", a task called "Chart Recorder Output" was using the output of the "Pressure Recording".

If we click <u>Yes</u>, the "Pressure Recording" will be deleted from the <u>Task Manager</u> list and references to it removed from both experiments. Furthermore, references to "Chart Recorder Output" will be removed from "Pressure Experiment".

If we click No, the delete operation will be abandoned and no changes will be made to the task list or any experiments.

3.5.1.4 Task Changes Will Cause Errors



Task Changes Will Break Experiment Message

It is possible to break a task simply by editing it. For example you may choose sensors that are incompatible with each other. Or you may change a stand-alone task which is in an experiment by itself to become dependent on output from another task.

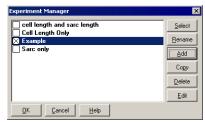
Any edit that results in such an error will result in the above *alert dialog*. You may get this *alert* immediately after editing the task, or you may get it when exiting the Task Manager dialog.

This alert provides a message that describes the detected error. Usually this message contains enough data to allow you to discern the rule that was broken. However in some of the more obscure corner cases, the message will be meaningful only to technicians. In that case, you should contact your system provider for more information.

3.6 Experiment Manager Dialog

The <u>Experiment Manager</u> is used to create a system-wide list of experiments and to select the current experiment. The current experiment can be edited in the <u>Parameters 20 dialog</u> and run with the <u>Start 37 function</u> in the <u>Collect menu</u>.

The main area of the <u>Experiment Manager</u> dialog shows a list of all experiments currently defined in the system. The blue background bar shows the highlighted experiment while the checked experiment is the current experiment that will be edited in the <u>Parameters</u> dialog or run with <u>Start</u>.



Experiment Manager Dialog

Here is a description of the functions of in the Experiment Manager dialog:

Select Marks the currently highlighted experiment as current (moves the check box to the

highlighted line).

Rename Allows the user to change the experiment name.

Add Adds a new experiment (see below).

Copy Adds a new experiment with the same settings as the selected experiment.

Delete Delete highlighted experiment, after confirmation.

Edit Opens the <u>Parameters</u> *dialog* to allow editing of the selected experiment.

Cancel Undo ALL changes and close dialog. Clicking the X in the upper-right corner is the

same as Cancel.

OK Save ALL changes and close dialog.

Adding a New Experiment

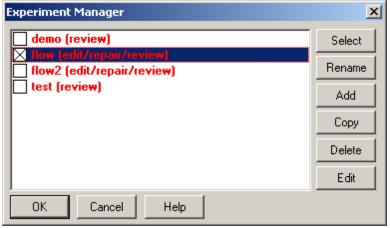
When you click <u>Add</u> in the <u>Experiment Manager</u> *dialog*, you will be asked to enter a name for your new experiment. When you click <u>OK</u>, the experiment will be added to the list, highlighted and made the current experiment. At this point, the <u>Parameters</u> *dialog* will be empty. Please see the Parameters section for a discussion of selecting the tasks and other options for this experiment.



Name New Experiment Dialog

Experiment Needs Editing or Review

When a task is changed, either directly by editing or indirect in response to changes in the hardware tree, each experiment that uses the task will be marked as "requiring review" by displaying the experiment name in red followed by "(review)" or "(edit/ repair/review)". When you select the experiment and click on the Edit button a message will be displayed showing you the reason for the error - see Parameters Altered 28 alert for more details.



Experiement Manager Dialog with Errors

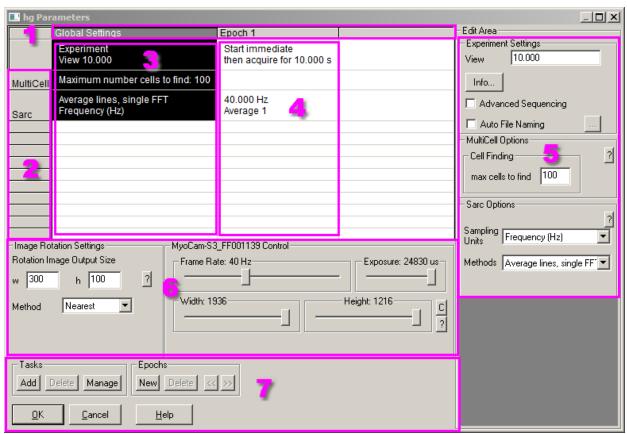
3.7 Parameters Dialog

The <u>Parameters</u> *dialog* is used to enter/edit the details of the current experiment selected in the <u>Experiment Manager</u>. The <u>Parameters</u> *dialog* is used to:

- Define general experiment information.
- Select specific tasks for this experiment from the list defined in the <u>Task Manager [13]</u> dialog.
- Create one or more epochs that set sampling duration, rates and average parameters for each task.

Clicking the <u>OK</u> button saves the parameters to the selected experiment. They will be recalled the next time the experiment is selected.

To access the <u>Parameters</u> dialog select "Parameters..." from the <u>Collect</u> menu or click the <u>Edit</u> button in the <u>Experiment Manager 18</u> dialog.



Parameters Dialog Areas

The <u>Parameters</u> dialog has the following main areas:

- 1. Column Headings The top gray row describes column contents.
- 2. Row Headings The leftmost gray column shows the names of tasks that have been added to this experiment.
- 3. Experiment Settings The first white column shows the experiment global settings in the first (unlabeled) row, followed by the experiment settings for each task.
- 4. Epoch Settings The remaining white columns show settings for one or more epochs. The first (unlabeled) row displays the epoch parameters that are consistent for all tasks. The following rows display epoch settings specific to each task.

- 5. Edit Area The group to the far right displays editing controls to allow you to change details associated with the currently selected column in the spread sheet.
- 6. Global Sensor Settings The group just below the white columns allows you to set the sensor option to use for this experiment if required by the current tasks.
- 7. Control functions The group at bottom left contains buttons used to add/delete tasks (rows), add/delete/move epochs (columns), close the <u>Parameters</u> dialog, cancel *ALL* changes or display help.

The <u>Parameters</u> dialog can be resized by dragging the lower right corner or the borders. If there are more rows and/or columns than can be displayed, scrollbars will appear to allow for scrolling through the main grid (areas #3 and #4) while the row and column headings (#1 and #2) remain to help you keep track of your position.

The <u>Control Functions</u> area (#7) contains buttons that allow you to add and remove rows (tasks) and columns (epochs) while the <u>Edit</u> area (#5) is used to edit values for the currently selected column (see below).



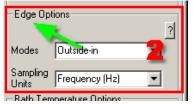
The exact contents of the <u>Parameters</u> dialog will vary depending on the options purchased with your system as well as the values you enter when adding and editing tasks using the task manager. The screen shots shown should be considered examples only.



The **?** mini-button in the <u>Edit</u> area groups will display specific help for the controls in that group.

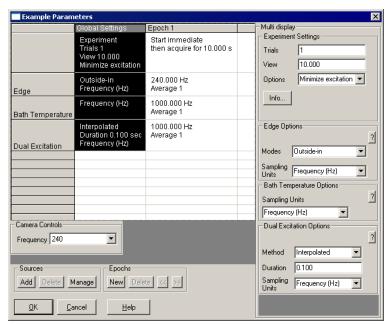
Edit Area

The <u>Edit area</u> is used to change any value in either the <u>Experiment Settings</u> or <u>Epoch Settings</u> columns. When you click on a column heading or a specific cell, the <u>Edit area</u> will display editing controls for the selected cells. For each selected cell, there will be a matching group in the <u>Edit area</u> which contains the input controls needed to change the values. Each group is labeled with the row heading unless it is the first row, in which case it is labeled with the column heading. In the example to the right, the group name shown by the green arrow ("Edge Options") matches the task name displayed in the row heading ("Edge").



Edit Area Group Label

Experiment Settings



Experiment Settings Column selected

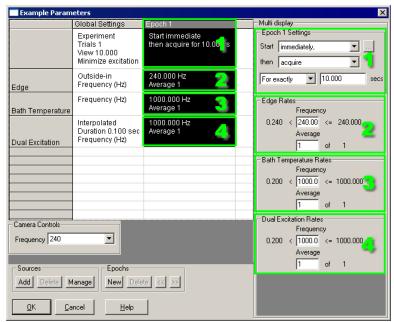
The figure shows the edit area when the <u>Experiment Settings</u> column is selected. The indicated areas show:

1. Experiment Global Settings: Experiment options global to all tasks in an experiment - See Experiment Global Settings 25 for details.

(the remaining items depend on the tasks in the experiment)

- 2. <u>Edge Options</u>: Experiment options for "Edge" task See <u>Cell Length Recording Task</u> Experiment Settings 57 for details.
- 3. <u>Bath Temperature Options</u>: Experiment options for "Bath Temperature" task See <u>Trace</u> <u>Recording Task Experiment Settings</u> 43 for details.
- 4. <u>Dual Excitation Options</u>: Experiment options for "Dual Excitation" task <u>Dual Excitation Trace</u>
 Recording Task Experiment Settings 49.

Epoch Settings



Epoch Settings Column selected

The figure shows the edit area when an "Epoch Settings" column is selected. The indicated areas show:

- 1. Epoch start and duration settings See Epoch Settings 26 for details.

 (the remaining items depend on the tasks in the experiment)
- 2. Edge Options: Epoch options for "Edge" task See Cell Length Recording Task Epoch Settings 57 for details.
- 3. Bath Temperature Options: Epoch options for "Bath Temperature" task See <u>Trace Recording</u> Task Epoch Settings 43 for details.
- 4. Dual Excitation Options: Epoch options for "Dual Excitation" task <u>Dual Excitation Trace</u>

 Recording Task Epoch Settings 49



If all the the Edit Area groups do not fit in the <u>Parameters</u> dialog, a scroll bar will be displayed that will scroll the task items groups only. The top group (#1) will always display.



If you click on a specific cell, the appropriate editing group will appear in the Edit Area.



The specific controls available in the Experiment and Epoch settings for each task depends on the task type of the task. Refer to the appropriate <u>Acquisition Tasks</u> 40 section for more information.

Global Sensor Settings Area

The <u>Global Sensor Options</u> area of the <u>Parameters</u> dialog contains controls for sensor options that are selected for the entire experiment. Controls will only appear in this area if one or more of the sensors selected in the task settings for any task in the current experiment have options that can be selected on an experiment-by-experiment basis. For example, the picture above shows the camera controls for the lonOptix MyoCam which allow you to select the current camera mode.

Control functions: Task Group

The <u>Tasks</u> group has the following functions for working with <u>Task</u> rows.

Add Task

When you click on the Add button in the Tasks group of the Parameters dialog, you will see a list of tasks that have been defined in the Task Manager dialog. If you select a task, it will be added to the next empty row and the Experiment Settings and Epoch Settings columns will be filled with default values. You can edit the default values by selecting the appropriate cell in the main grid then editing the values in the matching Edit Area [21] group.



Add Task Menu

Delete Task

Clicking the <u>Delete</u> button in the <u>Task</u> group will delete the current task (row) WITHOUT confirmation. This button is grayed out if the currently selected row is not a task row (i.e. the row heading or the global settings row).



When you delete a task from the experiment you are NOT deleting it from the system. Any other experiments using the same task will not be affected.



If you accidentally delete a task, you can press the <u>Cancel</u> button to restore ALL parameters to their previous values.

Control functions: Epochs Group

The Epochs *group* has the following functions for working with Epoch columns.

New Epoch

Clicking the <u>New button</u> in the <u>Epochs</u> group of the <u>Parameters</u> dialog will add a copy of the currently highlighted epoch to the end of the list. If this is the first epoch it will filled with default values. You can edit the new epoch by selecting the appropriate cell in the main grid and then editing the values in the matching <u>Edit Area</u> 71 group.

Delete Epochs

Clicking the <u>Delete...</u> button in the <u>Epochs</u> group of the <u>Parameters</u> dialog will remove the current epoch and shift any remaining epochs to the left over the deleted epoch.

Move Epochs

Clicking the << or >> buttons in the Epochs group of the Parameters dialog will move the current epoch to the left or right. When the experiment is run, epochs are run from left to right. So, moving an epoch allows you to change the order in which they are run.

Control functions: Ok, Cancel and Help

Cancel Undo ALL changes and close dialog. Clicking the X in the upper-right corner is

the same as Cancel.

OK Save ALL changes and close dialog.

Help Pulls up this help dialog.

Experiment Settings

3.7.1 Experiment Global Settings

The Experiment Global Settings group appears as the top group in the Edit Area of the Parameters 20 dialog when the Experiment Settings column, or a cell within it, is selected. The controls in this group let you select options that affect the entire experiment:

View The View window shows the number of seconds displayed in the

lonWizard trace viewer when the experiment is started.

Info The Info... button pulls up the Experiment Info dialog (see

below).

Auto File Naming ... If selected, Auto File Naming enables automatic generation of

> experimental filenames. The name is based on the current computer time (date:hours:seconds) plus a user supplied prefix and suffix. Clicking the ... button will display the Automatic File Naming Settings dialog (see below) that permits you to set the prefix and suffix. When this feature is enabled, a name will be generated for each new experiment. When you complete the experiment and save the resulting file in lonWizard, the save dialog will have the automatically generated name already entered and will be pointing to the last directory used for saving or opening a file. If those settings are correct, simply select

Save and the file will be saved.

DA Delay ... If one or more trace output tasks are part of the current

> experiment the Experiment Global Settings area will include a display of the current DA delay followed by a ... button that will

display the **DA Delay** dialog (see below)

Experiment Info

The Experiment Info dialog allows you to enter general information about the experiment. The information is intended to be used to help you document the conditions of the experiment for easy review at a later time.



Settings



Experiment Info Dialog

Automatic File Naming Settings Dialog

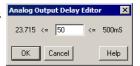
When you click on the ... button after "Auto File Naming" in the Experiment Global settings area you will open the Automatic File Naming Settings dialog. This dialog allows you to set prefix and suffix that will be attached to the automatically generated portion of the filename. The automatically generated portion is the current date in YYYY-MM-DD format plus the time in HHMMSS format.



Automatic File Naming Settings Dialog

DA Delay Editor

When you click on the ... button after "DA Delay" in the Experiment Global settings area you will open the DA Delay Editor dialog. This dialog allows you to set the delay between when data started acquiring and when the value will be output to the analog output.

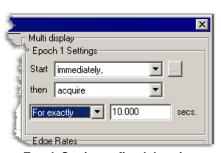


DA Delay editor

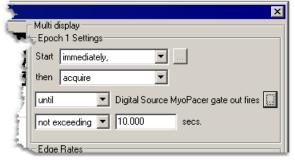


The minimum DA Delay amount is determined by the delay characteristics of the sensors selected in all trace output tasks in the current experiment. For instance, with a video camera the minimum delay is the a combination of the integration time AND the frame grabber acquisition time

3.7.2 Epoch Settings



Epoch Settings - fixed duration



Epoch Settings - until event or duration

The <u>Epoch Settings</u> *group* appears as the top group in the <u>Edit Area</u> of the <u>Parameters</u> dialog when an <u>Epoch Settings</u> *column*, or any cell within it, is selected. The controls in this group let you select options that control how the selected epoch is started and when it ends.

Start Determines when data collection starts after the epoch is started. Options are:

Immediately Data collection starts immediately.

After a key press Data collection starts after operator clicks "Click Ok to start" message box

that appears when epoch starts.

After a hardware event

Data collection starts based on trigger defined with the ... button. See <u>Trigger</u>

Options 27 (below).

Then Determines what is done during this epoch. Options are:

Acquire Data is collected from all tasks.

Delay No data is collected.

For Duration

For exactly Set duration of this epoch to the specified number of seconds.

Until When selected, the epoch will end before the specified not exceeding time if a

trigger occurs. Click the ... button to the right on this line to set up a trigger. S

ee <u>Trigger Options</u> 26 (below).

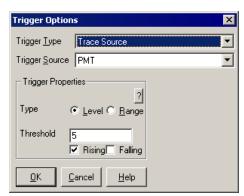
Not exceeding When using one or more <u>until</u> options, this determines the maximum duration.

3.7.2.1 Trigger Options

Trigger options are available to start data collection for an epoch (when start=Hardware) or to end data collection (when an "until" option is selected). In either case, you access the <u>Trigger Options</u> dialog by clicking on the ... button in the <u>Epoch Settings</u> group of the <u>Parameters</u> 120 dialog.



Epoch Trigger Options - Raw Digital Sensor



Epoch Trace Sensor - Raw Trace Sensor

Trigger Type Selects the trigger type that you are defining.

Raw Digital Sensor Trigger occurs when a digital event coming from the Trigger Source is

detected. Our System Interfaces define a digital event as the rising edge

of a TTL pulse.

Raw Trace Sensor Trigger occurs when the value of the raw trace selected in <u>Trigger Source</u>

matches the level(s) set in the Trigger Properties group.

Trigger Source Selects the device producing the digital event or raw trace that is used in the

trigger. lonWizard will scan through the hardware tree and will list all possible

hardware triggers in the drop down list.

Trigger Properties If appropriate for the trigger type, displays options to determine how trace

sensor generates triggers.

Trigger Type<u>Level</u> will test the signal against a single value, and <u>Range</u> will test the

signal against two values.

Upper Threshhold & Lower Threshold

Enter the test value in the "raw" units for the device selected in <u>Trigger Source</u>. When the input value "crosses" this value, a trigger will be generated if the corresponding rising/falling option is selected (see next).

Rising Select to generate a trigger when input value goes from below to above the

level entered above.

Falling Select to generate a trigger when input value goes from above to below the

level entered above.

3.7.3 Alerts

The <u>Parameter</u> dialog can display a number of *alert dialog boxes*. These *alerts* are documented in the following sections.

3.7.3.1 Parameters Altered



Parameters Altered Message

When entering the Parameter [27] dialog for an experiment that needs review (see Framework Repair Process) [29] you will see an alert dialog similar to that shown above. This alert is telling you all the task changes that prompted the experiment review. You should look at the experiment and the epochs for the tasks that remain in the experiment to be sure that the sampling rates and so forth are appropriate.

3.8 Framework Repair Process

As described in the Acquisition Framework Overview 4, the acquisition system has three layers: hardware, tasks and experiments. Edits to the either of the first two layers almost invariably impact subsequent layers to the extent that the experiment will no longer execute. While the acquisition framework prevents most improper experiment configurations, it is not possible to lock out every error and end up with a usable system. The number of lockouts needed put the user into what feels like a straightjacket. To balance between ease of use and correctness, the acquisition framework implements a repair process to help the user fix problems.

When the hardware or tasks are editing such that the experiments are broken or potentially changed in subtle ways, the acquisition framework enters the *repair state*. In this state, the experiment execution controls (Start, Pause, etc) are disabled until the issues are resolved. You must then repair each layer of the framework until all are fixed and reviewed at which point you will be able to run experiments again. The framework guides you by disabling access to higher layers while lower layers need repair. The below we discuss the elements of the repair state.

The first area monitored by the framework repair state mechanism is the hardware tree. When you select Ok from the Hardware \(^{\text{Particles}}\) dialog box, the acquisition framework validates the new hardware tree before the Hardware \(^{\text{Particles}}\) dialog exits to make sure that no tasks have been broken. If it finds a problem it presents an alert message \(^{30}\) warning you of the issue. Should you decide to proceed, the framework enters the first stage of the repair process - task repair. As long as tasks are broken, you will note the following changes:

- All experiment toolbars will be disabled and hidden.
- Experiment control user inputs (Start, Stop, etc) will be disabled.
- The Experiments dialog box will be disabled.
- The Parameters dialog box will be disabled.
- The Task Manager dialog will be enabled and will indicate tasks that need repair.

Once all the tasks are repaired, you now enter the experiment repair and review state. Typically during task repair, sensors are changed and perhaps entire tasks are deleted from the system. These actions will result in changes to the experiments that contained those tasks. These experiments will be marked as needing repair and/or review. Typically this amounts to simply going into the Parameter dialog for each experiment and making sure that the necessary data is being collected and that the task edits did not result in inappropriate sampling rates. Until the experiments are all reviewed you will note the following changes to the system:

- All experiment toolbars will be disabled and hidden.
- Experiment control user inputs (Start, Stop, etc) will be disabled.
- The Parameters dialog box will now be enabled.
- The Experiments dialog box will be enabled and will indicate experiments that need repair.

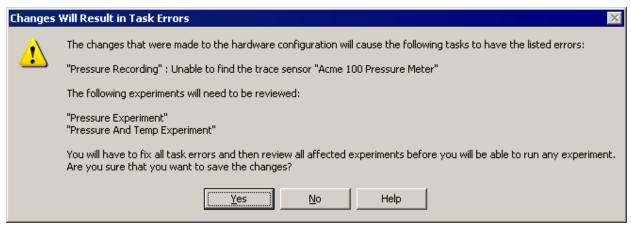
Note that you can enter the experiment repair state simply by editing tasks. If you make a change in a task that will impact sampling rates, all experiments that use that task are marked as "Need Review" and the framework enters the experiment repair state.

Finally, note also that if you exit the acquisition module while it is still in the repair state, it will return to the repair state the next time it is run. You will be reminded of this fact via an alert message.

3.8.1 Alerts

The following sections document the *alert dialog boxes* related to the framework repair process.

3.8.1.1 Changes Will Result in Task Errors



Hardware Changes Will Break Experiments Message

The above *alert* indicates that the recent changes made to the hardware will break your existing experimental setup. This is happening because the experiments in question are referencing hardware elements that no longer exist in the new hardware tree.

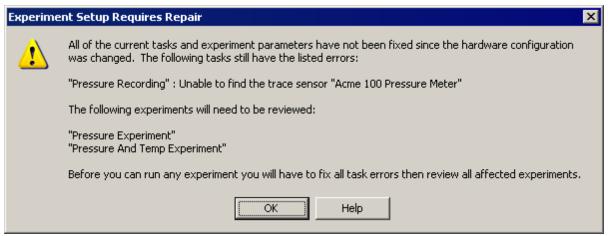
You may have edited the hardware tree because your hardware setup has in fact changed. Following the example in the figure above, perhaps you have upgraded your system by purchasing a new Acme 5000 Pressure Meter to replace your old model 100. In this case any errors introduced by a hardware edit would be legitimate and you would save your changes by clicking <u>Yes</u>. You then need to repair the effected tasks and review the experiments before being able to acquire data again.

If you are not editing your hardware tree in response to an actual change to your system and are seeing this *alert* unexpectedly, click <u>No</u> to return to the <u>Hardware Manager</u> *dialog* and then click <u>Cancel</u> to abandon the inadvertent changes.

3.8.1.2 Re-entering Repair State on Launch

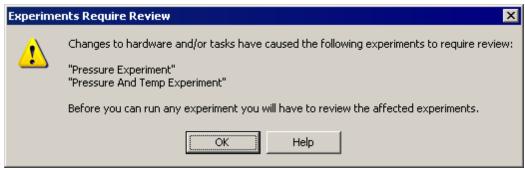
If the acquisition module was exiting while in the <u>repair state 29</u> , you will be reminded of the needed repairs the next time the module is <u>launched</u> 5 .

The below alert is shown if there are outstanding task repair issues.



Re-entry With Broken Tasks Message

If the tasks are alright but there remain experiments that require review or repair, the below alert will be displayed.



Re-entry With Experiments in Need of Review Message

In either event, you will need to repair the issues before data acquisition can proceed.

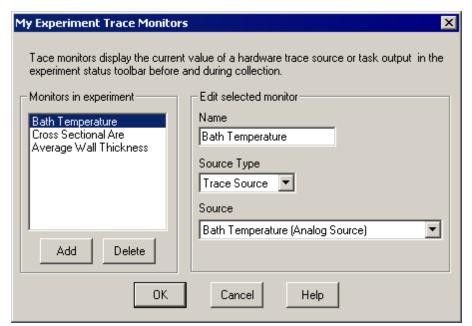
To repair tasks you will primarily use the <u>Task Manager [15]</u> dialog box. You may need to work with the <u>Hardware Manager 8</u> dialog if devices were deleted in inadvertently.



To fix some task errors you may have to add a device to the hardware tree in the <u>Hardware Manager</u> 8 before you can edit the task.

To review experiments, use the **Experiments** 18 dialog box.

3.9 Trace Monitors Dialog



Trace Monitors Dialog

The <u>Trace Monitors</u> *dialog* allows you to define one or more "trace monitors" that will display the current value from either a hardware trace sensor or from the output of any recording task in the current experiment. All trace monitors appear in a single <u>Trace Monitors Tool Bar [33]</u> in the <u>Experiment Tool Bars [35]</u> area at the bottom of the lonWizard window.

The <u>Monitors in experiment</u> *group* shows the list of monitors for the current experiment, allows you to select a monitor for editing in the <u>Edit selected monitor</u> *group* and add or delete monitors.

Monitors in experiment group

List Select an existing trace monitor name from the list will load the current definition

into the Edit selected monitor group so that you can edit its definition.

Add a new trace monitor to the list that is a copy of the currently selected trace

monitor. Add will be disabled if there are no trace sensors in the hardware tree and

no tasks in the current experiment.

Delete Delete the currently selected trace monitor from the list.

Edit selected monitor group

Name This is the name that will be used to label this trace monitor in the list and when it

is displayed in the Experiment Tool Bars [35] area. The system will set a default name as you change the Source or Source Type. Once you change the name from its default value it will no longer be changed when you change Source.

Source Type Selects between monitoring the output of a trace sensor in the Hardware Tree 8

or the output of a a task in the current experiment.

Source List all available sources for the current <u>Source Type</u>. <u>Name</u> will be changed based

on the source unless you have edited the name since the last time you changed

the Source Type.

3.9.1 Trace Monitors Tool Bar

П	Bath Temperature 2.052		Cross Sectional Are	1018.356	Average Wall Thickness	1.700	
Ш	2.052	Min	DI	233.182 Min 3926.240 Max	David	0.374 Min 7.113 Max	Reset
Ш	2.052	Max	neset	3926.240 Max	neset	7.113 Max	neset

Trace Monitors tool bar with three monitors

The <u>Trace Monitors</u> *tool bar* appears whenever one or more trace monitors have been added in the <u>Trace Monitors Dialog</u> 32. You may hide and show the <u>Trace Monitors</u> *tool bar* by *checking* and unchecking the "Trace Monitors" choice in the middle section of the <u>Collect Menu</u> 6.

3.10 Mark Text Dialog

The <u>Mark Text</u> dialog allows you to enter text for the mark shortcut keys that are entered using the <u>Mark</u> group of the <u>Experiment Status (35)</u> tool bar or by pressing the corresponding function key. The mark will be added when you click the button or press the function key and will have a description that was entered for the key in the <u>Mark Text</u> dialog.



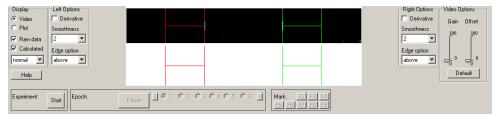
Experiment Status tool bar - mark group

To access the Mark Text dialog select "Marks..." from the Collect menu.



Mark Text Dialog

3.11 Experiment Tool Bars



Cell Length & Experiment Status Tool bars

The <u>Experiment Tool Bar</u> area at the bottom of the main lonWizard window displays one or more *tool bars* based on the tasks that are part of the current experiment. Every experiment will have an <u>Experiment Status</u> *tool bar* and certain tasks, such as Edge Detection, will have additional tool bars as shown in the figure above.

Tool bars can be hidden by un-checking them in the second group of controls in the <u>Collect menu</u> (show at right). When a *tool bar* is checked, it will be displayed at the bottom of the screen. Otherwise, it will be hidden.



Collect menu - Tool bars



All available *tool bars* are automatically displayed when you exit the <u>Parameters 201</u> dialog or change experiments in the Experiment Manager 181 dialog.

3.11.1 Experiment Status Tool Bar



Experiment Status Tool Bar - While running

The Experiment Status tool bar is available in the Experiment Tool Bars 35 area for every experiment. It appears at the bottom of the screen once the current experiment is not blank (that is has at least one task and epoch). The Experiment Status tool bar is used to provide quick access to the common experiment control functions. The three main areas of the tool bar provide the following functions:

Experiment group Start or stop 37 the current experiment and display remaining time in experiment.

Epoch groupPause or resume [37] an epoch, jump to epoch [37] number n and display time

remaining in current epoch.

Mark group

Add mark 37 at current time. Mark description will contain the phrase entered in

Mark Text 34 dialog.

3.11.2 Manual Control Tool Bar



Manual Control Toolbar with two manual controls

The <u>Manual Control</u> *tool bar* appears whenever one or more acquisition tasks in the current experiment provide manual control functions. For details on the operation of a specific manual control please refer to the "Manual Control" section of the appropriate acquisition task or acquisition device.

3.12 Running the Experiment

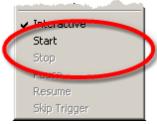
Once you have defined the current experiment parameters in the <u>Parameters 20 dialog</u>, you can perform the following functions.

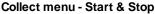
Start/Stop

The primary operation that you will want to perform is to start and stop your experiment. To start an experiment, either pick <u>Start</u> from the <u>Collect menu</u> or click the <u>Start</u> button in the <u>Experiment Status</u> 35 tool bar.

Once an experiment has started, it will run all the epochs defined in the Parameters 20 dialog, unless it is stopped or paused.

To stop an experiment, either pick <u>Stop</u> from the <u>Collect</u> *menu* or click on the <u>Stop</u> *button* in the <u>Experiment Status</u> 155 *tool bar.*





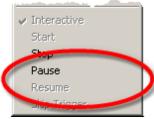


Once an experiment has been stopped it can NOT be restarted, only saved.

Pause/Resume

While an experiment is running, you can pause the experiment by either picking <u>Pause</u> from the <u>Collect</u> menu or clicking on the <u>Pause</u> button in the <u>Experiment Status [35]</u> tool bar.

Once an experiment is paused, you can resume the experiment by picking Resume from the Collect menu or clicking on the Resume button in the Experiment Status 35 tool bar.



Collect menu - Pause & Resume



Changing epochs will also resume data collection.

Changing Epochs

Normally, an experiment will run epochs from left to right.

You may restart any epoch by selecting it from the Epoch group in the Experiment Status 35 tool bar.



Experiment Status Tool bar - Epoch group

Adding Marks

To enter a mark along with its accompanying text (see the Mark Text 34 dialog), you either click on the corresponding button in the Mark group in the Experiment Status 55 tool bar or press the indicated function key.



You may also enter marks at any point in the past with the mouse. See the main lonWizard manual for details.

Experiment Status tool bar - mark group

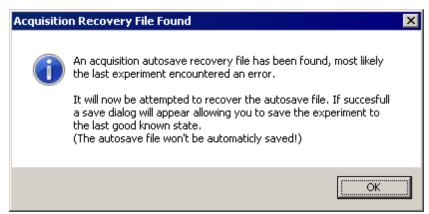
Saving/Closing

After you stop the experiment, you can can save it by picking Save from the Collect menu.

3.13 Autosave

lonWizard has a built-in autosave functionality which will run during an experiment. When an experiment is started the autosave file will be created automatically in which the acquired data will be saved at one second intervals. If lonWizard experiences a crash or freeze during an experiment it might be possible to recover the data acquired, however no guarantees if and how many data can be recovered are made.

When lonWizard is started and an autosave file is found this message will be displayed.



Autosave file found

After closing the window by pressing <u>Ok</u> the process of recovery will start. When the recovery is successful a <u>File Saving Dialog</u> will be presented allowing the experiment to be saved.



Once an autosave file has been deleted it is no longer possible to recover the data



After an experiment has been recovered and saved through an autosave file make sure all the data is correct, including any manual changes to the experiment such as notes, constant values and/or marks.

3.14 Acquisition program/data files

Acquisition support is delivered as a collection of files that are installed in the lonWizard program directory. In addition, the acquisition module writes the information that you enter into a series of configuration files that are also stored in the lonWizard program directory.

Program Files

High-level acquisition support is provide by the following files located in the lonWizard program directory:

IAB_D4.DLL High-level acquisition support

IAB_KW.SYS Low-level kernel mode acquisition support

HWMGR D4MT.DLL Hardware manager

The software required for each <u>device</u> are provided in additional files. Some standard <u>devices</u> are distributed as with the acquisition module (that is they are standard) while others, such as Edge Detection, are sold separately. The acquisition module scans the lonWizard program directory for these files:

*.IHC Tree component routines. For each .IHC file there will be a corresponding .

SYS file.

*.RSS Task routines. For each .RSS file there will be a corresponding .SYS file.

Configuration Files Created

The acquisition module stores the data you enter in the following files located in the lonWizard program directory:

IHWCFG.XML Hardware manager configuration data

IAB_D4.EST Task and experiment definitions

IAB_D4.GST Task control options entered via the tool bars



To copy your hardware, task and experiments settings between installations of IonWizard copy all three files.



If you delete IHWCFG.XML the other files will be unusable.

4 Acquisition Tasks

Before you can create an an experiment, you must define all of the tasks that you want to execute in the <u>Task Manager</u> dialog. To define a task, you will need to select from a list of the task types that are available. There are two main groups of task types: recording tasks and output/control tasks.

A recording task records data from one or more devices. Depending on the task type, the data will be either a collection of traces (values over time) or will be a list of events (times an event occurred). Recording tasks may also control devices as part of the recording task, such as moving a filter wheel to the correct excitation filter before reading the the emission output intensity.

Output/Control tasks output trace data to a device or provide control of a device separately from other experiment tasks.

Recording Task Types

Here is a list of common recording task types. Additional recording task types may be documented separately.

Trace Records values from a device at the rate specified in the current epoch.

Event Records the times that a signal is detected from a device.

Fluorescence* Controls an excitation light source and records resulting emission brightness.

Single excitation, dual excitation and dual emission are supported.

Cell Length* Detects left and right edge of cell image from video camera and calculates

resulting cell length.

Sarcomere Spacing* Calculates average inter-sarcomere spacing from an area of a camera image.

Vessel Dimension* Detects vessel wall characteristics from up to four areas of a camera image.

Vessel Flow Characteristics* Multiple high-level vessel specific calculations derived from other

vessel measurements.

Output/Control Task Types

Here is a list of common output/control task types. Additional output/control task types may be documented separately.

Trace OutputOutputs an analog signal reflecting data recorded by one device to another

device.

Signal Generator Provides the ability output specific values for each epoch and to optionally

provide manual control.

^{*} These tasks are extra-cost options and may not be present on your system.

4.1 Recording Tasks

Recording tasks are tasks that record data from one or more devices. They may also control additional devices needed for the production of the data. Recording tasks will produce one or both of the following types of data:

Trace Data A list of values sampled over time at a fixed rate. The exact rate as well as any

averaging is defined in each experiment epoch. Trace data is displayed as Value

vs time graphs.

Event Data A table of times that an event was detected. Events are recorded whenever they

occur and do not have any settings in an experiment epoch. Events are displayed

as "marks" along the time axis.

4.1.1 Trace Recording Task

A trace recording task records raw data values from the device selected in the <u>Trace Recording Task</u> 42 dialog using the rate and averaging parameters defined in the current <u>epoch settings</u> 43 l.

A simple multiply/offset scaling calculation (entered in the <u>Trace Recording Task [42]</u> dialog) is used to allow the raw data to be converted to the real world units of the connected device (ie temperature, pressure, etc...).

A trace recording task uses data entered in three separate places:

- <u>Task settings</u> 42] Settings that apply to all experiments using this task. Entered in the <u>Task</u> <u>Manager</u> 13] *dialog*.
- Experiment settings [43] Settings for this task that apply to all epochs in an experiment. Entered in the Parameters [20] dialog.
- Epoch settings [43] Settings for this task for a specific epoch in an experiment. Entered in the Parameters [20] dialog.

Task Output

The trace recording task produces the following output in lonWizard where "Name" is the description entered in the <u>Cell length recording task</u> dialog:

"Name-Raw" trace Displays the actual data collected from the selected device in the appropriate

units, usually volts.

"Name-Scaled" trace Displays data that has been scaled using the multiplier and offset values

entered in the <u>Trace Recording Task</u> 42 dialog. The graph vertical axis will be labeled using the <u>Full Description</u> string from the <u>Trace Recording Task</u> 42

dialog.

4.1.1.1 Task Settings

Clicking the <u>Edit...</u> button in the <u>Task Manager [13]</u> when a <u>Trace Recording Task [41]</u> is highlighted will display the task settings *dialog*. It has the following fields:

Name Name this task. Name is also used as the "Type"

in the <u>Trace Viewer</u> for data acquired using this

task.

Sensor Select the desired analog trace <u>Task Connection</u>

from the list available in the current hardware tree.

Image sensors can not be used.

Full Description Describe the parameter being recorded (eg.

"Temperature" or "Degrees C"). Used as the vertical axis label for data displayed in scaled

units.

Abbreviation Provide short hand notation of <u>Full Description</u> for

exported table headings. Again, for a temperature

probe you might enter "Temp" or "C".

Units Enter label for raw data trace. For analog inputs, it

would be volts. For PMT, it would be counts.

units/V Provide the calibration value that converts volts to

the desired units. You will probably need the manual of the device that puts out the analog

signal to find this value.

units@0 V
Provide the voltage at which the measurement

should be zero (for example, the voltage corresponding to a temperature of 0 degrees, a

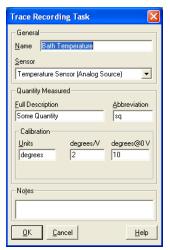
pressure of 0, etc.).

NotesEnter any notes to yourself about this recording

task.

In this example, the **degrees/V** parameter is the *slope* (S) parameter and

degrees@0V is the *y-intercept* (Y) parameter of the linear transformation function y = S * x + Y.



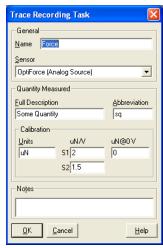
Trace Recording Task dialog

In some situations it is more convenient to enter the slope parameter as two values, values **\$1** and **\$2**. These two values are multiplied together to form the slope:

$$y = (S1 * S2) * x + Y$$

The selected <u>Sensor</u> tells the <u>Task Settings</u> *dialog* whether or not a two value calibration is appropriate.

For example, with the OptiForce, the slope value is the product of the probe stiffness in N/m (or nN/nm) and the readout calibration in nm/V. The product of these values is nN/V which is what you want. In this case you would enter *S1* and the stiffness and *S2* as the readout calibration. Since the probe stiffness only changes when you change the probe, you would probably want to enter it into the <u>Task Settings</u> dialog. The readout calibration changes with every quadrature however so you would probably be better off entering that in the <u>Task ToolBar</u> 44.



Trace Recording Task dialog with two slope entries



If you change the name of an existing task all saved user limits and templates will be reset.

4.1.1.2 Experiment Settings

Selecting the first column of a <u>trace recording task [41]</u> row in the <u>Parameters [20]</u> dialog will display the experiment settings *group* in the <u>edit area [22]</u>. The following values can be edited:

Sampling Units

Choose how you want to enter sampling rates in the epoch settings 43 dialog.

Frequency - Enter as Hertz.
Period - Enter as seconds.



Trace Recording Task experiment settings edit area

4.1.1.3 Epoch Settings

Selecting any epoch column of a <u>trace recording task [41]</u> row in the <u>Parameters [20]</u> dialog will display the epoch settings *group* in the <u>edit area [20]</u> for the selected epoch. The edit area will let you select the following values:

Frequency or Period Data sampling rate (Frequency vs Period

selection is made in the experiment settings [43] column). This is the rate at which a data point is added to the data set. This doesn't set the rate at which data is sampled from

Frequency 0.200 < 1000.0 <= 1000.000 Average the hardware. 1

Average

At a given frequency/period, there will be a maximum number of points (displayed as the "of n") that can be collected. Select the number of samples to average into a single

raw data point.

Trace Recording Task epoch settings edit area

Bath temperature Rates



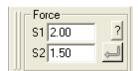
The minimum and maximum values for frequency or period are determined by the device capabilities and/or the current pacing frequency 101.



Frequency and period values are rounded to the nearest multiple of the pacing frequency 10 when focus is moved away from the control. This effect is especially noticeable when the entered frequency approaches the maximum.

4.1.1.4 Toolbar

The Trace Recording Task toolbar is only displayed when the slope of the linear transformation function is a product of two values entered separately:



Any of the two values can be modified individually. Both values take effect upon hitting the Enter key or pressing button.

4.1.2 Event Recording Task

An event recording task records the times a specific "event" is detected during acquisition as IonWizard event marks. You select the type of event to record in the Event Recording Task 45 dialog. Depending on the option selected, you may enter addition parameters that define the event in the experiment settings 45 edit area of the Parameters 20 dialog.

An event recording task uses data entered in two separate places:

- Task settings 45 Settings that apply to all experiments using this task. Entered in the Task Manager 13 dialog.
- Experiment settings 45 Settings for this task that apply to all epochs in an experiment. Entered in the Parameters 20 dialog.

Task Output

An event recording task produces a single mark for each event that is detected. The mark text is automatically set to the name of the task entered in the Event Recording Task 45 dialog.

4.1.2.1 Task Settings

Clicking the Edit... button in the Task Manager 3 when an Event Recording Task 4 is highlighted will display the task settings dialog. It has the following fields:

Name Name this task.

Source Type Select type of source:

Digital Source - An event is recorded for each positive pulse from the source selected in

the Source drop-down list.

Trace Source - An event is recorded each time the value read from the source selected in the Source drop-down list matches threshold/range values entered in the experiment settings 45 group of the

Parameters dialog.

Task Trace - In this case, both the source and the threshold/range values are defined in the experiment settings 45 group of the Parameters dialog. An event is recorded each time value in the task trace matches

the threshold/range values.

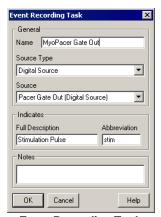
Source Select the desired digital or trace source to use

from a list of all that are available in the Hardware

Manager.

Full DescriptionDescribe the event that is being recorded.AbbreviationProvide short hand notation for Full Description.NotesEnter any notes to yourself about this recording

task.



Event Recording Task settings dialog



The <u>Source</u> drop down list is not used for the "Task Trace" source type as the trace is selected in the <u>experiment settings</u> 45 group of the <u>Parameters</u> dialog..

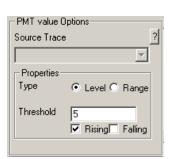
4.1.2.2 Experiment Settings

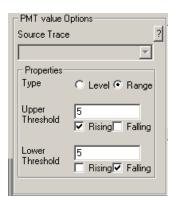
Selecting the first column of an <u>event recording task [44]</u> row in the <u>Parameters [20]</u> dialog will display the <u>experiment settings</u> group in the <u>edit area [21]</u>. The values that can be edited depend upon the <u>Source Type</u> selected in the <u>Event Recording Task [45]</u> dialog.

Event Type: Digital Source

There are no additional experiment settings required for the "Digital Source" source type.

Event Type: Trace Source or Task Trace





Single level threshold

Dual level threshold

For the "Trace Source" and "Task Trace" source types, you will need to specify the threshold value(s) that you would like to test to determine the event.

Type Choose "Level" for a single threshold value. Choose "Range" for two threshold

values.

Threshold Enter the threshold level in the raw units of the sensor.

Rising Create an event when the value of the sensor "rises" from below the threshold

value to above.

Falling Create an event when the value of the sensor "falls" from above the threshold

value to below.

Source Trace For a "Task Trace" source type, select from a drop-down list of all constructed

traces in the current experiment. For a "Trace Source" source type, the

control is disabled.



The specific raw sensor that is used for the "Trace Source" source type is set in the <u>Event Recording Task</u> 45 dialog.



You can select Rising, Falling or both for each threshold.

4.1.3 Fluorescence Photometry Recording Tasks

The Fluorescence Photometry Recording Tasks are a group of recording tasks that collect intensity of the emission output(s) that result from one or more wavelengths of excitation light. Each fluorescence task use a specific combination of input sensors and excitation wavelengths listed below. IonWizard manages access to the light source so that one complete set of raw data points is collected for all fluorescence recording tasks in the experiment.

Tasks Provided

The following fluorescence tasks are provided:

Single Excitation 52 Record single data trace from selected sensor excited with a single excitation

wavelength.

<u>Dual Excitation</u> Record two data traces from single selected sensor excited with two

alternating wavelengths.

Dual Emission 50 Record two data traces from the two selected sensors excited with a single

excitation wavelength.



The maximum sampling rate for all fluorescence recording sources in an experiment is the same as they all share a single light source.



Collecting from multiple fluorescence tasks may have a significant impact on the maximum rate if it introduces addition light source filter movement.

4.1.3.1 Dual Excitation Trace Recording Task

The dual excitation trace recording task controls a light source device to present alternating wavelengths of excitation light to a preparation. It then records the resulting emission light from each wavelength with a single sensor device into two separate output traces: raw numerator and raw denominator. In addition the task provides the ability to view background subtracted, ratio and calcium calculated traces

The dual excitation trace recording task uses data entered in three separate places:

- <u>Task settings</u> [48] Settings that apply to all experiments using this task, entered in the Task Manager *dialog*.
- Experiment settings 49 Settings for this task that apply to all epochs in an experiment, entered in the Parameters *dialog*.
- Epoch settings 49 Settings for this task for a specific epoch in an experiment, entered in the Parameters dialog.

Task Options

The dual excitation trace recording task supports two sperate methods of acquiring data: Interleaved and Interpolated.

The interleaved method alternates between the numerator and denominator filter positions for the duration of sampling.

The interpolated method starts by collecting a well resolved sample from the isosbestic filter, collects from the other filter position for the duration of sampling, then collects an ending sample from the isosbestic filter.



The Interpolated method is only available if an isosbestic filter is selected <u>Dual Excitation</u> <u>Trace Recording Task 48</u> dialog.



Selection of Interleaved or Interpolated for the current experiment is made in <u>Experiment</u> <u>Settings</u> 49.

Task Output

The dual excitation trace recording task produces the following output in lonWizard, where "Name" is the description entered in the <u>Dual Excitation Trace Recording Task</u> dialog:

"name-Raw" traces

Two channels of the actual data collected from the selected <u>sensor device</u>: **numerator** - data collected when filter in "numerator" position **denominator** - data collected when filter in "denominator" position

"name-Numeric Subtracted" traces

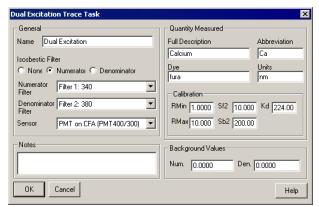
Four channels calculated from the raw data traces above: **numerator** - raw numerator value minus numerator background constant **denominator** - raw denominator value minus numerator background constant **ratio** - numeric subtract numerator divided by numeric subtracted denominator **calcium** - calcium calculation using numeric subtracted ratio and calcium

calibration constants



See IonWizard documentation for details on viewing traces

4.1.3.1.1 Task Settings



Dual Excitation Trace Recording Task dialog

Clicking the <u>Edit...</u> button in the <u>Task Manager [13]</u> when a <u>Dual Excitation Trace Recording Task [47]</u> is highlighted will display the task settings *dialog*. It has the following fields:

Name Name this task. Name is also used as the "Type" in the <u>Trace Viewer</u> for data

acquired using this task.

Isosbestic Filter Indicate whether the numerator or denominator filter is the Isosbestic filter for the

indicator dye. Select "none" if you are not using an Isosbestic filter.

Numerator Filter Select the filter used to provide the excitation light for the numerator trace.

Denominator Filter Select the filter used to provide the excitation light for the denominator trace.

Select the device that records the emission light from the preparation excited by

the excitation filters.

Notes Enter any notes to yourself about this recording task.

Full Description Describe the parameter being recorded (eg "Calcium" or "pH"). Used as the

vertical axis label for data displayed in scaled units.

Abbreviation Provide short hand notation for <u>Full Description</u> for exported table headings.

Dye Name the dye that you are using, for documentation purposes.

Background Values Provide the values to be used as the default background constants for new data

files.

<u>Num.</u> - Numerator background value <u>Den.</u> - Denominator background value

Calibration Values used as default values for Calcium Calibration constants.

RMin. - Ratio recorded using dye and zero calcium solution.

RMax. - Ratio recorded using day at saturating calcium solution.

Sf2 - Background-subtracted free calcium denominator.

<u>Sb2</u> - Background-subtracted bound calcium denominator value.

Kd - Dissociation constant.



Sensor

Use the IonWizard Constants... function to change background values or calibration constants for the current file.



If you change the name of an existing task all saved user limits and templates will be reset.

4.1.3.1.2 Experiment Settings

Selecting the first column of a <u>dual excitation trace recording task</u> row in the Parameters *dialog* will display the experiment settings *group* in the edit area. The following fields can be edited:

Method Select sampling method to use. (See <u>overview</u> 47)

for details.)

Interleaved - Ratiometric sampling method. **Interpolated -** Puesdo-ratiometric sampling

method.

Duration If you have selected the Interpolated method,

enter the duration, in seconds, to sample each

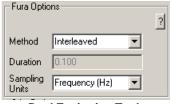
isosbestic point.

Sampling Units Chose how you want to enter sampling rates in

the epoch settings [49] dialog.

Frequency - Enter as Hertz.

Period - Enter as seconds.



Dual Excitation Task experiment settings edit area



When using the Interpolated method, the duration for the isosbestic point should be long enough to provide a well-averaged value. For most cases, 0.1 seconds (100 points at 1Khz) should be enough.



When using the Interpolated method, the epoch begins by sampling data at the isosbestic point. Trace data is collected after the isosbestic point has been collected and the filter has been moved.

4.1.3.1.3 Epoch Settings

Selecting any epoch column of a <u>Dual excitation trace recording task</u> (47) row in the Parameters dialog will display the epoch settings *group* in the edit area for the selected epoch. The edit area will let you select the following values:

Frequency or Period Data sampling rate (Frequency vs Period

selection is made in the experiment settings [49] column). This is the rate at which a data point is added to the data set. This does not set the rate at which data is sampled from

the hardware.

Average At a given frequency/period there will be a

maximum number of points (displayed as the "of n") that can be collected. Select the number of samples to average into a single

raw data point.



Dual Excitation Task epoch settings edit area



The minimum and maximum values for frequency or period are determined by the sensor and filter switching device capabilities and/or the current pacing frequency 10.



Frequency and period values are rounded to the nearest multiple of the <u>pacing frequency</u> 10 when you click on a different field. This effect is especially noticeable when the entered frequency approaches the maximum.

4.1.3.2 Dual Emission Trace Recording Task

The dual emission trace recording task controls a light source device to provide a single wavelength of excitation light to a preparation. It then simultaneously records the resulting emission light from two wavelength with two separate sensor devices into two separate output traces: raw numerator and raw denominator. In addition the task provides the ability to view background subtracted, ratio and calcium calculated traces

The <u>Dual emission trace recording task</u> uses data entered in three separate places:

- <u>Task settings</u> [51] Settings that apply to all experiments using this task, entered in the Task Manager *dialog*.
- Experiment settings 52 Settings for this task that apply to all epochs in an experiment, entered in the Parameters *dialog*.
- Epoch settings [52] Settings for this task for a specific epoch in an experiment, entered in the Parameters dialog.

Task Output

The dual emission trace recording task produces the following output in lonWizard where "Name" is the description entered in the <u>Dual Emission Trace Recording Task</u> dialog:

"Name-Raw" traces

Two channels of the actual data collected from each sensor device: numerator - data collected from the "numerator" sensor denominator - data collected from the "denominator" sensor

"Name-Numeric Subtracted" traces

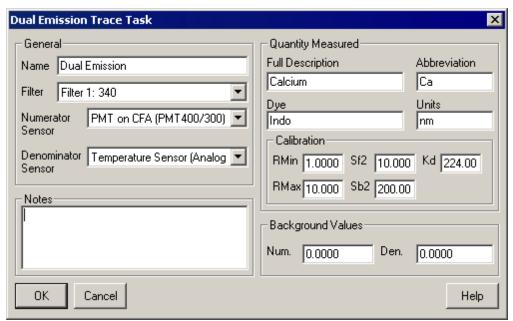
Four channels calculated from the raw data traces above: **numerator** - raw numerator value minus numerator background constant **denominator** - raw denominator value minus numerator background constant **ratio** - numeric subtract numerator divided by numeric subtracted denominator **calcium** - calcium calculation using numeric subtracted ratio and calcium

calibration constants



See IonWizard documentation for details on viewing traces

4.1.3.2.1 Task Settings



Dual Emission Trace Recording Task dialog

Clicking the Edit... button in the Task Manager 13 when a Dual Excitation Trace Recording Task 47 is highlighted will display the task settings dialog. It has the following fields:

Name Name this task. Name is also used as the "Type" in the <u>Trace Viewer</u> for data

acquired using this task.

Filter Select the filter used to provide the excitation light.

Numerator Sensor Select the device that records the emission light for the numerator trace. **Denominator Sensor** Select the device that records the emission light for the denominator trace.

Notes Enter any notes to yourself about this recording task.

Full Description Describe the parameter being recorded (eg "Calcium" or "pH"). Used as the

vertical axis label for data displayed in scaled units.

Abbreviation Provide short hand notation for <u>Full Description</u> for exported table headings.

Dye Name the dye that you are using, for documentation purposes.

Background Values Provide the values to be used as the default background constants for new data

files.

<u>Num.</u> - Numerator background value <u>Den.</u> - Denominator background value

Calibration Provide values to be used as default values for Calcium Calibration constants.

<u>RMin.</u> - Ratio recorded using dye and zero calcium solution. <u>RMax.</u> - Ratio recorded using day at saturating calcium solution.

Sf2 - Background-subtracted free calcium denominator.

Sb2 - Background-subtracted bound calcium denominator value.

Kd - Dissociation constant.



Use the IonWizard Constants... function to change background values or calibration constants for the current file.



If you change the name of an existing task all saved user limits and templates will be reset.

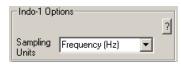
4.1.3.2.2 Experiment Settings

Selecting the first column of a <u>Dual excitation trace recording task</u> 47 row in the Parameters dialog will display the experiment settings group in the edit area. The following fields can be edited:

Sampling Units

Chose how you want to enter sampling rates in

the epoch settings 52 dialog. Frequency - Enter as Hertz. Period - Enter as seconds.



Dual Emission Task experiment settings edit area

4.1.3.2.3 Epoch Settings

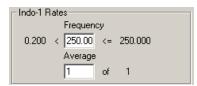
Selecting any epoch column of a <u>Dual excitation trace recording task</u> 47) row in the Parameters dialog will display epoch settings group in the edit area for the selected epoch. The edit area will let you select the following values:

Frequency or Period Data sampling rate (Frequency vs Period selection is made in the experiment settings 49 column). This is the rate at which a data point is added to the data set. This does not set the rate at which data is sampled from the hardware.

Average

At a given frequency/period there will be a maximum number of points (displayed as the "of n") that can be collected. Select the number of samples to average into a single

raw data point.



Dual Emmisions Task epoch settings edit area



The minimum and maximum values for frequency or period are determined by the sensor and filter switching device capabilities and/or the current pacing frequency 101.



Frequency and period values are rounded to the nearest multiple of the pacing frequency 10h when you click a different field. This effect is especially noticeable when the entered frequency approaches the maximum.

4.1.3.3 Single Excitation Trace Recording Task

The single emission trace recording task controls a light source device to provide a single wavelength of excitation light to a preparation. It then records the resulting emission light with a sensor device to create a single output trace: raw. In addition, the task provides the ability to view a background subtracted trace and, optionally, a background subtracted, normalized trace.

The single emission trace recording task uses data entered in three separate places:

- Task settings [53] Settings that apply to all experiments using this task, entered in the Task Manager dialog.
- Experiment settings 54 Settings for this task that apply to all epochs in an experiment, entered in the Parameters dialog.

• Epoch settings [54] - Settings for this task for a specific epoch in an experiment, entered in the Parameters dialog.

Task Output

The single emission trace recording task produces the following output in lonWizard where "Name" is the description entered in the <u>Single Emission Trace Recording Task</u> dialog:

"Name-Raw Intensity" trace

Raw data collected from the sensor device.

"Name-Numeric Subtracted" trace (if the <u>experiment settings [54] output option</u> is "BG Subtraction only")

Raw data value minus numerator background constant.

"Name-Numeric Subtracted" traces (if the experiment settings 54) output option is "BG and

normalized")

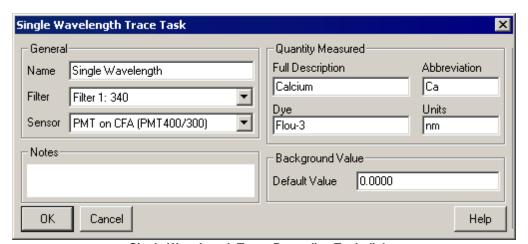
Two traces calculated from raw data:

intensity - raw data value minus numerator background constant. **normalized** - intensity value divided by the normalization constant.



See IonWizard documentation for details on viewing traces

4.1.3.3.1 Task Settings



Single Wavelength Trace Recording Task dialog

Clicking the <u>Edit...</u> button in the <u>Task Manager [13]</u> when a <u>Single Emission Trace Recording Task [52]</u> is highlighted will display the task settings *dialog*. It has the following fields:

Name Name this task. Name is also used as the "Type" in the <u>Trace Viewer</u> for data

acquired using this task.

Filter Select the filter used to provide the excitation light.

Sensor Select the device that records the emission light.

Notes Enter any notes to yourself about this recording task.

Full Description Describe the parameter being recorded (eg "Calcium" or "Ph"). Used as the

vertical axis label for data displayed in scaled units.

Abbreviation Provide short hand notation for <u>Full Description</u> for exported table headings.

Dye Name the dye that you are using, for documentation purposes.

Background Value Provide the value to be used as the default background constant for new data files.



The normalization constant defaults to "1" and can not be changed in the Task Settings dialog because you must manually calculate the each time the experiment is run



Use the IonWizard Constants... function to change background values or enter a normalization constant for the current file.



If you change the name of an existing task all saved user limits and templates will be reset.

4.1.3.3.2 Experiment Settings

Selecting the first column of a <u>single emission trace recording task 52</u> *row* in the Parameters *dialog* will display the experiment settings *group* in the edit area. The following fields can be edited:

Sampling Units Chose how you want to enter sampling rates in

the epoch settings 52 dialog.

Frequency - Enter as Hertz.

Period - Enter as seconds.

Output Options Select what output traces you want to view in

lonWizard (see overview 52 for details) **BG Subtraction Only** - The "Numeric

Subtracted" trace will contain the raw trace minus the background constant

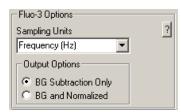
BG and Normalized - The "Numeric Subtracted" trace will have two channels:

Intensity - raw trace minus the background

constant

Normalized - Intensity trace divided by

Normalization constant



Single Emission Task experiment settings edit area

4.1.3.3.3 Epoch Settings

Selecting any epoch column of a <u>Single emission trace recording task [52]</u> row in the Parameters *dialog* will display <u>epoch settings</u> *group* in the edit area for the selected epoch. The edit area will let you select the following values:

Frequency or Period Data sampling rate (Frequency vs Period

selection is made in the experiment settings

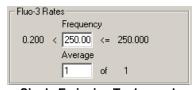
49 column). This is the rate at which a data point is added to the data set. This does not set the rate at which data is sampled from

the hardware.

Average At a given frequency/period there will be a

maximum number of points (displayed as the "of n") that can be collected. Select the number of samples to average into a single

raw data point.



Single Emission Task epoch settings edit area



The minimum and maximum values for frequency or period are determined by sensor device capabilities and/or the current pacing frequency 10.



Frequency and period values are rounded to the nearest multiple of the <u>pacing frequency</u> 10 when focus is moved away from the control. This effect is especially noticeable when the entered frequency approaches the maximum.

4.1.4 Cell Length Recording Task

The cell length recording task analyzes video images to find the left and right edges of an object. The task produces two raw data traces: left edge position and right edge position, relative to the left side of the image, in pixels. In addition to the raw data display, the task provides the ability to view the data in calibrated units using a user-supplied scaling factor and to display the difference between the edges, which is the cell length.

The edge detection recording task uses information entered in the following places:

- <u>Task settings</u> [56] Settings that apply to all experiments using this task, entered in the <u>Task</u> Manager [13] *dialog*.
- Experiment settings [57] Settings for this task that apply to all epochs in an experiment, entered in the Parameters [20] dialog.
- Epoch settings [57] Settings for this task for a specific epoch in an experiment, entered in the Parameters [20] dialog.
- Global Sensor Settings Area [23] Experiment configuration of camera parameters for some cameras (see documentation for selected camera for details), entered in the Parameters [20] dialog.
- Tool bar [58] Real-time display of images and control of cell length detection settings such as video line to analyze and the detection threshold, entered in the tool bar [35].

Task Modes

The cell length recording task supports two edge detection modes: Line Scan Mode and Box Mode.

The Line Scan Mode has two options:

- Outside-in detection, the selected video line is scanned from the edge of the image towards the
 center. Detection stops on the first edge that meets the criteria selected in the <u>cell length recording</u>
 task [58] tool bar.
- The inside-out mode starts scanning in the center and moves towards the outside.

In the Box Mode a snapshot from the object in rest should be taken and this snapshot is used to do the detection. It also offers an option to first average all the lines in the box together before doing the detection.



Task mode is selected in the Experiment Settings 57 area of the Parameters dialog



Both the Outside-In and Inside-Out Line Scan Modes are incompatible with the Multicell Task 113. If an experiment includes a Multicell Task 113, select Box Mode.

Task Output

The edge detection recording task produces the following output in lonWizard, where "*Name*" is the description entered in the <u>Cell Length Recording Task</u> 56 dialog:

"Name-Pixels" traces Two channels of the raw data collected from the selected camera device:

Left - left edge pixel position **Right** - right edge pixel position

"Name-Length" traces Three channels calculated from the raw data traces above:

Left - left edge pixel position scaled to specified units **Right** - right edge pixel position scaled to specified units **Length** - difference in edge positions scaled to specified units



See IonWizard documentation for details on viewing traces

4.1.4.1 Task Settings

Clicking the Edit... button in the Task Manager when a Cell Length Recording Task 55 is highlighted will display the task settings dialog. It has the following fields:

Name for this specific instance of the task. Also

used as part of the string in the first yellow box (type) in the <u>Trace Viewer</u> for data acquired using

this task.

Camera Select the source device for the transmitted light

images to be analyzed.

Full Description Describe the parameter being recorded (eg

"Length"). Used as the vertical axis label for data

displayed in scaled units.

Abbreviation Provide short hand notation for <u>Full Description</u> for

exported table headings.

Units Provide name of units for scaled length traces.

Units/Pixel Provide PScale factor used to convert from pixels

to units.

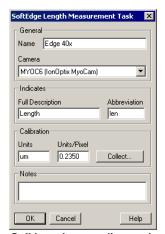
Collect... Run the <u>Video Calibration dialog</u> 127 to

automatically calculate the Units/Pixels from live

video.

Notes Enter any notes to yourself about this recording

task.



Cell length recording task settings dialog



Depending on the camera type selected, additional camera options for this task may be selected in the Global Sensor Settings Area 3 of the Parameters Dialog



If you change the name of an existing task all saved user limits and templates will be reset.

4.1.4.2 Experiment Settings

Selecting the first column of a cell length recording task [55] row in the Parameters 20 dialog will display the experiment settings group in the edit area 21h. The following fields can be edited:

Mode Select edge detection mode to use, see Edge

Options 59 section of the task tool bar for details.

Outside-in - Line Scan Mode Detect from edges towards center.

Inside-out - Line Scan Mode, Detect from center

towards edges.

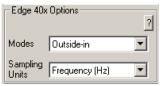
Box - Box Mode, Detect the movement of the edge based on the box method. When this option is selected an additional checkbox Average Lines becomes available which allows for averaging the

lines for the box method.

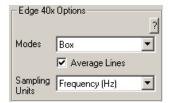
Sampling Units Chose how you want to enter sampling rates in

the cell length recording task epoch settings 57 group in the Parameters dialog edit area.

Frequency - Enter as Hertz. **Period** - Enter as seconds.



Cell Length Recording Task Line Scan Mode experiment settings edit area



Cell Length Recording Task Box Mode experiment settings edit area

4.1.4.3 Epoch Settings

Selecting any epoch column of a cell length recording task [55] row in the Parameters 20 dialog will display epoch settings group in the edit area 21 for the selected epoch. The edit area will let you select the following values:

Sampling frequency. (if "Frequency" selected in Frequency

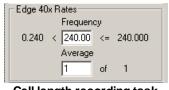
the experiment settings 57 column)

Period Sampling period. (if "Period" selected in the

experiment settings [57] column - not pictured)

Average At a given frequency/period there will be a

maximum number of points (displayed as the "of n") that can be collected. Select the number of samples to average into a single raw data point.



Cell length recording task gobal settings edit area



The minimum and maximum values for frequency or period are determined by the camera capabilities and settings

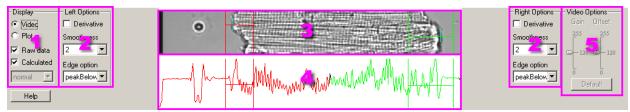


Frequency and period values are rounded to the nearest multiple of the pacer frequency when focus is moved away from the control. This effect is especially noticeable when the entered frequency approaches the maximum.

4.1.4.4 Tool Bar

Depending on the chosen **Mode** setting in the <u>Experiment Settings</u> 1571 the toolbar will appear either as shown in the **Line Scan Mode** or **Box Mode** version.

Line Scan Mode Toolbar



Line Scan Mode Tool Bar

The <u>Edge Detection Recording Task</u> *Line Scan Mode tool bar* provides an on-screen mechanism for visualizing the image that is being analyzed, to set dynamic detection options and to visually verify the detection results. It has the following main areas:

- Display options Determines what is displayed in the <u>Edge Detection Recording Task</u> Line Scan Mode tool bar.
- 2. Left and right edge detection options Presents options for creating calculated trace and threshold detection algorithms for left (green) and right (red) edges.
- 3. Video/Plot display Displays live video from selected camera with controls for left and right raw lines or oscilloscope-link display of detected left and right edge values.
- 4. Graph/Threshold display Displays live raw line intensity and/or calculated traces (depending on selected display options).
- 5. Video options Presents available parameters for current camera device selected in task settings. See the explanation at point 5 in the Box Subtraction Toolbar section <u>Video options</u>.

Display Options

VP 1.	D: 1 P		1 1	1 (
Video	Display II	ve images	and edge	selection	controls in

the Video/Plot area (#3).

Plot Display the detected edges in real time using an

oscilloscope-like display (not shown) in the Video/

Plot area (#3).

Raw data Display the raw line intensity data in black in the

Graph/Threshold area (#4).

Calculated Display the line intensity data after processing

(red=left, green=right) in the Graph/Threshold area

(#4).

Control Mode Select how the left (red) and right (green) edge

options are linked:

Normal - Left and right edge controls operate independently. The control is currently disabled because this is the only available option, which

will be selected as default.



Display options

Left/right edge detection options

Derivative Check to display derivative of calculated trace.

Smoothness Select the amount of smoothing applied by the calculation - higher numbers equal more smoothing.

Select how calculated trace is scanned to determine

Select how calculated trace is scanned to determine the final edge positions.

above - Edge is the first point within the detection limits where the value is above the threshold.

below - Edge is the first point within the detection limits where the value is below the threshold.

peakAbove - Edge is the maximum value of the first peak that crosses above the threshold. To be considered a peak, it must start below the threshold, cross above, then return below.

peakBelow - Edge is the minimum value of the first peak that crosses below the threshold. To be considered a peak, it must start above the threshold, cross below, then return above.

locked - Edge is fixed at the mid-point between the detection limits.



Left options

Video/Plot Area

Edge option

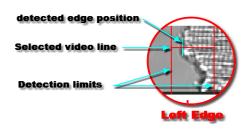
When "video" is selected in the <u>Display Options</u> group, a live video image will be displayed with edge controls. If the control mode is "normal" or "locked", separate left and right edge controls will be displayed (as shown). If the control mode is "single", a combined edge control will be display with two detected edge indicators. (not shown)

The edge control elements are used to select where in the image the edge detection takes place:

Selected video line - The location of the horizontal red and green selection lines determines the raw data lines to be used for edge determination. When the mouse is over the selection line, the cursor will change to a vertical double arrow. When the cursor is a vertical double arrow, you can click and drag the selection line to a new position.



Video display with edge controls

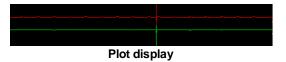


Edge control elements

Detection limits - The location of the vertical red and green limit lines determines the the range to be scanned to locate the edge. Scanning direction depends on which edge is being scanned and the edge mode selected in Experiment Settings [57] dialog. When the mouse cursor is over either of the vertical detection limits, the cursor will change to a horizontal double arrow. Click and drag to move the individual line. These lines continue down into the threshold area and can be adjusted from either display.

Detected edge position - This vertical mark will move along the selected video line to indicate the position of the detected edge on the actual image. See below for details.

When "plot" is selected in the <u>Display Options</u> group, a oscilloscope-like plot of the left (red) and right (green) detected edges is displayed. The horizontal line indicates the current data location and moves left-to-right every 5 seconds. This gives the user the opportunity to make sure the trace data looks good before starting the experiment.



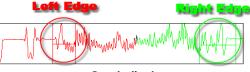
Graph/Threshold Area

The <u>Graph/Threshold</u> area displays the raw video intensity data as a black trace "beneath" the calculated intensity data in red and green (red=left and green=right). The <u>Edge Detection Options groups</u> control the parameters for how the calculated trace is created. The "Raw Data" and "Calculated" options in the <u>Display Options group</u> control which respective traces are displayed.

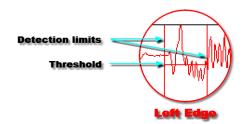
The threshold control elements are used to determine how the intensity trace from the video image line should be used to find the edge.

Detection limits - As with the <u>Video Display</u> area detection limits, these controls determine the area to be scanned to locate the edge. Scanning direction depends on which edge is being scanned and the edge mode selected in the <u>Experiment Settings</u> [57] dialog. These lines continue up into the video area and can be adjusted from either display.

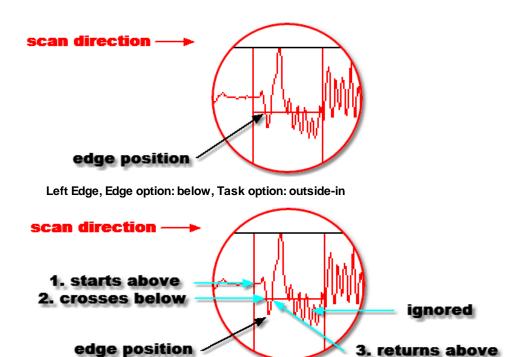
Threshold - The threshold is used to determine the intensity value that is used in the edge detection scanning algorithm. The pictures below show how the threshold is used with the two major edge and task options.



Graph display

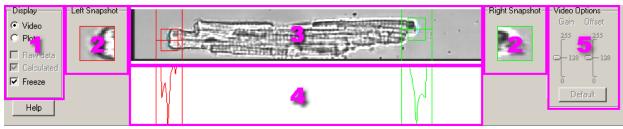


Threshold control elements



Left Edge, Edge option: peakBelow, Task Option: outside-in

Box Mode Toolbar



Box Mode Tool Bar

The <u>Edge Detection Box Subtraction Recording Task</u> Box Mode tool bar provides an on-screen mechanism for visualizing the image that is being analyzed, to set dynamic detection options and to visually verify the detection results.

Box Mode works by collecting a reference image, which is then compared to later images to that reference. Beyond moving the box over the area of the edge, no user threshold setting is required, which enables Box Mode to work with a Multicell Task experiment. The Box mode also has been shown to work better in cases where the object doesn't have a clear edge. The Edge Detection Box Subtraction Recording Task Box Mode tool bar has the following main areas:

- 1. Display options Determines what is displayed in the <u>Edge Detection Box Subtraction Recording Task</u> Box Mode tool bar.
- 2. Left and right edge snapshots Displays the current snapshot for the left and right edge.
- 3. Video/Plot display Displays live video from selected camera with controls for left and right controls or oscilloscope-link display of detected left and right edge values.
- 4. Graph display Displays live the calculated traces.

5. Video options - Presents available parameters for current camera device selected in task settings.

Display Options

Video Display live images and edge selection controls in

the Video/Plot area (#3).

Plot Display the detected edges in real time using an

oscilloscope-like display (not shown) in the Video/

Plot area (#3).

Raw data There is no raw data for the box subtraction mode.

This option is disabled.

 Calculated
 Display the box subtraction line from the current

snapshot taken (red=left, green=right) in the Graph area (#4). Only active option for the Box Subtraction

method.

Freezes the current image to allow easy selecting of

the left (red) and right (green) snapshots.



Display options

Left/right edge detection options

This area will show the current snapshot for the left and right boxes. A snapshot will be taken every time the red/green box is moved around with the mouse, or when any other adjustment is made to one of the controls. When used in combination with the Multicell Task 113 the controls will be disabled and the snapshot will be send from the Multicell Window.



Left snapshot

Video/Plot Area

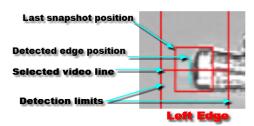
When "video" is selected in the <u>Display Options</u> *group,* a live video image will be displayed with edge box controls.

The edge box control elements are used to select where in the image the edge detection takes place:

Selected video line - The location of the horizontal red and green selection lines determines the raw data lines to be used for edge determination. When the mouse is over the selection line, the cursor will change to a vertical double arrow. When the cursor is a vertical double arrow, you can click and drag the selection line to a new position.



Video display with edge box controls



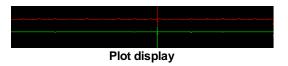
Edge box control elements

Detection limits - The location of the vertical red and green limit lines determines the the range to be scanned to locate the edge. When the mouse cursor is over either of the vertical detection limits, the cursor will change to a horizontal double arrow. Click and drag to move the individual line. These lines continue down into the threshold area and can be adjusted from either display.

Detected edge position - This vertical mark will move along the selected video line to indicate the position of the detected edge on the actual image.

Last snapshot position - Box indicating where the last snapshot was taken.

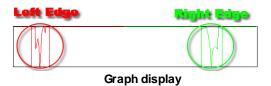
When "plot" is selected in the <u>Display Options</u> group, a oscilloscope-like plot of the left (red) and right (green) detected edges is displayed. The horizontal line indicates the current data location and moves left-to-right every 5 seconds. This gives the user the opportunity to make sure the trace data looks good before starting the experiment.



Graph Area

The <u>Graph</u> area displays the calculated box subtracted data in red and green (red=left and green=right).

Detection limits - As with the <u>Video Display</u> area detection limits, these controls determine the area to be scanned to locate the edge. These lines continue up into the video area and can be adjusted from either display.



Video Options Group

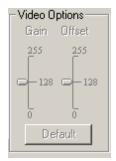
If supported by the camera selected in the <u>Task Setting [56]</u> dialog, the <u>Video Options</u> group will be displayed to allow you to change the image brightness (gain) and black level (offset).

Gain Increase brightness of the camera image

Offset Change the black level of the camera image



Gain and Offset are analog functions done before the video image is digitized. Setting Gain and Offset correctly will result in better images.



Video options

4.1.5 CytoMotion Experiment Task

The CytoMotion experiment task detects the contrast between a selected reference image and the live image stream. The task produces on or two raw data traces, depending on the chosen methods.

The CytoMotion experiment task uses information entered in the following places:

- <u>Task settings</u> [65] Settings that apply to all experiments using this task, entered in the <u>Task</u> <u>Manager</u> [13] *dialog*.
- Experiment settings 65 Settings for this task that apply to all epochs in an experiment, entered in the Parameters 20 dialog.
- Epoch settings [66] Settings for this task for a specific epoch in an experiment, entered in the Parameters [20] dialog.
- Global Sensor Settings Area 23 Experiment configuration of camera parameters for some cameras (see documentation for selected camera for details), entered in the Parameters 20 dialog.
- Tool bar 67 Real-time display of images and control of the region of interest (ROI) on which the CytoMotion measurement will be executed..

Task Options

The CytoMotion experiment task experiment settings 65 allows you to choose the CytoMotion method and also provides additional options when a Multi Cell Task 113 is present.

Task Output

The CytoMotion experiment task produces the following output in lonWizard, where "Name" is the description entered in the CytoMotion Experiment Task [65] dialog. The

output depends on the selected *CytoMotion Methods* in the <u>Experiment settings</u> 65°. It can be one or two of the following raw traces:

"Name-PixelIntensity" trace The pixel intensity at the selected region from the selected camera device.

"Name-PixelCorrelation" trace A correlation algorithm calculates the contrast at the selected region from the selected camera device.



See IonWizard documentation for details on viewing traces

4.1.5.1 Task Settings

Clicking the Edit... button in the Task Manager 13 when a CytoMotion Experiment Task 64 is highlighted will display the task settings dialog. It has the following fields:

Name Name for this specific instance of the task. Also

used as part of the string in the first yellow box (type) in the <u>Trace Viewer</u> for data acquired using

this task.

Camera Select the source device for the transmitted light

images to be analyzed.

Full Description Describe the parameter being recorded (eg

"CytoMotion"). Used as the vertical axis label for

data displayed in scaled units.

Abbreviation Provide short hand notation for <u>Full Description</u> for

exported table headings.

Units Provide name of units for the Units to Pixel ratio.*

Units/Pixel Provide Scale factor used to convert from pixels to

units.*

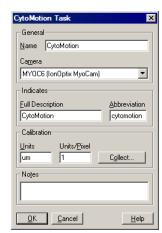
Collect... Run the <u>Video Calibration dialog</u> 127 to

automatically calculate the Units/Pixels from live

video

NotesEnter any notes to yourself about this recording

task.



CytoMotion experiment task settings dialog

* The Units and Units/Pixel fields are only used in combination with a Multi Cell Task 13, but need to be entered at all times.



Depending on the camera type selected, additional camera options for this task may be selected in the Global Sensor Settings Area 3 of the Parameters Dialog



If you change the name of an existing task all saved user limits and templates will be reset.

4.1.5.2 Experiment Settings

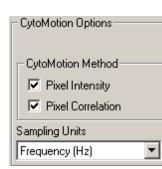
Selecting the first column of a CytoMotion experiment task [64] row in the Parameters [20] dialog will display the experiment settings group in the edit area [21]. The following fields can be edited:

CytoMotion Method

Select the CytoMotion methods which should be used. At least one has to be selected at all times.

Pixel Intensity - Average of the difference between all pixels of the captured reference frame against the current live image. **Pixel Correlation** - The correlation algorithm is used to calculate the correlation between the captured reference frame

and the current live image.



CytoMotion experiment experiment settings edit

Optional If you add a Multi Cell Task 113 into the same experiment these

additional options will be available:

Reference Frame Method - select the method how to determine the reference frame. Currently only the Max Relative

Correlation method is available.

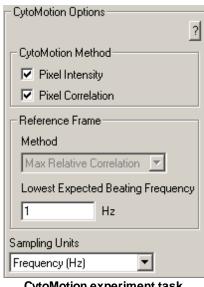
Lowest Expected Beating Frequency - minimum expected contraction frequency of the sample, used to find the best

reference frame.

Sampling Units Chose how you want to enter sampling rates in the <u>epoch</u>

settings 66 dialog.

Frequency - Enter as Hertz. **Period -** Enter as seconds.



CytoMotion experiment task experiment settings edit area when a Multi Cell task is present in the experiment

4.1.5.3 Epoch Settings

Selecting any epoch column of a CytoMotion experiment task [64] row in the Parameters [20] dialog will display epoch settings group in the edit area [21] for the selected epoch. The edit area will let you select the following values:

Frequency Sampling frequency. (if "Frequency"

selected in the experiment settings 65

column)

Period Sampling period. (if "Period" selected in

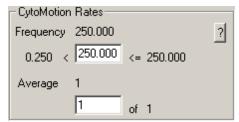
the experiment settings 65 column - not

pictured)

Average At a given frequency/period there will be a

maximum number of points (displayed as the "of n") that can be collected. Select the number of samples to average into a

single raw data point.



CytoMotion experiment task epoch settings edit area

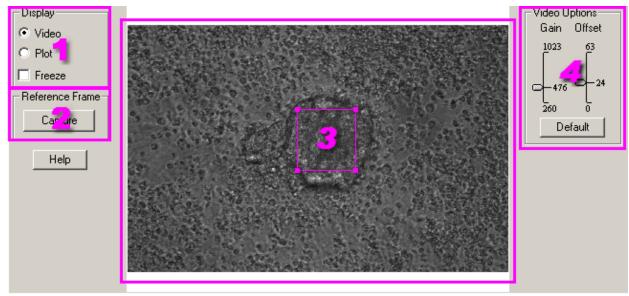


The minimum and maximum values for frequency or period are determined by the camera capabilities and settings



Frequency and period values are rounded to the nearest multiple of the pacer frequency and displayed above the input box. When focus is moved away from the control this value is moved into the input control.

4.1.5.4 Tool Bar



CytoMotion experiment task tool bar

The <u>CytoMotion Experiment Task</u> *tool bar* provides an on-screen mechanism for visualizing the image that is being analyzed, to set dynamic detection options and to visually verify the detection results. It has the following main areas:

- 1. Display options Determines what is displayed in main *area* (#3) of the <u>CytoMotion Measurement Task</u> tool bar.
- 2. Reference Frame Capture the reference frame.
- 3. Video/plot area Display live video with the region of interest controls or a live plot.
- 4. Video options Adjust parameters for current camera device selected in task settings, if any.

Display Options

Video Display live images and region *control* in the video/plot

area (#3).

Plot Plot the current result (#3).

Freezes the live image stream (#3) to make it easier to

capture a reference frame (#2).



Display options

Reference Frame Option

Capture Captu

Capture a reference frame to be used in the selected CytoMotion method. It will be disabled when a Multi Cell Task 13 is present and the experiment has been started, the Multi Cell Task 13 will capture and set the reference frame when needed. A reference frame will also be captured when the region of interest is moved or changed in size. It will capture a reference frame only when the mouse button is released.



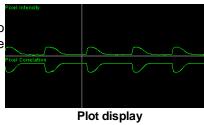
Reference Frame Options

Video/Plot Area

When "video" is selected in the <u>Display Options</u> *group*, a live video image will be displayed with region *control*. The image within the region is being used within the selected CytoMotion methods. When this region is moved or resized a new reference frame will be captured. The display won't be updated when the *Freeze* option in the <u>Display Options</u> is enabled.

Region control

When "plot" is selected in the <u>Display Options</u> *group*, an oscilloscope-like plot of the selected CytoMotion methods will be displayed. This function allows the user to preview trace data and ensure that it looks good before starting the experiment. The vertical indicates the current data location and moves left-to-right every 5 seconds.



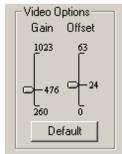
Video Options Group

If supported by the camera selected in the <u>Task Setting [65]</u> dialog, the <u>Video</u> <u>Options</u> group will be displayed to allow you to change the image brightness (gain) and black level (offset).

Gain Increase brightness of the camera image

Offset Change the black level of the camera image

Default Reset the Gain and Offset to the default camera values







Gain and Offset are analog functions done before the video image is digitized. Setting Gain and Offset correctly will result in better images.

4.1.6 Sarcomere Spacing Recording Task

The sarcomere spacing recording task analyzes images from a video device to find the average intersarcomere spacing within a user defined section. The task produces one raw data trace, average sarcomere spacing in pixels, and provides the ability to view the data in calibrated units using a user-supplied scaling factor.

The sarcomere spacing recording task uses information entered in the following places:

- <u>Task settings</u> 70] Settings that apply to all experiments using this task, entered in the <u>Task</u> <u>Manager</u> 13] *dialog*.
- Experiment settings 70 Settings for this task that apply to all epochs in an experiment, entered in the Parameters 20 dialog.
- Epoch settings 70 Settings for this task for a specific epoch in an experiment, entered in the Parameters 20 dialog.
- Global Sensor Settings Area 3 Experiment configuration of camera parameters for some cameras (see documentation for selected camera for details), entered in the Parameters 2 dialog.
- Tool bar 71 Real-time display of images and control of sarcomere spacing detection settings such as the region of interest and the detection limits.

Task Output

The sarcomere spacing recording task produces the following output in lonWizard where "*Name*" is the description entered in the <u>Sarcomere Spacing Recording Task</u> 70 dialog:

"Name-Pixels" traces Average sarcomere spacing for region of interest in pixels.

"Name-Length" traces Average sarcomere spacing in user-specified units.

Algorithm Notes

The sarcomere spacing recording task uses the Fast Fourier Transform (FFT) to determine the average sarcomere spacing of the region of interest (ROI) in one or more lines of a video image. The FFT is a calculation that inputs some waveform and outputs a power spectrum trace which shows the relative contribution of every frequency in that waveform. For example, if the waveform were a simple sine wave with a frequency of 1 Hz, the resulting power spectrum would have a single spike at 1 Hz. Because the striation pattern on the myocyte is fairly regular in frequency, a strong spike is created at that frequency in the power spectrum trace. Once the frequency of the spike is determined, a simple inversion results in the average spacing (1/frequency=period or length).

Since the sarcomere spacing recording task is using the FFT, there are a couple of FFT-related things that should be understood. First, in order to minimize processing artifacts, the original intensity trace data is multiplied by a Hamming window, which is a cosine function that decreases the intensity of the video images at the edges. The "windowed" trace (blue) in the Graph/Limits Tri area of the Tool Bar Tri is displayed to remind the user that the edges of the ROI carry very little weight in determining the final measurement.



If possible, always extend the ROI approximately 30% beyond the edges of "real" image data so that as much real data as possible is in the significant section of the Hamming window.

Second, the resulting FFT power spectrum traces show the **RELATIVE** contribution of frequencies in the original trace and therefore have no vertical units. This means that when displaying the power spectrum trace, the program has to determine the vertical scale based on the power spectrum data. One problem with this is that the first data point in the power spectrum represents the DC offset (technically 0Hz contribution) and is significantly higher than the other "real" data. To handle this problem, only a user-specified area of the power spectrum trace is scanned to determine vertical scaling. The detection limits are set using the green lines in the <u>Graph/Limits</u> 3 area of the <u>Tool Bar</u> 3.



You MUST move the left detection limit line far enough to the right so that the large spacing (low-frequency) values are not included when calculating the power spectrum vertical scale.

Finally, it should be noted that most discussions of the FFT power spectrum trace refer to the resulting frequencies not the resulting lengths as the frequency spectrum is the direct output. However, since the length is simply 1/frequency, they are directly related.



Because the FFT outputs frequencies from low to high and length is 1/frequency, sarcomere lengths are from high to low. This means that the maximum sarcomere spacing limit is on the LEFT and the minimum sarcomere spacing limit is on the RIGHT.

4.1.6.1 Task Settings

Clicking the Edit... button in the Task Manager when a Sarcomere Spacing Recording Task 8 is highlighted will display the task settings dialog. It has the following fields:

Name for this specific instance of the task. Also

used as part of the string in the first yellow box (type) in the <u>Trace Viewer</u> for data acquired using

this task.

Camera Select the source device for the transmitted light

images to be analyzed.

Full Description Describe the parameter being recorded (eg

"Length"). Used as the vertical axis label for data

displayed in scaled units.

Abbreviation Provide short hand notation for <u>Full Description</u> for

exported table headings.

Units Name of units for scaled length traces.

Units/Pixel PScale factor used to convert from pixels to units.

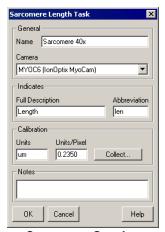
Collect... Run the <u>Video Calibration dialog</u> 127 to

automatically calculate the Units/Pixels from live

video

Notes Enter any notes to yourself about this recording

task.



Sarcomere Spacing Recording Task settings dialog



Depending on the camera type selected, additional camera options for this task may be selected in the Global Sensor Settings Area 3 of the Parameters Dialog



If you change the name of an existing task all saved user limits and templates will be reset.

4.1.6.2 Experiment Settings

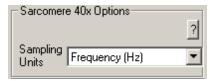
Selecting the first column of a <u>Sarcomere Spacing Recording Task [68]</u> row in the <u>Parameters [20]</u> dialog will display the experiment settings *group* in the <u>edit area [21]</u>. The following fields can be edited:

Sampling Units Choose how you want to enter sampling rates

in the <u>Sarcomere Spacing Recording Task</u>
<u>epoch settings</u> 70 group in the <u>Parameters</u>

dialog.

Frequency - Enter as Hertz. **Period -** Enter as seconds.



Sarcomere spacing recording task gobal settings edit area

4.1.6.3 Epoch Settings

Selecting any epoch column of a <u>Sarcomere Spacing Recording Task [68]</u> row in the <u>Parameters [20]</u> dialog will display the epoch settings *group* in the <u>edit area [21]</u>. The edit area will let you select the following values:

Frequency Sampling frequency (if "Frequency"

selected in the experiment settings 70

column).

Period Sampling period (if "Period" selected in

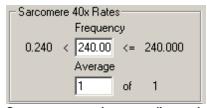
the <u>experiment settings</u> 70 column -

not pictured).

Average At a given frequency/period there will be

a maximum number of points (displayed as the "of n") that can be collected. Select the number of samples to average into a single raw

data point.



Sarcomere spacing recording task gobal settings edit area

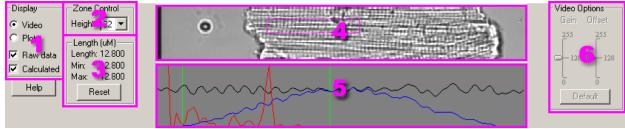


The minimum and maximum values for frequency or period are determined by the camera capabilities and settings.



Frequency and period values are rounded to the nearest multiple of the pacer frequency when focus is moved away from the control. This effect is especially noticeable when the entered frequency approaches the maximum.

4.1.6.4 Tool Bar



Sarcomere Spacing Recording Task Tool Bar

The <u>Sarcomere Spacing Recording Task</u> *tool bar* provides an on-screen mechanism for visualizing the image that is being analyzed, to set dynamic detection options and to visually verify the detection results. It has the following main areas:

- 1. Display options Determines what is displayed in the Sarcomere Spacing Recording Task tool bar.
- 2. Zone options Set the zone height.
- 3. Current length Shows value for current sarcomere length in scaled units
- 4. Video/Plot display Display live video from selected camera with region of interest control for current zone or oscilloscope-like display of sarcomere spacing.
- 5. Graph/Limits display Displays live raw line intensity and/or resulting FFT power spectrum trace (depending on selected display options) as well as control of detection limits.
- 6. Video options Select available parameters for current camera device selected in task settings.

Display Options Group

Video Display live images from the selected camera in real

> time along with the control for selecting the part of the image to scan for the sarcomere spacing in the

Video/Plot area (#4).

Plot Display the average sarcomere length using an

oscilloscope-like display (not shown) in the Video/

Plot area (#4).

Raw data Display the raw line intensity trace (black) and the

windowed intensity trace (blue) in the Graph/Limits

area (#5).

Calculated Display the FFT power spectrum trace (red) in the

Graph/Limits area (#5).



Display options

Zone Options Group

Height Display current zone height or change to specific

value.



Zone options

Video/Plot Area

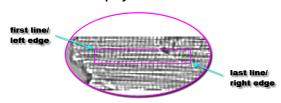
When "video" is selected in the Display Options group a live video image will be displayed with the ROI. The ROI control selects the area of the video image to process.

When the mouse cursor is inside the zone it will turn into a hand and you can move the entire box by clicking and dragging. When the mouse cursor is over the edges the cursor will change to a horizontal or vertical double arrow which will allow you to click and drag the edge.

When "plot" is selected in the <u>Display Options</u> group a oscilloscope-like plot of sarcomere spacing (green) is displayed. The horizontal line indicates the current data location and moves leftto-right every 5 seconds.



Video display with zone control



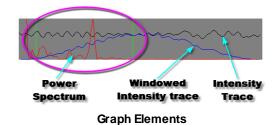
Sarcomere zone control

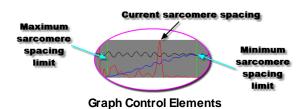
Graph/Limits Area

The <u>Graph/Limits</u> area displays the raw video intensity trace in black, a windowed intensity trace in blue and the resulting FFT power spectrum in red. The "Raw Data" and "Calculated" options in the <u>Display Options</u> group control whether the respective traces are displayed.

Min and Max sarcomere spacing limits -

These limit lines select the portion of the FFT power spectrum (red) used to scale the display and locate the peak frequency. The average sarcomere spacing is computed from the frequency with the maximum value within these limits.







Depending on the camera type selected, additional camera options for this task may be selected in the <u>Global Sensor Settings Areal 23</u> of the <u>Parameters</u> dialog.

Video Options

If supported by the camera selected in the <u>Task Setting</u> ⁷⁰ dialog, the <u>Video Options</u> group will be displayed to allow you to change the image brightness (gain) and black level (offset).

Gain Increase brightness of the camera image

Offset Change the black level of the camera image



Gain and Offset are analog functions done before the video image is digitized. Setting Gain and Offset correctly will result in better images.



Video options

4.1.7 Vessel Dimension Recording Task

The vessel dimension recording task analyzes video images to find the the characteristics of a vessel wall at up to four separate locations in the video image. The task collects raw pixel position of the left and right inner and/or outer wall positions. In addition to the raw data display, the task provides the ability to view the data in calibrated units using a user-supplied scaling factor and to perform addition calculations such as wall thickness, cross-sectional area and media/lumen ratio.

The vessel dimension recording task uses information entered in the following places:

- <u>Task settings</u> ⁷⁵ Settings that apply to all experiments using this task, entered in the <u>Task</u> Manager ¹³ dialog.
- Experiment settings 75 Settings for this task that apply to all epochs in an experiment, entered in the Parameters 20 dialog.
- Epoch settings 76 Settings for this task for a specific epoch in an experiment, entered in the Parameters 20 dialog.
- Global Sensor Settings Area 23 Experiment configuration of camera parameters for some cameras (see documentation for selected camera for details), entered in the Parameters 20 dialog.

• Tool bar 76 - Real-time display of images and control of cell length detection settings such as video line to analyze and the detection threshold, entered in the tool bar 35.

Task Options

The vessel dimension recording task <u>experiment settings</u> [75] allow you to select which raw traces are collected and configure which optional calculated traces will be shown.

Task Output

The vessel dimension recording task produces the following output in lonWizard, where "Name" is the description entered in the Vessel Dimension Recording Task 75 dialog:

"Name-Pixels" traces Raw data traces collected from the selected camera device:*

Inside Left - inside left edge pixel position
Inside Right - inside right edge pixel position
Outside Left - outside left edge pixel position
Outside Right - outside right edge pixel position

Inside Left - inside left edge position in scaled units
Inside Right - inside right edge position in scaled units
Outside Left - outside left edge position in scaled units
Outside Right - outside right edge position in scaled units

Lumen Diameter - inside diameter in scaled units

Vessel Diameter - outside diameter in scaled units

Left Wall Thickness - left wall thickness in scaled units

Right Wall Thickness - right wall thickness in scaled units

Average Wall Thickness - average wall thickness in scaled units

Cross Sectitonal Area - T.B.D. Media/Lumen Ratio - T.B.D.

^{*} Actual traces that you will see depend on options selected in the experiment settings 75.



See IonWizard documentation for details on viewing traces

4.1.7.1 Task Settings

Clicking the Edit... button in the Task Manager 3 when a Cell Length Recording Task 3 is highlighted will display the task settings dialog. It has the following fields:

Name Name for this specific instance of the task. Also

used as part of the string in the first yellow box (type) in the <u>Trace Viewer</u> for data acquired using

this task.

Camera Select the source device for the transmitted light

images to be analyzed.

Full Description Describe the parameter being recorded (eg

"Length"). Used as the vertical axis label for data

displayed in scaled units.

Abbreviation Provide short hand notation for <u>Full Description</u> for

exported table headings.

Units Provide name of units for scaled length traces.

Units/Pixel Provide Scale factor used to convert from pixels to

units.

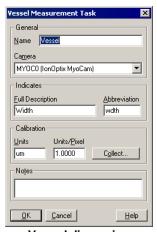
Collect... Run the <u>Video Calibration dialog</u> 127 to

automatically calculate the Units/Pixels from live

video

NotesEnter any notes to yourself about this recording

task.



Vessel dimension recording task settings dialog

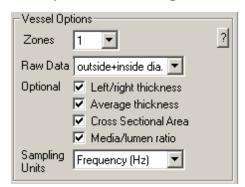


Depending on the camera type selected, additional camera options for this task may be selected in the Global Sensor Settings Area [23] of the Parameters Dialog



If you change the name of an existing task all saved user limits and templates will be reset.

4.1.7.2 Experiment Settings



Vessel Dimension Recording Task experiment settings edit area

Selecting the first column of a <u>cell length recording task [73]</u> row in the <u>Parameters [20]</u> dialog will display the experiment settings *group* in the <u>edit area [21]</u>. The following fields can be edited:

Zones Select the number of separate vessel measurements to collect (1-4)

Raw Data Select what raw data traces you would like to collect:

inside diameter - collect left and right inside position
outside diameter - collect left and right outside position

outside+inside dia. - collects both

Optional If you select "outside+inside dia" in the <u>Raw Data</u> option you can also select

which additional calculated traces will be available.

Left/right thickness - calculates thickness of left and right walls. **Average thickness -** calculates average of left/right thickness. **Cross Sectional Area -** calculates the area the vessel wall.

Media/lumen ratio - calculates ratio of the outer (media) and inner (lumen) ratio

Sampling Units Chose how you want to enter sampling rates in the epoch settings 76 dialog.

Frequency - Enter as Hertz.

4.1.7.3 Epoch Settings

Selecting any epoch column of a <u>vessel dimension recording task</u> [73] *row* in the <u>Parameters</u> [20] *dialog* will display <u>epoch settings</u> *group* in the <u>edit area</u> [21] for the selected epoch. The edit area will let you select the following values:

Frequency Sampling frequency. (if "Frequency"

selected in the experiment settings 75

column)

Period Sampling period. (if "Period" selected in

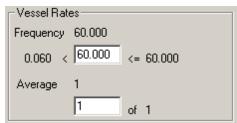
the <u>experiment settings</u> 75 column - not

pictured)

Average At a given frequency/period there will be a

maximum number of points (displayed as the "of n") that can be collected. Select the number of samples to average into a

single raw data point.



Vessel dimension recording task gobal settings edit area

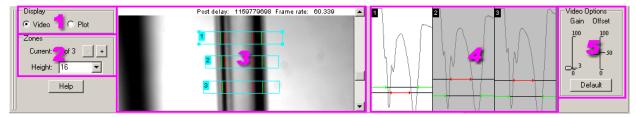


The minimum and maximum values for frequency or period are determined by the camera capabilities and settings



Frequency and period values are rounded to the nearest multiple of the pacer frequency and displayed above the input box. When focus is moved away from the control this value is moved into the input control.

4.1.7.4 Tool Bar



Vessel Dimension Recording Task Tool Bar

The <u>Vessel Dimension Recording Task</u> *tool bar* provides an on-screen mechanism for visualizing the image that is being analyzed, to set dynamic detection options and to visually verify the detection results. It has the following main areas:

- Display options Determines what is displayed in main area (#3) of the <u>Vessel Dimension Recording</u> <u>Task</u> tool bar.
- 2. Zone control Select current zone or set specific height of current zone
- 3. Video/plot area Display live video with zone controls or a live plot of current zone.

- 4. Zone graphs/thresholds Displays graphs of video data in each zone and control for inside and/or outside thresholds.
- 5. Video options Adjust parameters for current camera device selected in task settings, if any.

Display Options

Video Display live images and zone position

controls in the video/plot area (#3).

Plot Display the position of all edges for the

current zone in the video/plot area (#3).



Display options

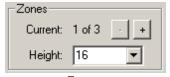
Zones Options

Current Use the plus and minus *buttons* to move

between zones, if there are more than one.

Height Select a specific height for the current

zone.



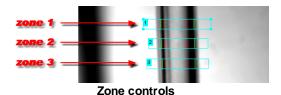




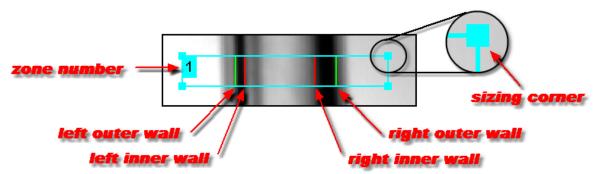
All lines in a zone are averaged to create the intensity trace used to scan for the wall locations. More lines will lessen the effect of artifacts such as blood and fat within the zone.

Video/Plot Area

When "video" is selected in the <u>Display Options</u> group, a live video image will be displayed with one to four zone *controls*. The exact number of zones that are available is set in the task <u>Experiment Settings</u> [75]. Each zone consists of a cyan box that indicates the area of the image that is being used to calculate the vessel dimensions.



In addition to the zone border there are the following addition elements:



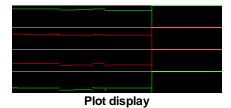
Zone control elements

Sizing corners - For the current zone only there are small boxes drawn on each corner of the zone border. These indicate that the zone can be resized by grabbing those boxes or the actual border lines of the zone. When you move the cursor over a sizing *element* the cursor will change into a two-headed arrow.

Zone number - The zone number is displayed in a solid cyan box in anchored to the upper-left corner of the *control*. If the height of the zone is small the box may extend below the border of the zone so that the entire number can be read.

Detected wall positions - The current inner and outer wall positions are indicated by red and green vertical lines that move within the zone rectangle. The green lines show the position of the outer walls and the red lines show the inner walls. You can select whether the inner, outer or both inner and outer positions are calculate in the task Experiment Settings 75

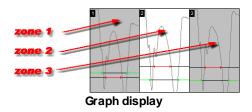
When "plot" is selected in the <u>Display Options</u> *group*, a oscilloscope-like plot of the all wall positions in the current zone. This function allows the user to preview trace data and ensure that it looks good before starting the experiment. The vertical indicates the current data location and moves left-to-right every 5 seconds. The number of plots displayed is dependent on <u>Raw Data</u> *option* selected in the task <u>Experiment Settings</u> 75 - Two plots will be displayed for inside or outside only and four plots will be displayed for inside+outside. The order of the plots from top to bottom is left outside, left inside, right inside, right outside.

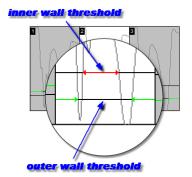


Zone Graph/Threshold Area

The Zone Graph/Threshold area displays a graph of the average intensity of all lines in each zone along with inner and outer threshold levels controls. The threshold controls allow you to tune where the vessel walls are "found" by the detection algorithm. The current zone, as set in the Zone Control area, is drawn with a white background while the others are drawn in gray.

The results of the detection algorithm are displayed on the corresponding threshold lines. For the outer wall the "outside" parts of the lines are drawn in green and for the inside wall the "inside" of the line is drawn in red. In addition there are small arrow heads that point in the direction the line was scanned. The tips of the arrows will point to the video intensity trace at the point where it crosses the threshold where the edge was found.





Threshold control elements

Video Options Group

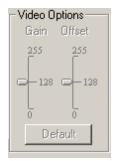
If supported by the camera selected in the <u>Task Setting [75]</u> dialog, the <u>Video Options</u> group will be displayed to allow you to change the image brightness (gain) and black level (offset).

Gain Increase brightness of the camera image

Offset Change the black level of the camera image



Gain and Offset are analog functions done before the video image is digitized. Setting Gain and Offset correctly will result in better images.



Video options

4.1.8 Vessel Flow Characteristics Recording Task

The Vessel Flow Characteristics Recording Task provides the ability to display one or more of the following vessel-centric traces:

- Mean Pressure
- Vascular Resistance
- Shear Stress
- Reynolds Number

The Vessel Flow Characteristics Recording Task uses information entered in the following places:

- Task settings 81 Settings that apply to all experiments using this task, entered in the Task Manager 13 dialog.
- Experiment settings [85] Settings for this task that apply to all epochs in an experiment, entered in the Parameters 20 dialog.
- Epoch settings 86 Settings for this task for a specific epoch in an experiment, entered in the Parameters 20 dialog.

Task Options

The vessel flow characteristics recording task experiment settings [85] allow you to select which raw traces are collected and configure which optional calculated traces will be shown.

Task Output

The vessel flow characteristics recording task produces the following traces in lonWizard, where "Name" is the description entered in the Task Settings 81 dialog. The actual traces that you will see depend on options selected in the Task Settings 81 and Experiment Settings 85.

"Name-Raw Inputs" traces

Raw data traces collected by Data Sources 83 that are defined using a hardware sensor.

Flow - flow rate

Diameter - vessel diameter Inlet Pressure - inlet pressure Outlet Pressure - outlet pressure

"Name-Inputs" traces "Raw Inputs" trace(s) scaled to calibrated units specified in Data Sources [৪3] group of Task Settings 81 PLUS area if diameter trace does not have zones

Flow - flow rate

Diameter - vessel diameter Inlet Pressure - inlet pressure Outlet Pressure - outlet pressure

Area - calculated from "Diameter" task output OR from "Diameter" raw input trace.

"Name-Inputs (zones)" traces

Calculated trace when diameter traces can have more than one zone Area - calculated from "Diameter" task output

"Name-Inputs (iunit)" traces

(hidden by default) All "Inputs" traces converted to intermediate units.

"Name-Inputs (iunit, zones)" traces*

(hidden by default) All "Inputs (zones)" traces converted to intermediate units.

"Name-Inputs (interpolated iunit)" traces*

(hidden by default) All "Inputs (iunit)" traces interpolated to the same times as the master

"Name-Inputs (interpolated iunit, zones)" traces*

(hidden by default) All "Inputs (iunit, zones)" traces interpolated to the same times as the master

"Name-Outputs (iunit)" traces*

(hidden by default) Results of calculations for all traces without zones selected in the Experiment Settings 1851 "Traces To Collect" group in intermediate units.

Flow Velocity - flow rate divided by lumen area

Wall Shear Stress - calculation of frictional drag exerted on arterial walls during flow

Vascular Resistance - calculation of force opposing the movement of solution through a vessel

Reynolds Number - calculation that describes whether the flow is either turbulent or laminar

Mean Pressure - average inlet and outlet pressure

Pressure Differential - difference between inlet and outlet pressure.

"Name-Outputs (iunit, zones)" traces*

(hidden by default) Results of calculations for all traces with zones selected in the Experiment Settings [85] "Traces To Collect" group in intermediate units Wall Shear Stress - calculation of frictional drag exerted on arterial walls during flow

Vascular Resistance - calculation of force opposing the movement of solution through a vessel

Reynolds Number - calculation that describes whether the flow is either turbulent or laminar

Reynolds Number -

"Name-Outputs" traces

All "Name-Output (iunit)" traces converted to output units specified in the <u>Task</u> <u>Settings</u>[81] <u>Unit Conversions</u>[84] *sub-dialog*

"Name-Outputs (zones)" traces

All "Name-Output (iunit,zones)" traces converted to output units specified in the Task Settings 81 Unit Conversions 84 sub-dialog

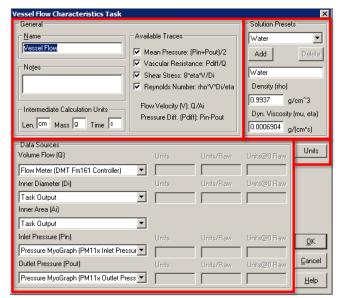


Refer to "Showing and Hiding Trace Bars" in the IonWizard documentation for details on viewing hidden traces

Vessel Characteristics

The vessel flow characteristics recording task produces the following traces in lonWizard, where "Name" is the description entered in the

4.1.8.1 Task Settings



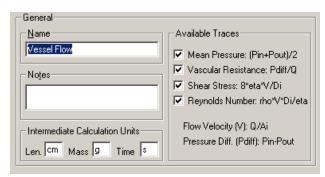
Vessel Flow recording task settings

Clicking the Edit... button in the Task Manager (13) when a Vessel Flow Characteristics Recording Task (79) is highlighted will display the task settings dialog. There are three major sections in the task settings dialog that are detailed below. In addition there is a Unit Conversions (84) sub-dialog accessed from the Units... button.



Each Trace lists the formula used to calculate the value based on the symbols defined in parenthesis after the cooresponding trace, preset or data source name.

General



Vessel Flow General Settings

The general section has the following fields:

Name Name for this specific instance of the task. Also used as part of the string in

the first yellow box (type) in the <u>Trace Viewer</u> for data acquired using this

task.

Notes Enter any notes to yourself about this recording task.

Intermediate Calculation Units All raw traces must be converted to a common set of intermediate units in order perform the calculations. Specify the units for Length (Len.), Mass and Time.

Available Traces

The first items select which traces will be available and determines which Data Sources 3 will be required:

Mean Pressure - requires Inlet Pressure and Outlet Pressure data sources **Vascular Resistance** - requires Volume Flow, Inlet Pressure and Outlet Pressure data sources

Shear Stress - requires Volume Flow, Inner Diameter and Inner Area data sources

Reynolds Number - requires Volume Flow, Inner Diameter and Inner Area data sources

The last two items indicate which additional available traces are automatically available based traces selected above:

Flow Velocity - available if Shear Stress or Reynolds Number trace is selected

Pressure Diff - available if Mean Pressure or Vascular Resistance trace is selected



The selection of intermediate units is personal preference. The samples shown use "cgs" units (i.e., centimeters, grams and seconds) as the intermediate units as most of the output values taken from the literature use cgs standards, such as dynes.



If you change the name of an existing task all saved user limits and templates will be reset.

Solution Presets

The <u>Presets</u> *group* allows you to define an arbitrary number of solutions that can be selected in the <u>Experiment Settings</u> to define the density and viscosity parameters required for the flow calculations.

Name (*drop down*) The name drop down allows you to

select from the list of previously created solution presets. When you select a new value the remaining fields in the <u>Presets</u> *group*. are loaded with values

that were previous entered.

Add Makes a copy of the current solution

which you can then edit using the remaining *controls* in the <u>Presets</u> *group*.

Delete Deletes the current solution and selects

the previous one for editing. Disabled if

there is only one preset defined.

Name (edit) Edit the name of the current solution.

Changes made here automatically appear in the **Name** *drop-down field* as

well.

Density Enter the density for the named

solution.

Dyn. Viscosity Enter the Dynamic Viscosity for the

named solution.



Vessel Flow Solution Presets

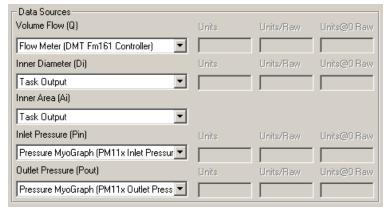


The units for the solution values are set in the <u>Intermediate Calculation Units</u> group in the <u>General</u> group



The values shown are for water at 37°C

Data Sources



Vessel Flow Data Sources

The Data Source group allow you to define the source of the data for each calculation parameter.

The first column lists the possible sources for each of the parameters. The specific parameter name is listed above the source *drop-down list*. The first choice, "Task Output" allows you to use any trace in any recording task in the current experiment (the specific trace is selected in Experiment Settings) 85.

For "Inner Area (Ai)" the other choice is "Calculate from diameter", for all other data sources the remaining choices are a list of all compatible sensors

in the current hardware tree.

Units Calibrated units for the selected source, e.g. Temperature, flow, etc...

Disabled when the sensor provides this information.

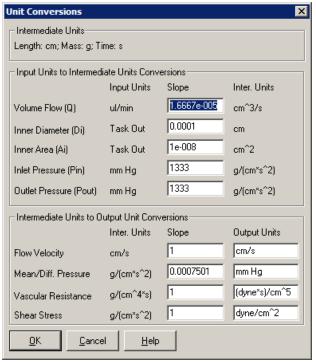
Units/Raw Scale factor to convert raw sensor values (e.g. volts) to calibrated units

specified in the **Units** field. Disabled when sensor provides this information.

Units@0Raw Calibrated units value to return raw sensor value is 0. Disabled when sensor

provides this information.

4.1.8.1.1 Unit Conversions



Vessel Flow Solution Presets

The Unit Conversions sub-dialog, accessed by pressing the <u>Units...</u> button in the <u>Task Settings</u> 81 dialog, provides the ability to set the conversion factors used to process the raw sources and provide the results in the desired output units.

Intermediate Units

This section shows the values entered in the main task settings dialog for reference.

Input Units to Intermedite Units Conversions

Specify the conversion factor (slope) need to convert the specified input units to the specified intermediate units (Inter. Units). If the input units are listed as "Task Out" you will need determine the actual input units yourself from the task that you select in the Experiment Settings 85.

Intermediate Units to Output Unit Conversions

This section allows you to convert the calculation results from intermediate units to any other unit desired. Enter the scale factor (**slope**) and the name for the **Ouptut Units** for each output trace.



Vascular Resistance is commonly reported in either $(dyne^*s)/cm^5$ or $(MPa^*s)/m^3$. 1 $(dyne^*s)/cm^5 = 0.1 (MPa^*s)/m^3$



If you want use the intermediate units as the output units enter a slope of 1 and type the inter. units values into the output units field.

4.1.8.2 Experiment Settings

Selecting the first column of a <u>Vessel Flow Characteristics Recording Task [79]</u> row in the <u>Parameters [20]</u> dialog will display the <u>experiment settings</u> group in the <u>edit area [21]</u>. The following fields can be edited:

Sampling Units Chose how you want to enter

sampling rates in the **Epoch**

Settings 86 dialog.

Frequency - Enter as Hertz. **Period -** Enter as seconds

Traces To Collect Check any optional traces that you

want to collect with this data.

Master Rate Select what data trace will provide

the output times for the calculated data. Choices are **Flow, Diameter,**

Inlet Pressure and Outlet

Pressure.

Solution Preset Select the solution preset to use

from the list defined in the <u>Solution</u>

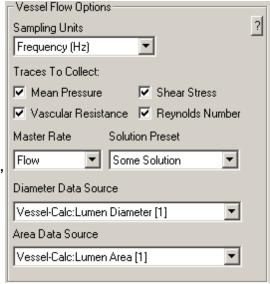
<u>Presets [82]</u> group of the <u>Task</u>

Settings 81 dialog.

Task Data Sources Select the specific task output trace for each "Task Output" data source

for each "Task Output" data source defined in the Task Settings 81

dialog.



Vessel Flow Recording Task experiment settings edit area



All output traces (eg. "Name-Outputs...") will have the same times (rate) as the data trace selected in "Master Rate."

4.1.8.3 Epoch Settings

Selecting any epoch column of a <u>Vessel Flow Characteristics Recording Task</u> ⁷⁹ row in the <u>Parameters</u> ²⁰ dialog will display the <u>epoch settings</u> group in the <u>edit area</u> ²¹ for the selected epoch. There will be a separate group for each **Data source** and the contents of that group will depend on how it is configured in the <u>Task Settings</u> ⁸¹ and <u>Experiment Settings</u> ⁸⁵

The edit area will let you select the following values:

If the data source is a hardware sensor...

Frequency* Sampling frequency. (if

"Frequency" selected in the experiment settings 85 column)

Period* Sampling period. (if "Period"

selected in the experiment

settings 85 column - not pictured)

Average At a given frequency/period there

will be a maximum number of points (displayed as the "of n") that can be collected. Select the number of samples to average into

a single raw data point.

If the data source is a task output...

Frequency* Sampling frequency. (if

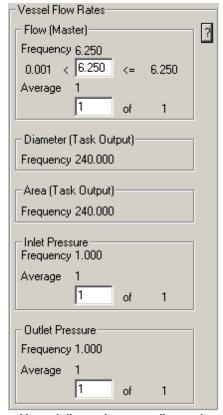
"Frequency" selected in the

experiment settings 85 column)

Period* Sampling period. (if "Period"

selected in the experiment

settings 85 column - not pictured)



Vessel dimension recording task gobal settings edit area



The minimum and maximum values for frequency or period are determined by the hardware capabilities and settings



Frequency and period values entered are rounded to the nearest multiple of the pacer frequency and displayed above the input box. When focus is moved away from the control this value is moved into the input control.

^{*} Only editable on the master data source

^{*} Only editable on the master data source

4.2 Output/Control Tasks

Output Tasks are tasks that output data to one or more devices. Output tasks can output:

Trace data

Any trace data that can be displayed in an lonWizard trace viewer can also be

output to any analog output device. An example usage would be to output the cell

length trace to a chart recorder

Signal Generator A task can output a different value to a device at the start of each epoch. An

example usage would be to change the temperature of a solution between each

epoch.

4.2.1 Trace Output Task

The trace output task scales values from the selected <u>Trace Data Source</u> and outputs them to the <u>Data Receiving Device</u>. The source can be any trace from the current experiment or any analog sensor in the current hardware tree. The range of source values that you specify in the <u>Experiment settings</u> 43 are output over the range of the <u>Data Receiving Device</u>.

New output values are calculated at the pacing rate set in the <u>Hardware Manager Timer Configuration Dialog</u> 10. The output is delayed by an amount specified in the <u>Experiment Parameters Global Settings DA</u> <u>Delay</u> 25 to allow data from sensors with different processing times to be synchronized.

A trace output task uses data entered in three separate places:

- <u>Task settings</u> 42] Settings that apply to all experiments using this task. Entered in the <u>Task</u> <u>Manager</u> 13] *dialog*.
- Experiment settings [43] Settings that apply to all tasks and epochs in an experiment. Entered in the Experiment Global Settings [25] section of the Parameters [20] dialog.
- Epoch settings [43] Settings for this task that apply to all epochs in an experiment. Entered in the Parameters [20] dialog.

4.2.1.1 Task Settings

Clicking the Edit... button in the Task Manager 3 when a Trace Output Task 87 is highlighted will display the task settings dialog. It has the following fields:

Name Name this task.

Trace Data Source Select the source for the data to be output.

Available choices are:

Trace Output A single choice that allows you to choose any trace in the current experiment. The specific trace is selected is selected in Experiment Settings [20] column of

the Parameters Dialog 20 .

Analog sensors All analog sensors in the

hardware tree are individually listed.

Data Receiving Device List of all devices in the Hardware Tree that can

receive (and then output) analog data.

NotesEnter any notes to yourself about this recording

task.



Trace Output Task dialog

The Calibration setup for a selected <u>Data Receiving Device</u> is being ignored. The outputted data should always be in the device raw units. (for example volts)

4.2.1.2 Experiment Settings

Selecting the first column of a <u>trace output task 87</u> row in the <u>Parameters 20</u> dialog will display the experiment settings *group* in the <u>edit area 22</u>. The following values can be edited:

Trace Rec Task If "Trace Output" was selected as the <u>Trace</u>

Data Source in the Task Settings 87 dialog,

this drop down list will show all

combinations of trace/channel/zones from all available tasks in the experiment. If a hardware source was selected, this box will

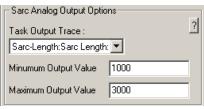
be grayed out.

Minimum Output Value Device or trace value that will result in

lowest output voltage.

Maximum Output Value Device or trace value that will result in

highest output voltage



Trace Output Task experiment settings



Adding one or more Trace Output tasks will enable the <u>DA Delay</u> 26 option in the <u>Experiment Global Settings</u> 25 area of the Parameters dialog



The trace output task uses the full voltage range of the data receiving device or, if supported, the "input range" set in the specification dialog. The lowest voltage will be output for the minimum value or below, the highest voltage will be output for the maximum value or above and the voltage will be linearly scaled between lowest and highest voltage if the value is between the minimum and maximum.

4.2.1.3 Epoch Settings

There are no editable parameters for the <u>Trace Out Task</u>.

Sarc Analog Output Rates

No editable parameters for Trace
Output Task.

Trace output task epoch settings

4.2.2 Signal Generator Task

The Signal Generator Task provides the ability to output separate voltages for each epoch in an experiment and to optionally provide manual control using the <u>Manual Control Tool Bar</u>[36].

An overview of the available waveform shapes and their specific settings is available in the Shapes [96] section.

The signal generator task is configured in three separate places:

- <u>Task settings</u> [89] Settings that apply to all experiments using this task. Entered in the <u>Task Manager</u> [13] *dialog*.
- Experiment settings [90] Settings that apply to all tasks and epochs in an experiment. Entered in the Experiment Global Settings [25] section of the Parameters [20] dialog.
- Epoch settings [92] Settings for this task that apply to all epochs in an experiment. Entered in the Parameters [20] dialog.

4.2.2.1 Advanced Features

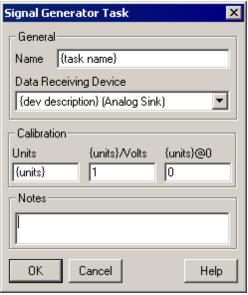
The Signal Generator Task has advanced features that are enabled with the purchase of the Advanced Signal Generator upgrade package. These features are transparently enabled by your product key. These features include a number of new waveforms and also new parameter specification modes for all waveforms. The following table compares the Standard Signal Generator with the Advanced Signal Generator

Feature	Standard Signal Generator	Advanced Signal Generator	
Waveform Shapes 96	Fixed	Fixed	
	Pulse	Pulse	
	Sawtooth	Sawtooth	
	Triangle	Triangle	
	Trapezoid	Trapezoid	
		Sine	
Entry Mode 101	Absolute	Absolute Only	
		Delta Only	
		Mixed	
Level Units 100	Device	Device	
		Percentage of Reference	
Repetition Counts 97	No	Yes	
Parameter Increments	No Yes		

4.2.2.2 Task Settings

Clicking the Edit... button in the Task Manager when a Signal Generator Task 88 is highlighted will display the task settings dialog. It has the following fields:

The input values shown in the example image ({task name} and {units}) are non-specific examples only. Please replace with values that are appropriate for your use.



Signal Generator Parameters

Name

Name for this specific instance of the task. Also used as the title for the Manual Control Tool Bar [93]

Data Receiving Device Select the device in the IonWizard Hardware Tree that will receive the output from

the signal generator. The value shown, {dev description} will be replaced by the description that you enter when the specifying the Analog Sink Device in the

hardware tree.

 Calibration
 The <u>Calibration</u> options are only available when the selected <u>Data Receiving Device</u>

is setup to be calibrated, otherwise this section will not be displayed and the raw

units from the device will be used. (e.g. "Volts")

Units Enter the units for what is being controlled.

Units/Raw The slope of the calibration equation that converts the raw device units (for example voltage) into the desired calibrated units. For example if your device is controlled by an analog voltage, this will be calibrated units per volt (um per volt, pascals per volt, etc). You will either need to run a calibration or consult the manual of the device that receives the raw signal data to find this value.

Units@0 Raw The calibrated output value when the traw device input value is zero. So for example if your device is controlled by an analog voltage, what is the

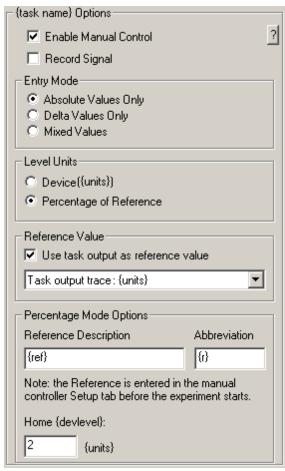
response of the device in calibrated units when the input voltage is 0.

Notes Enter any notes to yourself about this recording task.

4.2.2.3 Experiment Settings

Selecting the first column of a <u>Signal Generator Task</u> row in the <u>Parameters 20 dialog</u> will display the experiment settings *group* in the <u>edit area 22 dialog</u>.

In the image below text in braces, e.g. {units} are placeholders for test that you entered earlier. The {units} text is determined by the <u>Calibration</u> settings in the <u>Task Settings</u> while the {devlevel} text comes from the device you have selected for this task.



Signal Generator experiment settings

Enable Manual Control

If checked a <u>Signal Generator Manual Control</u> [93] will be added to <u>Manual Control Tool Bar</u> [36]. It will also enable Percentage of Reference Entry Level options.

Record Signal

If checked the waveform output will be recorded as a trace. The recorded trace will be in raw device units of the device to which they are output.

Entry Mode

The Entry Mode determines how a level will be treated. See for a extended discussion about this setting the Entry Modes 101 page.

Absolute Values Only The values are treated as absolute settings.

Delta Values Only

The values are applied to the current active signal (advanced feature | ss²).

Mixed Values Allows values to be entered either as absolute or delta. An additional

checkbox is provided to enable the delta mode on a per value base (advanced feature 88).

Level Units

The <u>Level Units</u> determines how levels (i.e. waveform amplitudes) are specified in both the <u>Epoch</u> <u>Settings</u> 92 and <u>Signal Generator Manual Control</u> 93. There are two options:

Device Units Enter levels in absolute device units.

Percentage of Reference Enter levels as a percentage of a Reference value. If this option is

selected the <u>Percentage Mode Options</u> section will be enabled (<u>advanced feature</u> 88). This option is described in detail in <u>Parameter Entry</u> 100.

The <u>Percentage of Reference Level Units</u> deserves a little more attention. This mode allows you to enter percentage values of some yet to be determined reference value when specifying waveform parameters. This is useful when you want to create consistent protocols in cases where the initial conditions of the preparation are not known until the experiment is actually running.

For example, consider using the Signal Generator to drive the length of a cell or the pressure in a vessel. Probably you would like to increase the cell length or vessel pressure in steps of 5%, 10% and 15% above some resting state to record the measured change in some other parameter. However, because the resting states change from one experiment run to another you cannot calculate the actual absolute step levels to program.

This is where percentage mode comes in. You specify the protocol naturally in terms of the percentage steps you want. Later, just before the experiment runs, you set the actual Reference value for the preparation via the <u>Signal Generator Manual Control</u> 3. The Signal Generator will then calculate the actual absolute device steps needed for you with no further intervention. The percentages entered are always based upon this 100% Reference value, no percentage of percentage calculation is applied. For further discussion, see <u>Parameter Entry</u> 1001.

Reference Value / Initial Value

This option will become available when <u>Delta Values Only Entry Mode</u> together with <u>Device Units Level Units</u> is selected or when the <u>Percentage of Reference Level Units</u> is enabled. It allows you to retrieve the initial delta value or reference value from another task output trace. It can further be setup in the <u>Signal Generator Manual Control</u> [93].

Use task output as reference/initial valueIf checked the the available task output traces will be selectable from the dropdown menu.

Percentage Mode Options

Reference Description A description of the Reference value. This string appears later when you are

entering the Reference and helps you to remember what to enter. If you were controlling cell length, for example, you might want to enter "Resting Cell

Length".

Abbreviation Enter an abbreviation for the above description. Continuing our example, you

might enter "CLr".

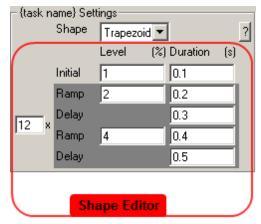
Home {devlevel} Set the Home level of the device in absolute units. The device is forced to the

Home level before entering the Reference value. This ensures that the device will optimally positioned for maximum excursions from preparation resting

state.

4.2.2.4 Epoch Settings

Selecting any epoch column of a <u>Signal Generator Task</u> row in the <u>Parameters [20]</u> dialog will display the <u>epoch settings</u> group in the <u>edit area [21]</u> for the selected epoch. The edit area will let you select the following values:



Signal Generator Epoch Settings

Shape Select the shape of the waveform that is outputted to the Data Receiving Device

during this epoch. See for the available shapes and their specific settings the

Shapes and Phases 96 page.

Shape Editor Depending on the selected shape type the appropriate shape parameters will be

displayed. See Shapes and Phases 96.



Values set by an epoch may be overridden via the <u>Manual Controller and Institute Settings</u> (manual control must be enabled in the <u>Experiment Settings</u> [90]).

4.2.2.5 Manual Control

The <u>Signal Generator</u> *task* has a manual control *group* that appears in the <u>Manual Control Tool Bar and States and States and States and States are at the bottom of the IonWizard window. It allows you to see and override the setting set by acquisition tasks.</u>

When you have selected <u>Percentage of Reference Level Entry</u> option the manual controller also serves as the place where you enter the Reference value as described below.

Basic Manual Control



Signal Generator Manual Control

Shape Selection Select the shape waveform from the tab. When the <u>Enter as</u>

percentages option is selected in the Experiment Settings 90 an

additional Home/Ref tab will be available:

Shape Editor Set the shape specific parameters. Look at Shapes and Phases

96 for how to set up each specific shape.

Current Status Displays the current active waveform information;

The <u>Source</u> indicates <u>Epoch</u> when the current epoch settings are

active and Manual when the settings have been manually

overruled from this tool bar.

Shape displays the current active waveform shape.

The other parameters displayed will vary depending on the active

shape.

Enable button When *pressed* enables editing and activating of "new" set values.

When released acquisition task(s) control of the device, if any,

will be enabled.

Lock button When *pressed* previously activated values will remain in effect as

long as manual override is enabled. When *released* (as shown) acquisition task(s) control of the device, if any, will return at the

end of the current epoch.

Activate button When clicked all "new" values will override any values set by

acquisition task(s), if any. Values will not return to acquisition task control until then end of the current epoch or until manual

override is disabled.

Stop Signal Generator Stops the current active waveform (if any) and output the last

active value until the button (now reading <u>Resume Signal Generator</u>) is pressed again. If no other waveform has become active it will be continued where it left off. When a new waveform was activated, either by an epoch switch or through the manual control, the new activated waveform will start executing from the beginning of the shape. The <u>Stop Signal Generator</u> button will be

inactive when the Home/Ref tab is selected.



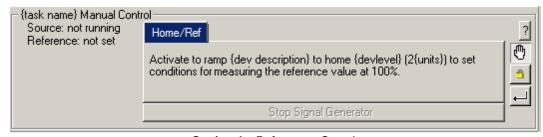
When manual override is enabled and you activate new settings the normal output by the signal generator task is disabled until the end of the epoch or, if the <u>Lock</u> button is pressed, until manual override is disabled.

Reference Entry

When you have enabled the <u>Percentage of Reference Level Entry</u> option the <u>Home/Ref</u> tab is added to the Manual Controller. If you have not yet entered a Reference value it will be the only tab as shown in the following figure.



You can not start an experiment or output shapes in manual control until you set the reference value



Setting the Reference: Step 1

When you click the <u>Activate</u> button in this state, the Signal Generator will move the device to the Home level.



Setting the Reference: Step 2

After the device is at the home level you will be prompted to enter the matching reference level. When you click the <u>Activate</u> button the reference value will be set. If the <u>Use task output as reference</u> option is enabled the <u>Setup...</u> button will be available, and the current trace value from the task output will be displayed behind it. The <u>Setup...</u> button will open a dialog in which it is possible to setup the way the reference value is acquired from the incoming task output trace. See <u>Signal Generator Monitor Setup</u> 6.

The value acquired by the monitor setup will be continuously updated in the reference edit field. This behaviour can be overruled by clicking in the edit field, this will allow you to manually enter a reference value. If you want to re-enable the automatic updating of the reference value again it is necessary to open the Setup... dialog and close it by pressing Ok.

It is not possible to select the <u>Home/Ref</u> tab when the signal generator is stopped with the <u>Stop Signal Generator</u> button.



The method for measuring the actual reference value that corresponds to the device home position will depend on your unique experiment setup.

Initial Delta Value Entry

When the <u>Delta Values Only Entry Mode</u> together with <u>Device Units</u> is selected the <u>Init/Delta</u> tab is added to the Manual Controller.



You can not start an experiment or output shapes in manual control until you set the initial delta value



Initial Delta Value

The first line shows the current (cur), minimum (min) and maximum (max) value acquired from the task output trace as it is setup. The <u>Setup...</u> button will open a dialog in which it is possible to setup the way the reference value is acquired from the incoming task output trace. See <u>Signal Generator Monitor Setup</u> 6. The value acquired by the monitor setup will be continuously updated in the initial delta edit field. This behaviour can be overruled by clicking in the edit field, this will allow you to manually enter an initial delta value. If you want to re-enable the automatic updating of the initial delta value again it is necessary to open the <u>Setup...</u> dialog and close it by pressing <u>Ok</u>.

It is not possible to select the <u>Init Delta</u> *tab* when the signal generator is stopped with the <u>Stop Signal</u> Generator button.

4.2.2.6 Monitor Setup

When the <u>Use task output as reference/initial value</u> is enabled the <u>Setup...</u> button in the <u>Signal Generator</u> <u>Manual Control</u> will give access to the dialog below.



Monitor Setup

The first field allows you to enter the time in seconds the task output trace will be monitored. It uses a shifting timeframe which means the minimum and maximum values are within time "now" minus the time entered.

You can choose if you want to automatically acquire the <u>Minimum</u> or <u>Maximum</u> monitored value. The last two options allow you to add or subtract a certain amount to the monitored value.

Absolute	The value will be added/subtracted as an absolute value.	
%	The value to be added/subtracted will be a percentage of the chosen minimum or maximum	
% of max-min	The value to be added/subtracted will be a percentage of the difference between the minimum and maximum.	

Exiting the dialog by pressing Ok will enable the monitor updating automatically.

4.2.2.7 Shapes and Phases

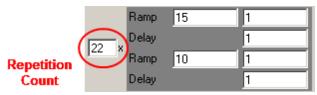
The Signal Generator Task can create a number of different pre-defined waveform *shapes*. Each shape consists of a series of *phases*. There are 4 phase types:

Level	Set the output level to the specified value for the specified duration
Ramp	Ramp from the current output level to the specified output level over the specified duration
Delay	Make no change to the output for the specified duration
Sine	Execute a single sine cycle (advanced signal generator 88) only).

Non-Repeating and Repeating Phases

Each shape begins with a non-repeating level phase that sets the initial output value and offset for the entire waveform. This initial level is then followed by zero or more repeating phases which constitute the periodic portion of the overall waveform. Depending on your settings, the repeating phases will repeat indefinitely or for some specific number of cycles.

Repetition Counts



Repetition Count Entry Field

If you have purchased the <u>Advanced Signal Generator [88]</u>, you will see a new field next to the repeating phases of all shapes except <u>Fixed</u>. This field allows you to specify the number times the waveform is repeated. If this field is blank, the waveform will repeat indefinitely.

Shape Phase Specifics

The sections below describe the phases used for each of the supported waveform shape. The exact layout of the parameter fields differs depending on whether your are editing via the <u>Epoch Settings</u> 192 in the Parameter Dialog box or via the <u>Manual Controller</u> 193. Both layouts are shown for each shape.

In all user interface elements we follow the convention that the repeating phases that make up the periodic waveform are always surrounded by a dark gray background.



The available shapes varies depending on your specific installation (see <u>Advanced Features</u> 88).



The specific syntax and options available for level and duration inputs will depend on the options selected in Experiment Settings 90.



A phase with zero duration will set the current level to the value specified and immediately execute the next phase.

Fixed

Sets a single value to be sent to the output device. This shape has only the initial level and no repeating phases.



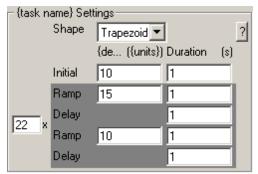
Fixed Epoch Settings

Initial

The level to be output.

Trapezoid

A trapezoid is characterized by a ramp to one level, a hold at that level followed by a ramp to a second level (typically the same as the Initial level).





Trapezoid Manual Control Settings

Trapezoid Epoch Settings

Initial

The initial non-repeating level; held for the time entered in the <u>Duration(s)</u> field.

Reps

The number of times to repeat the periodic phases (22 in this example).

Level to ramp to in the time entered in <u>Duration(s)</u>.

Hold at the Ramp value for the time entered in <u>Duration(s)</u>.

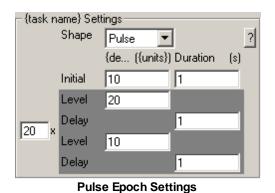
Level to ramp to in the time entered in <u>Duration(s)</u>.

Hold at the Ramp value for the time entered in <u>Duration(s)</u>.

Hold at the Ramp value for the time entered in <u>Duration(s)</u>.

Pulse

A pulse is a step to one level, a hold at that level followed by a step to a second level (typically the same as the Initial level).

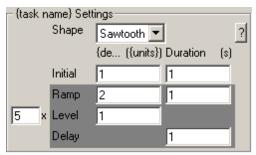


Pulse Manual Control Settings

Initial	The initial non-repeating level; held for the time entered in the <u>Duration(s)</u> field.
Reps	The number of times to repeat the periodic phases (20 in this example).
Level	First value level setting.
Delay	The time to hold the first level.
Level	Second value level setting.
Delay	The time to hold the second level.

Sawtooth

A sawtooth is a ramp to a level followed by an immediate step to a second level (typically the same as the Initial level).





Sawtooth Manual Control Settings

Sawtooth Epoch Settings

Initial The initial non-repeating level; held for the time entered in the <u>Duration(s)</u>

field.

Reps The number of times to repeat the periodic phases (5 in this example).

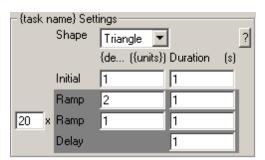
Ramp Enter value to ramp to in the time given in <u>Duration(s)</u>.

Level Value level setting to go at after the ramp ends.

Delay Enter the time it should stay at this level in the <u>Duration(s)</u> field.

Triangle

A triangle is a ramp to one level followed immediately by a ramp to a second level (typically the same as the Initial level).





Triangle Manual Control Settings

Triangle Epoch Settings

Initial The initial non-repeating level; held for the time entered in the <u>Duration(s)</u>

field.

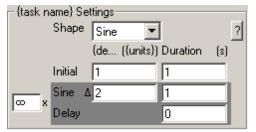
Reps The number of times to repeat the periodic phases (20 in this example).

Ramp Enter value to ramp to in the time given in <u>Duration(s)</u>. **Ramp** Value for the second ramp in the time given in Duration(s).

Delay Hold at the second ramp value for the time entered in <u>Duration(s)</u>.

Sine

A sine shape outputs a sine wave on each cycle with an optional delay between each sine. The sine shape is only available in the Advanced Signal Generator 88





Sine Manual Control Settings

Sine Epoch Settings

Initial	The initial non-repeating level; held for the time entered in the <u>Duration(s)</u> field.
Reps	The number of times to repeat the periodic phases (Infinite in this example).
Sine	The amplitude of the sine. This is inherently a delta value centered on the initial level. The <u>Duration(s)</u> determines the period of the sine wave.
Delav	Hold at the last value of the sine for the time entered in Duration(s).

4.2.2.8 Parameter Entry

There are three different components to the way the level and duration values that define the different waveforms are entered Signal Generator. Two of these components are the Entry Mode and Level Units options set in the Experiment Settings. The third component is the increment syntax that is entered into the parameter fields themselves.

Level Units

The Level Units selection in the Experiment Settings of enables value specification in either absolute Device units, or as a relative Percentage of a Reference units (Advanced Signal Generator 88) only).

Below are illustrations of how the parameter editor fields will appear for each setting.



30 Initial 30

Percentage Units

Level

(%)

When using Device units, the values you enter are directly written to the output device without translation. Thus if you enter 10 into a voltage level field, the value 10 is written directly to the voltage output device.

In contrast, when using Percentage units, you enter the percent value of some currently unknown reference value. The reference value itself is determined before the experiment starts and is almost certainly different from one experiment to another. The actual value written to the output device is the sum of the percentage and the Home value entered in the Experiment Settings. Thus if you enter 110% into a level field, the output will increase by 10% of the reference value from the Home value. To be formal, the value driven to the device for a given percentage value is given by the following equation:

DeviceValue = HomeValue + ReferenceLength*[(PercentValue-100)/100]

An example may be illustrative. Consider a stretching device that lets you stretch tissue by any amount in the range 0-50um. You are using Percentage units and choose a home value of 10. You now create a trapezoid that goes from 100% to 120% and back again. Having all that set up, you now go and mount your tissue sample and run through the Reference Entry protocol as described in Manual Control 4. You measure the length at rest as 150um and enter that as your reference. When you execute you stretch protocol, you first move to 100% which is defined as the home position and therefore the device will move to the 10um position. You then go to 120% (i.e. a 20% stretch). This is adding 20%, or 30um to the Home position. Since your Home position is 10um, the device will now move to the 40um position.

If you were to go to 80%, this would be a -20% stretch. Since your home position is 10um, the new position would be 10 minus 30um or -20um. However, since your device range is only 0-50um, it would clip at the 0 position.

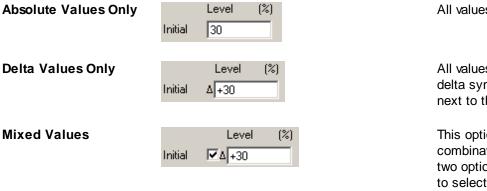
The level of indirection permitted by the Percentage units is required to specify protocols when you do not know the starting point. Two common experimental preparations that have this requirement are vascular experiments where you want to increase/decrease pressures from some unknown baseline and tissue stretching experiments were you want to increase/decrease tissue lengths from some unknown initial length.

Entry Mode

A second component of the parameter entry space is the Entry Mode. This determines whether you are entering values that are absolute values (of either Device or Percentage units) or if you are entering deltas. When using deltas (Advanced Signal Generator 88 only), the value you enter is added to the current value to generate the new output value. For example if you enter +5% as a delta value and the current output value is 110%, the new output value will be 115%. In contrast, 10% entered as an absolute with a current output value of 100% would drive the output to 10% of the reference value.

Deltas allow you to generate waveforms that can easily be shifted up and down by simply changing the initial level. They also permit you to easily match one epoch to another so as to avoid output discontinuities. This is achieved by entering 0 as the delta for the initial phase.

The Entry Mode option itself has three values as shown in the table below. These options permit different mixes of absolute and delta data entry.



All values are absolutes.

All values are deltas. A delta symbol will appear next to the delta fields.

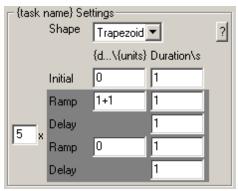
This option is simply a combination of the above two options. It allows you to select either the Absolute or Delta on a phase-to-phase basis with the aid of an additional checkbox.

Increments

The periodic portion of each waveform is repeated either indefinitely or as determined by the repeat count. It is possible on each repetition of the waveform to change the individual phase parameters (

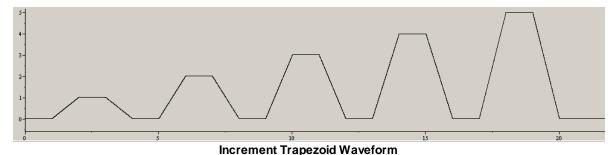
Advanced Signal Generator 88 only). Doing so permits very complex waveform generation.

Increments can be applied to both the level/ramp values and also to the duration of a phase. Both positive and negative values can be entered. The figure below shows the settings for a trapezoid. The user has chosen Device units with Absolute Only Entry Mode.

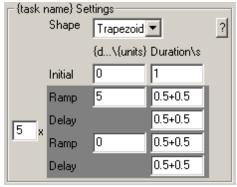


Increment Trapezoid Settings

They have added an increment to the amplitude of the initial ramp phase and indicated by the 1+1 syntax. The first "1" is the target level for the first pass. The +1 indicates that the amplitude will be increased with each subsequent repetition of the waveform. The resulting output looks like the following graph where each trapezoid has an amplitude of 1, 2, 3, 4, etc. If the user entered "5+1" the first trapezoid would have an amplitude of 5 followed by 6, 7, 8, etc.

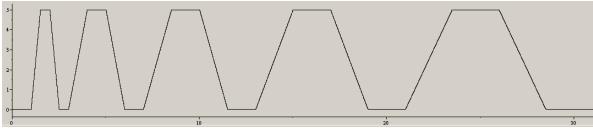


It is also possible to have increments on durations. Consider the following trapezoid settings:



Increment Duration Trapezoid Settings

Here the user has chosen a base value of 0.5 seconds for each phase with an increment of another 0.5 seconds for each repetition.



Increment Duration Trapezoid Waveform

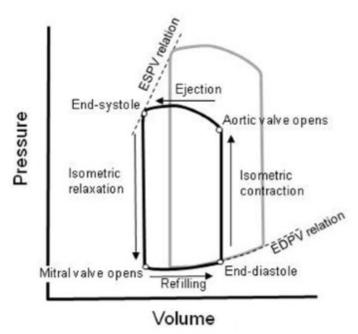
The <u>Initial</u> phase runs for 1 second which gives the offset seen at the beginning. Then the first waveform runs for 2 seconds (4 phases, each 0.5 seconds long). Every subsequent repetition will add another 2 seconds to the total waveform duration (0.5 seconds added to each phase). Thus the waveform durations will be 2, 4, 6, 8, 10, etc seconds.

4.2.3 Clamp Acquisition Tasks

The Clamp Acquisition Recording Task module provides a task, the <u>Work Loop Clamp [103]</u> task that controls a clamp device, such as the <u>FSI Clamp [146]</u>, to permit creating PV (or FL) loops on isolated cells or tissue strips.

4.2.3.1 Work Loop Clamp Task

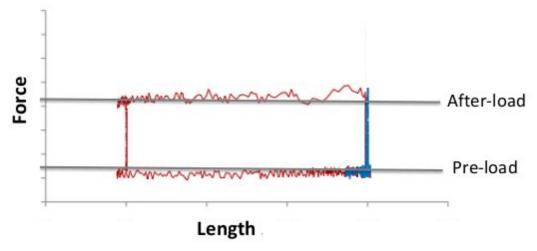
The Work Loop Clamp task is a control task that connects together a number of other tasks in order to permit simulating normal cardiac organ function in a single cell or in a tissue strip. If we take a heart and measure its pressure and volume as it beats and then plot the pressure versus the volume we would get the loops shown below. This is the so called PV plot which is widely used to characterize the whole heart.



If we consider how the pressure and volume changes in the whole heart are experienced by a small part of heart tissue or even by a single cell, it is apparent that pressure changes manifest themselves as varying forces applied to the tissue whereas the volume changes manifest themselves as changes in the length of the tissue. Therefore, by controlling the length and force levels experienced by a tissue sample, we can simulate real cardiac function in vitro. This is precisely what the Work Loop Clamp task does.

Work Loop Clamp Algorithm

The Work Loop Clamp task produces a force-length relationship in the tissue as shown in the figure below.



Force length loop data produced by the Work Loop Clamp Task

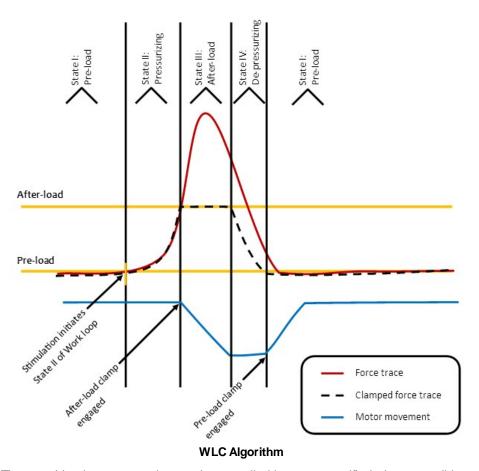
This figure is analogous to the PV loop in a real heart except pressure is replaced by force and volume is replaced by length. The length of the tissue is controlled by holding the tissue mechanically between two mounts. One of the mounts is stationary and the other is attached to a linear motor. By controlling the motor position, we control the tissue length.

The force level is read by a force transducer attached to the stationary mount. With the force input and the motor control output, the clamp device is able to make FL loops.

The clamp cycles through four states of execution to produce each loop:

State	Туре	Clamp Action
1. Filling	isotonic	The motor position is controlled to keep the tissue at the Pre-load force level.
2. Pressurizing	isometric	The motor is stationary.
3. Emptying	isotonic	The motor position is controlled to keep the tissue at the After-load force level.
4. Depressurizing	isometric	The motor is stationary.

The figure below provides a graphical representation of what is happening.



The transition between each state is controlled by user specified trigger conditions.

The clamp starts at the end of state 1. The tissue is at its maximum length and the force is clamped at the Pre-load level. When the clamp detects the onset of contraction (usually via a stimulation signal), it switches to state 2, which is the isometric Pressurizing state. We will describe how contraction is detected a little later after going through the loop.

While the clamp is in the Pressurizing state the tissue is internally contracting and, because the motor is not moving, the force (i.e. pressure) will go up and up. When the After-load level is reached the clamp switches to state 3, the Emptying state.

Because the tissue is contracting, the clamp has to move the motor so as to make the tissue shorter and shorter in order to keep the tissue's force level at the After-load level. The tissue will be allowed to contract until it has shortened so much it can no longer produce the After-load force level. At that point, the clamp will have start stretching the tissue to maintain the After-load force. This reversal of the motor from shortening to lengthening is detected by the clamp and triggers the switch into state 4, the Depressurizing state. The actual distance the motor must reverse itself to trigger the state change is determined by the **Begin diastole at** setting in the toolbar.

Once in the Emptying state, the tissue will internally relax and, because the motor is not moving, the force will drop. Once the force drops below the Pre-load level, the clamp switches into the Filling state.

Once in the Filling state, the motor must start stretching the cell. Otherwise, the cell would continue its internal relaxation and the force level would drop further. The motor will stretch the tissue until it is fully relaxed and is able to maintain the Pre-load force level based solely on its mechanical elastic properties. At this point, the clamp is waiting for the next contraction. As mentioned there are several way of doing this as determined by the **Begin systole at** setting in the toolbar.

The first **Begin systole at** option is **Event Trigger**. This option tells the clamp to sit at the end of the Filling state until it sees an electrical trigger signal. Presumably this signal has been generated by the electrical field stimulator and the tissue will start contracting almost immediately.

If the tissue is not paced, there is one option for trying to detect a new contraction based solely on looking at what the sample is doing. The non-pacing **Begin systole at** option is the **Position**Threshold option. This option is the same used to transition from Emptying to Depressurizing. The clamp looks at the motor position and when the motor starts having to move to shorter and shorter lengths to maintain the Pre-load force level, it assumes that the tissue has begun contracting and switches to the isometric Pressurizing state. The amount the motor has to move to trigger the state change is defined by the **Begin diastole at** setting.

Two Tasks

There are two Work Loop Clamp Tasks which both share a lot of in common; The new Work Loop Clamp Task and its predecessor called the Work Loop Clamp (Legacy) Task. In general it is advised to always use the newer Work Loop Clamp Task and only use the Legacy version when there is no other solution. Since the newer task allows for a better analysis too it is also advised to upgrade, when possible, a Legacy task to the new task.

Task Setup

The Work Loop Clamp task uses information entered in the following places:

- <u>Task settings [108]</u> Settings that apply to all experiments using this task, entered in the <u>Task Manager [13]</u> dialog. (For the Legacy Task see <u>Task settings (Legacy) [109]</u>).
- Experiment settings [110] Settings for this task that apply to all epochs in an experiment, entered in the Parameters [20] dialog. (For the Legacy Task see Experiment settings (Legacy)[111]).
- Epoch settings 111 Settings for this task for a specific epoch in an experiment, entered in the Parameters 20 dialog.
- Tool bar 111 Realtime control of the clamp.

Task Output

The Work Loop Clamp task produces the following traces in lonWizard, where "Name" is the description entered in the <u>Task Settings roal</u> dialog. The actual traces that you will see depend on options selected in the <u>Task Settings</u> roal and Experiment Settings.

"Name" traces

Force Raw - Displays the actual force data collected from the selected device in the appropriate units, usually volts.

Motor Position Raw - Displays the actual motor position data collected from the selected device in the appropriate units, usually volts.

State - The internal state of the two level clamp circuit. The trace data has only 4 values in the set [1..4]. Each value has the following meaning: 1=Filling, 2=Pressurizing, 3=Emptying, 4=Depressurizing.

Position Threshold - The current value of the position threshold as set via the manual control toolbar. This is a debugging aid.

Min Position - The current value of the internal minimum position variable. This is a debugging aid.

Max Position - The current value of the internal maximum position variable. This is a debugging aid.

"Name-ConsTraces" traces Force Scaled - Displays data that has been scaled using the multiplier and offset values entered in the Trace Recording Task 108 dialog.

Motor Position Scaled - Displays data that has been scaled using the multiplier and offset values entered in the <u>Trace Recording Task</u> 108 dialog.

Velocity - The velocity.

Power - The power.

Norm Power - The normalized power

Stress - The stress.

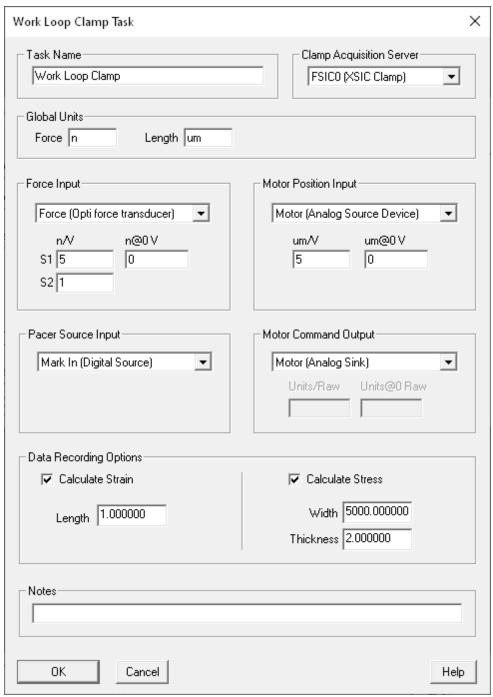
Strain - The strain.

Strain Rate - The strain rate.

Muscle Length - The muscle length.

4.2.3.1.1 Task Settings

Clicking the Edit... button in the Task Manager when a Work Loop Clamp Task of is highlighted will display the task settings dialog. It has the following fields:



Work Loop Clamp task dialog

Task Name Name for this specific instance of the task.

Clamp Acquisition Server The clamp acquisition server which will drive the work loop clamp

experiment.

Global Units Setup the different units.

Force - Units of the force.

Length - Units of the length/motor position.

Force Input The source of the force data.

Motor Position Input The source for the motor position data.

Pacer Source Input The source of digital events needed by the Begin systole at setting in the toolbar

111. The list of event sources is determined by the devices in the Hardware

Manager

Motor Command Output List of all devices in the Hardware Tree that can receive (and then output)

the data produced by the clamp.

Data Recording Options Allows for different types of data to be recorded.

Calculate Strain - Calculate the strain.

Length - Enter the length.

Calculate Stress - Calculate the stress.

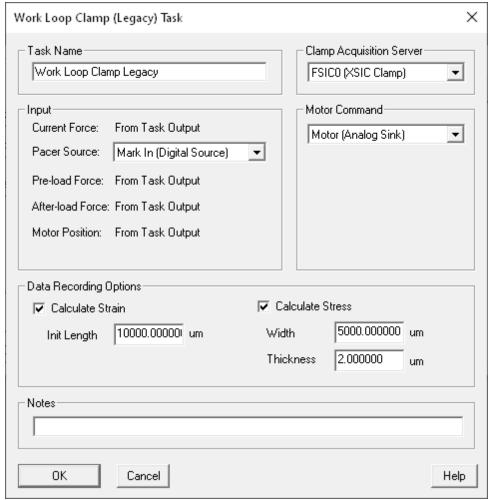
Width - The width of the tissue.

Thickness - The thickness of the tissue.

Notes Enter any notes to yourself about this recording task.

4.2.3.1.2 Task Settings (Legacy)

Clicking the Edit... button in the Task Manager 3 when a Work Loop Clamp Task of is highlighted will display the task settings dialog. It has the following fields:



Work Loop Clamp task dialog

Task Name Name for this specific instance of the task.

Clamp Acquisition Server The clamp acquisition server which will drive the work loop clamp

experiment.

Current Force The source of the force data. This data is provided by another task, typically a

Trace Recorder 41 task, and is selected via the task Experiment Settings 110

options in the Experiment Parameter 20 dialog box.

Pacer Source The source of digital events needed by the Event Trigger F/P Mode setting in

the toolbar 111. The list of event sources is determined by the devices in the

Hardware Manager

Pre-load Force The source of the Pre-load Force setting. This setting is provided by another task,

typically a Signal Generator task or possibly a Trace Recorder task, and is

selected via the task Experiment Settings 110 options in the Experiment Parameter

dialog box.

After-load Force This input needs to be setup in the Experiment Settings 110.

Motor Position This input needs to be setup in the Experiment Settings 110.

Motor Command List of all devices in the Hardware Tree that can receive (and then output) the data

produced by the clamp.

Data Recording Options Allows for different types of data to be recorded.

Calculate Strain - Calculate the strain.

Length - Enter the length.

Calculate Stress - Calculate the stress.

Width - The width of the tissue.

Thickness - The thickness of the tissue.

Notes Enter any notes to yourself about this recording task.

4.2.3.1.3 Experiment Settings

Selecting the first column of a Work Loop Clamp Task 103 row in the Parameters 201 dialog will display the experiment settings group in the edit area 211. The following fields can be edited:

Current Force Select the Force input. The inputs available

might be limited depending on the selected Clamp Acquisition Server in the Task Settings

108

Pre-load Force Select the Pre-load Force input. The inputs

available might be limited depending on the selected Clamp Acquisition Server in the Task

Settings 108

Record Additional State Info Select This option will record the

additional Position Threshold, Min Position and

Max Position traces.



Work Loop Clamp Task experiment settings edit area

Units

It is very important that the units of the various selections match so that the clamp can compare like numbers. The units for the **Pre-load Force** and **After-load Force** selections must match the units of the Current Force. The software will force these units to match and thus may result in selections that are incorrect. If this occurs, you will need to edit the recording tasks (e.g Trace Recorder or Signal Generator) you want to use as appropriate to assure these units are correct.

4.2.3.1.4 Experiment Settings (Legacy)

Selecting the first column of a Work Loop Clamp Task 103 row in the Parameters 201 dialog will display the experiment settings group in the edit area 21h. The following fields can be edited:

Current Force Select the Force input. The inputs available

> might be limited depending on the selected Clamp Acquisition Server in the Task Settings

Pre-load Force Select the Pre-load Force input. The inputs

> available might be limited depending on the selected Clamp Acquisition Server in the Task

Settings 108.

After-load Force Select the After-load Force input. The inputs

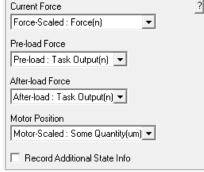
> available might be limited depending on the selected Clamp Acquisition Server in the Task

Settings 108.

Motor Position Select the Motor Position input. The inputs

> available might be limited depending on the selected Clamp Acquisition Server in the Task

Settings 108.



Work Loop Clamp Legacy Options

Work Loop Clamp Task experiment settings edit area

Record Additional State Info Select This option will record the

additional Position Threshold. Min Position and

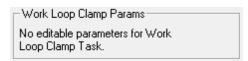
Max Position traces.

Units

It is very important that the units of the various selections match so that the clamp can compare like numbers. The Current Force and Motor Position selections drive the units and can be made freely. The units for the Pre-load Force and After-load Force selections must match the units of the Current Force. The software will force these units to match and thus may result in selections that are incorrect. If this occurs, you will need to edit the recording tasks (e.g Trace Recorder or Signal Generator) you want to use as appropriate to assure these units are correct.

4.2.3.1.5 Epoch Settings

There are no editable parameters for the Work Loop Clamp Task 103

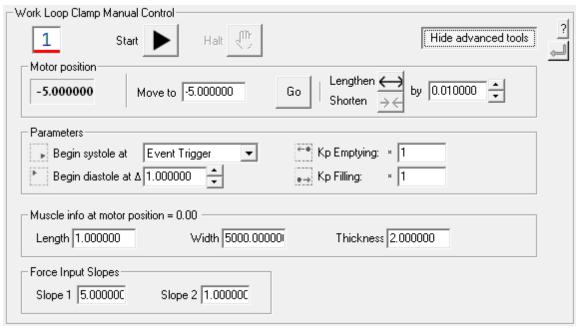


Work loop clamp task epoch settings

4.2.3.1.6 Tool Bar



Work Loop Clamp Task Tool Bar Simple View



Work Loop Clamp Task Toolbar Advanced View

The Work Loop Clamp Task tool bar provides realtime control for the clamp. You can toggle the toolbar between simple and advanced views by clicking on the **Show advanced tools** (or **Hide advanced tools**) button. The simple view is a strict subset of the functionality available in the advanced view.

State Indicator

The white box to the left of the Start button is the state indicator. There will be a red bar on the side of the box corresponding whichever of the four phases the clamp is currently operating in. In the figure above, the bar is at the bottom indicating that the clamp is currently in the *Filling* state. The number displayed also corresponds to the different states: 0=ldle, 1=Filling, 2=Pressurizing, 3=Emptying, 4=Depressurizing.

Start Starts the clamp.

Stop Stops the clamp on entry to the refilling state. The cell (or tissue) will be at its

shortest at this moment and will be unlikely to rip away from the attachment points

if it receives a stimulus pulse at this moment.

Halt This will stop the clamp immediately without finishing the protocol. This is not

recommended unless the cell or tissue has torn away from its attachment points. If this has happened, the feedback loop is open and the clamp will start running

between the extremes of the motor excursion.

Show/Hide advanced tools Toggle between simple and advanced view.

Motor Position

When the clamp is stopped, it is possible to directly control the position of the motor with the Motor Position controls. There are two options. The **Lengthen** and **Shorten** buttons will directly move the motor the amount as entered in the edit field. If you wish to make a larger move, the edit field to the left allows you to enter an arbitrary position. If you then hit the enter key (or click the **Go** button), the motor will ramp from its current location to the desired location. These controls are disabled when the clamp is running.

Parameters

Controls to setup the clamp process.

Begin Systole at The option to use when switching between the *Filling* and *Pressurizing* states. The choices are **Event Trigger** and **Position Threshold**. These options are explained in detail in the algorithm description 106.

Begin diastole at The threshold to use when the Begin Systole at is set to Position Threshold. This threshold is used unconditionally when switching between *Emptying* and *Depressurizing* states.

Kp Emptying Enter the Kp value for the emptying phase. **Kp Filling** Enter the Kp value for the filling phase.

Muscle info at motor position = 0.0 The muscle info when the motor is at position 0.0. It allows the user to enter a more precise value for these values as they were originally entered

in the Task Settings 108.

Length Enter the length.

Width The width of the tissue

Thickness The thickness of the tissue.

Force Input Slopes (Optional). When a sensor has been selected in the Task Settings 108 which

provides a calibration with two slopes the slopes can be adjusted here.

Motor Position Input Slopes (Optional). When a sensor has been selected in the <u>Task Settings</u> which provides a calibration with two slopes the slopes can be adjusted here.

4.2.4 Multi Cell Tasks

The Multi Cell Tasks module provides a single task, the Multi Cell (113) task, that uses available microscope automation to permit fully automatic data acquisition from multiple cells.

4.2.4.1 Multi Cell Task

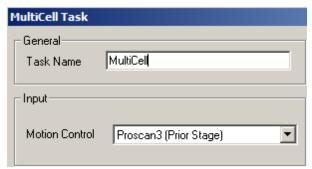
Task Setup

The Multi Cell task uses information entered in the following places:

- <u>Task settings</u> 114 Settings that apply to all experiments using this task, entered in the <u>Task</u> <u>Manager</u> 13 dialog.
- Experiment settings 114 Settings for this task that apply to all epochs in an experiment, entered in the Parameters 20 dialog.
- Epoch settings 114 Settings for this task for a specific epoch in an experiment, entered in the Parameters 20 dialog.
- Tool bar 115 Realtime control of the clamp.

Task Output

4.2.4.1.1 Task Settings

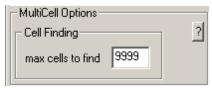


MultiCell Task Settings

Task Name Name for this specific instance of the task

Motion Control Select the hardware device that will be used for the MultiCell task

4.2.4.1.2 Experiment Settings



MultiCell Experiment Settings

The user can input the max amount of cells that they want to find.

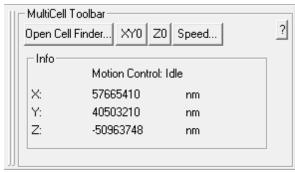
4.2.4.1.3 Epoch Settings



Epoch Settings

? The ? button redirects to a help file with more information on how to work the Epoch Settings

4.2.4.1.4 Tool Bar



MultiCell Toolbar

The Open Cell Finder opens a window that allows the user to do an analysis of cells.

The motion control is displayed in nanometers

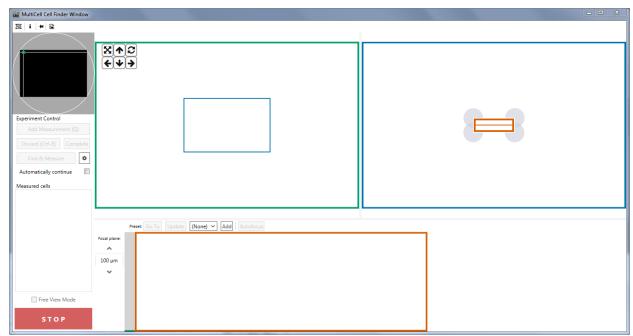
Open Cell Clicking on the Open Cell Finder opens the Cell Finder on another window. Once opened the Open Cell Finder button changes to Close Finder

XY0	Clicking on the XY0 button will allow the user to find the limit of the x and y axis
Z 0	Clicking on the Z0 button will allow the user to find the limit of the z axis
Speed	Clicking on the Speed button allows the user to make configurations to the Microscope Stage Motion Settings. Click on Microscope Stage Motion Settings 125 for more information
?	Clicking on the HeIp button will open a help file that gives instructions on how to use the MultiCell Toolbar

4.2.4.1.5 Cell Finder

Enter topic text here.

4.2.4.1.5.5 Cell Finder Settings



Cell Finder User Interface

The *Experiment Control* allows the user to do the following:

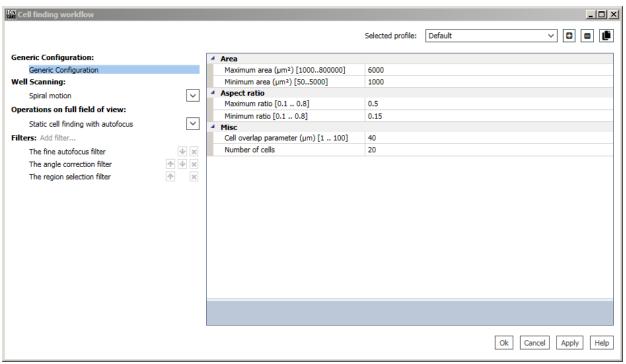
Add Measurement	The user is able to add proper measurement dimensions for the cell
Discard	The current measurement can be discarded
Complete Run	Runs the microscope to find any traces of cells on the screen
Find & Measure	This button allows the user to find some cells and measures them
Gear	This button refers to Cell Finder Preset 125 help file
Stop	The stop button is used to end the experiment control

4.2.4.1.5.5 Cell Finder Acquisition



Allows the user to add a new profile name that isn't already in use

Allows the user to remove the current selected profile

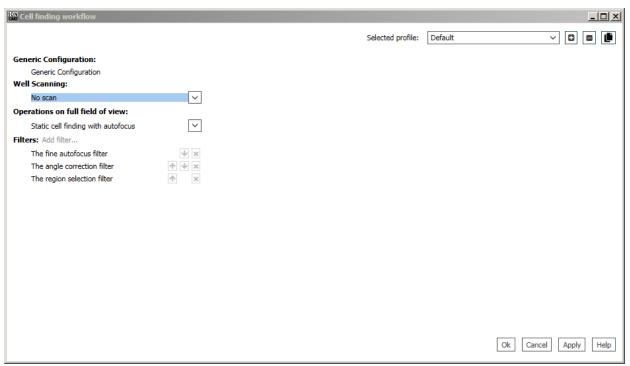


Cell Finder Generic Settings

Area The ranges for the smallest and largest area are displayed

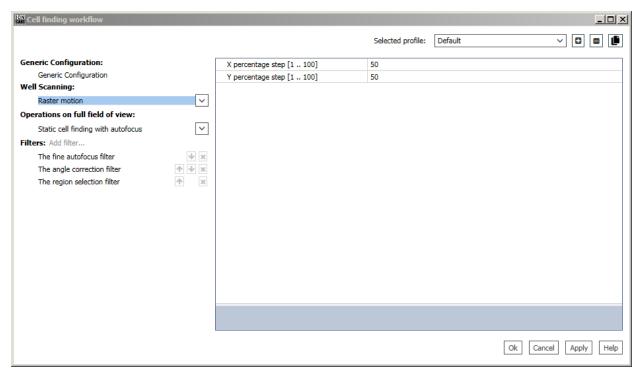
Aspect ratio The ranges for the smallest and largest ratio are displayed

Misc The cell overlap parameter and the number of cells are displayed



Cell Finder No Scan

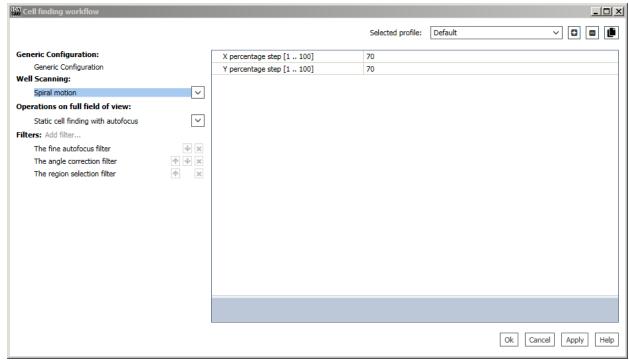
The system only finds cells in the current field of view instead of moving the objective



Cell Finder Raster Motion

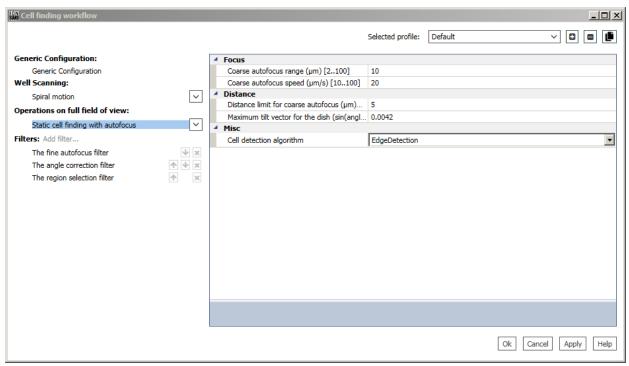
The objective is moved in a raster pattern from the current point and goes left. When the line is finished from which the motion started will continue by going one

YStep higher and reversing the direction of the X-axis



Cell Finder Spiral Motion

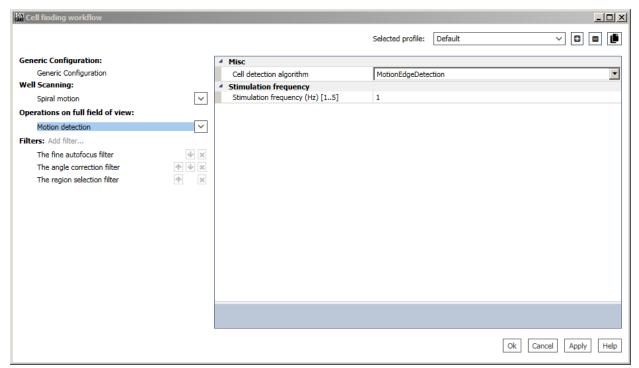
The objective is moved by the system in a spiral pattern



Static Cell Finding with autofocus

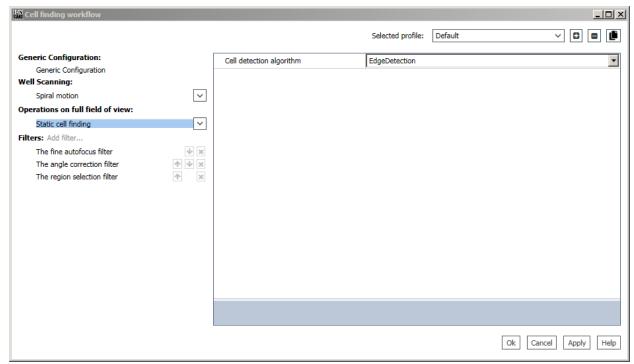
The system detects the single cells with a proper size and width/height ratio for each field of view. If we are out of focus at some point then the system will trigger

the coarse auto focus. The user can decide whether they want to use EdgeDetection or EnFCMEdgeDetection as the cell detection algorithm



Full field view: Motion Detection

The system detects whether the objects it found are beating. The user can decide whether they want to use MotionDetection or MotionEnFCMEdgeDetection as the cell detection algorithm



Full field view: Static Cell Finding

The system detects the single cells with a proper size and width/height ratio for each field of view. The user can decide whether they want to use EdgeDetection or

EnFCMEdgeDetection as the cell detection algorithm

Filters: Add filter...

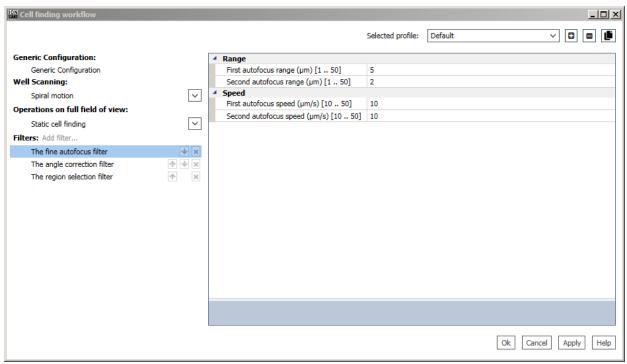
The fine autofocus filter The angle correction filter The region selection filter

Filters

The user can click on **Add filter...** to select from three different filter options. If the user decides that they don't want to use the filter they selected then they

can just click on the x button on the right side of it. Once the user is happy with the selection of the filters that they have picked then they can click on

Apply to save the changes



The fine autofocus filter

This filter is used to put the z-plane on the focus of the sarcomere structure rather than the cell itself. It contains two sweeps, checking both the peak and the signal to

noise ration in IDFFT frequency space

First autofocus range Defines the range that the objective sweeps over in the z direction for the

first sweep

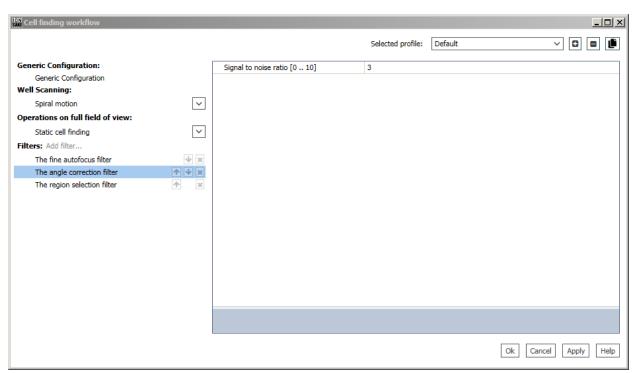
First autofocus speed Defines the speed of the objective movement in the z direction for the first

sweep

Second autofocus range Defines the range that the objective sweeps over in the z direction for the

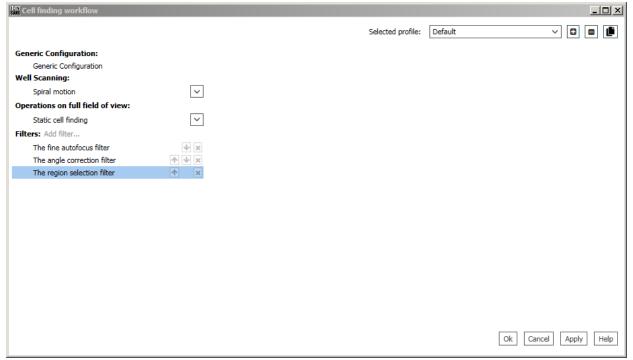
second sweep

Second autofocus speed Defines the speed of the objective movement in the z direction for the second sweep



The angle correction filter

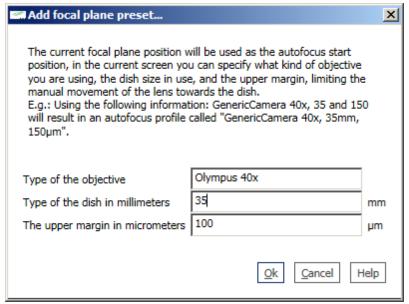
This filter can correct the orientation of the sarcomere structure if there is any mismatch between the orientation of the cell and the sarcomere structure.



The region selection filter

This filter selects the best region of the sarcomere structure in the cell

4.2.4.1.5.5 Cell Finder Preset



Focal plane preset

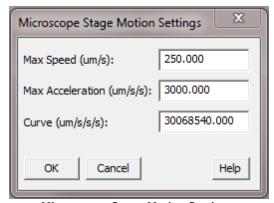
The autofocus start position uses the current focal plane position. An autofocus profile can be generated by providing the type of objective being used, the size of the dish, and the upper margin.

The manual movement of the lens towards the dish becomes limited.

4.2.4.1.6 Microscope Stage Motion

Enter topic text here.

4.2.4.1.6.6 Microscope Stage Motion Settings



Microscope Stage Motion Settings

Max Speed The user can adjust how fast the stage is moving

Max The user can adjust the rate of change for a speed in a certain direction

Curve The user can adjust the curve of the stage motion

Ok Clicking on Ok will apply any changes that occurred in any fields. If any fields are blank or

a non positive number, then an error message will appear asking the user to make a valid

input

Cancel Clicking on Cancel will disregard any changes made to the fields

Help Clicking on Help will provide the user a documented explanation on how to work the

Microscope Stage Motion Settings

4.3 Task Primitives

The following primitives are tools that are common to more than one task.

4.3.1 Video Calibration Dialog

The <u>Video Calibration</u> <u>dialog</u> is used to collect and calculate the scaling factor used to convert pixel dimensions into physical units from within the <u>Task Settings</u> <u>dialog</u> of any acquisition task that measures objects seen by a camera. The resulting value is automatically entered into the <u>Units/Pixel</u> <u>field</u> of the recording task when you click OK. This is done by placing an object with known physical dimensions on the microscope stage then using the mouse to indicate where on the video image the object is located.

The Video Calibration dialog has three main areas:

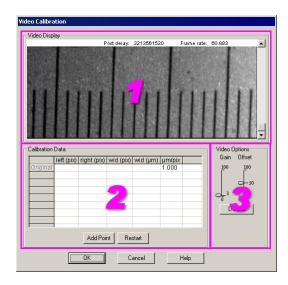
- 1. Video display and measurement area This area displays the live video from the camera.
- Calibration Data The original scaling value, all added calibration points and the new scaling value is displayed here. These values are continuously updated to reflect the current video image and position of the calibration markers.
- 3. Video Options Provides control of input gain and offset, If supported by the current sensor.

Note - you may resize the <u>Video Display</u> <u>dialog</u> by clicking and dragging the edge and corner of the <u>dialog</u> box.

Video Display Area

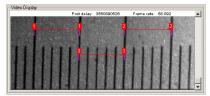
When you first open the <u>Video Calibration</u> <u>dialog</u> you will see live video displayed from the sensor **currently selected** in the recording task that you were editing when you pressed "Collect..." to start the <u>dialog</u>.

When you add a new calibration point a <u>calibration ruler control</u> will be displayed in the <u>Video Display area</u>. You use this control to indicate the position of an object that is the physical distance specified when you added the point.





Video Calibration Initial Video Display area



Video Calibration Video Display area with 3 points

Calibration Data Area

The <u>Calibration Data area</u> of the <u>Video Display dialog</u> displays all of the information used to calculate the new scaling value. The first row shows you the original value for the scaling factor and is not used in the new calculation. The next rows, if any, show the data for each calibration point that you add. If you have one or more calibration points the last row will show you the new value which is the average of all calibration points above.

The values in the columns for the calibration point rows allow you to see the intermediate values and calculations so you can double-check that everything is reasonable.. There are five columns:

left (pix)	Location	of the left	position of	f the

calibration ruler control in pixels.

right(pix) Location of the right edge of the

calibration ruler control in pixels.

wid(pix) Width in pixels, e.g. right(pix) - left(pix)
wid(µm) Width converted to microns using width

Width converted to microns using width entered when calibration point was

added.

μm/pix The resulting scale factor for this

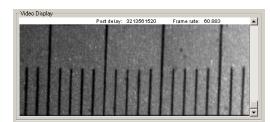
calibration point. e.g. wid(µm)/wid(pix)



Video Calibration Data area

4.3.1.1 Using The Video Calibration Dialog

- Place an object with known dimensions on the microscope stage. IonOptix provides a 10μm stage
 micrometer with each system for this purpose. The stage micrometer is a microscope-scale ruler
 mounted on a standard cover slip that has etched marks at precise 10μm increments with taller tics ever
 50μm.
- Position the slide and/or adjust the camera so that the ruler is parallel to the camera as shown. Make sure that the rules are in focus as much as possible and that there is reasonable contrast between the lines and the background.



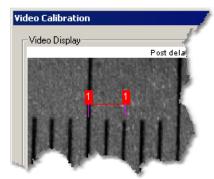
Video Calibration stage micrometer position

3. To add a new calibration measurement click the Add point button. A popup message box (shown at right) will prompt you to enter the length that you want to measure. The units, µm in this example, are taken from current value of the "units" field in the recording task that you are editing. The value that you enter here will be physical length of the calibration ruler control that you will position on the video screen in the next steps.



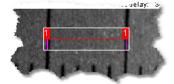
Video Calibration Enter unit spacing

 After you click the OK button a new <u>calibration ruler</u> <u>control</u> will be added in the <u>Video Display Area</u>. The <u>control</u> can be moved and resized by dragging different areas of the <u>control</u>.

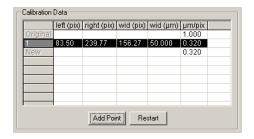


Video Calibration calibration ruler

- 5. To stretch or shrink the length click and drag the red box at either end to the left or right. When you click on the end a white box will appear to confirm that you have clicked in the correct location.
- elay: 34
- 6. The exact position of the edges are calculated by scanning the video image 'under' the box. The resulting position is displayed as a magenta line below the box. When positioning the left/right ends of the <u>calibration ruler control</u> move red box so that it is over the image of the line on the stage micrometer. If positioned properly the current position (magenta) line should be near the middle of the red box as shown here.
- current position
- 7. To move the ruler horizontally or vertically without changing the length click on the line between the boxes then drag the ruler to a new position. When you click on the line to move a white box will be drawn around the entire control as shown.



 As you move the <u>calibration ruler control</u> the values in the <u>Calibration data table</u> will change in the highlighted row and the resulting new scaling factor will be displayed in the last row.



You may added as many calibration points as you think are needed. When you are done press the <u>OK button</u> and the new calibration value (from the last row) will be entered into the Record task settings.

5 Acquisition Devices

In the context of the Hardware Manager, a device is software which provides support for a specific physical device. The Hardware Tree uses these devices to model the physical setup. Devices can be either root devices or attached devices. Root devices are interface cards plugged to a standard computer bus inside the computer or external port (see below). Attached devices are devices that are attached to root devices or other attached devices.

Eventually you attach a device that provides connections to the acquisition tasks that are used in an experiment. These connections between devices and acquisition tasks are called "task connections". When you edit the settings of a given task, such as the <u>Task Settings</u> 42 dialog of the <u>Trace Recording Task</u> 41, you will pick what you are recording by selecting a sensor from the list of all available analog trace <u>task connections</u> in the hardware tree.

In addition to providing a representation of the physical device interconnections, each device may have a specification and/or test function that can be accessed in the <u>Hardware Manager</u> *dialog*. The specification function provides the ability to indicate to lonWizard how the physical device is configured. If the device has options that can be controlled via computer, the specification function may also allow them to be set. The test function provides the ability to operate the physical devices from the <u>Hardware Manager</u> *dialog*. This is normally used to test that the device is able to control and/or read from the physical device.



Refer to specific device documentation for specific details on functions and connections provided by each device.

External Ports

Some devices attach to "standard" computer external ports. The device documentation will refer to the following external port types:

Serial Ports Legacy 9-pin serial-ports for slow speed communications with simple cables.

Parallel Ports Legacy 25-pin parallel ports for 'faster' communications.

USB The original USB port (usually called USB 1.0 or USB 1.1) was created to

replace legacy ports. It provided faster speeds as well as plug-and-play configuration and was originally designed for printers, keyboards etc...

USB 2.0 Second generation of USB that provides support for higher speed devices

such as hard drives and cameras.



If you are purchasing or upgrading a computer, you must make sure that the new computer has the type of ports needed by your devices.



IonWizard supports standard USB-to-Serial adapters that allow you to connect a serial device to a USB 1.1 or 2.0 port.



If your computer has extra slots but is missing a specific port type, you MAY be able to purchase an interface card (ie a PCI to USB 2.0) card.

5.1 Interface Card Devices

Interface Cards Devices correspond to physical cards that plug into a slots inside your computer of a specific type which is generally called a "bus". When you describe the type of interface card, you usually identify it by the type of bus that it is designed to work with. Currently lonWizard supports interface cards use either the ISA bus or the PCI bus.

The following computer buses may be present in your computer:

ISA Bus The ISA is the original PC interface slot that was present in the original PC AT

computers. The ISA bus was removed from main-stream computers around 2002. The only way to get a new computer with an ISA slot is to build your

own using a special motherboard.

PCI Bus The PCI bus was originally available with and has now replaced the ISA bus. It

provides faster performance and "plug-and-play" device configuration.

Other Buses As computers continue to evolve, new buses have been developed. Some of

these, such as PCI-Express, are now starting to appear in new computers along with or in place of PCI slots. Note that these new buses are NOT physically/electrically the same as the PCI Bus even though they have "PCI"

in their name.

No Slots As computers get cheaper and smaller, you may find computers that have no

available internal interface slots of any type.

5.1.1 Measurement Computing IO24 PCI Interface Card: MCIO24P

The Measurement Computing IO24 PCI interface card, or MCIO24P, is used by IonOptix to provide a communication link between the host computer and the Fluorescence System Interface 139 or the Data System Interface 146. (The System Interfaces that work with this card have a 37pin D-Sub connector and version numbers of FSI700 or DSI300 or higher. If your FSI or DSI does not have this connector and instead has a 50 pin and a 20 pin ribbon connector, the Real Time Devices AD2710 ISA Interface Card is the appropriate card).



MCC IO24 Digital I/O Card

Device Name

The MCIO24P appears as "MCC PCI-IO24 Cards" in the Add Root Device of Devices section. An instance of the device appears as "MCIO24Pn" in the Hardware Manager of dialog's Hardware Tree section.

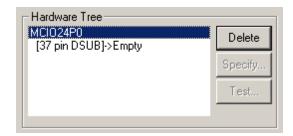


The "n" in the instance name (MCIO24Pn) will be 0 after computer is restarted and will increment each time the device is opened.

Requirements

The lonOptix IO24 Windows device driver must be installed as described in the IO24 hardware manual. This card requires that the computer has an available PCI slot 131.

5.1.1.1 Device Connections



Required connections

The Measurement Computing IO24 interface card is a root device that does not require any other device connection.

Provided connections

The MCIO24P provides the following connection:

37 pin DSUB

Control and data bus to connect to lonOptix IO24-compatible system interfaces (compatible interfaces have a 37 pin D-Sub connector).

5.1.1.2 Task Connections

The MCIO24P device does not provide any connections for acquisition tasks.

5.1.1.3 Specification Dialog

The MCIO24P *device* does not have a specification dialog. The <u>Specifiy...</u> *button* in the <u>Hardware Tree</u> *section* will be disabled when the MCIO24P is selected.

5.1.1.4 Test Dialog

The MCIO24P does not have a test dialog. The <u>Test...</u> button in the <u>Hardware Tree</u> section will be disabled when the MCIO24P is selected.

5.1.2 Mutech MV510 PCI Frame Grabber: MV510

The MuTech MV510 PCI frame grabber card, or MV510, is used by lonWizard to digitize RS-170 or PAL standard video from video devices such as the <u>lonOptix MyoCam</u> [168], standard consumer VCRs or any other video source.



Mutech MV510 Frame Grabber

Device Name

The MV510 device appears as "MuTech MV510" in the <u>Hardware Manager Add Root Dialog</u> 10 Type of <u>Devices</u> section. An instance of the device appears as "MV510 #n" in the <u>Hardware Manager Dialog</u> 10 Hardware Tree section.



The "n" in the instance name (MV510 #n) will be 0 after computer is restarted and will increment each time the device is opened.

Requirements

Version 2.12 of the MuTech Windows driver and application library software must be installed as described in the lonOptix MV510 hardware manual. This card requires that the computer has an available PCI slot 131.

5.1.2.1 Device Connections

The device connections provided by the MV510 are dependent upon the options defined in the <u>Specification</u> list dialog.



Note the MV510 can only sample from one camera at a time. If you have multiple cameras, the adapters will only save you the hassle of switching camera cables between experiments. Selection between cameras is based on the task that has been added to the current experiment.

No Cable (built in RCA connector)

The basic setup is that you have a single camera connected to the RCA connector of the board and no additional cable attached to the DB15 connector. This state is obtained by selecting "No Cable" in the <u>Specifications</u> *dialog*. One empty connection will appear in the Hardware Tree to which you can attach your camera.



Connections with no cable (built in RCA)

VC-DB15-4 V1 cable

The VC-DB15-4 V1 cable provides three color-coded BNC inputs and a 4-pin S-Video input to allow up to four cameras to be plugged in. The RCA connector and VID2 connector are connected internally. Therefore, a camera shown as being attached to the "VID2 - BLU BNC / RCA" connection may physically be plugged into either the RCA port or the blue BNC connector. This state is obtained by selecting "VC-DB15-4 V1 cable" in the <u>Specifications dialog</u>. Four empty connections will appear in the Hardware Tree to which you can attach your cameras to reflect the physical setup.



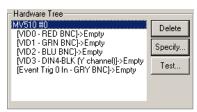
Connections with VC-DB15-4 V1 cable



The RCA connector and the VID2 (Blue) input are connected to the same input - do not attach a camera to VID2 and the RCA at the same time!

VC-DB15-4 V1.1 cable

The VC-DB15-4 V1.1 cable provides three color-coded BNC inputs and a 4-pin S-Video input to allow up to four cameras to be plugged in. This cable also has a general-purpose TTL input but it is not at this time supported in lonWizard. The RCA connector and VID2 connector are connected internally. Therefore, a camera shown as being attached to the "VID2 - BLU BNC / RCA" connection of the Hardware Tree may physically be plugged into either the RCA port or the blue BNC connector. This state is obtained by selecting "VC-DB15-4 V1.1 cable" in the Specifications dialog. Four empty connections will appear in the Hardware Tree to which you can attach your cameras to reflect the physical setup.



Connections with VC-DB15-4 V1.1 cable



The RCA connector and the VID2 (Blue) input are connected to the same input - do not attach a camera to VID2 and the RCA at the same time!



IonWizard does not currently support reading the MV510 general-purpose input.

Other cables

There are some additional cables that have there own connections that are not listed here as they are unlikely to be used with lonWizard.

5.1.2.2 Task Connections

The MV510 device does not provide any connections for acquisition tasks.

5.1.2.3 Specification Dialog



MV510 Specification Dialog

The MV510 specification dialog allows you to specify what, if any, addition adapter cable you have attached to the DB15 connector on the MV510. These adapter cables are only needed if more than one camera is going to be attached to the MV510.

Adapter Cable Part # options

The following adapter cable part numbers are commonly used with lonWizard.

No Cable This is the normal configuration for use with lonWizard. A single camera is

attached to the RCA connector.

VC-DB15-4 V1 This adapter provides the ability to connect multiple cameras to the MV510.

VC-DB15-4 V1.1 This adapter provides the ability to connect multiple cameras to the MV510 as

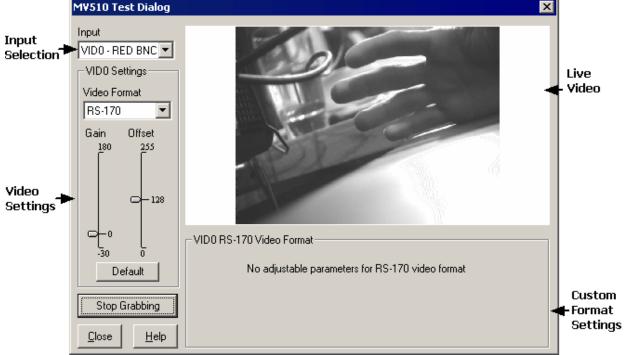
well as a general purpose TTL input (which is currently not supported by

IonWizard).

Other cables These are not likely to be used with lonWizard.

Refer to the Connections section for more information the exact connections that are provided by each adapter.

5.1.2.4 Test Dialog



MV510 Test Dialog

The MV510 Test Dialog permits independent testing of the MV510. It allows selecting between different inputs and configuring the device to handle different input formats. The *dialog box* is divided into a number of sections.

Input

Use the <u>Input combo box</u> to select between the different video inputs to the device. If your <u>specification [135]</u> indicates that you are only using the single RCA input, you will only have the single option for the RCA here. Any time you select an input and there is no detected video signal, you will be so warned and all input controls will be disabled.

Video Settings

The controls in the video settings determine the video format and the gain and offset for the input. The dialog box tracks these settings independently for each input. Use the <u>Gain</u> and <u>Offset</u> sliders to set the analog gain and offset for the input.

There are three options you can choose for the Video Format:

RS-170 The camera is an American 30Hz interlaced monochrome analog camera.

b&w PAL The camera is an European 25Hz interlaced monochrome analog camera.

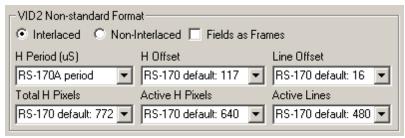
Custom The camera is outputting a custom analog signal. If this option is chosen, the

custom format settings section will be populated with controls to permit

advanced control of the frame grabber as described below.

Custom Format Settings

If the <u>Video Format</u> option in the video settings section is set to <u>Custom</u>, the controls in the custom format settings section become relevant.



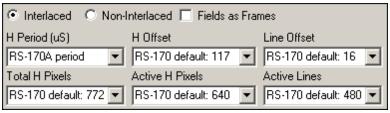
Test Dialog Custom Video Controls

These controls are the same as those documented in the next section, Frame Grabber Parameters 137

Live Video

The live video area lets you actually run the frame grabber. The <u>Start Grabbing/Stop Grabbing</u> *push button* in the lower left of the *dialog box* controls the video state. If the video format parameters are set incorrectly for the camera you will find that it takes a very long time for the <u>Start Grabbing/Stop Grabbing</u> to respond. Be patient as this is normal behaviour.

5.1.2.5 Frame Grabber Parameters



MV510 Frame Grabber Parameters

The <u>Frame Grabber Parameter</u> controls are used to set MV510 parameters as part of the connected camera's <u>Specification</u> dialog. This allows each camera to have different frame grabber parameters that are automatically set whenever the camera is used. Similarly, controls are used in the <u>Test 138</u> dialog to allow the MV510 to be tested. To open, select the camera in the hardware tree and click the <u>Specify</u> button. Now select the Framegrabber Parameters radio button to pull up the above interface.

The MV510 defines the following parameters:

Interlaced If <u>Fields as Frames</u> is *not checked*, the frame grabber will combine the odd/even

fields from camera into one output frame.

If Fields as Frames is checked, the frame grabber will synchronize to odd/even

fields but will return each field as a separate frame.

Non-interlaced When selected, the frame grabber will return each field as a separate frame. It will

ignore odd/even field information, if present.

Field as Frames This checkbox determines how interlaced images are processed. See above

"Interlaced" definition.

H Period This selection sets the sample rate for pixels along a horizontal line.

Total H Pixels This selection sets the number of pixels in a line, including unsampled border

oixels.

H Offset This selection sets the number of pixels from the horizontal sync to the start of

actual video data.

Active H Pixels This selection sets the number of pixels to acquire per full video line.

Line Offset This selection sets the number of lines to skip from the start of the video field to

the first line to save.

Active Lines This selection sets the number of lines to acquire in a complete frame.



When using a non-standard camera, such as the <u>MyoCam</u> 168, please use the settings described in the 'Specification Dialog' section for the specific camera.



Some cameras do not output odd/even fields. In this case, if you select <u>Interlaced</u>, the frame grabber will report an error when you attempt to start video.

5.2 IonOptix Interface Devices

System Interface Devices are a class of devices that provide the majority of the interface functions for the typical lonOptix acquisition system. They provide varying combinations of analog and digital io and light source control. The original system interfaces work with an ADA270 <u>ISA interface card [132]</u> while the current system interfaces work with an IO24 <u>PCI interface card [132]</u>.

5.2.1 Fluorescence System Interface (IO24): FSIC



FSI (IO24) front panel



FSI (IO24) back panel

The IO24 version of Fluorescence System Interface, or FSIC, is IonOptix's current full featured system interface. This FSI uses a 37-pin male-to-female DSUB cable to connect to a Measurement Computing IO24 PCI digital I/O card. All analog and digital inputs and outputs, TTL pulses from pmt tubes and control signals for fluorescent light sources run through this device.

Device Name

The IO24 based Fluorescence System Interface appears as "FSICn" in the <u>Hardware Manager</u> 8 dialog's <u>Hardware Tree</u> section.

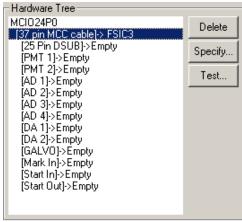


The "n" in the instance name (FSICn) will be 0 after computer is restarted and will increment each time the device is opened.

Requirements

The FSIC requires the proper installation of a Measurement Computing I024 PCI Interface Card (listed as the MCIO24P in the hardware tree). Please see the Measurement Computing I024 [132] PCI interface card, to see the requirements for that device. Please also see the Timer Settings [140] section of the FSIC's documentation for timer requirements.

5.2.1.1 Device Connections



FSI (1024) Connections

Required Connections

The FSIC must be connected to the Measurement Computing MCIO24P [132] device 37-pin MCC Cable port in the Hardware Tree [8].

Provided Connections

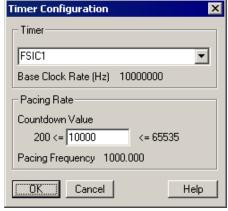
The FSIC provides the following connections for other devices:

25 pin DSUB	Control bus to connect to lonOptix excitation light sources.
PMT 1, 2	TTL inputs to count output of TTL output photomultipler tubes or equivalent.
AD 1 - 4	Analog inputs to read analog outputs of external devices.
DA 1, 2	Analog outputs to connect to analog inputs of external devices.
GALVO	TTL input for synchronizing HyperSwitch with other hardware.
Mark In, Start In	TTL inputs to read TTL outputs of external devices.
Start Out	TLL output to connect to TTL input of an external device and allow triggering from lonWizard.

5.2.1.2 Task Connections

The FSIC device does not provide any connections for acquisition tasks.

5.2.1.3 Timer Settings



Timer Configuration Dialog

When using the Fluorescence System Interface, you must select it as the system timer in the Hardware Manager's <u>Timer Configuration 101</u> *dialog* in order to properly sample PMT and analog signals.

You can increase the fundamental pacing frequency by changing the <u>Countdown Value</u> but it is STRONGLY recommended that you use the normal value of 10,000 which results in a 1KHz pacing frequency.



Consult IonOptix before using pacing frequencies greater than 1000Hz.

5.2.1.4 Specification Dialog



FSI (IO24) Specify Dialog

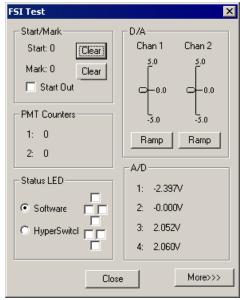
The FSIC specification dialog shows any accessory features the software finds in the device. These features are typically installed by lonOptix at the factory. The above figure shows the display for a stock FSI with no additional features installed.

If you happen to field upgrade an existing device by installing a daughter-card, the specification dialog serves as a "refresh" mechanism. Only after running the specification dialog and clicking <u>OK</u> will the hardware tree reflect the new functionality (e.g. additional PMT or AD channels).

5.2.1.5 Test Dialog

The FSI Test dialog allows you to exercise all of the hardware functions of the interface and all related driver software. The dialog initially displays the basic view 142 that allows simple tests to be performed. The advanced view 144 (pulled out by clicking the More button) gives you access to lower-level functions. The primary function of this dialog is to allow the user to confirm that the connected hardware device is operating properly.

Basic View



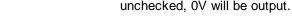
Basic 1024 FSI Test Dialog

The basic view gives you the ability to read the current values on the digital and analog inputs, control the values on the digital and analog outputs and control the status LEDs. The sections of the dialog are described below.

Start/Mark

The <u>Start/Mark</u> section of the test dialog allows you to read the current state of the start and mark inputs and set the state of the start output.

Start	Displays the current status of the Start In input. Sets to 1 when the signal goes from 0 to 5V. Clears to zero when the Clear button is pressed.
Mark	Displays the current status of the Mark In input. Sets to 1 when the signal goes from 0 to 5V. Clears to zero when the Clear button is pressed.
Start Out	Controls Start Out output. When checked, 5V will be output. When





An easy way to test the functioning of the Start and Mark inputs is to use a BNC cable to connect Start Out to Start In or Mark In. When you press the <u>Clear</u> button, the value should change to zero. When you check the <u>Start Out</u> box, the value should change to one. Note that unchecking <u>Start out</u>" will NOT change the value to zero. Only pressing the <u>Clear</u> button will do that.

PMT Counters

The <u>PMT Counters</u> section displays the current values for PMT inputs 1 and 2. The value automatically updates in response to changes in light "seen" by the connected photomultiplier tube.



When PMT tubes see too much light, they will shut down to protect themselves from damage. When this occurs, the counts will drop to zero.



If a PMT tube is not connected, the PMT counter usually reads 1.

Status LED

The Status LED section allows you to control the 7-segment LED number display on the FSI.

Software When selected, the FSI LED display can be controlled directly from the

FSI Test dialog. See "LED segments" definition below.

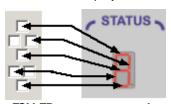
HyperSwitch When selected, the FSI LED is controlled by hardware. See <u>HyperSwitch</u>

145 section of the advanced view 144 of this test dialog for more

information.

LED segments When <u>Software</u> is selected, each check box controls one segment (bar) of

the LED display as shown in the figure below.



FSI LED segment controls

D/A

The <u>D/A</u> section allows you to control the analog outputs.

Chan 1, Chan 2 The Chan 1, Chan 2 sliders allow you to set the D/A output voltages. In

addition to moving the indicator with the mouse. you can use the page up/

down and arrow keys when the slider has focus.

Ramp The Ramp button will cause the D/A output to ramp from -5V to 5V over

approximately 1 second. When done, it will return to the value selected on the slider control. This function is mainly used with an oscilloscope to

assure that all output values are being output correctly.

A/D

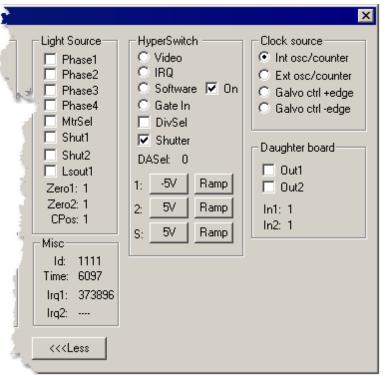
The A/D section displays the current voltage readings for each A/D input channel.

1, 2, 3, 4 Displays the current voltage on the corresponding A/D channel.



An easy way to test the functioning of the D/A outputs and A/D inputs is to use a BNC cable to connect a D/A channel to an A/D channel. The connected A/D channel should read close to the value set with the appropriate D/A slider.

Advanced View



Expanded I024 FSI Test Dialog

The advanced view (pulled out by clicking the <u>More</u> *button*) provides access to low-level functions. Most of the functions that can be tested here are internal FSI functions or external functions not available on BNC connections.

Light Source

The <u>Light Source</u> section allows you to control and read signals that are present on the 25-pin light source connector. Since it is not possible to read or set these signals without a special test cable, the specific function of these controls will not be discussed.

Misc

The <u>Misc</u> section displays status information from the FSI and its driver. Information here can be used to verify that the FSI hardware is properly connected and that the driver interrupt is functioning properly.

•	
ld	Displays the status of an internal device id register. You may be asked to provide this value to lonOptix while debugging a problem.
Time	Time is an internal counter that counts timing clock pulse received by the FSI. This value counts DOWN from the <u>Pacer Frequency</u> value entered in the <u>Timer Configuration [140]</u> dialog and automatically restarts when it reaches zero. If this value is not changing, check the <u>Clock Source [145]</u> section and/or the cable connections.
Irq1	This counts the number of times the FSI interrupt processing code has been called since the driver was first loaded. When operating correctly, the FSI driver should get one interrupt each time the <u>Time</u> value (above) changes. If this value is not incrementing, it means that interrupts are being blocked.
Irq2	This counts the number of times the FSI interrupt was received but not caused by the FSI. Unless the MCIO24P card is sharing an interrupt line with another card, this value will be zero.

HyperSwitch

The <u>HyperSwitch</u> section allows selection of the mode of HyperSwitch control. The FSI can control the HyperSwitch in four different modes. When running lonWizard, the selection of the proper mode is done automatically.

Video Not supported. Please ignore. IRQ Not supported. Please ignore.

Software When selected, the <u>On</u> check box to the immediate right selects between

path 1 and path 2.

Gate In When selected, the <u>Galvo In</u> hardware input selects between path 1 and

path 2.

DivSel When unchecked - a rising edge on the input associated with the selected

mode will cause the mirror to switch from the path it is currently on to the

other path.

When checked - the level of the input associated with the selected mode

determines position: low = path 1, high = path 2.

Shutter When checked, the mirror moves to shutter position REGARDLESS of

any path 1/path 2 selection input. In addition, if the Status LED |143 control is set to "HyperSwitch", the LED display will be set to "-".

1, 2, S These three sections allow you to test the circuitry that sets the precise

mirror position for each HyperSwitch light path: 1=Path 1, 2=Path 2,

S=Shutter.

-5V/0V/5V - Each click on this button will cause the output voltage to change to the next voltage in the -5V, 0V, 5V rotation. The current voltage

is indicated on the button.

RAMP - The Ramp button will cause the D/A output to ramp from -5V to

5V over approximately 1 second.

Clock source

The <u>Clock source</u> section selects the signal to provide the main FSI clock. On each clock pulse, the FSI will latch the current PMT counts and start A/D conversions on all input channels. When the A/D conversion is complete, an interrupt will be sent to the computer to inform the PC that new data is available.

Int Osc/Counter

Use an internal crystal oscillator (10MHz) and a programmable counter.

Ext Osc/Counter

Use a clock signal input via the Clock In BNC (on the rear panel) and the

programmable counter.

Galv ctrl/+edge Use the Galvo In BNC (on the rear panel) directly. Trigger on input

change from low-to-high (rising edge).

Galv ctrl/-edge Use the Galvo In BNC (on the rear panel) directly. Trigger on input

change from high-to-low (falling edge).



IonWizard currently only supports Int Osc/Counter during acquisition.

Daughter board

The <u>Daughter board</u> section provides access to two "spare" digital input/output bits internal to the FSI.

5.2.1.6 Options

The FSIC is available with several options as described below.

4 PMT Option

The 4 PMT Option adds two additional PMT inputs to FSIC bringing the total to 4. This can be very useful for certain multi-dye preparations. If this option is present, you will see "PMT Counters: 4" listed in the Specification Dialog [41] box.

High Speed Clamp Option

This option adds special circuitry to the FSIC that can be used to drive external devices via a proportional controller. Two controller options are available, a standard single level clamp and an advanced dual level clamp. The dual level clamp is used to create work loops (i.e. PV loops) in isolated cells or muscle strips.

This clamp hardware is used by special experimental tasks such as the Work Loop Clamp (103) task (available separately).

5.2.2 Data System Interface (IO24): PDSI



DSI (IO24) front panel



DSI (IO24) back panel

The IO24 version of Data System Interface, or PDSI, is the current version of IonOptix's basic function system interface. This PDSI uses a 37-pin male-to-female DSUB cable to connect to a Measurement Computing IO24 (132) PCI digital I/O card. All analog and digital inputs and outputs run through this device.

Device Name

The IO24 based Data System Interface appears as "PDSIn" in the <u>Hardware Manager 8</u> dialog's <u>Hardware Tree</u> section.

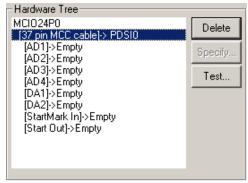


The "n" in the instance name (PDSIn) will be 0 after computer is restarted and will increment each time the device is opened.

Requirements

The PDSI requires the proper installation of a Measurement Computing I024 PCI Interface Card (listed as the MCIO24P in the hardware tree). Please see the Measurement Computing I024 PCI Interface Card 132 to see the requirements for that device. Please also see the Timer Settings 148 section of the PDSI's documentation for timer requirements.

5.2.2.1 Device Connections



DSI (IO24) connections

Required connections

The PDSI is connected to the Measurement computing MCIO24P interface card via a 37-pin DSUB cable.

Provided connections

The PDSI provides the following connections:

AD 1 - 4 Analog inputs to read analog outputs of external devices.

DA 1, 2 Analog outputs to connect to analog inputs of external devices.

Start/Mark In TTL input to read TTL output of an external device.

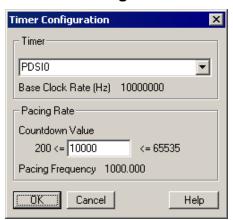
Start Out TLL output to connect to TTL input of an external device and allow triggering

from IonWizard.

5.2.2.2 Task Connections

The PDSI device does not provide any connections for acquisition tasks.

5.2.2.3 Timer Settings



Timer Configuration Dialog

When using the PDSI, you must select it as the system timer in the Hardware Manager's <u>Timer</u> <u>Configuration</u> 10 dialog in order to properly sample PMT and analog signals.

You can increase the fundamental pacing frequency by changing the <u>Countdown Value</u> but it is STRONGLY recommended that you use the normal value of 10,000 which results in a 1KHz pacing frequency.



Consult IonOptix before using pacing frequencies greater than 1000Hz.

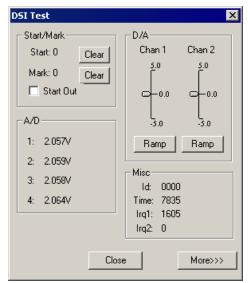
5.2.2.4 Specification Dialog

The PDSI does not have a specification dialog. The <u>Specifiy...</u> button in the <u>Hardware Tree</u> section will be disabled when the PDSI is selected.

5.2.2.5 Test Dialog

The <u>DSI Test</u> dialog allows you to exercise all of the hardware functions of the interface and all related driver software. The test dialog initially displays the <u>basic view [148]</u> that allows simple tests to be performed. The <u>advanced view [150]</u> (pulled out by clicking the <u>More button</u>) gives you access to lower-level functions. The primary function of this dialog is to allow the user to confirm that the connected hardware device is operating properly.

Basic View



Basic I024 DSI Test Dialog

The basic view gives you the ability to read the current values on the digital and analog inputs and control the values on the digital and analog outputs. The sections of the dialog are described below.

Start/Mark

The <u>Start/Mark</u> section of the test dialog allows you to read the current state of the Start/Mark input and set the state of the Start output.

Start	Displays the current status of the Start In input. Sets to 1 when the signal
	goes from 0 to 5V. Clears to zero when the Clear button is pressed.
Mark	Displays the current status of the Mark In input. Sets to 1 when the signal

Displays the current status of the Mark In input. Sets to 1 when the signal goes from 0 to 5V. Clears to zero when the Clear button is pressed.

Start Out Controls Start Out output. When checked, 5V will be output. When

unchecked, 0V will be output.



Note the <u>Stark/Mark</u> input on the <u>DSI front panel[146]</u> is connected to both the start and mark registers in the DSI. Since both are driven by a single input, they will be set and cleared simultaneously.



An easy way to test the functioning of the Start and Mark inputs is to use a BNC cable to connect Start Out to Start/Mark In. When you press the <u>Clear</u> button for either Start or Mark, both values should change to zero. When you check the <u>Start Out</u> box, the values should change to one. Note that unchecking <u>Start out</u>" will NOT change the values to zero. Only pressing the Clear button will do that.

A/D

The A/D section displays the current voltage readings for each A/D input channel.

1, 2, 3, 4 Displays the current voltage on the corresponding A/D channel.

D/A

The <u>D/A</u> section allows you to control the analog outputs.

Chan 1, Chan 2 The Chan 1, Chan 2 sliders allow you to set the D/A output voltage. In addition to moving the indicator with the mouse you can use the page up/down and arrow keys when the slider has focus.

Ramp

The <u>Ramp</u> button will cause the D/A output to ramp from -5V to 5V over approximately 1 second. When done, it will return to the value selected on the slider control. This function is mainly used with an oscilloscope to assure that all output values are being output correctly.



An easy way to test the functioning of the D/A outputs and A/D inputs is to use a BNC cable to connect a D/A channel to an A/D channel. The connected A/D channel should read close to the value set with the appropriate D/A slider.

Misc

The <u>Misc</u> section displays status information from the DSI and its driver. Information here can be used to verify that the DSI hardware is properly connected and that the driver interrupt is functioning properly.

d Displays the status of an internal device id register. You may be asked to

provide this value to lonOptix while debugging a problem.

Time is an internal counter that counts timing clock pulse received by the DSI. This value counts DOWN from the <u>Pacer Frequency</u> value entered in the <u>Timer Configuration</u> dialog and automatically restarts when it

reaches zero. If this value is not changing, check the <u>Clock Source</u> [145]

section and/or the cable connections.

Irq1 This counts the number of times the DSI interrupt processing code has

been called since the driver was first loaded. When operating correctly, the DSI driver should get one interrupt each time the <u>Time</u> value (above) changes. If this value is not incrementing, it means that interrupts are

being blocked.

Irq2 This counts the number of times the DSI interrupt was received but not

caused by the DSI. Unless the MCIO24P card is sharing an interrupt line

with another card, this value will be zero.

Advanced View



Advanced I024 DSI Test Dialog

The advanced view (pulled out by clicking the More button) provides access to low-level functions.

Clock source

The <u>Clock source</u> section selects the provider of the main DSI clock. On each clock pulse, the DSI will latch the current PMT counts and start A/D conversions on all input channels. When the A/D conversion is complete, an interrupt will be sent to the computer to inform the PC that new data is available.

Int Osc/Counter

Use an internal crystal oscillator (10MHz) and a programmable counter.

Ext Osc/Counter

Use clock signal input via the Clk In BNC (on the rear panel 146) and the

programmable counter.

Galv ctrl/+edge Use the Trig In BNC (on the rear panel 146) directly. Trigger on an input

change from low-to-high (rising edge).

Galv ctrl/-edge Use the Trig In BNC (on the rear panel 146) directly. Trigger on an input

change from high-to-low (falling edge).



IonWizard currently only supports Int Osc/Counter during acquisition.

Daughter board

The <u>Daughter board</u> section provides access to two "spare" digital input/output bits internal to the DSI.

5.3 IonOptix Flow Meter Devices

IonOptix Flow Meter Devices provide support for IonOptix flow meters.

5.3.1 Flow Meter: FM100

The <u>lonOptix FM100</u> software *device* allows flow readings from the lonOptix Flow Meter FM100 shown at right.

Device Name

The <u>IonOptix FM100</u> device appears as "Flow Meter" in the <u>Hardware Manager</u> 8 dialog's <u>Hardware Tree</u> section. The name can be changed in the <u>Specification Dialog</u> 153.

Requirements

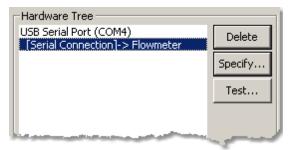
The <u>lonOptix FM100</u> device requires an available <u>serial port port</u> in the hardware tree. This serial port will automatically appear when you install the FM100 as described in the FM100 hardware manual.

Thus to use this device, you must first add a <u>Serial Port 228</u> device to the hardware tree that corresponds to the COM port given to the FM100 when you installed it. Then you attach the <u>lonOptix FM100</u> software device to that serial port.



IonOptix FM100 Flow Meter

5.3.1.1 Device Connections



IonOptix FM100 Device Connections

Required connections

The "Flow Meter" device must be connected to a <u>Serial Port 28</u> device. As described earlier, you must add the serial port root device with the same COM port value that was given to your FM100 when you installed it.

Provided Connections

The <u>lonOptix FM100</u> device does not provide any connections for other devices.

5.3.1.2 Task Connections

The <u>lonOptix FM100</u> device provides an analog sensor that can be selected as an input in acquisition tasks such as the <u>Trace Recording Task</u> 41. In the following list "*Name*" is the description entered in the <u>Specification</u> dialog.

Device Sensor

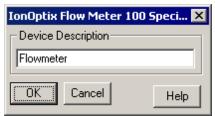
The following sensor can be selected in any acquisition task that monitors or records analog values.

"Name (IonOptix FM100)" Current flow reading from device

Device Inputs

The <u>lonOptix FM100</u> device does not provide any device inputs.

5.3.1.3 Specification Dialog

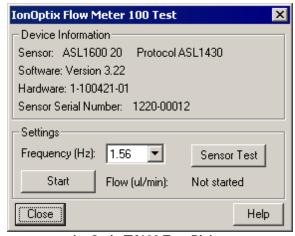


IonOptix FM100 Specification Dialog

The <u>lonOptix FM100 Specification</u> *dialog* provides the mechanism to set basic information about the connected device.

Description Enter "friendly" name used to identify this specific flow meter.

5.3.1.4 Test Dialog



IonOptix FM100 Test Dialog

The <u>lonOptix FM100 Test</u> dialog box provides real-time display and of data from the attached flow meter device.

Device Information: Display information about the attached device.

Frequency (Hz): Select the sampling frequency to use when the Start button is pressed. This

control is disabled unless update is stopped.

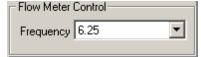
Start: Pressing the Start button starts real-time update of the Flow (ul/min) value. Once

started the Start button changes to "Stop".

Stop:

Pressing the **Stop** *button* stops real-time update of the <u>Flow (ul/min)</u> *value*. Once stopped the **Stop** *button* changes to "Start".

5.3.1.5 Global Sensor Settings



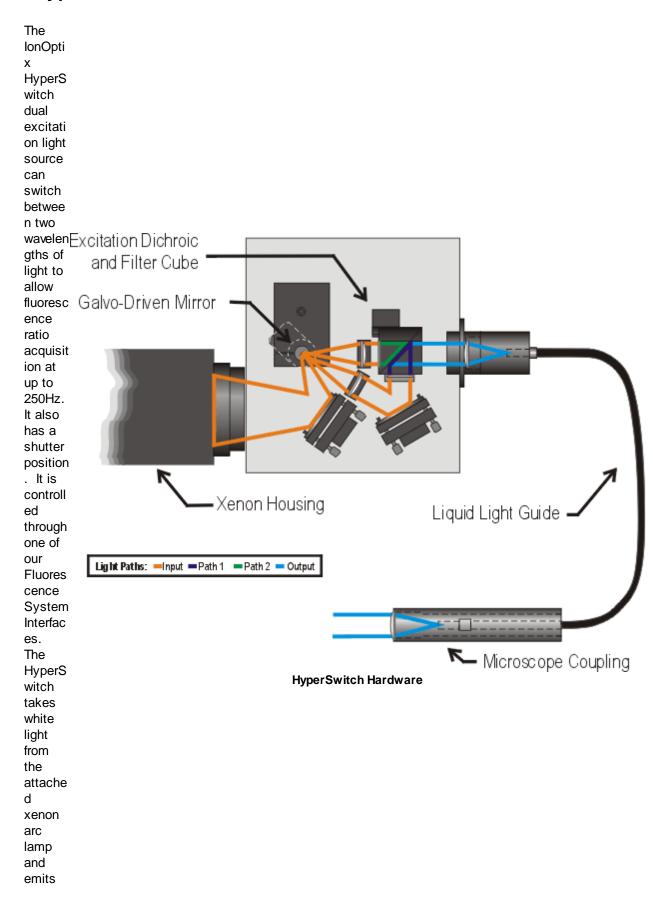
Global Sensor Settings

When the current experiment includes one or more tasks that use the <u>lonOptix FM100</u> *device*, a <u>Flow Meter Control</u> *group* will be added to the <u>Global Sensor Settings</u> 22 area of the <u>Parameters</u> 20 dialog. This control allows you to set the base acquisition rate that will be used when reading flow data from the device.

5.4 IonOptix Light Source Devices

lonOptix Light Source Devices provide the ability to deliver one or more excitation wavelengths to the epifluorescence port of a microscope. They are usually connected to a lonOptix System Interface Device [139].

5.4.1 HyperSwitch: HYPER



filtered light via a liquid light guide to the micros cope.



IonWizard does not support more than one excitation light source in hardware tree at the same.

Device Name

The HyperSwitch appears as "HYPER_n" in the <u>Hardware Manager 8</u> dialog <u>Hardware Tree</u> section.

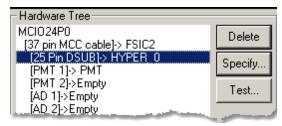


The "n" in the instance name (HYPER_n) will be 0 after computer is restarted and will increment each time the device is opened.

Requirements

Use of a HyperSwitch requires that one of our Fluorescence System Interfaces (either the FSIB or FSIC 139) has been properly installed. Please see the documentation for the Fluorescence System Interface for a list of its requirements.

5.4.1.1 Device Connections



HyperSwitch Connections

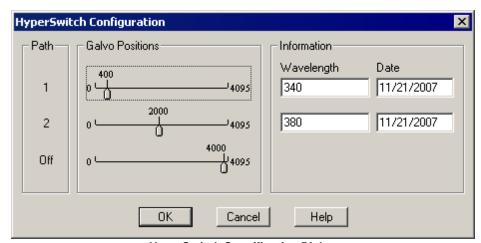
Required connections

The HyperSwitch must be connected to a <u>25 Pin DSUB</u> port on a Fluorescence System Interface.

5.4.1.2 Task Connections

The HYPER device does not provide any connections for acquisition tasks.

5.4.1.3 Specification Dialog



HyperSwitch Specification Dialog

The <u>HyperSwitch Configuration</u> *dialog* provides the mechanism to set the rotational position of the galvonometer mirror for the two excitation paths and the shutter position.

Galvo Positions Set mirror position for each of the 3 positions: path 1, path 2 and shutter.

Wavelength Describe the filter in the corresponding path of the excitation cube. The name can

include any alphanumeric characters such as 340DF10.

Date Enter the date or other note to help track filter source. It may be left blank.

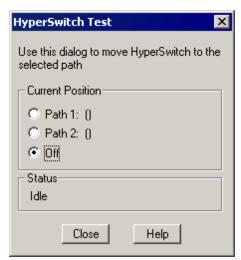


Refer to the Hardware manual for instructions on how to install filters and other device details.

Galvo Positions

The HyperSwitch switches wavelengths by moving a galvanometer mounted mirror to direct white light down one of three paths. Two of those paths (1&2) will eventually encounter filters that select the desired wavelengths, while the third (off) is a position that effectively shutters the light source. In order to work correctly, the galvanometer needs to be driven to a specific location for each path. For new light sources the values should be as shown above: 400, 2000, and 4000. However, if you have a very old light source or you wish to optimize your newer light source, you will need to manually set the mirror positions for each of the three positions in the Specification field dialog with the Galva position sliders.

5.4.1.4 Test Dialog



HyperSwitch Test Dialog

The <u>HyperSwitch Test</u> dialog allows you to manually move the position of the galvonometer mirror to the positions set in the <u>Specification Dialog</u> For <u>Path 1</u> and <u>Path 2</u>, the wavelength and date information will be displayed.

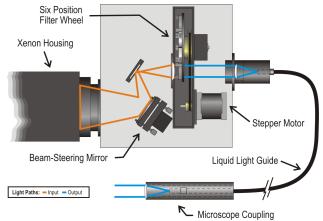
Path 1: Moves to the path 1 position.

Path 2: Moves to the path 2 position.

Off Moves to the shutter position.

Status Shows status of device: busy (while moving) or idle.

5.4.2 StepperSwitch (micro-stepping): USTEP

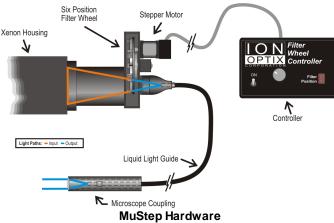


StepperSwitch (new micro-stepping) Hardware

The <u>StepperSwitch (new micro-stepping</u>) is functionally identical to the newer style MuStep. Please refer to the <u>MuStep</u> documentation.

5.4.3 MuStep: USTEP

The lonOptix MuStep and the older StepperSwitch (new micro-stepping) 159 are dual excitation light sources which have six optical filters and three shutter positions. They take white light from the attached xenon arc lamp and emit filtered light via a liquid light guide to the microscope. The separate Filter Wheel Controller allows remote manual control of the wheel position or control by lonWizard when connected to Fluorescence System Interface Devices.





IonWizard does not support more than one excitation light source in hardware tree at the same.

Device Name

The MuStep/StepperSwitch appears as "USTEP_n" in the <u>Hardware Manager</u> and <u>Hardware Manager</u> dialog's <u>Hardware Manager</u>. Tree section.

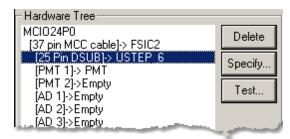


The "n" in the instance name (USTEP_n) will be 0 after computer is restarted and will increment each time the device is opened.

Requirements

Use of either a MicroStepper or <u>StepperSwitch (new micro-stepping)</u> requires that one of our Fluorescence System Interfaces (either the FSIB or <u>FSIC [139]</u>) has been properly installed. Please see the documentation for the Fluorescence System Interface for a list of its requirements.

5.4.3.1 Device Connections



MuStep* Connections

Required connections

The MuStep/StepperSwitch must be connected to a <u>25 Pin DSUB</u> port on a Fluorescence System Interface..

5.4.3.2 Task Connections

The USTEP device does not provide any connections for acquisition tasks.

5.4.3.3 Specification Dialog



MuStep* Specification Dialog

The <u>MuStep/StepperSwitch Specification</u> dialog allows you to describe what is installed in each position of the filter wheel.

Status Basic information about the filter position

Open - nothing is installed at this position so all light from the xenon light source

will be transmitted.

Blocked - a solid slug is installed to block all light (equivalent to a shutter

position).

Filtered - a filter is installed as described in <u>Wavelength</u> and <u>Date</u> *fields*.

Wavelength Description of filter installed if status is "Filtered". Can include any alphanumeric

characters such as 340DF10.

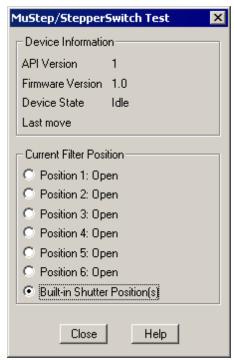
Date Date or other note to help track filter source if status is "filtered". It may be left

blank.



Refer to the Hardware manual for instructions on how to install filters and other device details.

5.4.3.4 Test Dialog



MuStep* Test Dialog

The <u>MuStep/StepperSwitch Test</u> dialog allows you to move the filter wheel to a specific position. The status, wavelength and date information for each position (set in the <u>Specification Dialog</u>) felt is described in the <u>Current Filter Position</u> area.

API Version Shows the command version used to communicate with the MuStep/

StepperSwitch.

Firmware Version Identifies the specific version of the software in the MuStep/StepperSwitch.

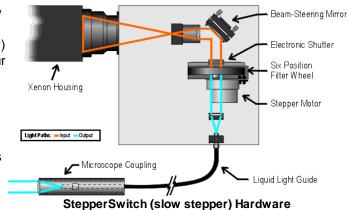
Device State Shows status of device: busy (while moving) or idle.

Last move Displays the time (in milliseconds) that it took to complete the last movement.

Current Filter Position Changes wheel to the specified position.

5.4.4 StepperSwitch (slow stepper): OSTEP

The older, original lonOptix StepperSwitch (slow stepper) is dual excitation light sources which has either six or eight optical filters and (usually) an electronic shutter. It is controlled through our original RTD style Fluorescence System Interface. It should receive white light from a xenon arc lamp and emit colored light into a liquid light guide. In the Hardware Manager, attachment of the device "OSTEP" to the "25 Pin DSUB" connection point on the FSI ensures proper lonWizard support.



Device Name

The StepperSwitch (slow stepper) appears as "OSTEP_n" in the <u>Hardware Manager 8 dialog's Hardware Tree</u> section.

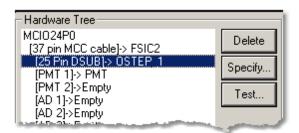


The "n" in the instance name (OSTEP_n) will be 0 after computer is restarted and will increment each time the device is opened.

Requirements

Use of a <u>StepperSwitch stepperSwitch</u> requires that our RTD style Fluorescence System Interfaces (the FSIB) has been properly installed. Please see the documentation for the Fluorescence System Interface for a list of its requirements.

5.4.4.1 Connections

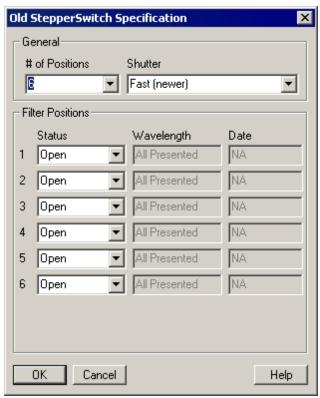


StepperSwitch (slow stepper) Connections

Required connections

The StepperSwitch (slow stepper) must be connected to a <u>25 Pin DSUB</u> port on an RTD style Fluorescence System Interface.

5.4.4.2 Specification Dialog



StepperSwitch (slow stepper) Specification Dialog

The <u>Old StepperSwitch Specification</u> dialog allows you do describe what is installed in each position of the filter wheel.

of positions Number of filter positions in wheel

6 - Six positions (3 pairs) with built-in shutter positions

8 - 8 positions with no shutters

Shutter Determine characteristics of separate shutter, if any

slow (old) - original slow shutter driver faster (new) - newer fast shutter driver

disable - no external shutter

Status Basic information about the filter position

Open - nothing is installed at this position so all light from the xenon light source

will be transmitted.

Blocked - a solid slug is installed to block all light (equivalent to a shutter

position).

Filtered - a filter is installed as described in <u>Wavelength</u> and <u>Date</u> *fields*.

Wavelength Description of filter installed if status is "Filtered". Can include any alphanumeric

characters such as 340DF10.

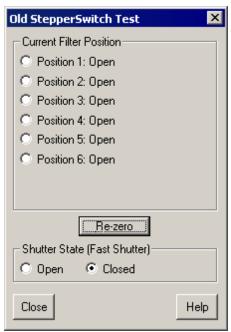
Date Date or other note to help track filter source if status is "filtered". It may be left

blank.



Refer to the Hardware manual for instructions on how to install filters and other device details.

5.4.4.3 Test Dialog



StepperSwitch (slow stepper) Test Dialog

The <u>Old StepperSwitch Test</u> dialog allows you to move the filter wheel to a specific position. The status, wavelength and date information for each position (set in the <u>Specification Dialog</u>) 16 is described in the <u>Current Filter Position</u> area.

Current Filter Position Change wheel to specified position.

Re-zero Run routine to find "home" position for wheel.

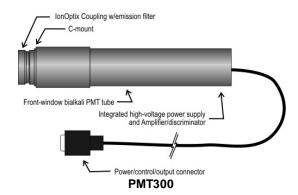
Shutter State Open or close separate mechanical shutter if present.

5.5 IonOptix Light Sensor Devices

Standard Light Sensor Devices provide the ability to view the overall intensity of fluorescence emission or a transmitted light image.

5.5.1 Photomultipler Tube (PMT400/300)

The PMT300 or PMT400 is photomultiplier tube (PMT) with an integrated amplifier/descriminator that outputs TTL pulses in proportion to the the amount of photons seen by the detector at the end of the device. They connect to a Fluorescence System Interface via the 9-pin DSUB connector.





The PMT400/300 software will work with any device that outputs TTL pulses at a rate proportional to the amount of light with a proper electrical connector or adapter.

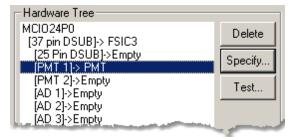
Device Name

The Photomultiplier Tube device appears as "PMT" in the <u>Hardware Manager and Jacobs Specification Dialog</u> 167. The name can be changed in the <u>Specification Dialog</u> 167.

Requirements

Use of a PMT requires a Fluorescence System Interfaces (either the FSIB or FSIC [139]) has been properly installed. Please see the documentation for the appropriate Fluorescence System Interface for a list of its requirements.

5.5.1.1 Device Connections



PMT400/300 Connections

Required connections

The PMT400/300 must be connected to a TTL PMT counter connector on a Fluorescence System Interface...

5.5.1.2 Task Connections

The <u>PMT400/300</u> *device* provides a sensor that can be selected in acquisition tasks. In the following list *"Name"* is the description entered in the <u>Specification 167</u> *dialog.*

Device Sensor

The following sensor can be selected in any acquisition task that monitors or records analog values.

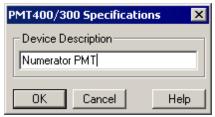
"Name (PMT400/300)"

Last PMT count acquired

Device Inputs

The PMT400/300 device does not provide any device inputs.

5.5.1.3 Specification Dialog



PMT400/300 Specification Dialog

The specification dialog for the PMT400/300 allows you to enter an arbitrary description for the photomultiplier tube. The following values may be entered:

Device Description String displayed when selecting this devices in the Task Manager.

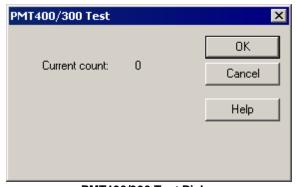


If you have two or more PMTs in your system (such as dual emission), it is better to name them by function, such as "Numerator PMT", instead of by number such as "PMT #1". When you follow this suggestion, the hardware tree will better document your setup. Eg "[PMT 1] -> Numerator PMT" and "[PMT 2] -> Denominator PMT" instead of "[PMT 1] -> PMT #1" and [PMT 2] -> PMT #2".



If you only have a single PMT in your system, the simple generic name "PMT" is all that is needed

5.5.1.4 Test Dialog



PMT400/300 Test Dialog

The <u>PMT400/300 Test</u> *dialog* will display the current live count from the attached photo multiplier tube. The <u>Current count</u> will automatically update.



When PMT Tubes see too much light, they will shut down to protect themselves from damage. When this occurs, the count will drop to zero.



If a PMT tube is not connected, the PMT counter usually reads 1.

5.5.2 Variable field-rate Video Camera (MyoCam): MYOC

The MyoCam is a variable field-rate camera used in the acquisition of length or sarcomere spacing data. It is physically connected to a framegrabber installed in the computer and to a power supply that provides gain, offset and rate control. In the Hardware Manager, attachment of the device "MYOC" to the MV510 root device ensures proper lonWizard support.



MyoCam Camera and Controller

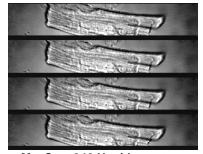


If you a have problem getting MyoCam images to work in IonWizard, the first thing that you should check is that the MyoCam specifications are correct.

MyoCam Variable Field-Rate Details

There are two difference between the MyoCam and a standard black-and-white RS170 camera. The first difference is that the MyoCam always samples the "odd" lines resulting in a 640x240 image 60 times per second - this is usually called "non-interlaced video." Because there is no formal standard for non-interlaced video, we refer to the MyoCam as a pseudo-standard RS170 camera.

The second difference is the technique used to achieve frame rates of 120Hz and 240Hz. For 120Hz, the MyoCam samples the top half of the CCD image sensor twice per frame. For 240Hz, the MyoCam samples the top quarter of the CCD image four times per frame. The images are "stacked" into a normal video frame which means the MyoCam video output can be treated as "normal" 60Hz video. It can be displayed on monitors and, more importantly, acquired by a standard frame grabber. When you view the output in 120Hz mode, you will see two half-height images in the MyoCam Specification Dialog 170 or in the MyoCam Test Dialog 172. At 240Hz, you will see four quarter-height images.



MyoCam 240 Hz video output



Each half- or quarter-height part of the full image is actually a unique image sampled at a different point in time.



The only place that you will see the "stacked" images are in the <u>MyoCam Specification</u> dialog, the <u>MyoCam Test</u> dialog or on an external video monitor directly connected to the MyoCam video output.

Device Name

The MyoCam appears as "MYOCn" in the Hardware Manager 8 dialog's Hardware Tree section.



The "n" in the instance name (MYOCn) will be 0 after computer is restarted and will increment each time the device is opened.

Requirements

Use of a MyoCam requires that a MuTech MV510 Framegrabber has been properly installed. Please see the documentation for the MuTech MV510 Framegrabber [133] for a list of its requirements.

5.5.2.1 Device Connections



MyoCam Connections

Required connections

The "Video" BNC connector on the MyoCam must be connected to a frame-grabber video input connection such as the Mutech MV510 and the 9 pin D-Sub must be connected to an IonOptix Video Power Supply.

Provided connections

The MyoCam also provides the following connections.

NA Unused connection

AUX TTL synchronization output sent at the start of each video field.

5.5.2.2 Task Connections

The MYOC device provides a singled device sensor that can be selected in acquisition tasks.

Device Sensor

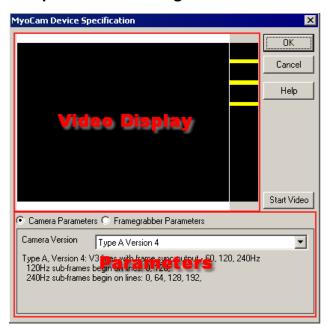
The following sensor can be selected in any acquisition task that monitors or records images.

"MYOCn (IonOptix MyoCam)" Current image (for explanation of "MYOCn" see Device Name) বিজী

Device Inputs

The MYOC device does not provide any device inputs.

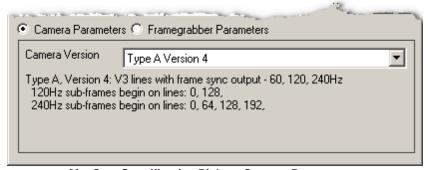
5.5.2.3 Specification Dialog



MyoCam Specification Dialog

The <u>MyoCam Device Specification</u> dialog allows you to select the <u>MyoCam camera version</u> 1701, set the <u>frame grabber parameters</u> 1711 and <u>Verify Camera/Frame grabber parameters</u> 1711.

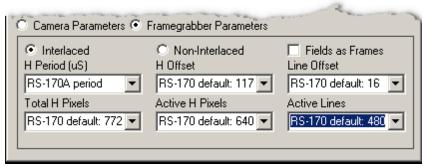
Camera Parameters



MyoCam Specification Dialog - Camera Parameters

When <u>Camera Parameters</u> is selected, the bottom part of the dialog will allow you to specify the version of the MyoCam that is connected. When you select a version from the drop-down menu, information about the camera parameters will be displayed below. Unless otherwise instructed, you should always select "Type A Version 4".

Framegrabber Parameters



MyoCam Specification Dialog - MV510 Frame Grabber Parameters

When <u>Framegrabber Parameters</u> is selected, the bottom part of the dialog will show controls for the video acquisition parameters that can be configured in the framegrabber. For the MyoCam to work properly, **YOU MUST** select "Non-Interlaced" mode and type in "240" for Active Lines. Leave all other parameters in their default state.



The specific settings that can be changed depend on the capabilities of the frame grabber in your system - refer to the 'Frame grabber Parameters' section of the frame grabber documentation for more details on the meaning of each field.



You MUST set 'Non-Interlaced' and '240 Active Lines' in the Framegrabber Parameters for the MyoCam to work properly!

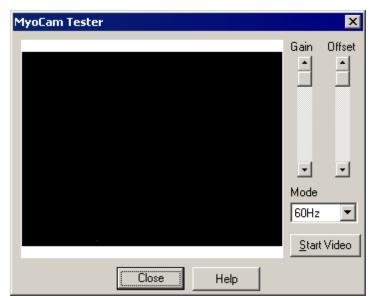
Verifying Camera and Framegrabber parameters

When you click the <u>Start Video</u> button, you should see a live picture from the camera displayed in the Video Display section of the dialog (see above). When the MyoCam video format switch (circled right) is set to 240Hz, the three black lines between the four parts of the image (see picture in <u>MyoCam Variable Field-Rate Details</u> 168) should be perfectly aligned with the three yellow lines drawn on the right edge of the image.



MyoCam 60/120/240 switch

5.5.2.4 Test Dialog



MyoCam Test Dialog

The <u>MyoCam Test Dialog</u> allows you to view live video from the camera and experiment with basic adjustments to the framegrabber parameters.

Gain Adjust the frame grabber gain.

Offset Adjust the frame grabber offset (also known as the black level).

ModeConfigures how software "cuts apart" the camera image (the setting here should

match the switch on MyoCam CCD Control box).

Start/Stop Video Starts and stops live video display in the test dialog



Gain/Offset values set in the test dialog do not have any effect on other parts of IonWizard

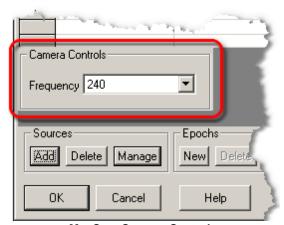


As a general rule, you should leave the software gain/offset controls at their default values and use the hardware gain and offset controls on the MyoCam CCD Control box.



To check if the camera is outputting the proper information, set Mode to 60Hz. When the switch on the video power box is set to 60Hz, you should see one full-height image. When it is set to 120Hz, you should see 2 half-height images. When it is set to 240Hz, you should see 4 quarter-height images.

5.5.2.5 Global Sensor Settings



MyoCam Camera Controls

When the current experiment includes one or more tasks that use the MyoCam, a <u>Camera Controls</u> group will be added to the <u>Global Sensor Settings</u> 22 area of the <u>Parameters</u> 20 dialog. This control allows you to set the frame mode that you will using when running this experiment.

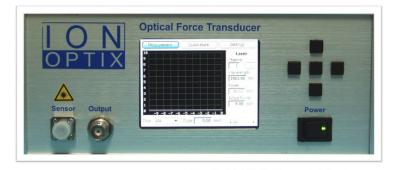
Refer to the Parameters 20 dialog documentation for more details.



You MUST set the mode switch on the MyoCam CCD Control Box to match the value you select in the Parameters *dialog* Camera Control when you start this experiment.

5.5.3 Optical Force Transducer

The lonOptix OptiForce is an optical fiber interferometry-based force transducer specially designed to detect the nanoscopic forces from single isolated cardiac cells. The OptiForce must be connected to an analog input port.



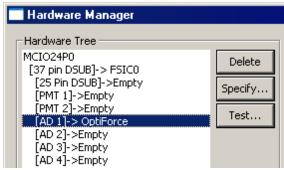


IonOptix OptiForce

Device Name

The lonOptix OptiForce device appears as "OptiForce" in the <u>Hardware Manager (8)</u> dialog's <u>Hardware Tree</u> section. The name can be changed in the <u>Specification Dialog</u> (167).

5.5.3.1 Device Connections



OptiForce Connections

Required connections

The <u>OptiForce</u> must be connected an analog input port such as the "AD 1" port of a <u>Fluorescence</u> System Interface 140.

5.5.3.2 Task Connections

The OptiForce Device provides an analog sensor that can be selected as in input in acquisition tasks such as the Trace Recording Task 41. In the following list "Name" is the description entered in the Specification dialog.

<u>OptiForce Device</u> requests the selected Task to have a two slope calibration. However this option might be ignored by the selected Task.

Device Sensor

The following sensor can be selected in any <u>acquisition task</u> that monitors or records analog values.

"Name (Opti force transducer)" Current value from device

Device Inputs

The OptiForce Device does not provide any device inputs.

5.5.3.3 Specification Dialog



OptiForce Specification Dialog

The Opti Force Device Specification dialog for the OptiForce Device allows you to enter an arbitrary description for the optical force transducer. The following values may be entered:

Device Description

Enter string to uniquely identify the device and output connection that you are defining.

Parent Device Settings

The options shown in the <u>Parent Device Settings</u> group are dependant on the parent device the <u>OptiForce Device</u> is connected to. If the input range of the parent device can not be changed (shown above), the input range will be displayed. If the input range of the parent device can be changed (as shown below), select the input voltage range.



5.5.3.4 Test Dialog



OptiForce Test Dialog

The <u>OptiForce Device Test</u> *dialog* displays the voltage being output by the external device. The value updates automatically until the *dialog* is closed.



Make sure the optiforce device is physically connected to the indicated port on the parent device and is switched on.

5.6 Windows Video Devices

Windows Videos Devices use the Microsfot Windows DirectShow API to communicate with the camear. Cameras which support this API are usually labeled as "DirectX-compatible," "DirectShow-compatible" or as having "WDM driver". Because these devices use the DirectShow API they can be physically connected by any method supported by the device's software drivers.

The <u>Generic DirectX Camera [182]</u> device provides basic video control and capture for any DirectShow camera while other devices such as the <u>MyoCamS USB 2.0 Camera [176]</u> device provide access to vendor-specific enhanced features such as faster frame rates and increased bit-depth.

5.6.1 MyoCamS USB 2.0 Camera

The MyoCamS *root device* provides device-specific support for the lonOptix MyoCamS USB 2.0 camera. In addition to providing the basic image acquisition functions of the Windows DirectShow compatible camera it provides access to the lonOptix camera extensions.

Device Name

The MyoCamS appears as "Aemics VI80U USB Camaera" in the <u>Hardware Manager 81</u> dialog's Hardware Tree section.

Requirements

The computer must have DirectX version 9 or later

5.6.1.1 Device Connections



MyoCamS Connections

The MyoCamS root device does not provide connections for other devices.

Required connections

The MyoCamS is a root device and is not connected to another device in the Hardware Tree 8.

5.6.1.2 Task Connections

The MyoCamS device provides a single device sensor that can be selected in acquisition tasks.

Device Sensor

The following sensor can be selected in any <u>acquisition task</u> that monitors or records images.

"Aemics Current image VI80U USB Camera (VI80u)"

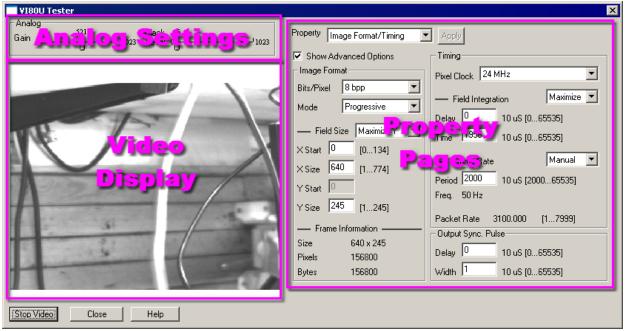
Device Inputs

The MyoCamS device does not provide any device inputs.

5.6.1.3 Specification Dialog

The MyoCamS *root device* does not have a specification *dialog*. The <u>Specifiy...</u> button in the <u>Hardware Tree</u> section will be disabled when the MyoCamS is selected.

5.6.1.4 Test Dialog



MyoCamS Test Dialog Overview

The MyoCam-S Test dialog is used to verify that the camera is operating properly and to provide the ability to experiment with various camera settings. There are three main areas:

Analog Settings

The <u>Analog Settings</u> controls adjust how the raw video signal is processed before it is digitized. You should set the gain and black level controls so that the dark areas of your image appear black and the brightest images are near-white.

Gain - Controls the overall brightness of the video image. If gain is too high bright areas will "wash out" to solid white, if too low the image will be dark.

Black Level - Controls the level that is digitized as black. Decreasing the black level make the entire image darker. If the black level is too low many dark areas will be solid black, if its too high "black" areas will appear gray.



If Gain and Black Level are set incorrectly it will hard, if not impossible, to see the video image. If this happens reset set gain and black level to the default values, gain=500, black level=10

Video Display

Displays live video after <u>Start Video</u> button pressed, stops when <u>Stop Video</u> button pressed.

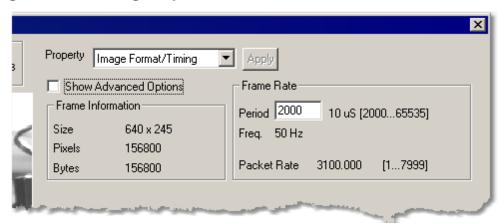
Property Pages

The test dialog has two main "property pages" selected by the <u>Property</u> *drop-down list:*

Image Format/Timing - Show properties that control image format (size) and capture rate. There are two version of this page, based on whether the <u>Show</u> <u>Advanced Options</u> check box is checked.

Trigger/Output - Show properties that control camera trigger and output options.

Image Format/Timing Properties - Basic



MyoCamsS Basic Image Format/Timing Properties

When Image Format/Timing is selected as the current property AND Show Advanced Options check box is NOT CHECKED the basic version of the image Image Format/Timing controls (shown above) will be displayed. In basic mode you enter the desired frame rate and the system will set the maximum number of lines (y size) and integration time that the camera will support at the given rate.

Frame Information

The <u>Frame Information</u> *group* displays information about the resulting image given the values that you have selected.

Size Number of pixels and number of lines in each image (frame) acquired

Pixels Total number of pixels in each image

Bytes Total number of bytes in each image

Frame Rate

Period Enter the number 10µs clock periods per frame (i.e. frame period in

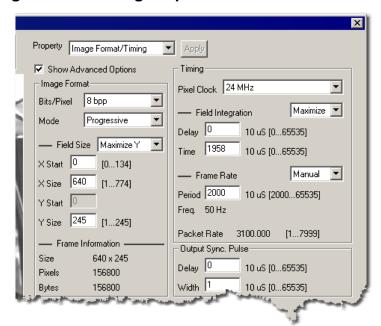
milliseconds times 100).

Freq. Displays the resulting frame frequency for the entered <u>Period</u>.

Packet Rate Displays the USB packet rate. This information is useful for debugging

purposes.

Image Format/Timing Properties - Advanced



MyoCamsS Advanced Image Format/Timing Properties

When Image Format/Timing is selected as the current property AND Show Advanced Options check box IS CHECKED the advanced version of the image Image Format/Timing controls (shown above) will be displayed. The advanced controls give you control of all software-adjustable MyoCamS parameters.

Image Format

The main (unlabeled) *controls* in the <u>Image Format</u> *group* determine the major characteristics of the image that will be acquired:

Bits/Pixel Select number of bits to store for each pixel

8 bpp - 8 bits/pixel, smallest pixel size, needed to achieve maximum frame-rates and smallest images

12 bpp - 12 bits/pixel, more detail for slower frame-rates, doubles size of resulting images

Mode Selects camera acquisition mode

Progressive - Only even lines are acquired which doubles the available frame rate and halves the number of lines per field.

Interlaced - Each image is acquired in two halves, even lines then odd lines, and then combined into a single image. This results in all lines being acquired but a decrease in the maximum frame-rate.



When acquiring interlaced images the odd and even lines are acquired at different points in time which can result in "comb" effects if the image moves between odd and even frames. This may make interlaced mode inappropriate in some situations.

Image Format - Field Size

The <u>Field Size</u> section of the <u>Image Format</u> group allows you to specify the specific dimensions of the image given the constraints of the main <u>Image Format</u> options entered above:

drop down

Control how field size parameters are adjusted when values in OTHER controls are changed:

Maximize Y - As values are changed in other parts of the <u>Property</u> area the <u>Y Size</u> value will be recalculated to the maximum possible value given other parameters.

Manual - The Y Size value will not be changed which may limit the

maximum values of other parameters.

X Start First pixel to acquire in line. To center the acquired image on the sensor

chip enter half of maximum value

X Size Number of pixels to acquire in a line, use <u>X Start</u> to offset pixels in line.

The primary reason to decrease X Size is to reduce the size of the resulting image files which is only significant if the images are saved.

Y Start
Y Size
Starting line to acquire. The value is fixed at zero for the MyoCamS
Total number of lines to acquire. The maximum value automatically

accounts for Mode selection in Image Format group as well as requested

Frame Rate if Frame Rate is set to "Manual"



Decreasing <u>Y Size</u> will result in higher maximum frame rates while changes in <u>X Size</u> do not have a significant effect in the maximum frame rate

Image Format - Frame Information

The <u>Frame Information</u> section of the <u>Image Format</u> group displays information about the resulting image given the values that you have selected.

Size Number of pixels and number of lines in each image (frame) acquired

Pixels Total number of pixels in each image

Bytes Total number of bytes in each image

Timing

The main (unlabeled) *control* in the <u>Timing</u> *group* allows you to select the clock used to read the image data from the CCD sensor.

Pixel Clock Selects the CCD pixel (read-out) clock frequency:

24 MHz - high speed read-out clock resulting in largest y-size for a given frame-rate

12 MHz - medium speed read-out clock decreases CCD read-out noise while maintaining "reasonable" rates

1 MHz - high quality read-out clock minimizes CCD read-out noise to maximize the amount of "real" data available when saving 12-bit data.

This option dramatically reduces the maximum frame-rate.



Delay

The qualitative difference between read-out clocks may not be noticeable and/or measurable unless you are in a low light (high gain) situation

Timing - Field Integration

The <u>Field Integration</u> section of the <u>Timing</u> group allows precise control when the CCD is sensitive to light

drop downControl how field size parameters are adjusted when values in OTHER

controls are changed:

Maximize - As values are changed in other parts <u>Property</u> area the <u>Time</u> field will be recalculated to the maximum possible value given other parameters

Manual - The Time field value will not be changed which may limit the

maximum values of other parameters.

Number of 10µs clock periods to delay from frame "start" before

Time Number of 10µs clock periods to "expose" CCD

"exposing" CCD.



Changing the Field Integration <u>Time</u> (either manually or via Maximize) effects the brightness of the acquired image in the same way that changing the shutter speed does on a 35MM camera.



If you set the Field Integration <u>Time</u> to a fixed value (drop down=manual) the overall brightness of the image will not change as you pick different frame-rates.



If you have enough light a shorter Field Integration <u>Time</u> can be used to decrease the amount of motion blur caused by the image moving while the CCD is exposed. Again similar to using fast shutter settings on a 35MM camera

Timing - Frame Rate

The <u>Frame Rate</u> section of the <u>Timing</u> group allows you to specify specific camera frame rates.

Period Enter the number 10µs clock periods per frame (i.e. frame period in

milliseconds times 100).

Freq. Displays the resulting frame frequency for the entered <u>Period</u>.

Packet Rate Displays the USB packet rate. This information is useful for debugging

purposes.

Output Sync Pulse

The Output Sync Pulse group allows control of a output pulse that occurs for each frame acquired

Delay Number of 10µs clock periods from frame "start" to setting output pulse to

active.

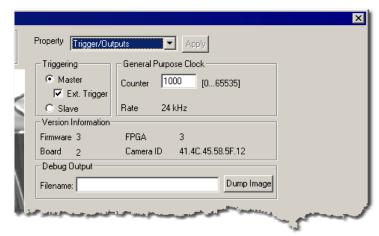
Width Number of 10µs clock periods before pulse is set to inactive. Set to zero

to disable.



<u>Delay</u> happens BEFORE the CCD is sampled and increases the amount of time required to sample each frame which decreases the maximum frame-rate

Trigger/Outputs Properties



MyoCam-S Test Dialog -Trigger/output Controls

When Trigger/Outputs is selected as the current property the following *controls* will be displayed:

Triggering

The <u>Triggering</u> *group* allows you to configure multiple MyoCamSs to operate in a Master/Slave relationship so that images acquired between the two cameras are phase-locked.

Master Camera generates all timing and clocks required for operation.

Ext. Trigger - If selected the MyoCamS outputs signals needed to provide

timing and clock signals to a 2nd, slave, MyoCamS

Slave Camera uses timing and clock signals from first, master, MyoCamS



Contact IonOptix for more information on this function

General Purpose Clock

The <u>General Purpose Clock</u> *group* allows an arbitrary divisor to be entered to create a slower frequency clock signal that is phase-locked to the MyoCamS <u>Pixel Clock</u>.

Counter Count-down value from pixel clock to output clock

Version Information

Firmware MyoCamS firmware version

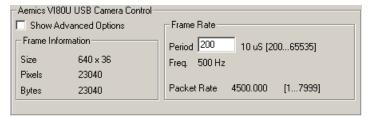
FPGA MyoCamS programmable logic code version

Board MyoCamS board version
Camera ID Unique camera ID value

Debug Output

Dumps a raw image for debugging purposes.

5.6.1.5 Global Sensor Settings



MyoCamS Global Sensor Options - Basic

When the current experiment includes one or more tasks that use the MyoCamS, a <u>Camera Controls</u> group will be added to the <u>Global Sensor Settings</u> 22 area of the <u>Parameters</u> 20 dialog. This control allows you to set the frame mode that you will use when running this experiment.

Refer to the <u>Image Format/Timing Properties - Basic 178</u> and the <u>Image Format/Timing Properties - Advanced 179</u> groups in the Test *dialog* for details on the operation of the Camera controls.

5.6.2 Generic DirectX Camera

The Generic DirectX Camera *root device* provides support for any Windows device that supports the Windows DirectX 9 "DirectShow" interface. This can include frame grabber cards, USB cameras or any other devices that provides the required functions.



IonOptix does not guarantee that the Generic DirectX Camera device will work with every Windows video device.

Device Name

The Generic DirectX Camera *device* appears as "Generic DirectX KS Camera" in the <u>Hardware Manager Add Root Dialog</u> 10 Type of Devices section. An instance of the device appears using same name as is used in Windows in the <u>Hardware Manager Dialog</u> 18 Hardware Tree section. That is if the camera appears a "USB Camera with mic" in My Computer it will appear as "USB Camera with mic" in the lonWizard hardware tree.

Requirements

The computer must have DirectX version 9 or later and the required device must support the DirectShow functions.

5.6.2.1 Device Connections



MyoCamS Connections

The Generic DirectX Camera root device does not provide connections for other devices.



"Orange Micro iBOT2 USB 2.0 Camera" is the Windows DirectX name of one specific camera used. You will only see the DirectX devices that exist in your computer, if any.

Required connections

The Generic DirectX Camera is a root device and is not connected to another device in the Hardware Tree 8).

5.6.2.2 Task Connections

Generic DirectX Camera device provides a singled device sensor that can be selected in acquisition tasks.

Device Sensor

The following sensor can be selected in any acquisition task that monitors or records images.

"Windows Directx Name (Generic Current image DirectX Camera)



"Windows Directx Name" is the DirectX device name assigned to the camera in Windows and will vary depending on the make and model of the camera

Device Inputs

The Generic DirectX Camera device does not provide any device inputs.

5.6.2.3 Specification Dialog

The Generic DirectX Camera root device does not have a specification dialog.

5.6.2.4 Test Dialog



Generic DirectX Camera Test Dialog

The Generic DirectX Camera *root device* allows you to view live video from the camera and experiment with basic adjustments to the video acquisition parameters.

Mode If supported by the Windows device, selects the Windows video mode to use.

Gain Adjusts the video gain.

Offset Adjusts the video offset (also known as the black level).

Start/Stop Video Start and stop real-time update of live video image in test dialog.



Video settings in the test dialog do not affect other parts of IonWizard

5.6.2.5 Global Sensor Settings

The Generic DirectX Camera root device does not have any experiment-adjustable sensor settings.

5.7 Miscellaneous Devices

Miscellaneous Devices are simple devices that provide task connections as well as other random devices that don't fit in other categories.

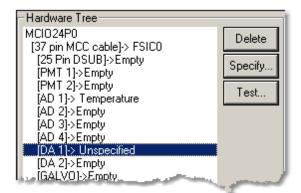
5.7.1 Analog Sink Device

The <u>Analog Sink Device</u> allows you to define the characteristics of an analog input of an external device. The range of voltages that can be output is dependent on the capabilities of the parent device and the settings in in the <u>specification dialog</u> [186].

Device Name

The <u>Analog Sink Device</u> appears as "Unspecified" in the <u>Hardware Manager 8 dialog's Hardware Tree</u> section. The name can be changed in the <u>Specification Dialog 186</u>.

5.7.1.1 Device Connections



Analog Sink Device Connections

Required connection

The <u>Analog Sink Device</u> can be connected to any available analog output port in the hardware tree such as the "DA 1" port of a <u>Fluorescence System Interface 140</u> as shown above.

Provided connections

The Analog Sink Device does not provide connections to other devices.

5.7.1.2 Task Connections

The <u>Analog Sink Device</u> device provides an analog output that can be selected as destination in acquisition tasks such as the <u>Trace Output Task</u> 87. In the following list "*Name*" is the description entered in the <u>Specification</u> 186 dialog.

Device Sensor

The Analog Sink Device device does not provide any device sensors.

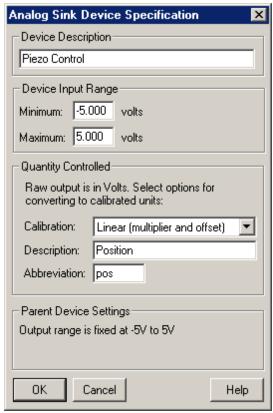
Device Inputs

The following device can be selected in any acquisition task that outputs analog values.

"Name (Analog Sink)"

Voltage specified by acquisition task will be output to device

5.7.1.3 Specification Dialog



Analog Sink Device Specification Dialog

The <u>Analog Sink Device Specification</u> dialog allows you describe the external device input port and specify the range of voltages that can be accepted.

Device Description Enter string to uniquely identify the device and input connection that you are

defining.

Device Input Range Define the minimum and maximum voltages that the external device input can

accept.

Quantity ControlledHere you choose the calibration needed to convert from usable units into raw volts and also, if relevant, the terms that describe the quantity controlled.

There are two <u>Calibration</u> options available:

None - No calibration is applied to the data controlled. This implies that the device in question is a voltage device. In this case the <u>Description</u> and <u>Abbreviation</u> *fields* will be unavailable and are assigned the values Electric Potential and Ptnl. respectively.

Linear - A linear calibration is applied to convert units into the final raw voltage data. This calibration is run and the resultant calibration constants are entered in the acquisition program. The text entered in the <u>Description</u> and <u>Abbreviation</u> *fields* here will be presented in the acquisition program to help label the calibration constants. This text should indicate the quantity controlled, not the units of what is being controlled. The actual units (Pascals, degrees Celsius, mm Hg, etc.) will be defined by the standard used in the calibration. Thus here you should choose Temperature over C and Pressure over Pascals.

Parent Device Settings

The options shown in the <u>Parent Device Settings</u> group are dependant on the parent device the <u>Analog Sink Device</u> is connected to. If the parent device has a programmable range (shown below), set the output voltage range of parent device output. If the parent device's output range can not be changed (shown above), the <u>Parent Device Settings</u> group will display the output range of the parent.





The device input range is used to prevent IonWizard from sending voltages that may harm the device. It does NOT affect how values are scaled to volts.



The device description should describe the external device and its input connection so that the hardware tree will document your setup. Eg "[DA 1] -> Chart Recorder" or "[DA 1] -> P-Clamp input 2".

5.7.1.4 Test Dialog



Analog Sink Device Test Dialog

The <u>Analog Sink Device Test</u> dialog does not currently function. Eventually it will allow you to set the voltage output to the device.

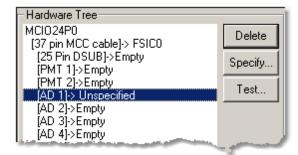
5.7.2 Analog Source Device

The <u>Analog Source Device</u> allows you to define a connection to an analog output of an external device. The range of voltages that can be input from the external device is dependent on the capabilities of the parent device and the settings in the <u>Specification dialog</u> [189].

Device Name

The <u>Analog Source Device</u> appears as "Unspecified" in the <u>Hardware Manager (8)</u> dialog's <u>Hardware Tree</u> section. The name can be changed in the <u>Specification Dialog</u> (189).

5.7.2.1 Device Connections



Analog Source Device Connections

Required connections

The <u>Analog Source Device</u> must be connected an analog input port such as the "AD 1" port of a Fluorescence System Interface 140.

Provided connections

The Analog Source Device does not provide connections to other devices.

5.7.2.2 Task Connections

The <u>Analog Source Device</u> device provides an analog sensor that can be selected as in input in acquisition tasks such as the <u>Trace Recording Task</u> [41]. In the following list "*Name*" is the description entered in the <u>Specification</u> [186] dialog.

Device Sensor

The following sensor can be selected in any acquisition task that monitors or records analog values.

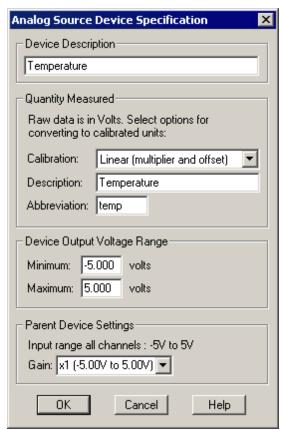
"Name (Analog Source)"

Current analog value from device

Device Inputs

The Analog Sink Device device does not provide any device inputs.

5.7.2.3 Specification Dialog



Analog Source Device Specification Dialog

The <u>Analog Source Device Specification</u> *dialog* allows you describe an external device's output port and specify the range of voltages that it can produce.

Device Description Enter string to uniquely identify the device and output connection that you are defining.

Quantity Measured

Here you choose the calibration needed to convert raw volts into usable units and also, if relevent, the terms that describe the quantity measured. There are two Calibration options available:

None - No calibration is applied to the data acquired. This implies that the sensor in question is a voltage sensor. In this case the <u>Description</u> and <u>Abbreviation</u> *fields* will be unavailable and are assigned the values Potential and Pot. respectively.

Linear - A linear calibration is applied to convert raw data into the final output units. This calibration is run and the resultant calibration constants are entered in the acquisition program. The text entered in the <u>Description</u> and <u>Abbreviation</u> *fields* here will be presented in the acquisition program to help label the calibration constants. This text should indicate the quantity measured, not the units of measurement. The actual units (Pascals, degrees Celsius, mm Hg, etc.) will be defined by the standard used in the calibration. Thus here you should choose Temperature over C and Pressure over Pascals.

Device Output Voltage Range Enter the minimum and maximum voltages that the external device can output.

Parent Device Settings

The options shown in the <u>Parent Device Settings</u> *group* are dependant on the parent device the analog source device is connected to. If the input range of the parent device can be changed (as shown above), set the input voltage range. If the input range of the parent device can not be changed (shown below), the input range will be displayed.





The device output range is used to document what voltages the external device will output, they don't not affect how voltages are converted to units in Acquisition Tasks.



The device description should describe the external device and its output connection so that the hardware tree will document your setup. Eg "[AD 1] -> Temperature" or "[AD 1] -> Pressure monitor 2".

5.7.2.4 Test Dialog



Analog Source Device Test
Dialog

The <u>Analog Source Device Test</u> *dialog* displays the voltage being output by the external device. The value updates automatically until the *dialog* is closed.



Make sure the analog source device is physically connected to the indicated port on the parent device.

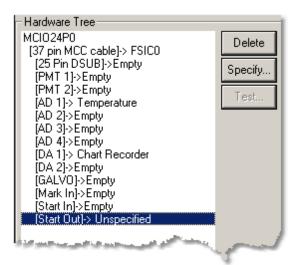
5.7.3 Digital Sink Device

The <u>Digital Sink Device</u> allows you to define the characteristics of a digital input of an external device.

Device Name

The <u>Digital Sink Device</u> appears as "Unspecified" in the <u>Hardware Manager 8 dialog's Hardware Tree</u> section. The name can be changed in the <u>Specification Dialog 19 1</u>.

5.7.3.1 Device Connections



Digital Sink Device Connections

Required connections

The <u>Digital Sink Device</u> must be connected to a digital output *port* such as the "Start Out" port of a <u>Fluorescence System Interface</u> 140.

Provided connections

The <u>Digital Sink Device</u> does not provide connections to other devices.

5.7.3.2 Task Connections

The <u>Digital Sink Device</u> provides a digital input that can be selected in acquisition tasks. In the following list "*Name*" is the description entered in the <u>Specification</u> dialog.

Device Sensor

The <u>Digital Sink Device</u> device does not provide any device sensors.

Device Inputs

The following device can be selected in any <u>acquisition task</u> that outputs digital values.

"Name (Digital Sink)"

Value specified by acquisition task will be output to device

5.7.3.3 Specification Dialog



Digital Sink Device Specification
Dialog

The <u>Digital Sink Device Specification</u> dialog allows you describe an external device's input port.

Device Description Enter string to uniquely identify the device and input connection that you are



The device description should describe the external device and its input connection so that the hardware tree will document your setup. Eg "[Start Out] -> Stimulator Trigger".

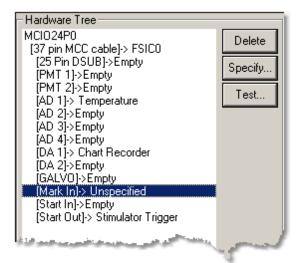
5.7.4 Digital Source Device

The <u>Digital Source Device</u> allows you to define the characteristics of a digital output of an external device. In the Hardware Manager, attachment of the device "Digital Source" to either the "Start In" or "Mark In" connection point on a DSI or FSI ensures proper IonWizard support.

Device Name

The <u>Digital Source Device</u> appears as "Unspecified" in the <u>Hardware Manager</u> 8 dialog's <u>Hardware Tree</u> section. The name can be changed in the <u>Specification Dialog</u> 189.

5.7.4.1 Device Connections



Digital Source Device Connections

Required connections

The <u>Digital Source Device</u> must be connected to a digital input *port* such as the "Mark In" port of a Fluorescence System Interface 140.

Provided connections

The <u>Digital Source Device</u> does not provide connections to other devices.

5.7.4.2 Task Connections

The <u>Digital Source Device</u> provides a digital sensor that can be selected in acquisition tasks such as the <u>Event Recording Task</u> 4. In the following list "*Name*" is the description entered in the <u>Specification</u> 186 dialog.

Device Sensor

The following sensor can be selected in any acquisition task that monitors or records analog values.

"Name (Digital Source)"

Current digital value from device

Device Inputs

The <u>Digital Source Device</u> device does not provide any device inputs.

5.7.4.3 Specification Dialog



Digital Source Device Specification Dialog

The <u>Digital Source Device Specification</u> dialog allows you describe an external device's output port.

Device Description Enter string to uniquely identify the device and output connection that you are defining.



The device description should describe the external device and its output connection so that the hardware tree will document your setup. Eg "[Mark In] -> MyoPacer Gate Out".

5.7.4.4 Test Dialog



Digital Source Device Test
Dialog

The <u>Digital Source Device Test</u> dialog displays the digital value being output by the external device. The value updates automatically until the dialog is closed.



Make sure the digital source device is physically connected to the indicated port on the parent device.

5.7.5 Miscellaneous Microscope Light Source Device

The <u>Miscellaneous Microscope Light Source Device</u> is used to describe any excitation light source that can not be controlled by the computer. For lonWizard's purpose, any non-computer controlled device becomes a single-excitation light source that is viewed as "always on" and with a fixed excitation filter.



IonWizard does not support more than one excitation light source in hardware tree at the same.

Device Name

The <u>Miscellaneous Microscope Light Source Device</u> appears as "Misc. Microscope Light Source" in the <u>Hardware Manager Add Root Dialog</u> 10 Type of Devices section. An instance of the device appears as "Generic Microscope Light Source" in the <u>Hardware Manager Dialog</u> 1 Hardware Tree section.

Requirements

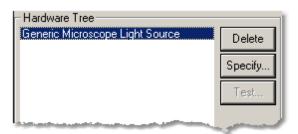
This device has no requirements.

5.7.5.1 Device Connections



Miscellaneous Microscope Light Source Add Root

The <u>Miscellaneous Microscope Light Source Device</u> is a root device which is added to the hardware tree using the <u>Add Root</u> 10 function of the Hardware Manager *dialog*. In Add Root dialog select "Misc. Micorscope Light Source" from in the <u>Type of Device</u> *list* then "MiscMicroscopeLS" in the <u>Instance of Device</u> *list*.



Miscellaneous Microscope Light Source Connections

Once the device has been added, it will show in the hardware tree with the description entered in the <u>Specification Dialog</u> 195].

Required connections

The <u>Miscellaneous Microscope Light Source Device</u> is a root device and does not have any required connections

5.7.5.2 Task Connections

The Miscellaneous Microscope Light Source Device does not provide any connections for acquisition tasks.

5.7.5.3 Specification Dialog



Miscellaneous Microscope Light Source Specification Dialog

The <u>Miscellaneous Microscope Light Source Specification</u> *dialog* allows you to enter the description of the device that appears in the hardware tree.

5.7.6 RS-170 Camera

The <u>RS-170 Camera Device</u> allows you to connect a standard RS-170 black-and-white interlaced camera to a compatible video input device.

Device Name

The RS-170 camera appears as "RS-170 Camera" in the <u>Hardware Manager and Jacobs Hardware Tree</u> section. The name can be changed in the <u>Specification Dialog</u> [196].

Requirements

A device (frame-grabber) which provides a RS-170 video input connection is required.

5.7.6.1 Device Connections



RS-170 Connections

Required connections

The RS-170 camera device requires a RS170 standard video input connection on a video input device such as the Mutech MV510 [133] [RCA] input (shown above).

Provided connections

The RS-170 camera device does not provide any connections.

5.7.6.2 Task Connections

The RS-170 Camera Device provides a single device sensor that can be selected in acquisition tasks.

Device Sensor

The following sensor can be selected in any acquisition task that monitors or records images.

"RS-170 Camera (Generic RS-170 This is a connection to the image stream. Note: "RS-170 Camera" is the name entered in the Specification Dialog 1961.

Device Inputs

The RS-170 Camera Device device does not provide any device inputs.

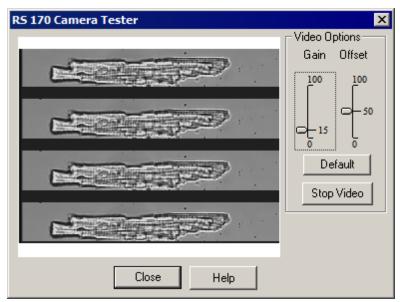
5.7.6.3 Specification Dialog



RS-170 Device Specification Dialog

The RS-170 Device Specification dialog has the following fields:

5.7.6.4 Test Dialog



RS-170 Device Test Dialog

The <u>RS-170 Device Test</u> dialog allows you to view live video from the camera and experiment with basic adjustments to the frame grabber parameters if supported by the parent device

Gain Adjust the frame grabber gain.

Offset Adjust the frame grabber offset (also known as the black level).

Default Set gain/offset fields to default values

Start/Stop Video Starts and stops live video display in the test dialog



Gain/Offset values set in the test dialog are local and do not have any effect outside the dialog.

5.7.7 CCIR Camera

The <u>CCIR Camera Device</u> allows you to connect a standard CCIR/PAL black-and-white interlaced camera to a compatible video input device.

Device Name

The CCIR camera appears as "CCIR Camera" in the <u>Hardware Manager and Jacobs Section</u>. The name can be changed in the <u>Specification Dialog</u> 1981.

Requirements

A device (frame-grabber) which provides a CCIR video input connection is required.

5.7.7.1 Device Connections



CCIR Connections

Required connections

The CCIR camera device requires a CCIR standard video input connection on a video input device such as the Mutech MV510 [133] [RCA] input (shown above).

Provided connections

The CCIR camera device does not provide any connections.

5.7.7.2 Task Connections

The CCIR Camera Device provides a single device sensor that can be selected in acquisition tasks.

Device Sensor

The following sensor can be selected in any <u>acquisition task</u> that monitors or records images.

"CCIR Camera (Generic CCIR Camera)"

This is a connection to the image stream. Note: "CCIR Camera" is the name entered in the Specification Dialog 198.

Device Inputs

The CCIR Camera Device device does not provide any device inputs.

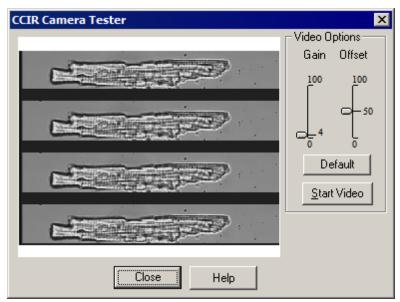
5.7.7.3 Specification Dialog



CCIR Device Specification Dialog

The CCIR Device Specification dialog has the following fields:

5.7.7.4 Test Dialog



CCIR Device Test Dialog

The <u>CCIR Device Test</u> dialog allows you to view live video from the camera and experiment with basic adjustments to the frame grabber parameters if supported by the parent device

Gain Adjust the frame grabber gain.

Offset Adjust the frame grabber offset (also known as the black level).

Default Set gain/offset fields to default values

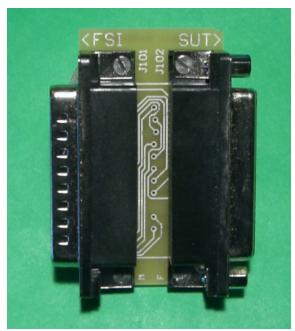
Start/Stop Video Starts and stops live video display in the test dialog



Gain/Offset values set in the test dialog are local and do not have any effect outside the dialog.

5.7.8 LS Port to Parallel Port Adapter

The <u>Parallel Port Adapter Device</u> is a simple device, shown below, that makes the standard lonOptix Fluorescence System Interface 25-pin light source port compatible with most devices that were designed to be connected to a standard PC printer port.



IonOptix Light Source Port to Parallel Port Adapter

For more information on installing the adapter see the FSI to Parallel Port Adapter Hardware Manual.



The signals supported by the adapter are a subset of the complete PC parallel port. Specifically only the 8 data outputs (pins 2-9) and the Busy (pin 11) and Paper-out (pin 12) input bits are supported.

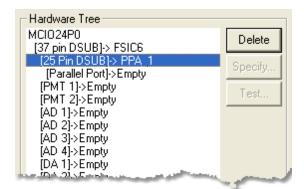
Device Name

The Parallel Port Adapter appears as "PPA_n" in the <u>Hardware Manager</u> 8 dialog's <u>Hardware Tree</u> section.



The "n" in the instance name (PPA_n) will be 0 after computer is restarted and will increment each time the device is opened.

5.7.8.1 Device Connections



Parallel Port Adapter Connections

Required connections

The <u>Parallel Port Adapter</u> must be connected to a <u>25 Pin DSUB</u> port on a Fluorescence System Interface..

Provided connections

The <u>Parallel Port Adapter</u> provides the following connections:

Parallel Port Subset of standard PC parallel printer port connections

5.7.8.2 Task Connections

The Parallel Port Adapter Device does not provide any connections for acquisition tasks.

5.7.8.3 Specification Dialog

The <u>Parallel Port Adapter Device</u> does not have a specification dialog. The <u>Specifiy...</u> button in the <u>Hardware Tree</u> section will be disabled when <u>Parallel Port Adapter Device</u> is selected.

5.7.8.4 Test Dialog

The <u>Parallel Port Adapter Device</u> does not have a test dialog. The <u>Test...</u> button in the <u>Hardware Tree</u> section will be disabled when <u>Parallel Port Adapter Device</u> is selected.

5.7.9 LS Port to DA Adapter

The <u>DA Port Adapter Device</u> is a simple device, really just a special cable as shown below, that makes the standard lonOptix Fluorescence System Interface 25-pin light source port compatible with most devices that can be driven by an analog voltage.



IonOptix Light Source Port to DA Adapter

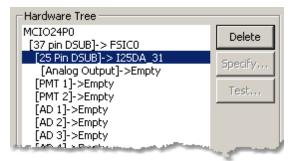
Device Name

The <u>DA Port Adapter</u> appears as "I25DA_n" in the <u>Hardware Manager</u> 18 dialog's <u>Hardware Tree</u> section.



The "n" in the instance name (I25DA_n) will be 0 after computer is restarted and will increment each time the device is opened.

5.7.9.1 Device Connections



DA Port Adapter Connections

Required connections

The DA Port Adapter must be connected to a 25 Pin DSUB port on a Fluorescence System Interface..

Provided connections

The <u>DA Port Adapter</u> provides the following connections:

Analog Output A voltage output which can drive analog input light sources (or other devices).

5.7.9.2 Task Connections

The <u>DA Port Adapter</u> does not provide any connections for acquisition tasks.

5.7.9.3 Specification Dialog

The <u>DA Port Adapter</u> does not have a specification dialog. The <u>Specifiy...</u> button in the <u>Hardware Tree</u> section will be disabled when <u>DA Port Adapter</u> is selected.

5.7.9.4 Test Dialog

The <u>DA Port Adapter</u> does not have a test dialog. The <u>Test...</u> button in the <u>Hardware Tree</u> section will be disabled when <u>DA Port Adapter</u> is selected.

5.8 The Imaging Source (TIS) Devices

The TIS devices provide support for cameras and frame grabbers manufactured by The Imaging Source, GmbH.

5.8.1 TIS DMK Camera

The TIS DMK Camera *root device* provides device-specific support for any monochrome camera that is supported by the The Imaging Source Imaging Control library. In addition to providing the basic image acquisition functions of the Windows DirectShow compatible camera it provides access to the enhanced TIS functions.

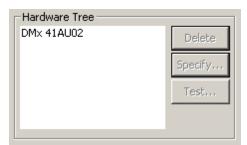
Device Name

The camera model number (e.g. "DMx 41AU02") is used as the default name in the <u>Hardware Manager</u> 8 dialog's <u>Hardware Tree</u> section. The name can be changed in the <u>Specification Dialog</u> 204 which is presented when you add the device.

Requirements

An available USB 2.0 or Firewire port.

5.8.1.1 Device Connections



TIS DMK Camera Connections

Required connections

The TIS DMK Camera root device is not connected to another device in the Hardware Tree 8.

Provided connections

The TIS DMK Camera root device does not provide connections for other devices.

5.8.1.2 Task Connections

The TIS DMK Camera root device provides a single device sensor that can be selected in acquisition tasks.

Device Sensor

The following sensor can be selected in any <u>acquisition task</u> that monitors or records images.

"DMx 41AU02 (The Image Source DMK)" Current image.

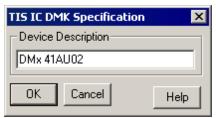


"DMx 41AU02" is the default name for one specific camera. The default will be different for other cameras and may also be changed in the <u>Specification Dialog</u> 2041.

Device Inputs

The TIS DMK Camera root device does not provide any device inputs.

5.8.1.3 Specification Dialog

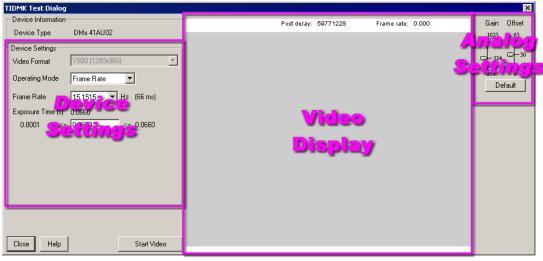


TIS DMK Camera Specification Dialog

The <u>TIS DMK Camera Specification</u> *dialog* has the following field:

Camera Description Enter string to uniquely identify the camera, default value will be the model number of the camera

5.8.1.4 Test Dialog



TIS DMK Camera Test Dialog

The <u>TIS DMK Camera Test</u> dialog allows you to view live video from the camera and experiment with the settings that you will be able to select in the <u>Global Sensor Settings</u> 2061. There are three main areas:

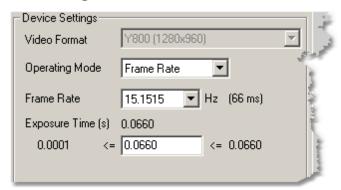
Device Settings The device settings are allows parameters for the specific camera

Video Displays live video after <u>Start Video</u> button pressed, stops when <u>Stop Video</u> button

pressed.

Analog Settings Adjusts how the raw video signal is processed before it is digitized.

Device Settings



TIS DMK Camera Test Dialog - Device Settings

The <u>Device Settings</u> *group* allows you to change camera parameters and view the results in the <u>Video</u> <u>Display</u> *area.*

Video Format Select the desired video format from the list of formats supported by the camera. If there is only one format the control will be disabled as shown above camera

Operating Mode Choose between the <u>Frame Rate</u> and <u>Exposure</u> mode. <u>Frame Rate</u> mode is most useful when you are interested in the frame rate - i.e. the number of images per second - and there is plenty of signal. Exposure mode is most useful when looking at dimmer signals, for example fluorescence, and you need to optimize the exposure time to capture a good image.

Frame Rate The behavior of this control depends on the <u>Operating Mode</u>. In <u>Frame Rate</u> mode, you chose the number of images per second from the combo-box. In <u>Exposure</u> mode, this become a read-only display of the frame rate resulting from the chosen exposure time.

Exposure Time In both operating modes, use this text field to enter the exposure time for each image. The range of values you can enter changes based on operating mode. In <u>Frame Rate</u> mode, the range is dictated by the chosen frame rate. In <u>Exposure</u> mode, the range is dictated by the camera itself.



In "Exposure" operating mode the resulting frame rate is limited by timing details in the camera. Changing exposure time may not always result in different frame rate.

Video Display

The Video Display area displays video from the camera when enabled

Start Video Starts display of live video from camera using settings in the <u>Device Settings</u> *group* . Once pressed *button* will change to "Stop Video"

Stop Video Stops display of live video leaving last image in the <u>Video Display</u> *area*. Once pressed *button* will change back to "Start Video"

Analog Settings

You should set the gain and black level controls so that the dark areas of your image appear black and the brightest images are near-white.

Gain Controls the overall brightness of the video image. If gain is too high bright

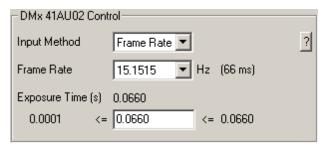
areas will "wash out" to solid white, if too low the image will be dark.

Black Level Controls the level that is digitized as black. Decreasing the black level make

the entire image darker. If the black level is too low many dark areas will be

solid black, if its too high "black" areas will appear gray.

5.8.1.5 Global Sensor Settings



TIS DMK Camera Global Sensor Options

When the current experiment includes one or more tasks that use the TIS DMK Camera, a <u>Camera Controls group</u> will be added to the <u>Global Sensor Settings</u> [22] area of the <u>Parameters</u> [20] dialog. This control allows you to set the frame mode that you will use when running the experiment.

Refer to the <u>Device Settings</u> [205] *group* in the <u>Test Dialog</u> [204] for details on the operation of the Camera controls.

5.8.2 TIS DFG Frame Grabber

The TIS DFG Frame Grabber *root device* provides the ability to record video images from a The Imaging Source frame grabber device that is supported by their IC Imaging Control library.

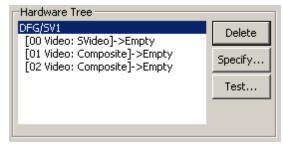
Device Name

The frame grabber model number (e.g. "DFG/SV1") is used as the default name in the <u>Hardware Manager</u> 3 dialog's <u>Hardware Tree</u> section. The name can be changed in the <u>Specification Dialog</u> which is presented when you add the device.

Requirements

An available interface slot or port compatible with the frame grabber device.

5.8.2.1 Device Connections



DFG/SV1 TIS DMK Camera Connections

Required connections

The TIS DFG Frame Grabber root device is not connected to another device in the Hardware Tree 8 h.

Provided connections

The TIS DFG Frame Grabber *root device* provides connections based on the information provided by the IC Capture Library for the frame grabber that is installed. The sample above, for the DFG/SV1 frame grabber, shows the following connections:

00 Video: SVideo Connection for camera via s-video connector

01 Video: Composite Connection for composite color or black-and-white camera via 1st RCA

composite input

02 Video: Composite Connection for composite color or black-and-white camera via 2nd RCA

composite input

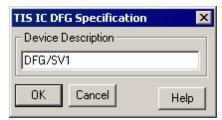


The camera connections are different for each frame grabber model

5.8.2.2 Task Connections

The TIS DFG Frame Grabber root device does not provide any connections for acquisition tasks.

5.8.2.3 Specification Dialog

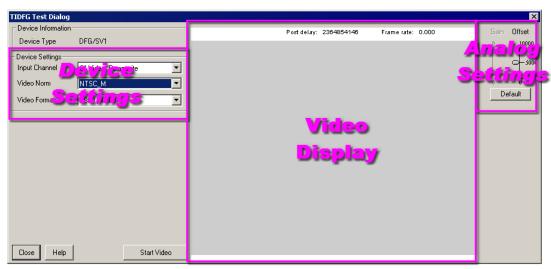


TIS DFG Frame Grabber Specification Dialog

The TIS DFG Frame Grabber dialog has the following field:

Device Description Enter string to uniquely identify the frame grabber, default value will be the model number of the frame grabber.

5.8.2.4 Test Dialog



TIS DFG Frame Grabber Test Dialog

The <u>TIS DFG Frame Grabber Test</u> *dialog* allows you to view live video from cameras attached to any of the frame grabber's inputs and then experiment with the settings that you will be able to select in the <u>Global Sensor Settings</u> [206]. There are three main areas:

Device Settings The device settings are allows parameters for the specific camera

Video Display Displays live video after Start Video button pressed, stops when Stop Video button

pressed.

Analog Settings Adjusts how the raw video signal is processed before it is digitized.

Device Settings



TIS DFG Frame Grabber Test Dialog - Device Settings

The <u>Device Settings</u> *group* allows selects options that will be used to display to display video in the <u>Video Display</u> *area*.

Input Channel List of available input channels (ports) on the frame-grabber

Video Norm Video timing standard to use: NTSC (North American) or PAL (European)

Video Format All available combinations of available color space (Y800, RGB24, etc) and zoom amounts for the given <u>Input Channel</u> and <u>Video Standard</u>.



The numb



The test dialog allows view video with different zoom amounts experiments will always run in the highest resolution for a given color space, ie Y800 (640x480)

Video Display

The <u>Video Display</u> area displays video from the camera when enabled

Start Video Starts display of live video from camera using settings in the <u>Device Settings</u> *group*. Once pressed *button* will change to "Stop Video"

Stop Video Stops display of live video leaving last image in the <u>Video Display</u> *area*. Once pressed *button* will change back to "Start Video"

Analog Settings

You should set the gain and black level controls so that the dark areas of your image appear black and the brightest images are near-white.

Gain Controls the overall brightness of the video image. If gain is too high bright

areas will "wash out" to solid white, if too low the image will be dark.

Black Level Controls the level that is digitized as black. Decreasing the black level make

the entire image darker. If the black level is too low many dark areas will be

solid black, if its too high "black" areas will appear gray.



While the test dialog allows for you to use different zoom amounts experiments will always run in the highest resolution for a given color space (ie Y800 (640x480)

5.9 IonOptix MyoCam-S3 Devices

IonOptix MyoCam-S3 Devices provide support for cameras manufactured by IonOptix, LLC.

5.9.1 IonOptix MyoCam-S3 Camera



The lonOptix MyoCam-S3 Camera *root device* item allows you to connect the namesake to the hardware tree.

Device Name

The camera model number (e.g. "MyoCamS3_M2024G_FF000773") is used as a default name in the Hardware Manager hardware Tree section. The name can be changed in the Specification Dialog [213] which is presented at the moment of adding the device.

Requirements

- 1. Available USB3 (preferred) or USB2 port.
- FPGA-equipped FSI device

Features

- Maximal frame rate for USB3 connection is 3500Hz, but that rate may not be achieved on every system due to multiple factors. With USB2 the frame rate can go up to 800Hz.
- · All images are recorded in grayscale 8 bit format
- Maximal exposure time for each given combination of frame rate and image dimensions.
- Maximal image size is 1936 pixels wide and 1216 pixels high for lower frame rates (up to 160Hz for USB3, or up to 10Hz for USB2).
- Maximal image height for higher frame rates depends on frame rate and average connection bandwidth. Camera can be calibrated to represent the actual limit for a given installation.
- Camera frame timestamps are synchronized with the internal clock of the attached FSI.

5.9.1.1 Device Connections

Required connections

The "IN" connector of the lonOptix MyoCam-S3 camera has to be attached to the START output port of a configured FPGA-equipped FSI with a BNC cable.



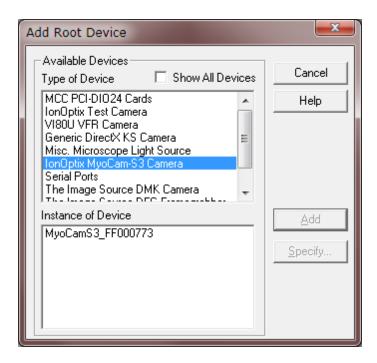
5.9.1.2 Task Connections

The <u>lonOptix MyoCam-S3 Camera</u> provides a single device sensor that can be selected in acquisition tasks.

Device Sensor

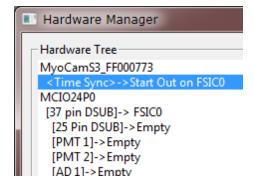
The following sensor can be selected in any acquisition task that monitors or records images:

MyoCamS3_ This name can be modified in the Specification Dialog 213. serial# (for example, MyoCamS3_FF0 00773)

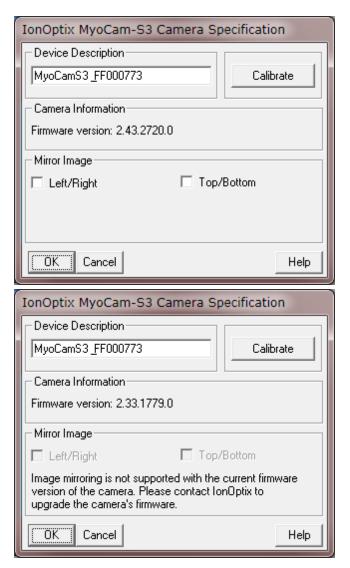


Device Inputs

The IonOptix MyoCam-S3 camera *root device* requires a connection to a <u>Start Out</u> port of a FPGA-equipped FSI device:



5.9.1.3 Specification Dialog



Device Description Enter a string to uniquely identify the camera. Default value is composed of a shortened camera model name and its serial number.

Calibrate Calibrate camera settings for the current USB port type. See Calibration for more information.

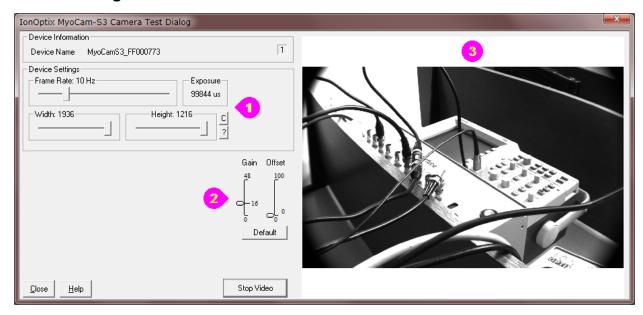
Camera Information Shows the current firmware version installed on the camera.

Mirror Image Left/Right: will mirror the image left/right.

Top/Bottom: will mirror the image top/bottom.

NOTE: Calibration is disabled unless the camera is connected to an FSI. See <u>Device Connections</u> [211] and <u>Task Connections</u> [211] to see how to establish a physical and logical link between a camera and an FSI. **NOTE**: The <u>Mirror Image Settings</u> are not available when the camera's firmware version is too low. Please contact lonOptix to upgrade the camera's firmware.

5.9.1.4 Test Dialog



The lonOptix MyoCam-S3 Test *dialog* allows to view live video from the camera and experiment with settings that can be selected later in the <u>Global Sensor Settings</u> 2151 dialog. There are three main areas:

Device Settings

]

The <u>Device Settings</u> group allows to change camera parameters and immediately see results in the <u>Video Display</u> area.

Fra Number of images per second. Maximum frame rate depends on the USB port used and the calibration limits (see <u>Calibration 216</u>) for more information).

Rate

Expo(*read-only*) Exposure time for this setup. The lonWizard automatically picks a maximal value that **sure** can be achieved for given frame rate and image dimensions. **[time**]

Widt Width of the image in pixels. This parameter can be varied from camera's minimum (256px) to h maximum (1936px) in 20% increments.

Heig Height of the image in pixels. The value depends on the frame rate, image dimensions, USB port **ht** used, and other parameters.

- Restore image width and height back to the calibrated values. See Calibration 216 for more information.
- ? Context help for this Device Settings dialog.

Analog Settings

Gain

Controls the overall brightness of the video image. If gain is too high bright areas will "wash out" to solid white, if too low the image will be dark.

Offset

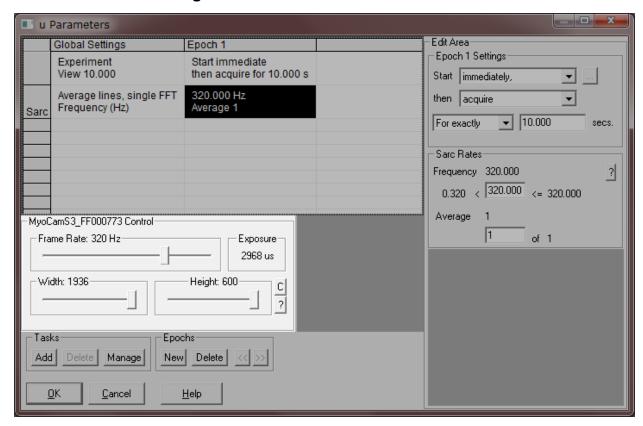
Controls the level that is digitized as black. Decreasing the black level make the entire image darker. If the black level is too low many dark areas will be solid black, if its too high "black" areas will appear gray.

It is recommended to set the gain and black level controls so that the dark areas of the image appear black and the brightest areas appear near-white.

Video Display

Start Video / Stop Video Starts or stops the live video from the camera.

5.9.1.5 Global Sensor Settings



Fra Number of images per second. Maximum frame rate depends on the USB port type and the calibration limits (see <u>Calibration 216</u>) for more information).

Rate

Expo(*read-only*) Exposure time for this setup. The lonWizard automatically picks a maximal value that **sure** can be achieved for given frame rate and image dimensions.

[time

]

Widt Width of the image in pixels. This parameter can be varied from camera's minimum (256px) to **h** maximum (1936px) in 20% increments.

Heig Height of the image in pixels. The value depends on the frame rate, image dimensions, USB port **ht** used, and other parameters.

- Restore image width and height back to the calibrated values. See Calibration 216 for more information.
- ? Context help for this Device Settings dialog.

5.9.1.6 Calibration

Introduction

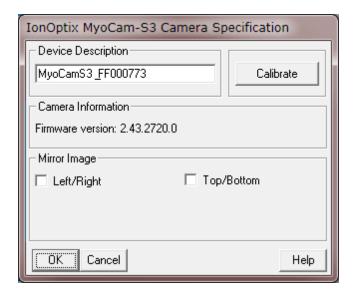
The purpose of camera calibration is to determine the dynamic limits on video streaming from the configured camera on a *specific* system. Generally, there are no additional limitations on video streaming at lower frame rates (125Hz and less). However, at the higher frame rates camera's own performance may become undermined by other factors like the USB port average bandwidth, CPU speed, etc.

The calibration process produces presets for a specific system and writes them into the lonWizard's XML configuration file. After that those presets are used throughout the system, for instance, in <u>Test Dialog [214]</u> or during the experiment. lonWizard ships with some reasonable default calibration parameters for the camera, so it may well just work out of the box. It is recommended however to run the calibration once after the lonOptix MyoCam-S3 camera device has been installed into the system, and every time when the system's hardware has been modified (for instance, the camera was plugged into a new USB controller card).

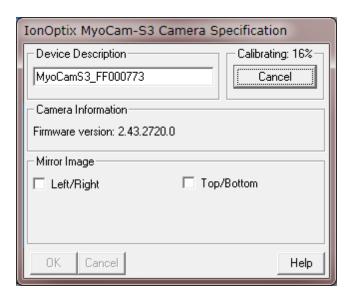
Since the USB2 and USB3 ports have different bandwidths, the lonWizard keeps two separate presets for each type. This means, for instance, that each time the calibration is launched from the Specification Dialog it only updates the presets for the USB port type that the camera is plugged into.

Launching The Calibration

The calibration starts from the Specification Dialog 213:



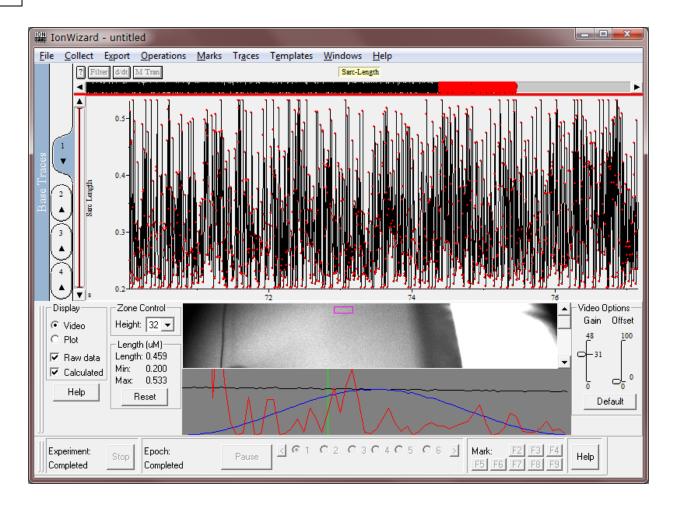
The calibration process may be canceled at any time.



NOTE: When the calibration is complete, it is important to save the results by pressing **OK** in the the <u>Specification Dialog</u> [213], while pressing **Cancel** will keep the previous calibration presets.

Overbuffering

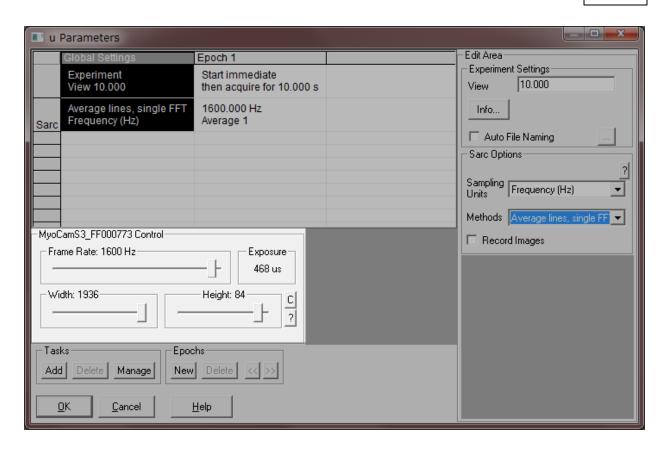
Normally, a camera device may be intermittently buffering up to a few frames while recording. However, if the camera settings are such that the required data throughput is consistently exceeding the USB port's bandwidth (which may be shared with other devices), the camera will accumulate time delays that may eventually become significant. This condition is called "overbuffering" (as opposed to sporadic buffering that doesn't exceed a certain small limit). When the overbuffering happens during an experiment, the lonWizard reports it by adding red dots to the trace. For example:



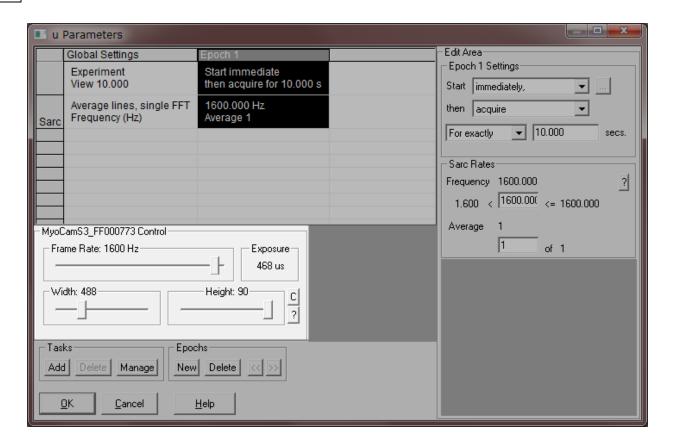
One way to recover is to run the calibration again. That will re-examine the system and most likely tighten the constraints on image dimensions or even reduce the maximal frame rate that is allowed to be set for that system. Another way is to manually reduce height or/and width in the <u>Global Sensor Settings</u> [215].

Calibrated Presets

It is common for higher (above 125Hz) frame rates to have a calibrated height value to be lower than the maximal value that the camera can achieve without accounting for the bandwidth. For instance, the calibrated image height for frame rate 1600Hz and width 1936px below is 84px, while the camera could potentially make 90px with these settings:



Simply setting the Height to 90px with the rest of the settings staying the same may cause the overbuffering while running the experiment. However, doing so while simultaneously reducing the image width will reduce the required throughput and therefore may be fine:



The decision of whether to reduce width or height generally depends on the nature of the experiment.

The button calibrated presets.

5.10 Danish Myo Techology Devices

The Danish Myo Technology devices provide support for equipment manufactured by Danish Myo Technology, Inc.

5.10.1 DMT Pressure Myograph: PM11X

The <u>DMT Pressure Myograph</u> <u>device</u> provides support for pressure myograph systems from Danish Myo Technologies. This device provides support for the temperature, pressure, force and optional pH functions of the system.



DMT Pressure Myograph

DMT Myograph controller

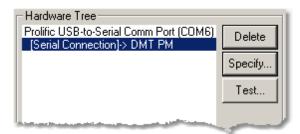
Device Name

The <u>DMT Pressure Myograph device</u> appears as "DMT PM" in the <u>Hardware Manager 8 dialog's Hardware Tree</u> section. The name can be changed in the <u>Specification Dialog [222]</u>.

Requirements

The <u>DMT Pressure Myograph device</u> requires an available <u>serial port</u> in the hardware tree.

5.10.1.1 Device Connections



DMT Pressure Myograph Connections

Required connections

The DMT Pressure Myograph device must be connected to a Serial Port *device* in the <u>Hardware Tree</u>

Provided Connections

The DMT Pressure Myograph device does not provide any connections for other devices.

5.10.1.2 Task Connections

The <u>DMT Pressure Myograph</u> device provides both sensors and inputs that can be selected in acquisition tasks. In the following list "*Name*" is the description entered in the <u>Specification</u> dialog.

Device Sensors

The following sensors can be selected in any <u>acquisition task</u> that monitors or records analog values.

"Name (temperature)" Current temperature (°C)

"Name (inlet pressure)" Current inlet pressure (mm Hg)

"Name (outlet pressure)" Current outlet pressure (mm Hg)

"Name (force)" Current force (mN)

"Name (pH)" Current pH (if "Has pH probe connection" is selected in the

Specification 227 dialog.)

"Name (target temperature)" Target temperature (°C)

"Name (target inlet pressure)" Target inlet pressure (°C)

"Name (target outlet pressure)" Target outlet pressure (°C)

Device Inputs

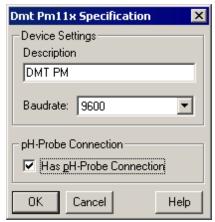
The following inputs can be selected in acquisition tasks that output analog values:

"Name (temperature)" Set target temperature (°C)

"Name (inlet pressure)" Set target inlet pressure (°C)

"Name (outlet pressure)" Set target outlet pressure (°C)

5.10.1.3 Specification Dialog



DMT Pressure Myograph Specification Dialog

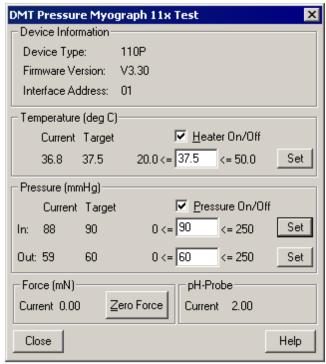
The <u>DMT Pm11x Specification</u> *dialog* provides the mechanism to set basic information about the connected device.

Description Enter "friendly" name used to identify this specific pressure myograph.

Baudrate Select baudrate used to communicate with pressure myograph.

Has pH-Probe Connection Enable display of readings from attached pH probe.

5.10.1.4 Test Dialog



Pressure Myograph Test Dialog

The <u>DMT Pm11x Test</u> dialog provides real-time display and control of the attached pressure myograph device.

Device Information: Display information about the attached device.

Temperature (deg C): Display current temperature and set temperature control options

Current - actual temperature

Target - the current target temperature

New - Enter new target temperature between displayed range then press Set

button to send to device.

Control Temperature - Check to enable heater control

Pressure (mmHg): Display current inlet and output pressure and set target pressure options.

Current - actual pressure

Target - the current target pressure

New - Enter new target pressure with indicated range then press the <u>Set</u> button to

make send to device.

Control Temperature - Check to enable pressure control.

Force (mN): Display current force and zero control.

Current - actual force

Zero Force - reset current force as zero force.

pH-Probe: Display current pH reading.

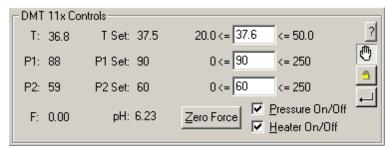
Current - Actual pH reading or "No pH -meter attached" if enabled in Specification

227 dialog



The initial values in the <u>Test</u> dialog are read from the Pressure MyoGraph device when the <u>Test</u> dialog is opened. After the dialog is opened changes made using the Myograph controller keypad will NOT be reflected in the <u>Test</u> dialog and will be replaced if changed using the <u>Set</u> button.

5.10.1.5 Manual Control



DMT Pressure Myograph Manual Control

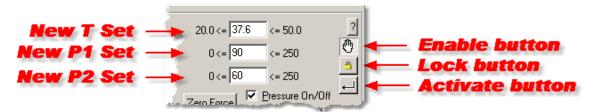
The <u>DMT Pressure Myograph</u> device has a manual control *group* that appears in the <u>Manual Control Tool Bar 36</u> displayed in the in the <u>Experiment Tool Bar 36</u> area at the bottom of the lonWizard window. It allows you to see the current hardware settings and to override settings set by acquisition tasks. The manual control *group* appears whenever any <u>DMT Pressure Myograph Task Connection 222</u> is used in any acquisition task that is included in the current experiment.

Display Values

The following values are displayed in the left side of the tool bar:

"T"	Current temperature (°C)
"P1"	Current pressure 1 (mm Hg)
"P2"	Current pressure 2 (mm Hg)
"F"	Current force (mN)
"рН"	Current pH (if "Has pH probe connection" is selected in the Specification Delta Delta De
"T Set"	Current target temperature (°C)
"P1 Set"	Current target pressure 1 (mm Hg)
"P2 Set"	Current target pressure 2 (mm Hg)

Manual Override



DMT Pressure Myograph Manual Toolbar Group - manual override

The top right section of the tool bar allows you to enter new "set" values, send them to the hardware and control how the long the stay activated.

New T SetEnter new value for "T Set" that will be set when Activate button is clicked

New P1 Set Enter new value for "P1 Set" that will be set when Activate button

is clicked

New P2 Set Enter new value for "P2 Set" that will be set when Activate

buttonis clicked

Enable button When *pressed* (as shown) enables editing and activating of "new"

set values. When released acquisition task(s) control of the

device, if any, will be enabled.

Lock button When *pressed* previously activated values will remain in effect as

long as manual override is enabled. When *released* (as shown) acquisition task(s) control of the device, if any, will return at the

end of the current epoch.

Activate button When clicked all "new" values will override any values set by

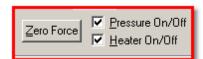
acqusition task(s), if any. Values will not return to acquisition task control until then end of the current epoch or until manual

override is disabled.



When manual override is enabled and you activate new settings the normal control of the DMT device by any acquisition tasks is disabled until the end of the epoch or, if the <u>Lock</u> button is pressed, until manual override is disabled.

Manual Settings



DMT Pressure Myograph Manual Toolbar Group - manual settings

The remaining *controls* in the toolbar give you manual control of parameters that can only be set here or with the front panel controls on the device. The <u>Enable</u>, <u>Lock</u> and <u>Activate</u> *buttons* operate differently for these controls, see below

Pressure On/Off Automatically changes to reflect current hardware setting. When

the <u>Enable</u> button is pressed you can turn on pressure control by checking the box and turn off by unchecking the box.

Heater On/Off Automatically changes to reflect current hardware setting. When

the Enable button is pressed you can turn on heater control by

checking the box and turn off by unchecking the box.

Zero Force When the <u>Enable</u> button is pressed clicking the button will make

the current force reading zero.

5.10.2 DMT Flow Meter: 161FM

The <u>DMT Flow Meter</u> *device* allows flow readings from the 161FM flow meter from Danish Myo Technologies.



DMT 161FM Flow Meter

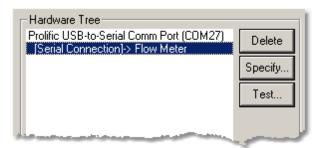
Device Name

The <u>DMT Flow Meter</u> device appears as "Flow Meter" in the <u>Hardware Manager and Jacobs Section</u>. The name can be changed in the <u>Specification Dialog</u> 127.

Requirements

The <u>DMT Flow Meter</u> device requires an available <u>serial port port</u> in the hardware tree.

5.10.2.1 Device Connections



DMT Flow Meter Device Connections

Required connections

The "Flow Meter" device must be connected to a "Serial Connection" port.

Provided Connections

The <u>DMT Flow Meter</u> device does not provide any connections for other devices.

5.10.2.2 Task Connections

The <u>DMT Flow Meter</u> device provides an analog sensor that can be selected as an input in acquisition tasks such as the <u>Trace Recording Task</u> 41. In the following list "Name" is the description entered in the <u>Specification</u> 227 dialog.

Device Sensor

The following sensor can be selected in any acquisition task that monitors or records analog values.

"Name (DMT Fm161 Controller)" Current flow reading from device

Device Inputs

The DMT Flow Meter device does not provide any device inputs.

5.10.2.3 Specification Dialog

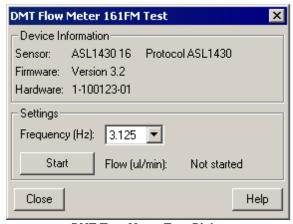


DMT Flow Meter Specification Dialog

The <u>DMT Flow Meter Specification</u> *dialog* provides the mechanism to set basic information about the connected device.

Description Enter "friendly" name used to identify this specific flow meter.

5.10.2.4 Test Dialog



DMT Flow Meter Test Dialog

The <u>DMT Flow Meter Test</u> *dialog* provides real-time display and of data from the attached flow meter device.

Device Information: Display information about the attached device.

Frequency (Hz): Select the sampling frequency to use when the **Start** *button* is pressed. This

control is disabled unless update is stopped.

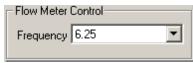
Start: Pressing the Start button starts real-time update of the Flow (ul/min) value. Once

started the **Start** button changes to "Stop".

Stop: Pressing the Stop button stops real-time update of the Flow (ul/min) value. Once

stopped the Stop button changes to "Start".

5.10.2.5 Global Sensor Settings



Global Sensor Settings

When the current experiment includes one or more tasks that use the <u>DMT Flow Meter device</u>, a <u>Flow Meter Control group</u> will be added to the <u>Global Sensor Settings</u> 22 area of the <u>Parameters</u> 20 dialog. This control allows you to set the base acquisition rate that will be used when reading flow data from the device.

5.11 Standard PC Port Devices

Standard PC Port Devices provide lonWizard the ability to control devices attached to <u>Acquisition Devices</u> External Ports 131.

5.11.1 PC Serial Port

The PC Serial Port device allows lonWizard to access to any standard serial port that is available to Windows.



The PC Serial Port device also supports all Windows-compatible USB-to-serial adapters.

Device Name

PC Serial Port devices appear as "Serial Ports" in the Add Root Device 10 dialog's Type of Devices section. When "Serial Ports" is selected each available serial port appears as "Communiction Port (COMn)" in the Instance of Device section. When added to the Hardware Manager 1 dialog's Hardware Tree the device will appear as "Communiction Port (COMn)".

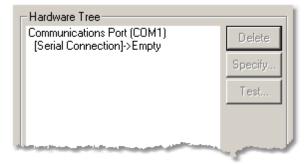


The "n" in the instance name ("Communiction Port (COMn)") is the serial port number assigned by Windows.

Requirements

There are no additional requirements as serial support is built in to Windows.

5.11.1.1 Device Connections



PC Serial Port Device Connections

Required connections

The PC Serial Port Device is a root device that does not require an other device connection.

Provided connections

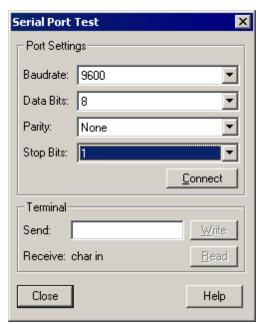
The PC Serial Port Device provides the following connection:

Serial Connection Serial port to connect to any serial port device

5.11.1.2 Task Connections

The PC Serial Port Device device does not provide any connections for acquisition tasks.

5.11.1.3 Test Dialog



PC Serial Port Device Test Dialog



The PC Serial Port Test Dialog does not currently function.

The PC Serial Port Test Dialog allows you to set the serial port communications parameters and send and receive characters.

5.12 Sutter Excitation Light Source Devices

The Sutter Excitation Light Source devices provide support for equipment manufactured by Sutter Instruments.

5.12.1 Sutter Lambda DG-4: DG4

The Sutter Lambda DG-4 is a 4-position excitation light source manufactured by Sutter Instruments (http://www.sutter.com), This hardware component gives the acquisition software the ability to run this light source at its fully-rated speed when attached to the FSI light source port using the Parallel Port Adapter

DG-4 Switching Times vs Pacer Frequency

The specification from Sutter Instruments for the amount of time that the DG-4 requires to move between filters is not very precise. The best information that we were able to obtain is that switching between adjacent positions is done in "less than 1ms" and switching longer distances happens in "less than 1.2ms". We have programmed the software to guarantee a **minimum** of 1ms and 1.2ms as appropriate. Operationally this is done by sending the position command during one pacer interrupt then waiting until the next pacer interrupt to start sampling data. What this means is that the pacer frequency determines the exact amount of time between changing the filter position and sampling the data

The filter movement time is part of what determines the maximum sampling rate of the experiment. For example if you are sampling dual excitation data each ratio point will consist of the following steps

- 1. Move to numerator filter position, wait for movement to complete
- 2. Sample numerator data point
- 3. Move to numerator filter position, wait for movement to complete
- 4. Sample denominator data point

At the default pacer frequency of 1KHz a pacer interrupt occurs once every 1ms. As discussed above this means that steps #1 and #3 will EACH take either 1ms ("less than 1ms" rounded up to the nearest millisecond) or 2ms (1.2ms rounded UP to the nearest millisecond). When using a PMT or Analog input sensor steps steps #2 and #4 will be 1ms each. So if you use adjacent filters for your dual excitation recording, you will collect a ratio pair every 4ms, or 250Hz. If your filters are not adjacent, the total time to sample one ratio pair will be 6ms or 166 ratios/sec with 0.8ms wasted on each filter move (2ms delay - 1.2ms needed).

You can reduce this wasted time by increasing the pacer frequency which is set in the Hardware Manager Timer Configuration *dialog*. By increasing the pacer frequency from 1Khz to 2KHz (which is done by halving the count down value) you will get the following values for a non-adjacent move: filter movement time: 1.5ms each (1.2ms rounded up to the nearest 0.5ms), data sampling 0.5ms each. This means that the total time to sample a complete ratio point will drop to 4ms (1.5*2+0.5*2) which results in a rate of 250 ratios/second. An adjacent move will not improve as much because you still need 1ms to move. Thus an adjacent ratio pair will take a total of 3ms for a data rate of 333Hz.



Pacer Frequencies between 1KHz and 5KHz are not guaranteed to work on all computers. Rates over 5KHz seldom work on any computer.

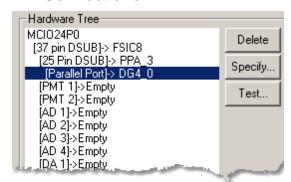
Device Name

The Sutter Lambda DG-4 appears as "DG4_n" in the <u>Hardware Manager and Jacobs Hardware Tree</u> section.



The "n" in the instance name (DG4_n) will be 0 after computer is restarted and will increment each time the device is opened.

5.12.1.1 Connections



Sutter Lambda DG-4 Connections

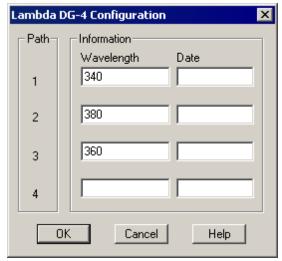
Required connections

The Sutter Lambda DG-4 must be connected to a <u>25 Pin DSUB</u> port **PARALLEL PORT** connection such as the one provided by the <u>Parallel Port Adapter [199]</u>.



Do NOT connect the Sutter Lambda DG4 directly to the FSI light source port connector!!

5.12.1.2 Specification Dialog



Sutter Lambda DG-4 Specification Dialog

The <u>Lambda DG-4 Configuration</u> dialog provides the mechanism to identify the filters that are loaded into the device.

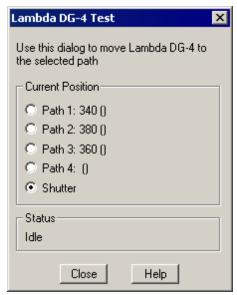
Wavelength Describe the filter in the corresponding filter path(position) of the Lambda DG-4

Date Enter the date or other note to help track filter source. It may be left blank.



Refer to the Sutter hardware manual for instructions on how to install filters and other device details.

5.12.1.3 Test Dialog



Sutter Lambda DG-4 Test Dialog

The <u>Lambda DG4 Test</u> dialog allows you to manually move the position of the DG-4 to the specific positions set in the <u>Specification Dialog [231]</u>. For <u>Path 1</u> through <u>Path 4</u>, the wavelength and date information will be displayed.

Path 1-4: Moves to the filter 1, 2, 3 or 4 position.

Shutter Moves to the "off" position.

Status Shows status of device: busy (while moving) or idle.

5.13 TILL Excitation Light Source Devices

The TILL Excitation Light Source Devices provide support for excitation light sources manufactured by TILL Photonics (www.till-photonics.com).

5.13.1 Polychrome V (analog)

The TILL Polychrome V is a galvanometer mounted monochromator based position excitation light source manufactured by TILL Photonics (www.till-photonics.com), This hardware component gives the acquisition software the ability to run this light source at its fully-rated speed when attached to any DAC port available on the system (for example on an FSIB, FSIC 138), DSIB, DSIC 148) or Light Source Port DA Adapter 207)).

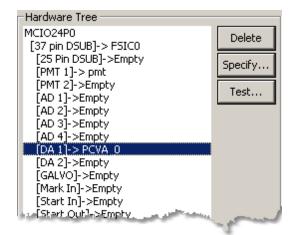
Device Name

The <u>Till Polychrome V Analog</u> appears as "PCVA_n" in the <u>Hardware Manager 181</u> dialog's <u>Hardware Tree section</u>.



The "n" in the instance name (PCVA_n) will be 0 after computer is restarted and will increment each time the device is opened.

5.13.1.1 Connections

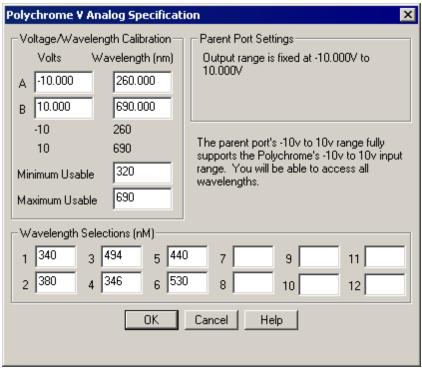


TILL Polychrome V Analog Connections

Required connections

The <u>TILL Polychrome V Analog</u> must be connected to a BNC voltage source connection such as the one provided by the FSIB, FSIC [139], DSIB, DSIC [146] or Light Source Port DA Adapter [201].

5.13.1.2 Specification Dialog



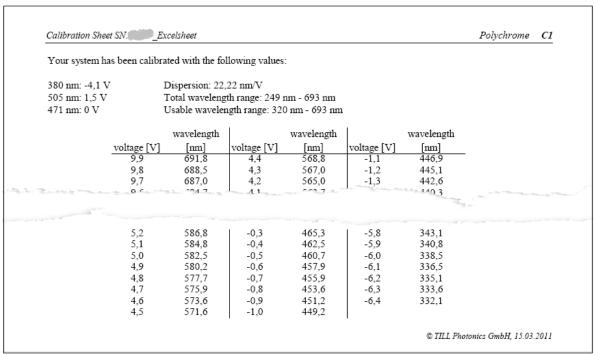
TILL Polychrome V Analog Specification Dialog

The <u>TILL Polychrome V Analog Specification</u> *dialog box* provides the mechanism to enter the device calibration constants, configure the parent device D/A port and preselect the wavelengths of interest. All three of these tasks must be completed before using the device.

Voltage/Wavelength Calibration

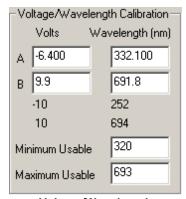
In order for the software to drive the Polychrome V to the correct wavelengths you must enter the calibration values from your device calibration sheet. TILL provides this information for each device based on the device serial number. If you have lost your calibration sheet or never got one, you can contact TILL directly and they will send you a new one.

Below is an example sheet which we have cut the middle out of to make it a bit smaller.



TILL Polychrome V Factory Calibration Sheet

You can see there are two sections, the header and the calibration table. You will need to enter information from both sections into the <u>Voltage/Wavelength Calibration</u> section of the <u>TILL Polychrome V Analog Specification</u> dialog box shown below.



Voltage/Wavelength Calibration Section

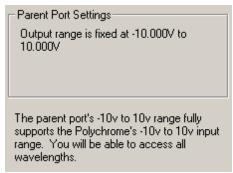
Here we have entered two pair of voltage/wavelength information from the large table in the calibration document. We simply chose the end points of -6.4 volts and 9.9 volts. You can chose any pair so long as they are more than 5 volts apart.

You also need to enter the minimum and maximum usable wavelengths given in the calibration document header as <u>Usable wavelength range</u>. This will determine the range of values you can enter later in the <u>Wavelength Selections</u> section.

If you enter values that are obviously incorrect, the *dialog box* will alert you by turning the *edit fields* red and displaying a red message at the bottom of the *dialog box*.

Parent Port Settings

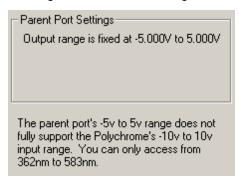
If you have attached your Polychrome V to a DA port that can be programmed, you will need to select the appropriate voltage range via the Parent Port Settings section shown below.



Parent Port Settings

The options inside the rectangle are supplied by the port itself and differ from device to device. Currently all devices supported by lonOptix have fixed DA port ranges so you will see information to that effect as displayed in the figure above.

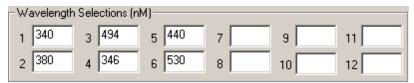
The Polychrome V has a +10v to -10v input range. If the parent port cannot generate that range of voltages, you will be notified that your ability to select wavelengths will be reduced. The reduced range will be given as shown in the figure below.



Parent Port with Restricted Range

Wavelength Selections

The final element of the <u>TILL Polychrome V Analog Specification</u> *dialog box* is the Wavelength Selections section shown below.



Wavelength Selections

Here you may enter up to 12 different wavelength selections. These wavelengths will appear in the various dialog boxes used to set up fluorescence recording tasks later. Empty selections will still be available for selection, but all map to the same neutral wavelength. The switching time of the light source is entirely dependent upon the difference in wavelengths and therefore the order of entry here is not important.

If you attempt to enter a wavelength outside the Usable Range, it will turn red and be flagged as an error as shown below. All errors (indicated in red) must be corrected before exiting the *dialog box*.



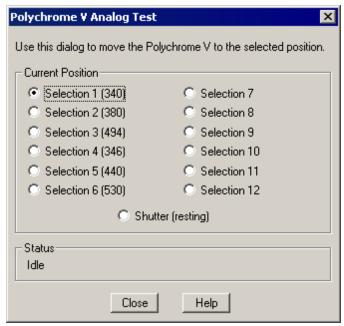
Wavelength Selection Error

If you attempt to enter a wavelength outside the range that the DA port can produce, it will turn yellow and flagged with a warning message as shown in the figure below.



Wavelength Selection Warning

5.13.1.3 Test Dialog



TILL Polychrome V Analog Test Dialog

The <u>TILL Polychrome V Analog Test</u> dialog allows you to manually move the monochrometer the one of the specific wavelengths selected in the <u>Specification Dialog</u> 234.

Selection 1-12: Moves to one of the 12 pre-selected wavelengths.

Shutter Moves to the "shuttered" position (red light).

Status Shows status of device: busy (while moving) or idle.

5.14 Motorized Microscopes

Enter topic text here.

5.14.1 CytoCypher CYCY100 Microscope



CytoCypher CYCY100 Microscope and Controller

The CYCY100 is the first generation fully robotic microscope from CytoCypher b.v. This device permits 100% automated cell finding and measurement.

Device Name

To add a CYCY100 to the system, you must select Add Root... To from the Hardware Manager To dialog and add the desired device from the list. Once added, the CYCY100 will appear with the name (e.g "Proscan3") in the Hardware Manager To dialog's Hardware Tree section. The name can be changed in the Specification Dialog 39.

Requirements

The CYCY100 requires that the appropriate Galil drivers are installed on the computer before running lonWizard. Please contact your support person should you have trouble finding the CYCY100 in the Hardware Manager (8) dialog's Add Root... (10) feature.

5.14.1.1 Device Connections

[insert screen shot of hardware tree with CyCy scope in it.]

Required connections

The <u>CytoCypher CYCY100 Microscope</u> device must be connected to your computer via a network cable to a dedicated network card. This connection is invisible to the lonWizard software and the CYCY100 is installed via the <u>Add Root...[10]</u> in the <u>Hardware Manager[8]</u> dialog.

Provided Connections

The CYCY100 does not provide any connection points for attaching other devices.

5.14.1.2 Task Connections

The CytoCypher CYCY100 device provides a motorized microscope connection that can be selected into a Multicell experiment task. In the following list "*Name*" is the description entered in the Specification Dialog [239]

5.14.1.3 Specification Dialog



CytoCypher Specification

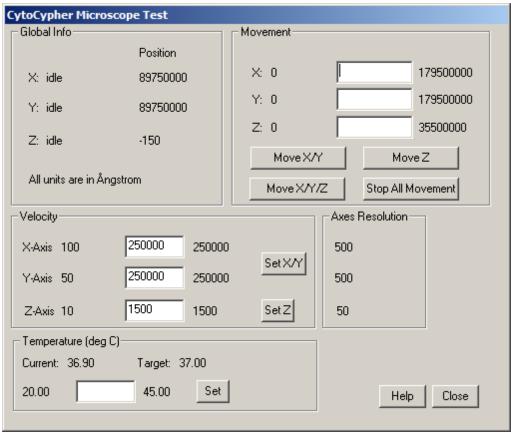
Device Settings

Information

Input a string for the description that uniquely identifies the instance of the device

Displays information about the attached device

5.14.1.4 Test Dialog

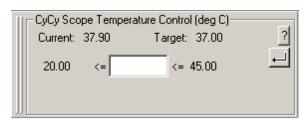


CytoCypher testing

The CytoCypher Microscope Test allows control movements through the test tools.

Global Info	Displays the current position in angstrom and shows whether its idle or not
Movement	Updates the Global Info when the user changes the movement in the x y or z position. The user also has the option to stop any movement currently happening
Velocity	Input a number that will go at a certain speed in either the x y or z direction
Axes Resolution	Displays the number of pixels contained for the x y and z axis
Temperature	Input a number between the min and max as the target temperature

5.14.1.5 Manual Control



CyCy Scope Manual Control

The CytoCypher CYCY100 microscope has a manual control *group* that appears in the Manual Control Tool Bar displayed in the in the Experiment Tool Bar area at the bottom of the lonWizard window. It allows you to see the current hardware settings and set some parameters directly from the software while experiments are running. The manual control *group* will appear whenever a CYCY100 microscope is selected into any acquisition task that is included in the current experiment.

- Allows you to input a number that will later become the target temperature if its within the parameters. If its not within the scope then the number will turn red

 When clicked allows you to set the target temperature
- when clicked allows you to set the target temperature
- Context help for the Manual Control of the CYCY100 Microscope

5.14.2 Prior Stages

The prior stages...

Device Name

An attached <u>Prior Stage</u> will appear as the descriptive name of the controller (e.g "<u>Proscan3</u>") in the <u>Hardware Manager 8</u> dialog's <u>Hardware Tree</u> section. The name can be changed in the <u>Specification Dialog [242]</u>.

Requirements

The <u>Prior Stage</u> device requires an available <u>serial port port</u> in the hardware tree.

5.14.2.1 Connections

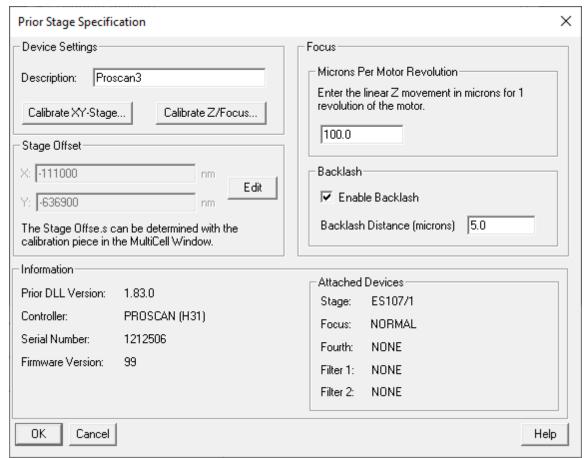


Prior Stage Connections

Required connections

The Prior Stage device must be connected to a "Serial Connection" port.

5.14.2.2 Specification Dialog



Prior Stage Specification Dialog

The Prior Stage Specification dialog shows the Device Settings, Information, and Attached Devices.

Calibrate XY-Stage... Clicking **Calibrate XY-Stage...** allows the user to calibrate on the XY plane. See Prior XY Calibration Spec 244 for additional information

Calibrate Z/ Focus...

Clicking **Calibrate Z/Focus...** allows the user to calibrate on the Z plane. See <u>Prior Z</u> Calibration Spec 245 for additional information

Stage Offset

The Stage Offset X and Y can be set through the calibration procedure in the MultiCell Cell Finder Window 115. However for manual editing it is

possible to press the **Edit** button and change the values. The **Edit** button will change to **Reset** in order to allow to restore the originally saved values.

When the values have been changed they will be saved when exiting the <u>Specification</u> <u>Dialog</u> by pressing **Ok**.

Focus Microns
Per Motor
Revolution

Enter the linear Z movement in microns for 1 revolution of the motor.

Focus Backlash

Help

Allowing the **Backlash** on the Z/Focus to be enabled or disabled. When enabled it is

possible to set the **Backlash Distance** to the correct

distance in microns. It is best to set the **Backlash Distance** equal to the true

mechanical backlash of the focus mechanism.

Information Global information about the controller and stage(s) attached to it.

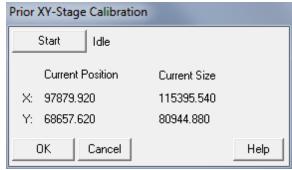
Ok Clicking the **Ok** button closes out of the dialog box with the new applied changes

Cancel Clicking the Cancel button exits out of the dialog box without applying any changes

Clicking on the **Help** button will open a help file that gives instructions on how to use

the Prior Stage Calibration features

5.14.2.2.1 Prior XY Calibration Spec

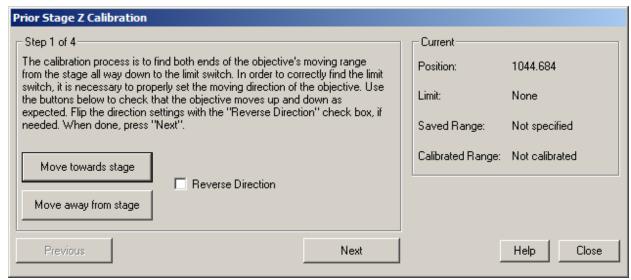


Prior XY Calibration Dialog

When opened, the dialog box shows the state, current position and current size of the stage. Once **Start** is clicked, the calibration process will commence and the state of the stage changes from idle to a moving state. During this time the user is able to click **Cancel** if they no longer wish to do the calibration. However they won't be allowed to click on **Ok** until after the calibration is stopped. Eventually the stage will move towards the center. During this process, the user will not be allowed to click on either the **Ok** or **Cancel** button. Once finished calibrating, the new size will replace the old current size.

Start	Clicking on the Start button begins the calibration process in the xy direction. Once started the Start button changes to Stop
Stop	Clicking on the Stop button ends the calibration process. Once stopped the Stop button changes to Start
Ok	Clicking on the Ok button exits the XY Stage Calibration dialog box
Help	Clicking on the HeIp button will open a help file that gives instructions on how to use the Prior XY-Stage Calibration features

5.14.2.2.2 Prior Z Calibration Spec



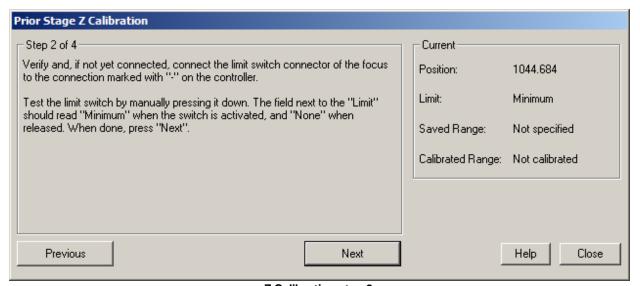
Z Calibration step 1

Step 1

Find the limit switch by setting the moving direction of the objective with clicking on either **Move towards** stage

or **Move away from stage**. The directions can be flipped by clicking on the **Reverse Direction** box. The current

position, limit, saved and calibrated range can be viewed on the right hand side. Once done, click on Next

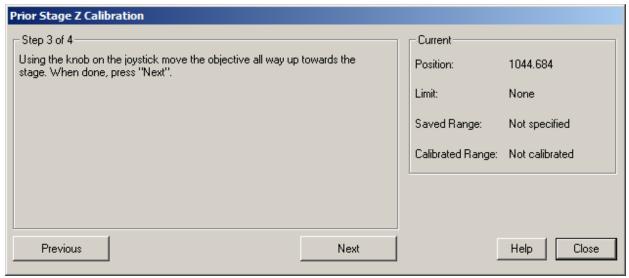


Z Calibration step 2

Step 2

The limit switch connector of the focus should be connected to the controller marked '-' to ensure its connected.

If the switch is activated, then the field on the right side of limit will read "Minimum" otherwise it will display "None". Click on **Next** for step 3.

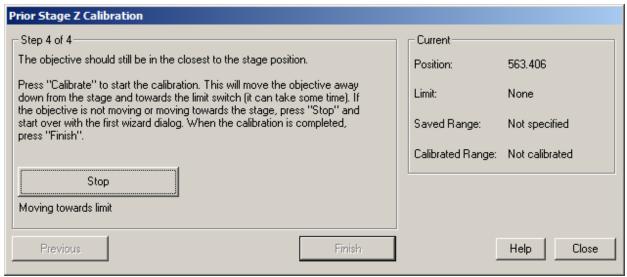


Z Calibration step 3

Step 3

Use the knob on the joystick to move the objective up until it reaches the closest stage position stage. Click on

Next for the last step.



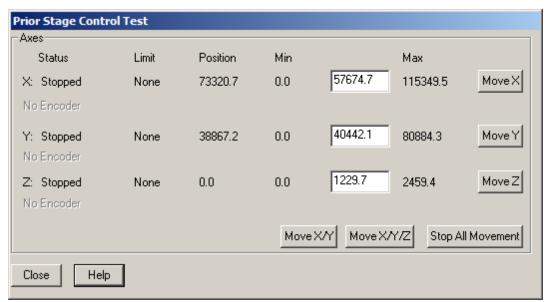
Z Calibration step 4

Step 4

Press **Calibrate** to start the calibration. Press **Stop** if the objective is idle or moving towards the stage. Click **Finish**

when the calibration is completed

5.14.2.3 Test Dialog



Prior Stage Test Dialog

In the *Prior Stage Test Dialog*, the user inputs a positive number into one of the three fields in order to move the objective towards a position in a certain axes.

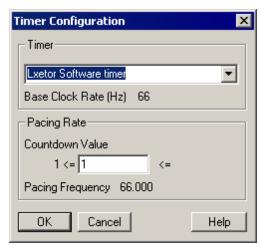
The user must make sure the number is between the Min and Max.

Move X	Clicking on the $\textbf{Move}\ \textbf{X}$ button moves the objective in the x direction based on the number entered on the field
Move Y	Clicking on the ${\bf Move}\ {\bf Y}$ button moves the objective in the y direction based on the number entered on the field
Move Z	Clicking on the $\textbf{Move}\ \textbf{Z}$ button moves the objective in the z direction based on the number entered on the field
Move X/Y	Clicking on the $\textbf{Move X/Y}$ button moves the objective simultaneously in the x and y direction based on the numbers entered in the fields
Move X/Y/Z	Clicking on the $\textbf{Move X/Y/Z}$ button moves moves the objective simultaneously in the x, y, and z direction based on the numbers entered in the fields
Stop All Movement	Clicking on the Stop All Movement button halts any current movement happening in the $x/y/z$ axes
Close	Clicking on the Close button exits out of the dialog box
Help	Clicking on the HeIp button will open a help file that gives instructions on how to use the Prior Stage Control Test features

5.15 Lxetor Software Timer

The <u>Lxetor Software Timer</u> is part of the Hardware manager and implemented using resources that are builtin to the computer. It is a Timer of "last resort" and should not generally be used.

5.15.1 Timer Settings



Lextor Timer Dialog

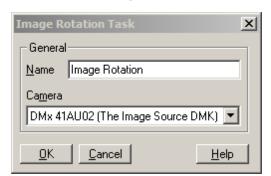
If you select the <u>Lxetor Software Timer</u> as the system timer in the <u>Hardware Manager Configure Timers</u> <u>Dialog [249]</u> you will be able to adjust the pacing frequency by changing the Countdown Value. As the <u>Base Clock Rate</u> is very slow you will probably always use a <u>Countdown Value</u> of one.

6 Acquisition Filters

Enter topic text here.

6.1 Image Rotation Filter

6.1.1 Filter Settings

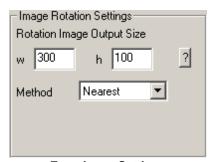


Filter Settings

Name for this specific instance of the task

Camera Select the camera device that will be used for image rotation

6.1.2 Experiment Settings



Experiment Settings

w The user can input a positive number on the field as the width for the rotation

image

h The user can input a positive number on the field as the height for the rotation

image

Method A range of procedures are available for picking

? Clicking on the ? button will open a help file that gives instructions on

understanding the Image Rotation Settings

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