



IonWizard 6.3

Core & Analysis Functions

IonWizard 6.3

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Document date: January 31, 2012

Printed: January 2012 in Milton, MA USA

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1 What is IonWizard?

IonWizard is a software program that can be used to acquire and analyze information about a cell, vessel or other preparation.

The acquisition portion acts as central control for IonOptix hardware to produce real time plots of fluorescence and dimension and other data traces. It may also be used to integrate data coming from non-IonOptix hardware.

The analysis portion supports averaging and curve fitting of the traces created by the acquisition package. It can also read data files saved by old IonOptix programs such as IonWizard 4.4, IMAQ, or the photon counter acquisition program PIA.

IonWizard allows the careful examination of fluorescence-time relations through the graphic scale manipulations that are unique to IonWizard. IonWizard permits the user to examine and edit experiment annotation, determine the fluorescence parameters at particular time points, perform averages over arbitrary periods, print data plots, and export ASCII versions of the data. IonWizard also allows the viewing and analysis of imaging data.

2 Manual Convention

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

- Underlined 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 Installing IonWizard

IonWizard is installed, configured and removed using a Microsoft Installer (MSI) file. This file contains all IonWizard files as well as the information needed to install it on your computer.

To run the installer simply double-click on the .MSI file.

Once you have started the installer you will have the option of installing either IonWizard core and analysis only or a complete IonWizard installation with core, analysis and all acquisition features.



You can run the MSI file directly from removable media. It is not necessary to copy the MSI file to your computer.



Acquisition functions CAN NOT BE INSTALLED on any 64-bit version of Windows.

3.1 Program Requirements

IonWizard can be installed and run on Windows XP, Windows Vista and Windows 7. The core program and analysis tools can be installed on either 32-bit or 64-bit versions of Windows however the acquisition functions may only be installed on **32-bit versions** of Windows

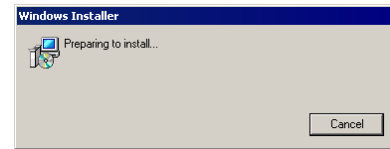


Acquisition functions CAN NOT BE INSTALLED on any 64-bit version of Windows.

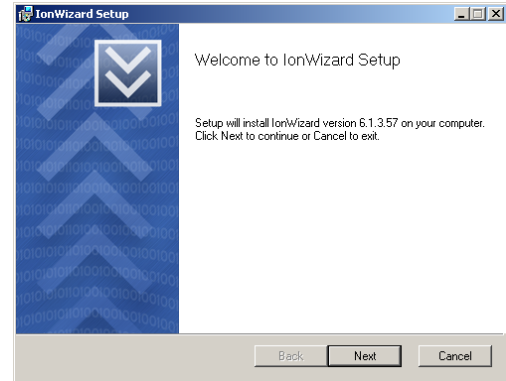
3.2 Normal Installation

A Normal Installation is when you run the installer and there is not a version of IonWizard currently installed.

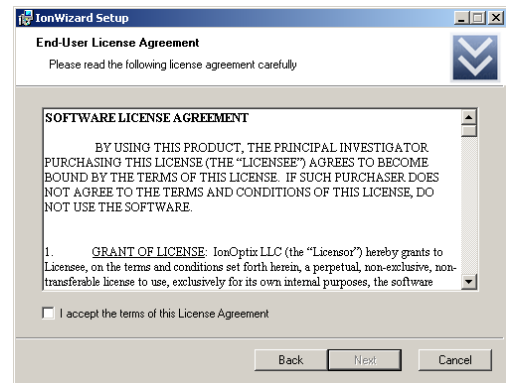
To start the installer **double-click** on the MSI file from the Windows explorer (My Computer)



The welcome dialog will display information about the install. If no previous version was found you will see the pictured dialog. If you see a different message then you should refer to either [Upgrading previous versions](#)^[8] or [Changing, Repairing and Removing](#)^[11] sections.

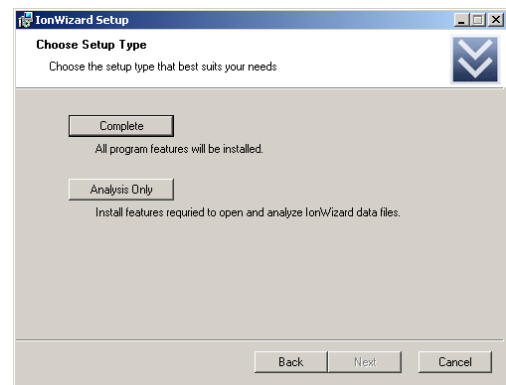


Check the box to confirm that you accept the license terms then **click Next** to continue.



Click on the Complete button to install IonWizard Core, Analysis and Acquisition functions. This button will be disabled when you install on a 64-bit version of Windows as acquisition functions are not supported.

Click the Analysis Only button to install IonWizard Core and Analysis functions only.



If you have previously installed a version of IonWizard, removed it and are now installing the same version again you will see this dialog.

Click the Use Archived *button* to use the configuration files that that were last used with this version of IonWizard

Click the Use Blank *button* to overwrite the archived configuration files for this version with blank (empty) configuration files.



Use Blank will replace the archived configuration for the version that is being installed. Configuration files for other versions will NOT be affected.

This screen is the final screen that you see before installation begins. If you need to review your choices you can **click** the Back *button* to return to previous screens or **click** the Cancel *button* to exit the installer.

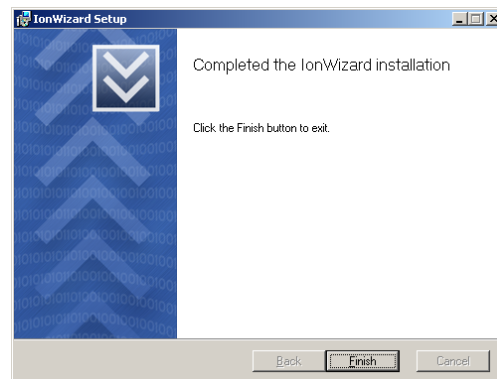
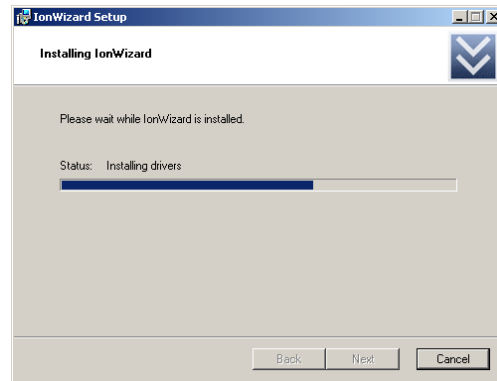
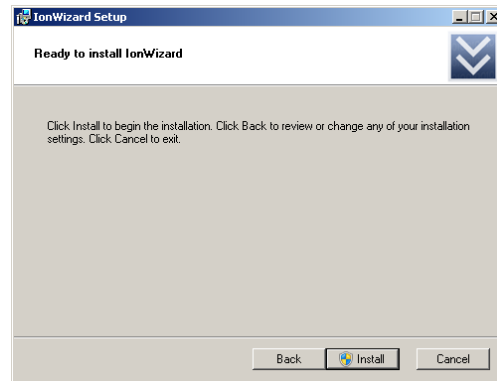
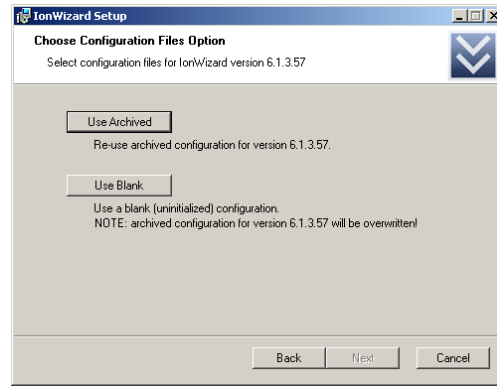
Click the Install *button* when you are ready to start the installation

As IonWizard is installing you will see this progress screen. The Status text ("Copying new files" in the sample picture) will change to indicate the current operation and the bar underneath will animate to show progress.



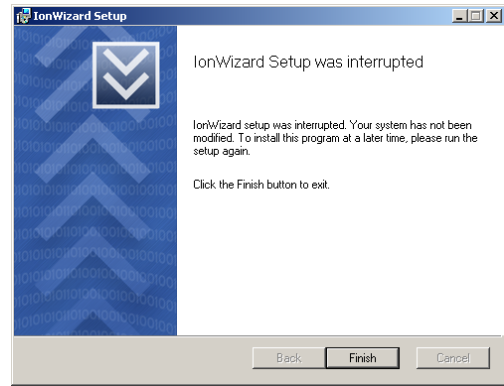
The progress bar will fill multiple times during installation.

If the installation was completed without error then you will see this dialog. **Click** the Finish *button* to exit the installer. You may then run IonWizard using the Windows Start Menu. See [Start menu and Control Panel options](#) ^[13]



If an error is encountered during installation the installer will stop and display this message. **Click** the **Finish** button to exit the installer.

If you encounter an error please try running the installer a second time **AFTER** restarting Windows before contacting support for help.



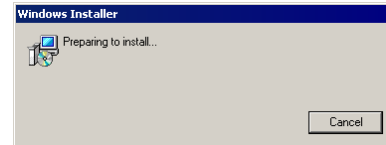
3.3 Upgrade Installation

A Upgrade Installation is when you run a installer that is newer than the version of IonWizard that is currently installed.



If you want to install an old (earlier) version that what is currently installed you must remove the current (newer) version and then do a normal installation of the older version.

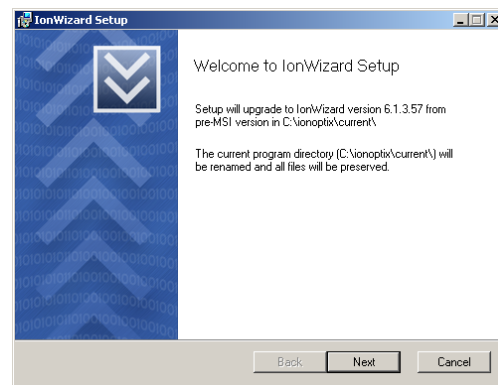
To start the installer **double-click** on the MSI file from the Windows explorer (My Computer)



If you are upgrading a version of IonWizard that was installed in c:\ionoptix\current you will see a dialog that explains you are upgrading a "pre-msi" version.

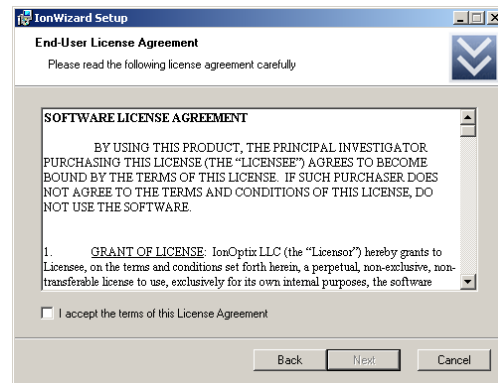


After upgrading a pre-msi installation you should NOT try to run IonWizard from c:\ionoptix !



If you are upgrading a version of IonWizard that was installed in c:\ionoptix\current you will see the software license agreement

Check the box to confirm that you accept the license terms then **click** the Next button to continue.



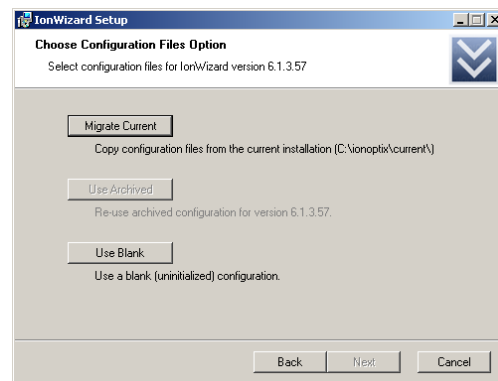
If this is the first time that you have installed this version of IonWizard you will see this dialog.

Click the Migrate Current button to copy the configuration files from the current installation.

Click the Use Blank button to set the configuration for this version to blank (empty) configuration files.



If you chose Use Blank the configuration files from the current version will NOT be erased - they will just not be copied to the new version.



If you have previously installed a version of IonWizard, removed it and are now installing the same version again you will see this dialog.

Click the Migrate Current *button* to copy the configuration files from the current installation.

Click the Use Archived *button* to use the configuration files that that were last used with this version of IonWizard

Click the Use Blank *button* to overwrite the archived configuration files for this version with blank (empty) configuration files.



Migrate Current and **Use Blank** will replace the archived configuration for the version that is being installed. Configuration files for other versions will NOT be affected.

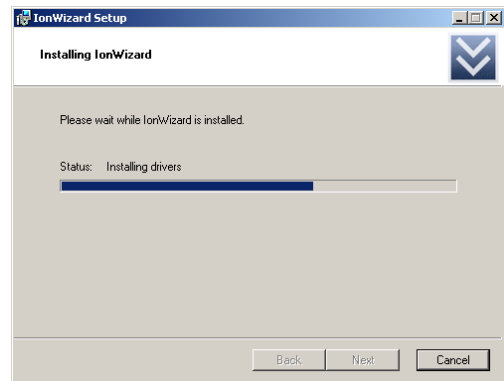
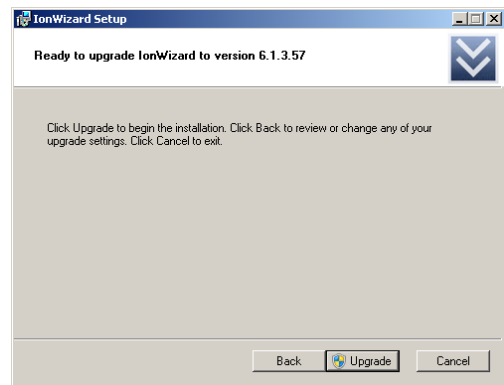
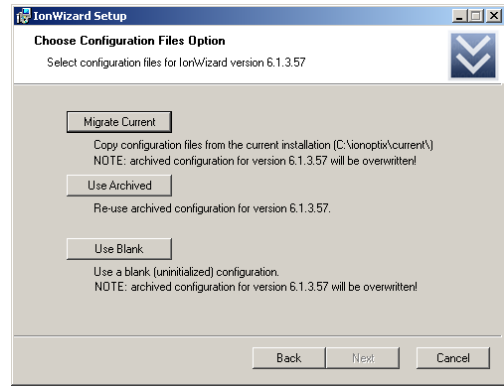
This screen is you final screen that you see before upgrade begins. If you need to review your choices you can **click** the Back *button* to return to previous screens or **click** the Cancel *button* to exit the installer.

Click the Upgrade *button* when you are ready to start the upgrade

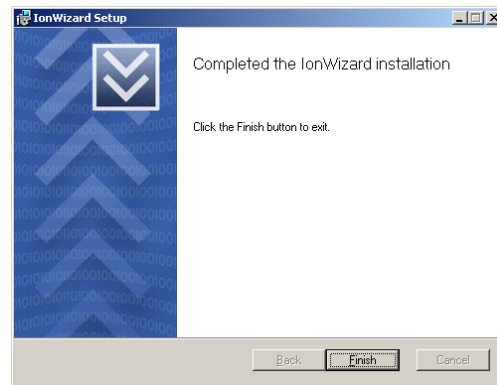
As IonWizard is upgarding you will see this progress screen. The Status text ("Copying new files" in the sample picture) will change to indicate the current operation and the bar underneath will animate to show progress.



The progress bar will fill multiple times during installation.

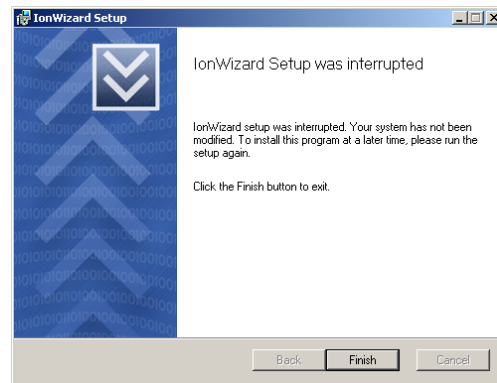


If the installation was completed without error then you will see this dialog. **Click** the Finish button to exit the installer. You may then run IonWizard using the Windows Start Menu. See [Start menu and Control Panel options](#) ^[13]



If an error is encountered during upgrade the installer will stop and display this message. **Click** the Finish button to exit the installer.

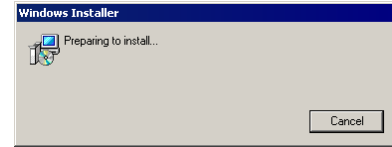
If you encounter an error please try running the installer a second time **AFTER** restarting Windows before contacting support for help.



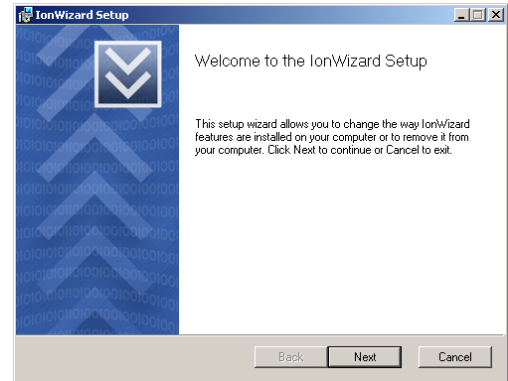
3.4 Removing, Repairing or Changing installation

Once you have installed a version of IonWizard you can remove, repair or change the installed features.

To start the installer click on "Remove, Repair or Change IonWizard" item in the "IonWizard" folder of "All Programs" in the Windows Start menu.



If you are running "Remove, Repair or Change" you will see this welcome dialog.

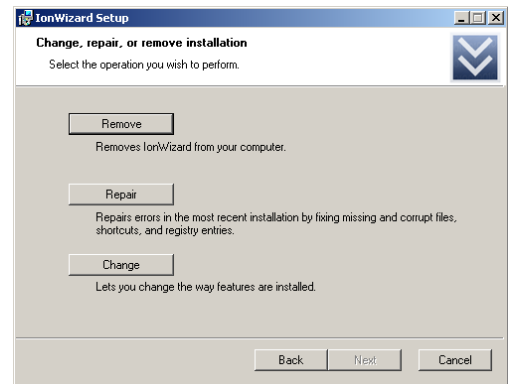


Select the maintenance function that you would like to perform.

Click the Remove button to uninstall the current version.

Click the Repair button to reinstall all of the files and drivers for the current version.

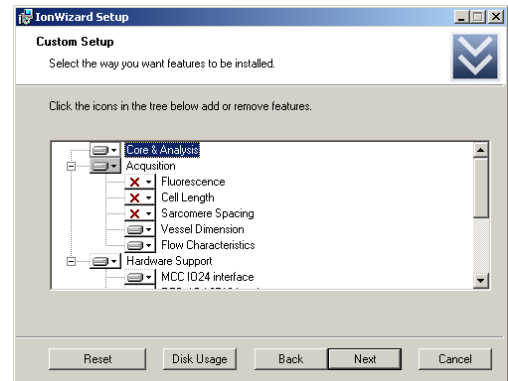
Click the Change button to add or remove features for the current version.



If you select change...

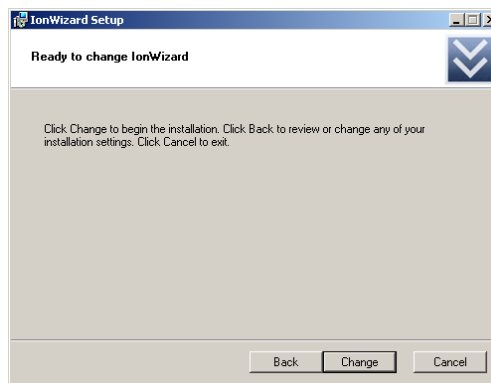
You will then have the option to add or remove features to/from the current installation of IonWizard. The dialog shows a IonWizard system in which optional components have not been installed (indicated by red "X").

Click the Next button to continue once you have made your selections.



Only use the change function when instructed. It is not needed in normal use.

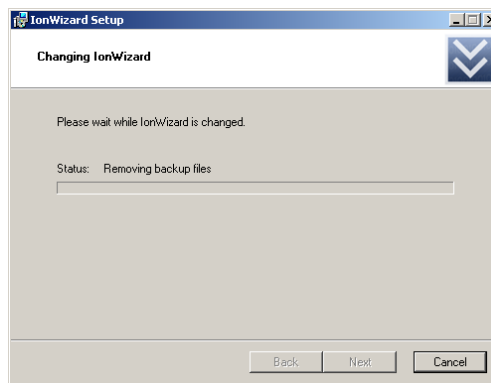
This screen is your final screen that you see before upgrade begins. If you need to review your choices you can **click** the **Back** button to return to previous screens or **click** the **Cancel** button to exit the installer. The exact text of the screen will depend on whether you selected **Remove**, **Repair** or **Change**. **Click** the **Remove**, **Upgrade** or **Change** button when you are ready to start.



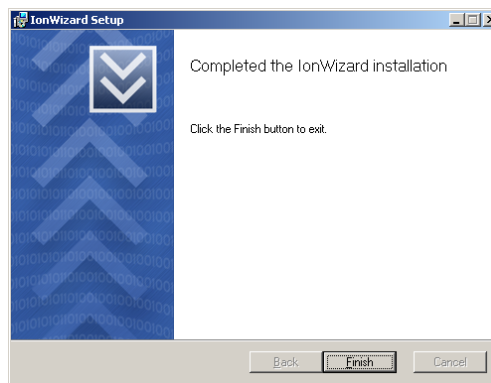
As IonWizard is being removed/upgraded/repared you will see this progress screen. The **Status** text ("Copying new files" in the sample picture) will change to indicate the current operation and the bar underneath will animate to show progress.



The progress bar will fill multiple times during installation.

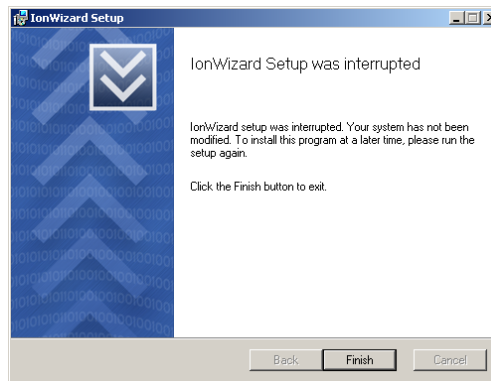


If the remove/update/repair was completed without error then you will see this dialog. **Click** the **Finish** button to exit the installer.

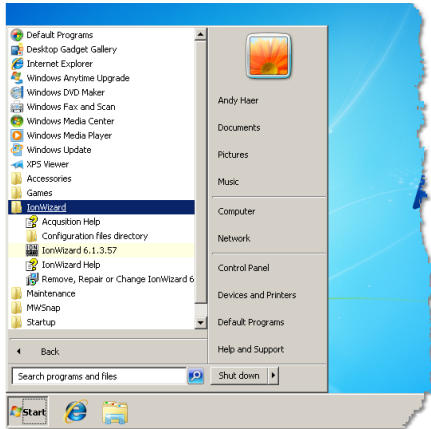


If an error is encountered during remove/update/repair the installer will stop and display this message. **Click** the **Finish** button to exit the installer.

If you encounter an error please try running the installer a second time **AFTER** restarting Windows before contacting support for help.



3.5 Start menu, Control Panel options and File Associations



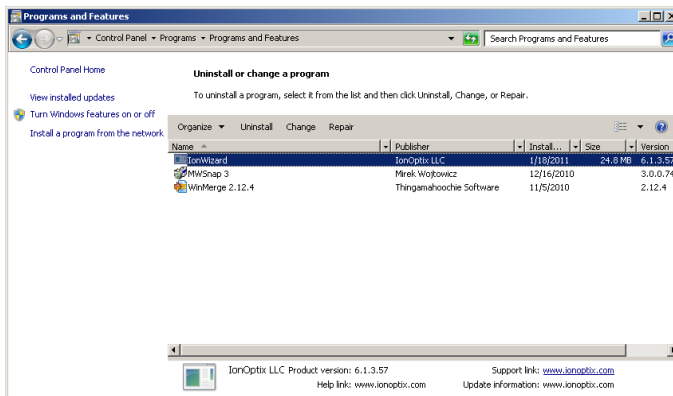
Windows start menu

The IonWizard installer adds a *folder* named "IonWizard" under the Windows Start Menu. It has the following items:

- **Acquisition Help** - Opens the on-line help file without starting IonWizard (only present if you do a "complete" installation)
- **Configuration files directory** - Opens the directory containing the program configuration files. See [Configuration file location, Backup & Restore](#)^[14] for details.
- **IonWizard** - Runs the IonWizard program.
- **IonWizard Help** - Opens the on-line help file without starting IonWizard
- **Remove, Repair or Change IonWizard** - Runs the installer to allow you to remove, repair or change installed features for IonWizard. See [Removing, Repairing or Changing installation](#)^[11] for details.



In Windows XP the IonWizard folder appears in the "All Programs" group of the start menu



Windows 7 Program Feature

The IonWizard installer also adds a "IonWizard" entry in the "Programs and Features" control panel that runs the same functions as the "Remove, Repair or Change IonWizard" item in the [Windows Start menu](#)

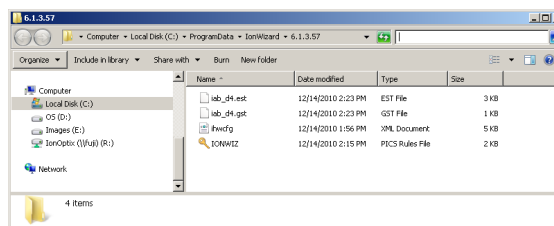


In Windows XP these functions will be in the "Add or Remove Programs" section of the Windows Control Panel.

3.6 Configuration File Location

IonWizard stores the configuration file(s) that hold users settings made in various parts of the program in a single configuration files directory. There is a separate directory for each version of IonWizard that has been installed on the computer.

To view the configuration files **click** on the " Configuration files directory" item from the IonWizard folder in the Windows Start menu.



The exact location of the files depends on the version of Windows that you are running and the version of IonWizard. The exact files depend on what options you have installed.

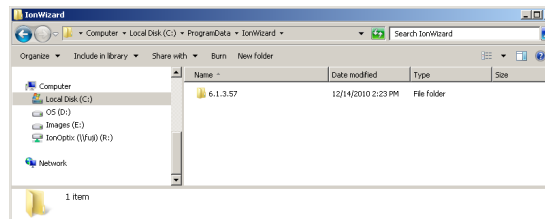


Always use the "Configuration files directory" start menu item to view the configuration files for the current version!

3.6.1 Backup & Restore

If you go up one level from the configuration files directory you will see that they are stored in a folder that is named using the IonWizard version number. If you have installed multiple versions there will be a separate folder for each version that you have installed.

You can make copies of these folders for backup using normal windows copy/paste tools.



If you are using a backup utility you can backup ALL configuration files sets by including the folder above the configuration files directory. In Windows XP the folder is usually C:\Documents and Settings\All Users\Application Data\IonWizard, in Windows 7 it is usually C:\ProgramData\IonWizard.



The exact location of the parent directory depends on the version of Windows that you are using. Use the "Configuration files directory" start menu shortcut to find the exact directory for your system.



Always backup/restore the complete set of configuration files in one directory! Mixing files from different times may cause the entire configuration to be unusable.



It is not necessary to backup the IonWizard program directory as no files are added or changed after it is installed.

4 Program Basics

The Program Basics chapter is a discussion of program control both common to other Windows® programs and specific to IonWizard.

4.1 User Interface Basics

This chapter outlines basic features of the IonWizard interface. Many of the issues discussed here are basic to the Windows operating system. If you have not used a Windows program before, have trouble getting a Windows program to operate correctly, or encounter language elsewhere in the document that requires explanation, then keep reading. If you are familiar with Windows, skip to the IonOptix Controls section.

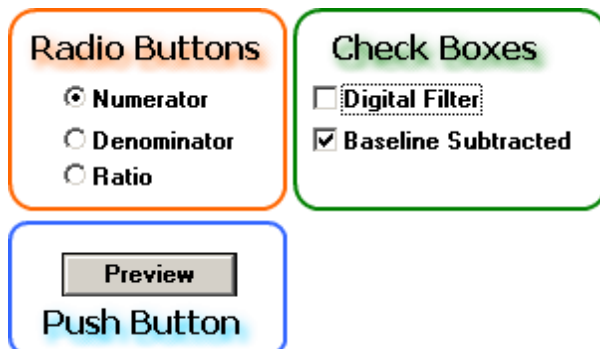
4.1.1 Controls and Input Focus

Control is a generic name for the graphical input elements that are part of the Windows® user interface. Controls include *radio buttons*, *check boxes*, and *scroll bars*. All controls accept input from the keyboard or a pointing device such as a mouse. In order for a control to accept commands, it must have input focus. The way that a control indicates that it has focus depends on the control. *Edit fields*, which allow the inputting of text, have a blinking caret, whereas *push buttons* outline themselves with a dotted line. Moving the cursor over a control and clicking the left mouse button is the easiest way to force a control to have focus. In *dialog boxes*, the tab key will move focus from one control to the next. The most important thing to remember is that if a control does not have focus it cannot accept keyboard commands. A common miscommunication occurs when the user attempts to operate one control while a different control has input focus. An example is typing into an edit field when the menu has focus. This frequently results in the inadvertent selection of a menu option, rather than the intended text insertion. If the computer is not behaving as it should, check to make sure the appropriate control has focus.

4.1.2 Windows® Controls

Windows® provides a series of different controls. While these controls are available to all application writers, and used by many programs, people still find Windows® to be a challenging working environment. Even experienced users discover new things about controls they have been using for years. The following topics outline the basic Windows® controls.

4.1.2.1 Check Boxes and Radio Buttons and Push Buttons



Collection of radio buttons, check boxes and push buttons.

Check boxes, *radio buttons*, and *push buttons* are all forms of buttons. The figure above is a hypothetical group of all three that could be used to select data for a display. Focus for buttons is indicated by a dotted rectangle, as shown by the *check box* Digital Filter. Once a button has focus, the **space bar** will cause it to operate. In this case, it would toggle Digital Filter into the selected state. A mouse click (**left button**) will perform the same function.

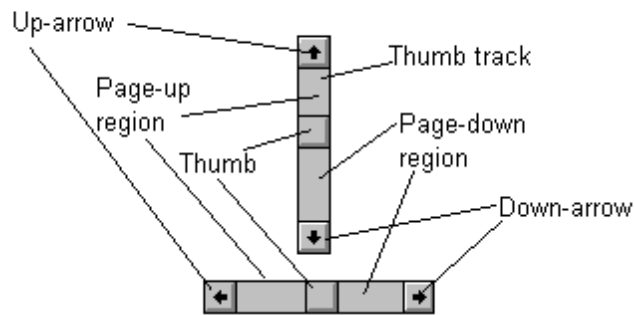
Check boxes and *radio buttons* are used for selecting options. A *check box*, when filled with an x, indicates the option has been selected. There is no interdependence between options in a list of *check boxes*. Any *check box* option can be selected regardless of the state of other options.

Radio buttons select between options that are mutually exclusive. While they still indicate selected

options, *radio buttons* do so with a black circle instead of an x. Only one of the options in a list of radio buttons may be selected at any one time. The data in the example above can only be Numerator, Denominator, or Ratio.

Push buttons perform an action. The name of the *push button* indicates its action. Preview shows that clicking on that push button would display a figure using the current options.

4.1.2.2 Scroll Bars



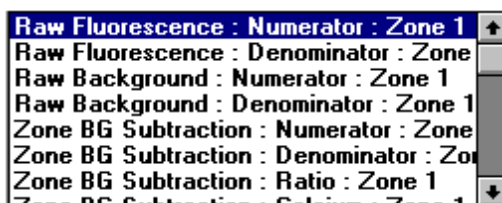
Scroll Bars and their components

Scroll bars are a standard Windows® control that allows the user to select, in a variety of ways, some offset into a range. *Scroll bars* are most frequently encountered in conjunction with word processing. Here the offset is the line number of the first row of visible text. The offset can also be a selection in a list, for example, of fonts. In a few instances, scroll bars select a numeric value from some range.

A *scroll bar* consists of four parts: two scroll arrows, a thumb, and the thumb track. The page up and page down regions further subdivide the thumb track. The extreme ends of the thumb track represent the maximum and minimum values that the data can attain. In the case of a word processing, these values would range from one to the number of lines of text. The center of the thumb represents the offset into that range. Clicking on a scroll arrow will make the current offset increase or decrease by one unit. The thumb will automatically move, in the appropriate direction, to show the change. Clicking the **left mouse button** down on the thumb and not letting up, the user can arbitrarily drag the thumb to a new location. A gray outline of the thumb will appear as the user begins to move the mouse. When the mouse button is released, the thumb will jump to the location of the gray outline. If the pointer is moved outside the thumb track, the gray outline will jump back to the current position of the thumb. This allows the scrolling operation to be aborted.

The last two ways to use a scroll bar is via the page up and page down regions. Clicking in the page up and page down region will cause the thumb, and hence the offset, to change by one page. How far that is depends on the application. In text editors, a page is the number of lines of text visible in the window that the scroll bar controls. In some numeric applications, the page distance may be fixed number like five or ten to allow rough variations in the value with fine tuning being done by the scroll arrows.

4.1.2.3 List Boxes



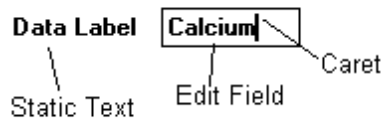
List box control

A *list box* is a control that has a list of text strings and, optionally, a *scroll bar*. The list of text represents options the user may choose. The highlighted list item is the currently selected option. If there are more options available than will fit in the area provided for the *list box*, a *scroll bar* will be present to allow scrolling through the other hidden items.

The **up arrow** and **down arrow** keys will select the item preceding or following the currently selected item.

The **page up** and **page down** keys will move the selection backward or forward in the list by the number of items shown. Typing a letter from the keyboard will jump the selection to the next item that begins with that letter.

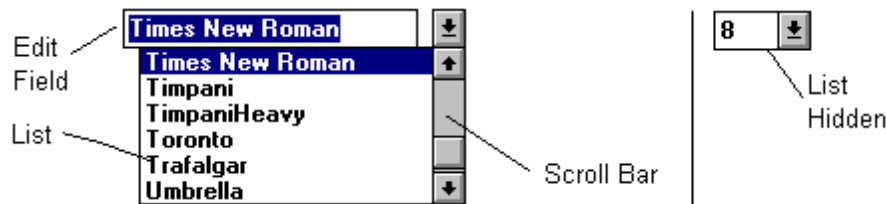
4.1.2.4 Edit Fields



Edit Field

An *edit field* allows the user to input text, often consisting of either labels or numbers. Names should not be entered into a numeric *edit field*. *Edit fields* indicate focus by a blinking vertical bar, called a caret. Generally, only a limited number of characters may be recorded by an *edit field*, so it is wise to keep names reasonably short.

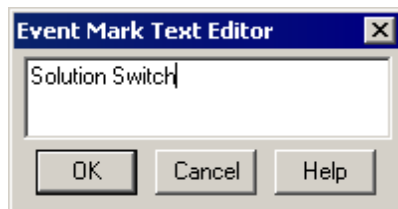
4.1.2.5 Combo Boxes



Combo-box control

A *combo-box* is a combination of a *list box* and an *edit field*. The *edit field* displays the current selection and the list shows options that are available. The highlighted text, or the blinking caret in the edit field, indicates focus. Clicking on the arrow to the right of the *edit field* toggles the *list box* between extended and retracted states. In the example above, the combo-box on the left is in the extended state. Clicking on the arrow will cause the *list box* to become hidden, as in the example on the right. Typing **ALT-down arrow** has the same effect as clicking on the arrow. Another option is to type the first few letters of the desired selection. This causes the list box to automatically jump to that item, facilitating the search process. Additionally, the scroll bar can be used to browse through the *list box* options.

4.1.2.6 Dialog Boxes



Dialog Box Example

Dialog boxes are a special type of window that may contain any number of the different controls previously described.

Dialog boxes behave in a well-defined fashion. Once in a dialog box, the user can move the input focus from control to control with the **tab** key. **Shift-tab** will move the focus through the controls in the opposite direction. Almost all dialog boxes typically contain three *push buttons*: OK, Cancel, and Help.

OK Button

The OK *push button* closes the dialog box and causes the options that were selected to be applied to the rest of the program.

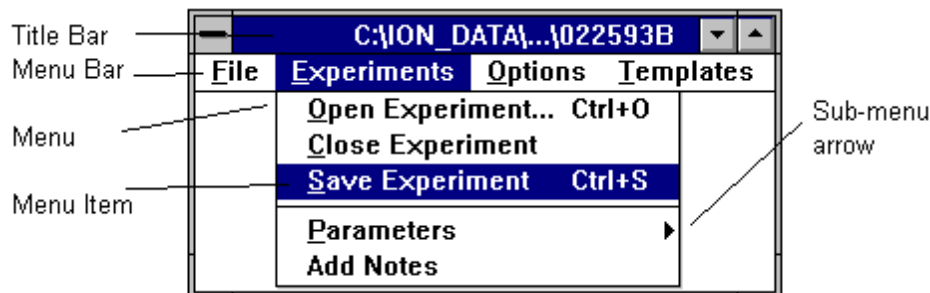
Cancel Button

The Cancel *push button* closes the dialog box changing nothing.

Help Button

The Help *push button* will bring up a help window with information specific to that *dialog box*. The help program presents expanded information to assist the user in making rational choices.

4.1.2.7 Menus



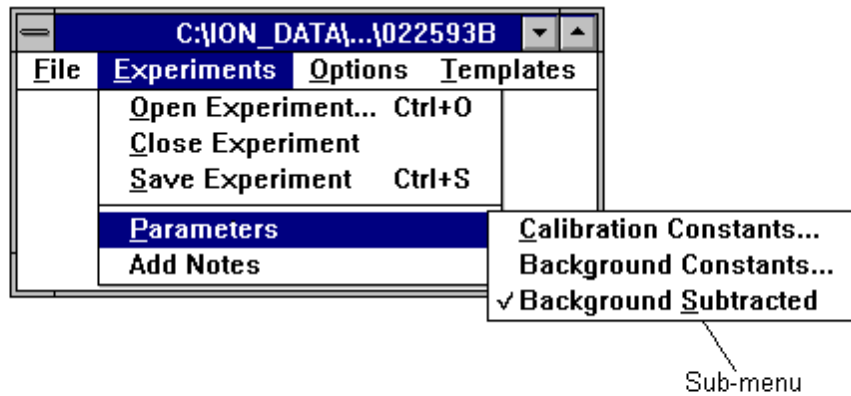
Menu bar and menu

Menus are at the top level of the interface hierarchy available in the Windows® environment. *Menus* generally call *dialog boxes* or alter global options.

By convention, a *menu item* that will invoke a *dialog box* will have an ellipsis following the text, indicating that there is more to do before the event will happen. Thus, Open Experiment..., as shown above, indicates that a *dialog box* will appear so the user can choose an experiment to view, whereas Close Experiment indicates that the current experiment will be closed with no further input.

Visible *menus* are always attached to a top level window just below the window's title bar. There are generally several different categories of functions listed, for example, File, Edit, or Tools. This area is called the *menu bar*. Clicking with the **left mouse button** on a word in the *menu bar* will cause the listing of another group of functions under the *menu bar*. Clicking on any of the items in the *menu* will cause the program to perform the action described.

Some *menu items* have a small arrow off to one side. This indicates that that item contains a *sub-menu*. The *sub-menu* will appear when the user clicks on the *menu item*, as illustrated below.



Sub-menu

For the purposes of this manual, the words appearing in the *menu bar* will be called *menus*. Thus, there are four *menus* in the example above: File, Experiments, Options, and Templates. Any word appearing in the list under a *menu* is referred to as a *menu item* or *menu selection*. In the case of *sub-menus*, a forward slash (/) will be used to separate the *sub-menu item* from the *menu item* housing the *sub-menu*. Therefore "Parameters/Calibration Constants..." from the Experiments *menu*" indicates that under the Experiments *menu* there is an item called Parameters which has a *sub-menu* containing the Calibration Constants... *menu item*.

In addition to using the mouse, users may also control menus from the keyboard. Most items in a menu bar have one of the characters underlined. By typing **ALT** and the underlined character, the *menu* will drop down just as if it had been mouse clicked. The **left arrow** and **right arrow** keys allow movement from *menu* to *menu* in the *menu bar*, while the **up arrow** and **down arrow** keys allow movement up and down the active *menu*. Once the desired *menu item* is highlighted, press **enter**. *Menu items* often have characters underlined just as the words in the *menu bar* do. Typing an underlined character (without the **ALT** key) will cause the *menu item* to be selected and executed. For example **ALT f, x** will cause most Windows® programs to exit.

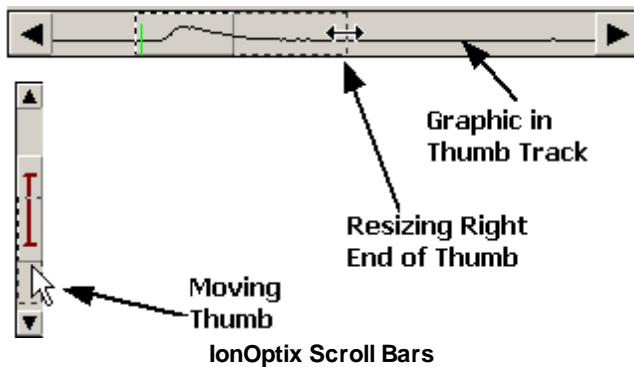
In addition to *menu bars* attached to windows, the Windows® environment also supports the concept of free floating *pop-up menus*. A *pop-up menu* is a *menu* (not a *menu bar*) that appears under the cursor after the user clicks the mouse in a specified region. After the *pop-up menu* appears, it behaves the way same as a regular *menu*. Clicking the mouse outside the *pop-up menu* causes the *pop-up menu* to disappear.

4.1.3 IonOptix Controls

In addition to the Windows® controls outlined in the previous section, IonOptix has a variety of its own controls.

4.1.3.1 IonOptix Scroll Bars

In order to facilitate the examination of data traces, IonOptix has developed a control adapted from the standard scroll bar.



One difference between IonOptix *scroll bars* and Windows® *scroll bars*, is that graphics (i.e. data traces) are drawn in the *thumb track* of the IonOptix version. Another and more important difference is that the *thumb* is more than an offset into a range represented by the ends of the *thumb track*. The beginning and end of the *thumb*, which the user can change at any time, represent the beginning and end of an arbitrary sub-range of the full data limits. By clicking the **right mouse button** anywhere in the *thumb track* and dragging the cursor along the *thumb track*, the user can re-size and move the *thumb*. The gray outline of the *thumb* indicates its new position once the right button is released.

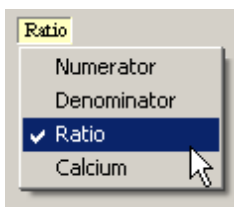
The offset changes possible with IonOptix *scroll bars* are nearly the same as those of regular *scroll bars*. The user can still drag the *thumb* about arbitrarily, and the *page up* and *page down* regions function the same. The *scroll arrows* are somewhat different, however, in that instead of moving one unit, the change is by one fifth of the length of the thumb. This difference is necessary due to the granularity of the data. The smallest unit of change is generally far too small to progress through the data at any reasonable rate.

When the cursor is a single pointer, the thumb can be moved using the **left mouse button**. The cursor changes from a regular pointer to a double ended arrow when it is moved over the ends of the *thumb*. This indicates that, with the **left mouse button**, the user may click and grab that end of the *thumb* and resize the thumb.

Maximizing The Thumb

Occasionally the user may want to force the *thumb* to its maximum extents. Holding down the **Ctrl** key while clicking the **left mouse button** anywhere in the *thumb track* will cause the *thumb* to assume its maximum values.

4.1.3.2 Yellow Buttons

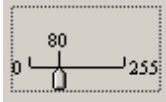


Yellow Button Menu

Yellow buttons are controls that look like labels, but when clicked reveal a *pop-up menu*.

The example above comes from the *information region* of a [trace viewer](#)³⁶, a composite control described later. The region with the text Calcium is the *yellow button*. The menu below Calcium appeared when the user clicked on the *yellow button* with the **left mouse button**. In this example, the user has the option to change the data trace shown in the trace viewer. Clicking anywhere outside the *menu* and the *yellow button* dismisses the *menu*.

4.1.3.3 Sliders



Slider Control

A *slider* resembles a *scroll bar* but is more appropriate for adjustment of a single numeric value. *Sliders* are often used in conjunction with a display that gives dynamic feedback of the effect of the new value. The slider indicates input focus with a dotted line around the perimeter, as shown above. The numbers on each end indicate the possible range of values. The number over the pointer is the current value.

If the slider has focus, the **arrow keys** will change the value in increments of one. The **page up** and **page down** keys change the value in increments of five.

Click the *slider pointer* with the **left mouse button** to change the value. As long as the **left mouse button** is down, the *slider pointer* will follow the mouse pointer movement. If the mouse pointer goes outside the *slider* area, the value will jump back to its original position. The slider value is updated when the **left mouse button** is released.

4.2 Logging on

IonWizard is designed to serve the various needs of multiple users on a single computer system. Personally defined configurations of the data display parameters are stored in association with the user's name. These display configurations are called *templates* and are discussed in detail below.



Log-on Dialog Box

The diagram above shows the *dialog box* that appears after launching IonWizard. If the user has run IonWizard before, their name will appear in the list of the *combo-box* titled Select who you are. Select the appropriate name and click OK.

If the user has not used IonWizard before, click Add User and the *combo-box* will change to an *edit field* where a new user name may be entered. Click OK to enter the new user.

Take notice of the version and revision number present in the dialog box. In this example, the full version of IonWizard is 6.0 revision 1. Any DLL with version 6.0 should run with this version of IonWizard, regardless of its revision.

4.3 Exiting

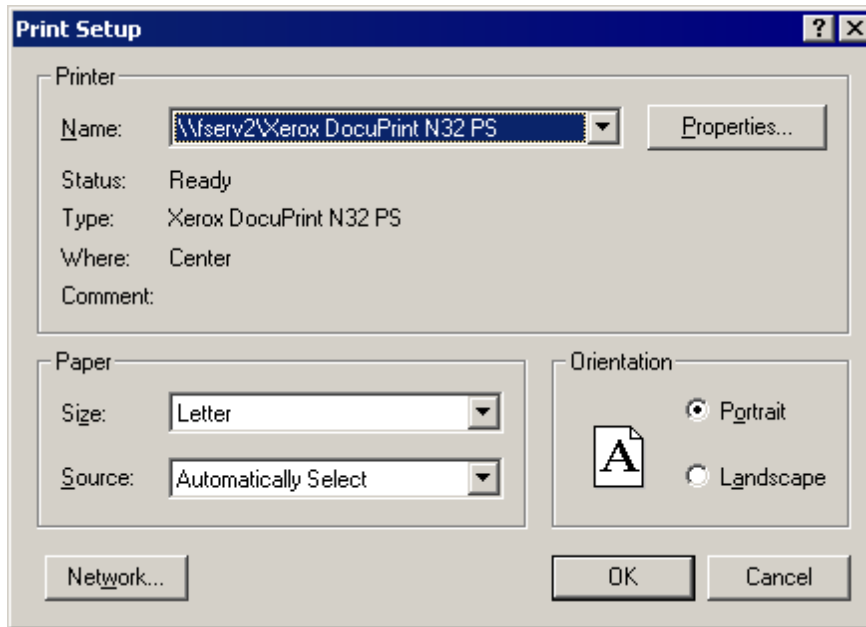
Exit from the data file you are working on by selecting Exit from the File *menu*. If you have opened more than one window, a second exit option will exist, Exit All. This has the same effect as closing each experiment individually from the menus. In either case, warnings will appear prompting the user to save any files that have been modified.

4.4 Selecting a Printer

IonWizard uses the same printer setup data for all printing. Therefore, make sure that the configuration setup on IonWizard is correct. Select Printer Select... from the *File menu*. This will invoke a standard *dialog box* with a list of available printers. This dialog box can also be reached from the *Trace Printing Options dialog box*.

Choose either the default printer, or one of the printers available to your machine. The *Network... push button* enables connection to any shared printer on the network.

Clicking the *Properties... push button* will bring up a *dialog box* supplied by the printer manufacturer allowing configuration of printer specific options.



Printer Selection Dialog Box

When you click OK, the selected printer will become the destination for all printing from IonWizard.

This information is saved in the current users [profile section](#)^[105].

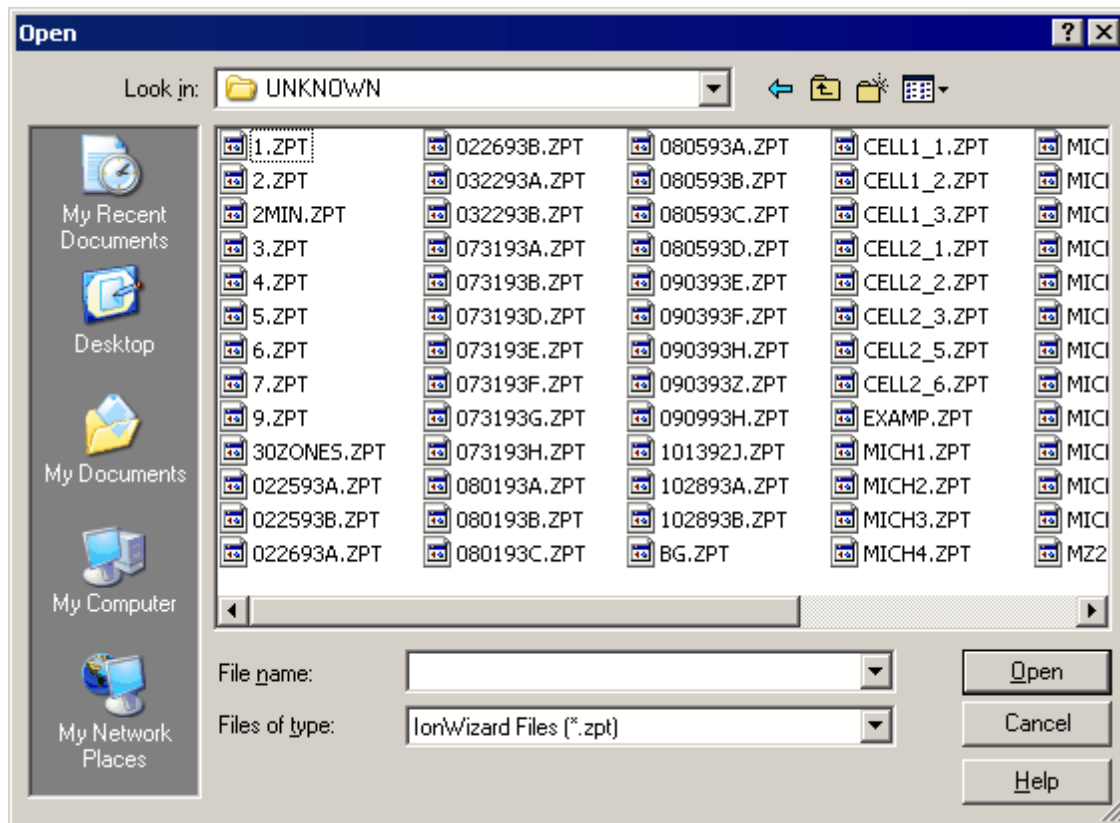
4.5 Handling Data Sets

The central IonWizard concept related to data containment is the experiment [data set](#)^[29]. Each top level IonWizard window presents a single data set. Each data set is accessed through a single root file which may or may not have associated auxiliary files (images, etc.). This section discusses opening, closing, saving, and creating (i.e. acquiring) data sets. We will cover data sets in more detail in later chapters.

4.5.1 Opening a Data Set

To open an existing data set, select Open... from the File menu. This will invoke the standard Windows Open *dialog box* as shown below.

Select the file search extension from the Files of type: *combo-box* in the lower center of the *dialog box*. The number of different types of files that can be opened depends on the number and type of Open DLLs present in the IonWizard directory. Typically *.zpt (Zone Plot files) is the only option.



Using the Look in: *combo-box* and the search locations on the left, navigate to the desired directory on the local or on a remote machine. Select the desired file from the listed options and click the Open *push button*.

IonWizard maintains a list of the last four files opened for quick access. This list is located at the end of the File menu.

4.5.2 Saving a Data Set

To save changes to a data set (for example new marks, notes, or calibration values) select Save from the File menu. The data set will be written to the disk. In the interest of data safety, any file opened in an old format will be renamed with a .bak extension prior to saving the file in the newest format. IonWizard will inform the user when this is about to occur.

4.5.3 Closing a Data Set

After examining one experiment and before examining another, it is a best (though not necessary) to close the current experiment in order to free up memory. Effects of this are most noticeable if the traces are very long or have lots of associated images. To close an experiment, select Close from the File menu. If changes have been made to the experiment, IonWizard will query whether or not to save the file. At this point, there will also be the option to cancel the closing operation which returns IonWizard to the pre-close state.

If you close a file in the last existing IonWizard top-level window, an empty window will appear so that you can load a new file. If there is more than one top-level IonWizard window, selecting Close will close the file and the window (the same behaviour as Exit).

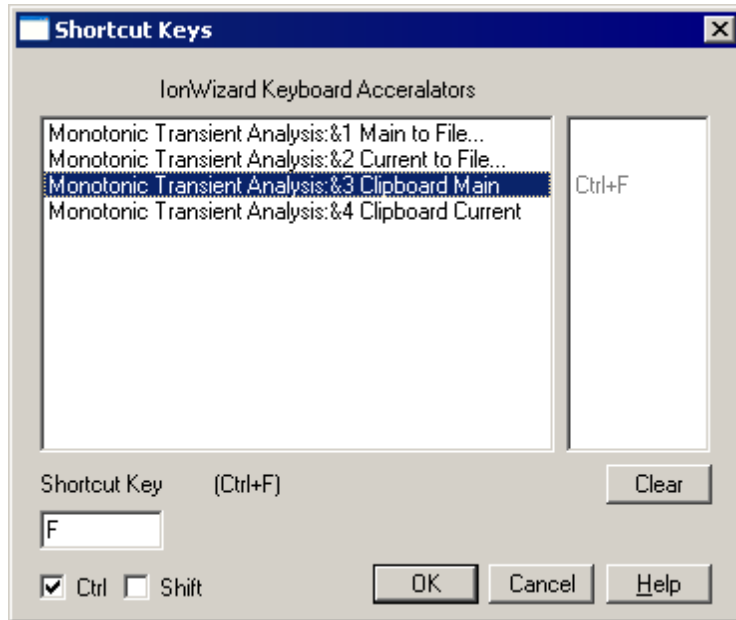
4.5.4 Creating a New Data Set

If the acquisition modules have been installed, IonWizard adds a *menu item* called New to the File menu. Selecting this *menu item* will begin a new data acquisition session.

Please see the separate acquisition help file for further information.

4.6 Shortcut Key Manager

IonWizard permits many of its plug-ins to add *items* to its *menus*. To assign short cut keys to these *items*, select Assign Shortcut Keys... from the Operations menu. This invokes the Shortcut Keys dialog box shown below.



Shortcut Keys Dialog Box

Select the command you wish to assign a shortcut key from the *list box* on the left. The *list box* to the right shows any current assignment. Use the *edit field* and the Ctrl and Shift *check boxes* in the lower left corner to select a key combination.

Use the Clear *push button* to clear any current assignment.

5 Data Sets

As touched on earlier in [Handling Data Sets](#)^[26], the central IonWizard concept related to data containment is the experiment data set. The raw data set as acquired directly from hardware contains the following broad classes of data:

Hierarchical Data	Data that is organized and accessed in a hierarchical structure. This includes trace arrays, image arrays, constant values and image points.
Marks	Transient and Event marks that indicate points of interest within time resolved data.
Notes	Free form notes and other descriptive text associated with the data set.
Zones	Information regarding regions of interest and sub-regions of interest as related to image data.

Further analysis function such as trial and event averages will create additional views of the base data. We call these views *data representations*.

While most of this information arguably falls somewhat under the category of trivia, there are two topics to discuss in a little more detail as they pertain pretty directly to the layout and behaviour of the program. Those topics are the [Hierarchical Data](#)^[30] and [Data Representations](#)^[31].

5.1 Hierarchical Data

The vast majority of the data in each data set falls under the category of *hierarchical data*. This includes trace arrays

image arrays, constants and image points. This is data that naturally lends itself to separation and classification in a hierarchical tree. For example, given four arrays of trace data:

- fura2 raw numerator,
- fura2 raw denominator,
- cell length left edge and
- cell length right edge

rather than to simply present a flat list as above, it is useful to group them :

- fura 2 raw,
 - numerator,
 - denominator,
- cell length,
 - left edge,
 - right edge.

We adopted a paradigm called data hierarchies to handle this grouping. This hierarchical tree has three levels called type, channel, and position.

Type

The hierarchy type represents a top-level logical grouping of data. Examples are [Raw Fluorescence](#), [Analog Data](#), or [Background Values](#). Each hierarchy type has two related sub-levels, the channel and the position.

Further in the text you will see references to trace types and image types. This is what they are referring to.

Channel

The first sub-level is the channel. Examples of channels for a [Raw Fluorescence](#) type might be [Numerator](#) and [Denominator](#). Channels within a type are related either by being part of a sampling set, or by being related calculations. Therefore, we see [Numerator](#) and [Denominator](#), or [Ratio](#) and [Calcium](#) together, but not [Numerator](#) and [Analog Channel 1](#).

Position

The second sub-level is the position. Positions indicate the location of a particular data item, if such information is applicable. All channels in a given type have the same number of positions, and presumably come from the same location. For example Numerator and Denominator data from Zone 1 is expected to be collected from the same region of interest in a fluorescence image. Thus the concept of positions is not abstract, it is truly the locations from which data was sampled. Any data that is not spatially related or has an implied spatial definition will not have positions.

Address

A full triplet of <type, channel, position> represents the hierarchy address of a particular bit of data. All hierarchical data in IonWizard is referenced via its address. When you use the *yellow buttons* in the *trace viewer information region* (see [§Trace Viewers](#)^[36]) to pick what trace to display, you are directly choosing the hierarchy address of the trace. Likewise, in the *Constants dialog box*, you directly pick the address of the constant value to edit as you select from each *list box* (see [§Constants](#)^[57]). Therefore the underlying concept of the data hierarchy very much reveals itself in the actions you must take when working with data in IonWizard.

5.2 Data Representation

IonWizard has the ability to perform high level analyses such as event averaging and trial averaging that globally effect all the time based hierarchical data (i.e. the trace arrays and the image arrays). For example, if you perform an event average on a data set that initially has Length and Calcium data, you will end up with a new, averaged, "version" of the Length and Calcium data. This new "version" is called a *data representation*. (If you wish to skip ahead, see [§Ensemble Averaging](#)^[83] for more information on averages).

Every data set has at least one data representation, namely the base representation that is the raw data as acquired by the acquisition module. This data representation is imaginatively named Base Traces. Each new trial or event average operation will create a new data representation with the name you specified as part of the operation.

In the following chapters we will explain in greater detail how to [create](#)^[85], [manage](#)^[51] and [view](#)^[33] data representations.

6 Basic Data Handling

In this chapter we will discuss the fundamental methods of visualizing and editing the data set. We will defer to later chapters topics such as advanced analysis, printing, exporting and so forth. Here we aim to familiarize you with simply looking at the data and basic data manipulations.

6.1 Trace and Image Arrays

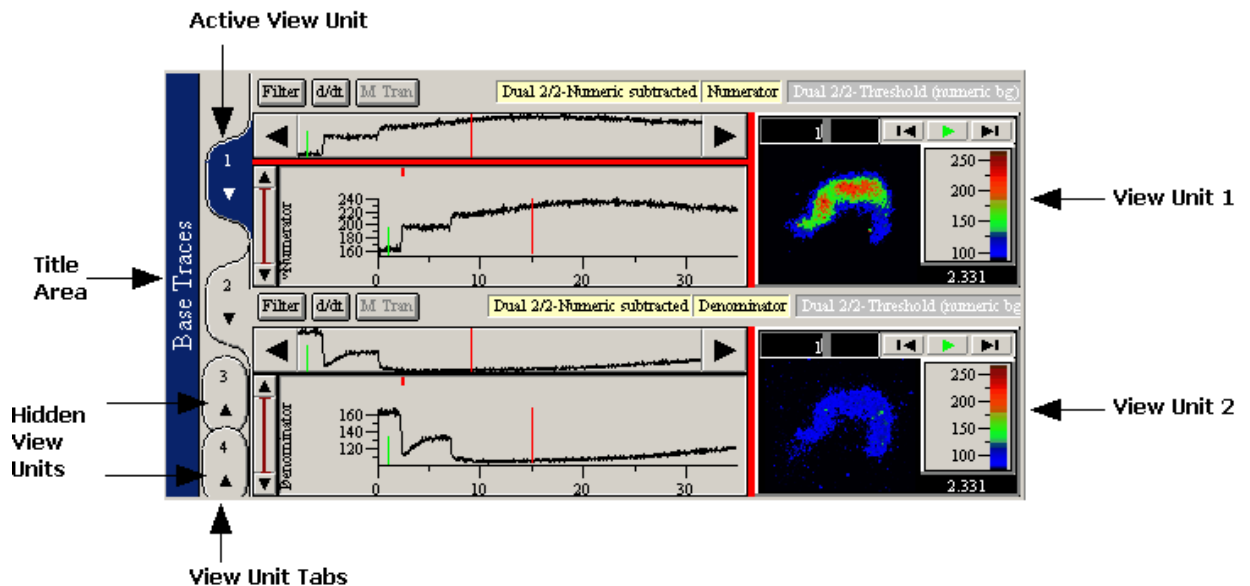
As discussed earlier, time based data, namely the trace and image arrays, are handled by an overarching concept called a [data representation](#)^[31]. Each data set will have at least one, and possibly many, different data representations. In this chapter, we will discuss visualizing the trace arrays and image arrays in a data representation via the trace bar and its constituent [trace viewer](#)^[36] and [movie player](#)^[42] components. We will also discuss [managing data representations](#)^[51].



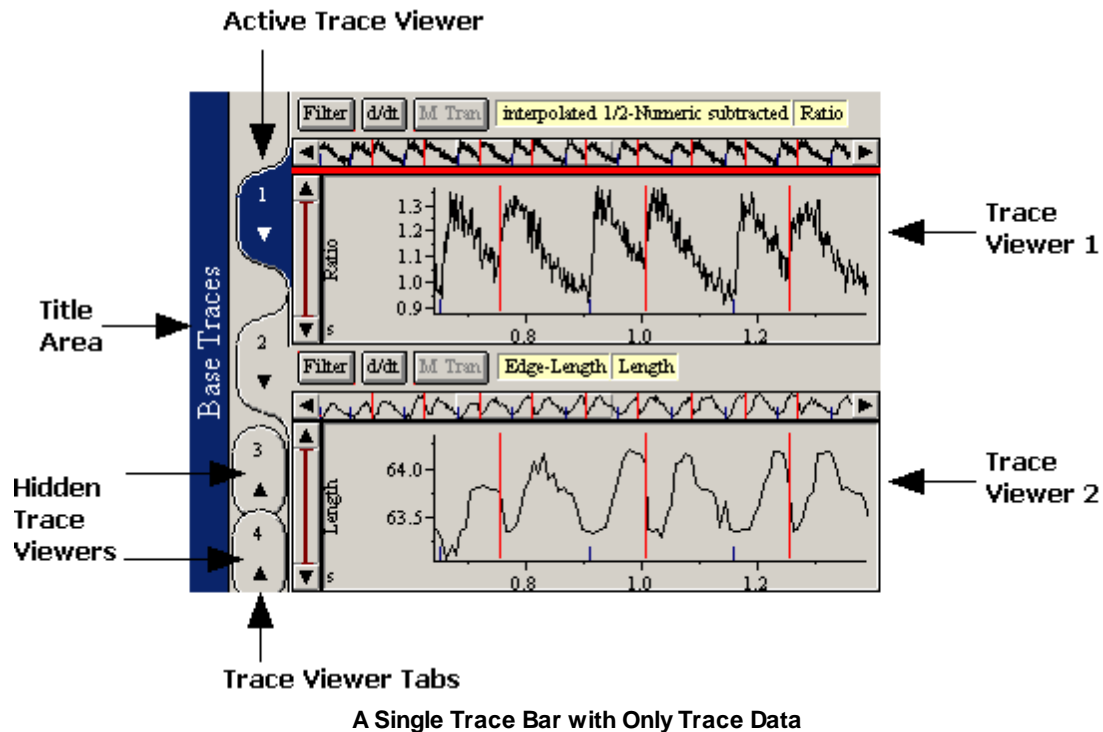
Image Arrays and related options are only available when a recording task saves the images. Please refer to the acquisition manual for details

6.1.1 Trace Bars

IonWizard contains a specially developed composite control dubbed the *trace bar* that displays and allows interaction with the data traces and images within a specific [data representation](#)^[31]. The primary purpose of the *trace bar* is to examine a large quantity of data as easily and intuitively as possible. The *trace bar* consists of four *view units*. The figure below shows a trace bar with two visible view units. Each view unit is able to display trace data via its *trace viewer*, and image arrays via its *movie player*. These components will get full attention in their own chapters later (see [§ Trace Viewers](#)^[36] and [§ Movie Players](#)^[42]).

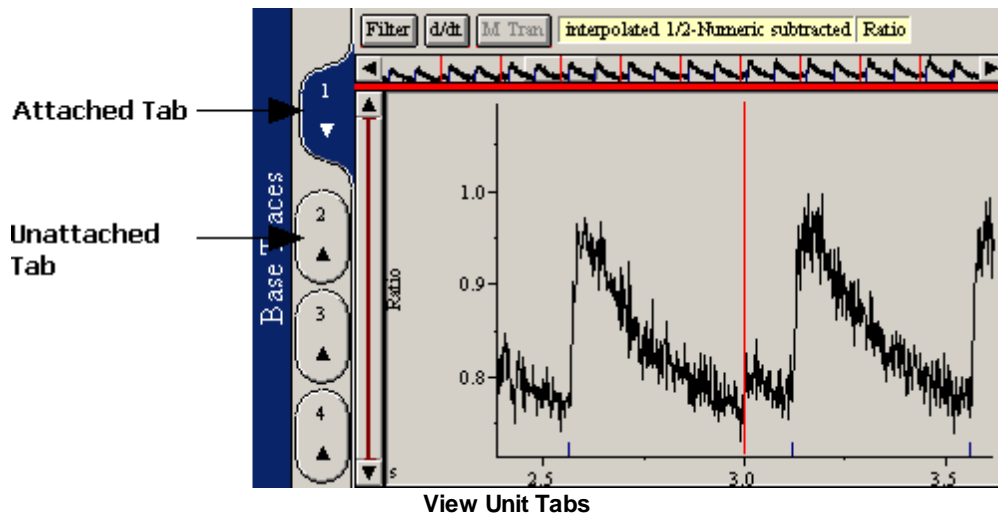


It is quite common for a data set to have no image data at all. In that case the *movie player* will not be available and the *trace bar* will look more like the figure below.



Since *view unit* is a somewhat cumbersome term, it is quite common to refer simply to *trace viewer 1* or *move player 1* rather than the "*trace viewer in view unit 1*". The figure above uses such shorthand, referring to everything as *trace viewers*. This manual attempts to use the proper terms in all cases, but keep in mind the shorthand usage.

View Unit Tabs

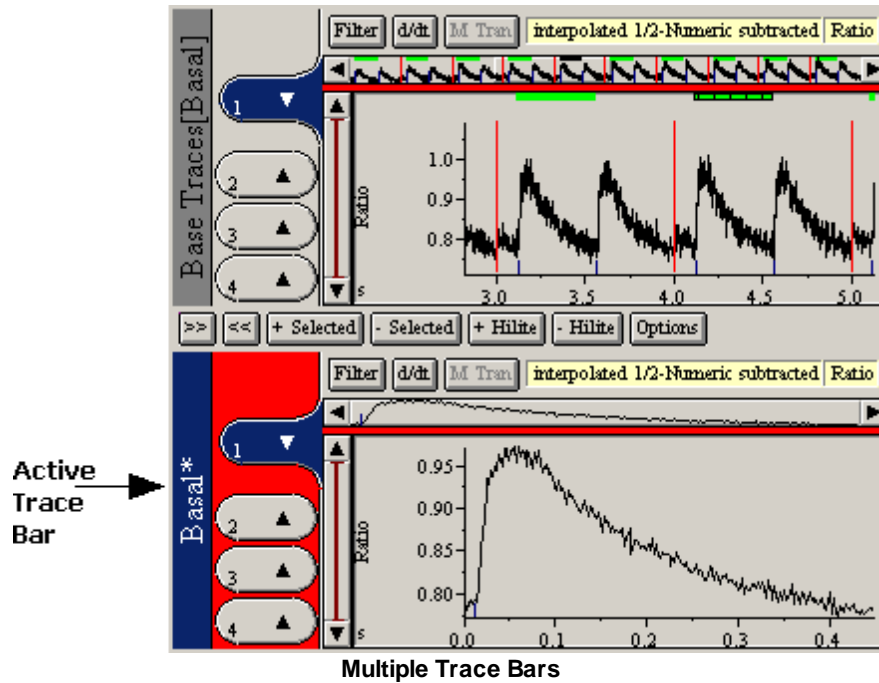


View unit tabs are custom *push buttons* that allow the user to quickly show or hide *view units*. Clicking on an attached *view unit tab*, shown above, will hide that *view unit*. Conversely, clicking on an unattached *view unit tab* will show the given *view unit*.

Multiple Trace Bars

In data sets where there is more than one data representation, there will more than one *trace bar*. Indeed there is exactly one trace bar for every data representation. The examples above illustrate single *trace bars* showing the Base Traces, the representation of the data as it was collected. If you

perform event or trial averages, each of those new data representations will be presented in its own *trace bar*. Each will be appropriately labeled in the title area on the left as shown in the figure below.

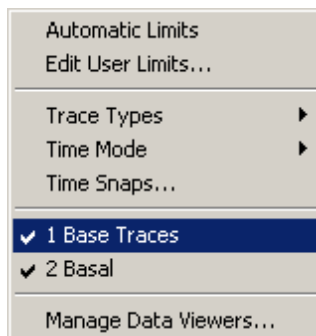


Operations such as printing, exporting, or trace analysis may be applied to the data in any *trace bar*. IonWizard looks for the active *trace bar* to determine which data representation to use in a given situation. In the illustration above, the bottom *trace bar* is active, as indicated by its highlighted title area. The upper *trace bar* is inactive. If the user selects Print... at this point, the Ratio trace from the Basal *trace bar* will be printed. IonWizard uses the same color schemes to indicate active *trace bars* as Windows® uses to indicate active windows. Clicking anywhere in a *trace bar* will cause that *trace bar* to become active. If there are no active *trace bars*, any *menu item* that requires an active *trace bar* will be disabled.

As mentioned earlier, there is also an active status at the level of individual *view units*. In the illustration above, *view unit #1* is active in the Basal *trace bar*. The active status of *view units* is shown by the color of the tab. Again, the coloring reflects the coloring used by the main window titles. The active *view unit* is always in the active *trace bar*. The trace visible in the active *view unit* is the object for operations that require a single trace, for example automatic transient mark insertion (this operation is detailed in [§ Transient Mark Acquisition](#)^[64] below).

6.1.1.1 Showing and Hiding Trace Bars

For each different *trace bar*, there will be a section in the Trace *menu* as shown below that allows showing or hiding that *trace bar*.



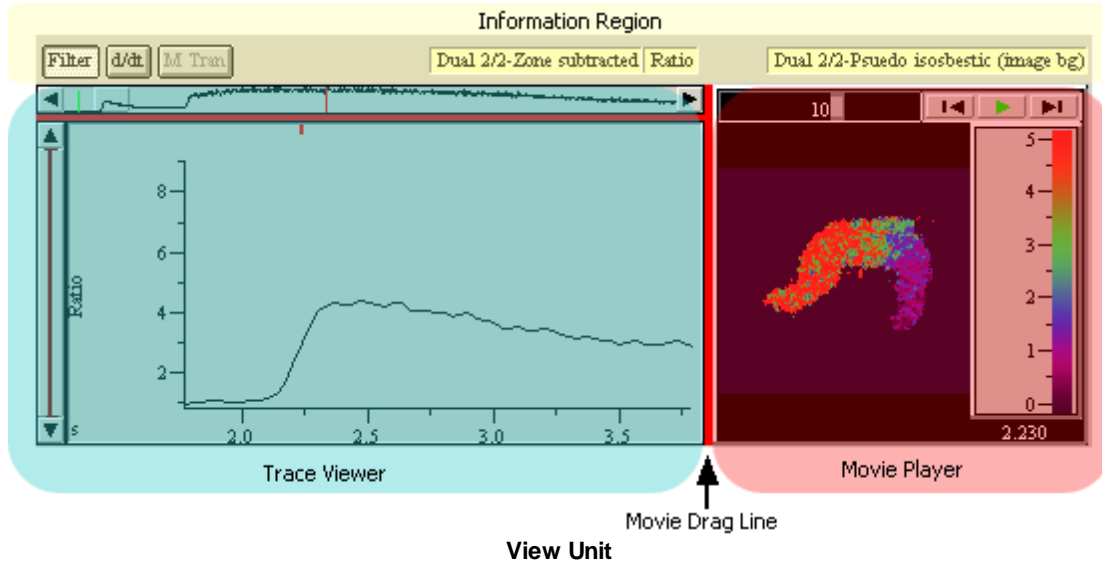
Trace Bar visibility menu

Selecting the NAME *menu item* (where NAME is the name of the [data representation](#)^[51]) will switch the visibility state of that data representation's *trace bar*. A check mark next to the *item* indicates that the

trace bar is visible.

6.1.1.2 View Units

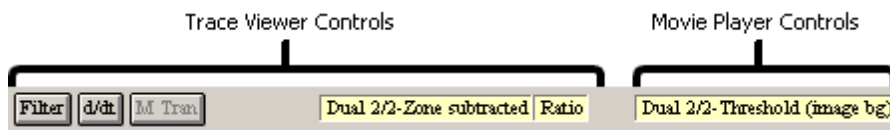
Each *trace bar* has four *view units*. A *view unit* consists of an *information region*, a *trace viewer* and a *movie player* along with a drag line to size the individual components. The *movie player* and its associated drag line will only be present if the data set has images. The figure below illustrates a *view unit*.



Each of the regions is explained in detail in dedicated chapters later in this document however we will give a brief overview here.

Information Region

The *information region* holds controls in the form of push buttons and [yellow buttons](#)^[21] that control the data shown in the *trace viewer* and the *movie player*. The controls on the left are for the *trace viewer* and those on the right are for the *movie player* as shown in the figure below.



Trace Viewer

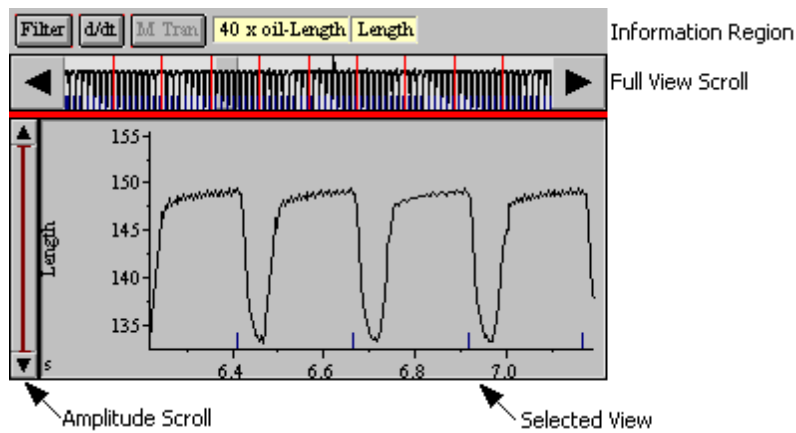
The [trace viewer](#)^[36] is the primary tool for visualizing and interacting with all the time resolved data except images. This includes not only the data traces themselves, but [event and transient marks](#)^[62] and also [time discontinuities](#)^[53].

Movie Player

The [movie player](#)^[42] permits visualizing any time resolved image data that is associated with a given trace and also provides methods to query the image data.

6.1.1.2.1 Trace Viewers

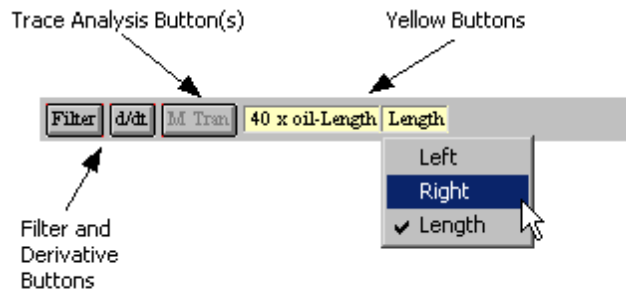
There are three regions in a trace viewer: (i) the *full view*, which gives a global view of the data; (ii) the amplitude scroll; and (iii) the selected view, which gives a detailed look at a segment of the full view. The trace viewer also injects a number of user interface elements into the information region that control what trace is being shown and permit analyses to be run on that trace data.



Trace Viewer

We will now discuss each of these regions and controls in detail.

Information Region



Information Region Trace Viewer Controls

The *information region* contains a number of controls that impact the *trace viewer*: the filter and derivative *buttons*, the trace analysis *buttons* and the *trace viewer yellow buttons*. The leftmost *yellow button* controls data type, the next *yellow button* controls the channel, and the rightmost, if present, controls the position. The figure above illustrates those controls.

Yellow Buttons

The *yellow buttons* allow the user to select any trace that is available for viewing. There are a maximum of three *yellow buttons* visible. They control the type, channel, and position of the currently viewed trace. For more information about the <type, channel, position> terminology see [\\$Hierarchial Data³⁰](#). To change any of the fields, click on the *yellow button* and a menu will appear to permit selection of a new trace. Changes of data type, made with the leftmost *yellow button*, will change the contents of the remaining *yellow buttons* and may cause the remaining channel and position buttons to be hidden or disabled.

In the example above, the user has clicked on the channel *yellow button* with the **left mouse button** to reveal the channel *pop-up menu*. The checked *menu item* indicates that the current trace is Length, which we can also see in the *yellow button* label. The highlighted *menu item*, Right, indicates we will change from <40 x oil - Length, Length> to <40 x oil - Length, Right> when the user releases the left mouse button.

If more than one *trace viewer* is visible in a *trace bar*, it may be desirable to have changes made in one *trace viewer* occur in the other *trace viewers*. For example, assume the user is viewing Zone 1 of multiple traces: Numerator, Denominator, and Ratio. To change all these traces to Zone 4, hold down the **Ctrl** key prior to making the selecting Zone 4 in the position *yellow button*. All the other *trace viewers* will attempt to change to Zone 4.

This operation can partially fail. Assume that one of the trace viewers was showing a [trace type³⁰](#) that had only two positions, Zone 1 and Zone 2, while the trace types in the other trace viewers all had four positions. When the user tried to change to Zone 4 with the

Ctrl key, this *trace viewer* would not be able to make that change. It would bound the desired new position (Zone 4) to the maximum position available for the selected trace type (Zone 2). The restriction that effected this one *trace viewer* would not be transmitted to any other *trace viewer*. They would still all change to Zone 4.

IonWizard allows the user to globally specify which types show up in the type yellow button menu. This is done via the Trace Types sub-menu under the Traces menu. This sub-menu lists all the available trace types. Items with checks are currently included in the type yellow button menus. Selecting one of the items toggles its inclusion. If only one type is selected, the type yellow button will disappear all together.

Filter and Derivative Buttons

At the far left of the *information region* are two *push buttons* that permit application of a trace filter and/or calculation of the derivative of the trace. The Filter push button will apply a digital filter of the users choosing. Trace filters are explained in [§Trace Filtering](#)^[79]. The filter state of a given *trace viewer* can also be controlled via the Raw Trace Filtering item under the Operations menu.

The d/dt push button causes the trace to be displayed as its first derivative. This is useful when working with transient marks. To apply the desired operation, click on the appropriate button. The button will then be drawn in a depressed state as shown below.



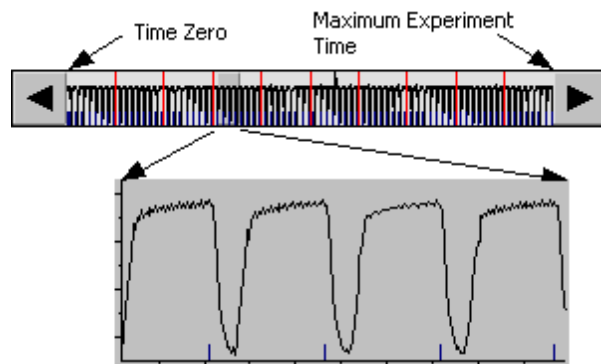
Clicking the depressed button again will clear the operation. These two operations can be applied in any combination.

Trace Analysis Buttons

If you have any trace analysis plug-ins installed, it is possible that the plug-in will place a button in the information region to allow you to invoke the analysis directly without having to go through a menu. If so, the trace analysis button will appear between the Filter/Derivative buttons and the yellow buttons. In the Information Region figure above, we see a M Tran push button. This is from the Monotonic Transient Analysis plug-in and would invoke that analysis if clicked. For more information, see [§Trace Analysis Plug-ins](#)^[80].

Full View Scroll

The *full view scroll* is an *IonOptix Scroll Bar* that allows the user to zoom in on specific time periods of the entire experiment. (For a review of *scroll bar* terminology and manipulation see the [§Scroll Bar](#)^[17] and [§IonOptix Scroll Bar](#)^[20] above). In the thumb track of the *full view scroll* is a picture of the entire data trace from time zero to the maximum experiment time as seen in the figure below. The left edge of the *full view scroll* thumb track represents experimental time zero while the right edge represents the end of the data run. The data trace shown in the *full view scroll* shows gross data changes, thus enabling the user to direct attention toward some interesting occurrence.



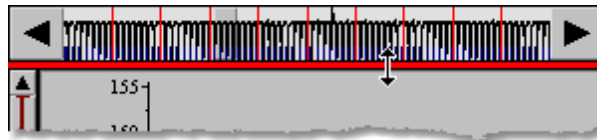
Full View Scroll and relation to Selected View

The thumb of the *full view scroll* represents the time range of the full trace displayed in the *selected view* as illustrated by the arrows in the figure above.

The *full view scrolls* of all the trace viewers in a given trace bar are locked together. Changes made to one *full view scroll* will effect the other *trace viewers* in that *trace bar*. This allows straight-forward correlation of events in one trace to events in another. There is no such communication between *trace bars*.

Drag Line

Regardless of the number of *trace viewers* showing, one red bar will be present in the first *trace viewer*. This bar is the drag line which allows the user to size the *full view scroll*. The cursor will turn into an "up/down arrow" as shown in the figure when the mouse pointer is moved over the line. Click and hold down the **left mouse button** and as the mouse is moved, a highlighted version of the drag line will follow the cursor.

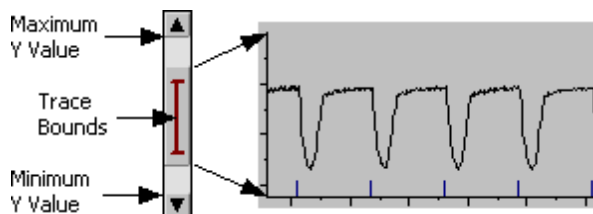


Drag Line

When the mouse button is released, the position of this highlighted line will become the new bottom edge of the *full view scroll*. A minimum and a maximum height exist for the *full view scroll bar*. The highlighted rectangle will stop and no longer follow the mouse when the user has reached one of these bounds. Releasing the mouse button will cause the rearrangement of all *trace viewers* to reflect this new sizing. The height of the *full view scroll* is stored in the [screen template](#)^[107].

Amplitude Scroll

The *IonOptix scroll bar* to the left of the *selected view* is the *amplitude scroll*. As with the *full view scroll*, the *amplitude scroll* controls the presentation of the trace data in the *selected view*. Instead of controlling the x axis however, the *amplitude scroll* controls the y axis. The top and bottom of the *amplitude scroll* indicate the maximum and minimum display limits for the given trace.



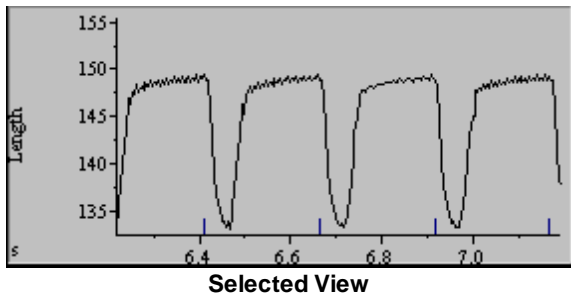
Amplitude Scroll

The display limits themselves have two possible sources. They can be derived from the data range of the trace itself, or from some user supplied boundaries. We refer to these as Automatic Limits or User Limits respectively. For more information regarding display limits and the editing of user limits, see the [Display Limits](#)^[40] section.

In the thumb track of the amplitude scroll itself is an \perp shaped symbol which indicates the range of data present for the given trace. If the display bounds are set to Automatic Limits, then this \perp will exactly cover the entire range of the thumb track. In the figure above, we see that the display bounds are set to User Limits and thus the \perp does not fill the entire thumb track.

Selected View

The *selected view* presents a view of the data trace that is zoomed in both time and in amplitude as specified by the *full view scroll* and the *amplitude scroll* respectively. The *selected view* is thus the main tool for visualizing the data trace. It is also the main control for performing simple analysis, for interacting with event and transient marks, for interacting with time discontinuities, and for working with event and trial averages.



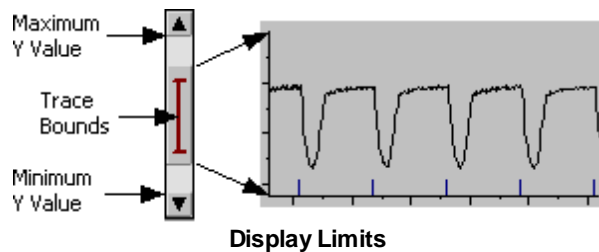
See the following sections for details on each of the operations carried out using the selected view:

1. [§Range Averages](#)^[41]
2. [§Adding Marks](#)^[63]
3. [§Editing/Viewing Existing Mark Information](#)^[68]
4. [§Querying Time Discontinuities](#)^[54]

In addition, trace analysis plug-ins can add graphical elements to the selected view to help with visualizing the analysis. Please see the specific trace analysis plug-in documentation for more information.

6.1.1.2.1 Display Limits

Recall that the amplitude scroll bar selects the current range for the y-axis of the selected view from a maximum range as indicated by the [Maximum Y Value](#) and [Minimum Y Value](#) in the figure below. This maximum range, the display limits, is determined by the choice of *automatic limits* or *user limits*.



Automatic Limits

Automatic limits forces the range of the amplitude scroll display limits to mirror that of the current trace data. This range is the same as that indicated by the [Trace Bounds](#) indicator in the figure above.

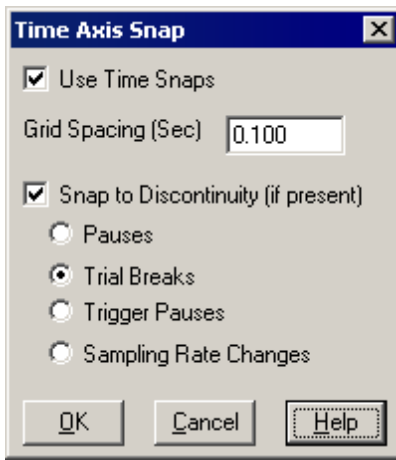
To change the the display limits to *automatic limits*, select [Automatic Limits](#) from the [Traces menu](#). A check next to this *menu item* indicates that *automatic limits* are being used. Otherwise, the display limits are set to *user limits*.

User Limits

If the current display limits are set to *user limits*, the maximum and minimum values of the amplitude axis are always the same for a given [trace type](#)^[30] and are chosen by the user. This is very helpful for comparing traces between experiments as it permits absolute comparisons and thus unusually large or small responses will be quite obvious. The *user limits* are arbitrary and user editable as described in [§User Limits](#)^[77].

6.1.1.2.1.1 Time Snap

By default, there are no restrictions on the thumb beginning and ending values of the [full view scroll bar](#)^[38]. That is, the time range of the [selected view](#)^[39] can begin and end on arbitrary values. However, it may be desirable to have greater control over the selected view beginning and end values. Select the [Time Snaps...](#) menu item from the [Traces](#) menu to bring up the [Time Axis Snap dialog box](#) as shown below.



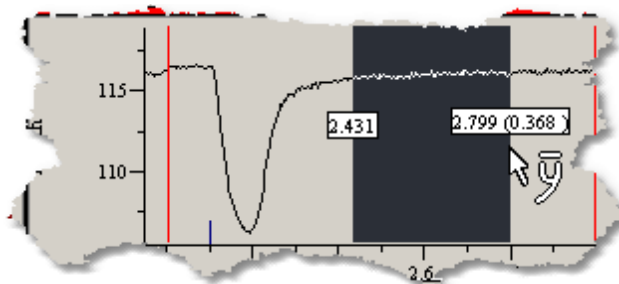
Time Snap Dialog Box

Time snaps work by creating an invisible grid along the full view. When the *full view thumb* is moved, it will snap to the nearest grid point. The grid can be either regularly spaced, or based on time discontinuities. To use any snap, select the [Use Time Snaps](#) check box. If you wish to snap to a specific discontinuity type, check the [Snap to Discontinuity](#) check box and then select the specific discontinuity type from the list. If not, leave [Snap to Discontinuity](#) unchecked and the thumb will snap to the nearest grid point as specified in the [Grid Spacing](#) edit field. Regardless of whether or not you intend to employ discontinuity snaps, you must enter a grid spacing. This allows a fallback in the event that the file does not have the discontinuity you have chosen.

All time snap options are saved in the [screen templates](#)^[107].

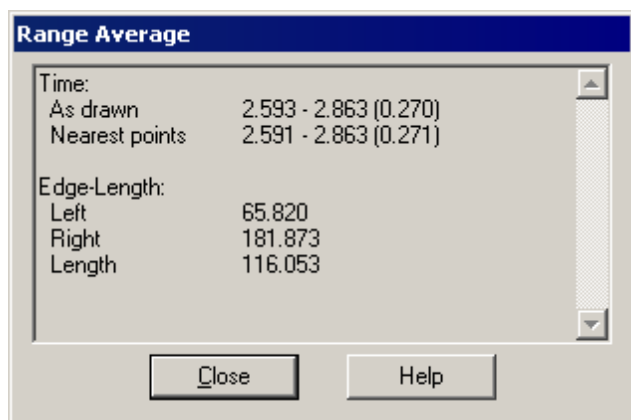
6.1.1.2.1.1 Range Averages

The user may desire to evaluate the average data value over an arbitrary time period within a trace. This is called a range average and is performed by clicking and dragging with the **left mouse button** in a *trace viewer's selected view*. Move the cursor to the beginning of the range and click the *left mouse button* down. While holding the button down, drag the cursor to the end of the range. A highlighted area will appear indicating the current selection as shown below.



Range Average Cursor and Highlighted Region

When the **left mouse button** is released, the following *dialog box* will appear.

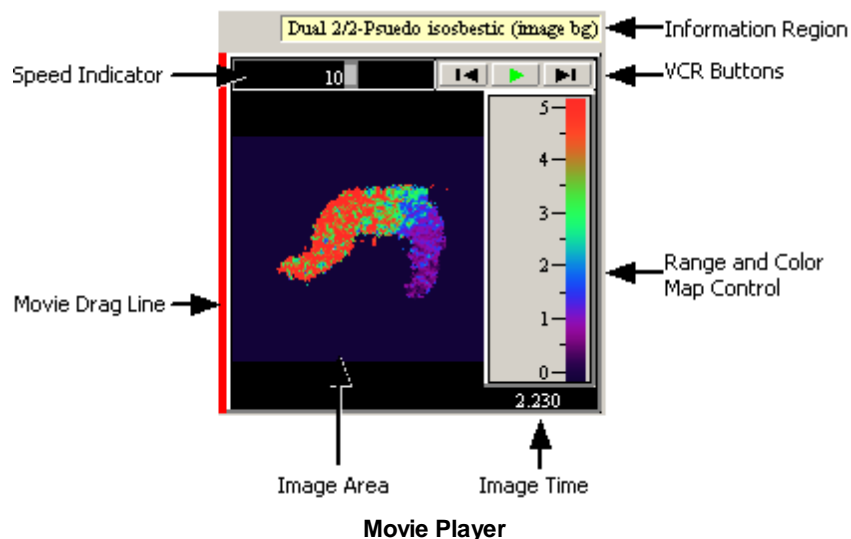


Range Average Dialog Box

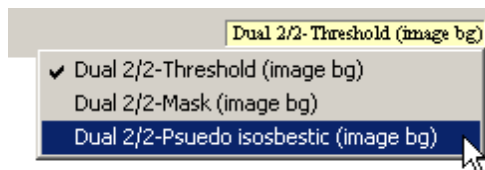
The average values of all channels for the given [trace type](#)^[30] will appear. Releasing the mouse button with the cursor outside the *selected view* cancels the operation. For more information on trace types and channels see [Hierarchical Data](#)^[30].

6.1.1.2.2 Movie Players

Movie players, which share the screen with the *trace bar*, are the primary means by which images are examined in IonWizard. When images are present, the right edge of the trace viewer is moved to the left and the *movie player* occupies the space thus created. The figure below shows a *movie player*. The *movie player* only displays images from the time period delimited by the *selected view*. *Movie players* inherit their active status from the *viewer unit* in which they reside. See [Trace Bars](#)^[33] and [View Units](#)^[36] for more information.



Information Region



Movie Player Yellow Buttons

When a *movie player* is present, it inserts *yellow buttons* in the *information region* to the right of the *trace viewer* yellow buttons (see [View Units](#)^[36] for more information). These *yellow buttons* allow the user to switch between any available image variations. Typically, these are variations in noise reduction techniques, also called thresholding. If there is only one image array associated with a given

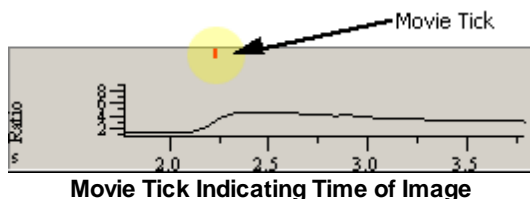
trace, the *yellow button* will be disabled. Otherwise, the user will be able to switch between the different images available. As with traces, holding down the **ctrl** key while switching will force the other visible *movie players* to make the same change.

Image Area

The image displayed is dictated by the trace displayed and also by the selection in the *yellow buttons* as described below. In addition to showing the image, the image area displays other information, can be used to access the movie menu, and can be used to zoom the image.

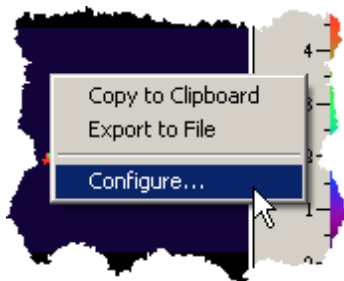
Image Time

The time of the image is displayed in the image area just below the *color map/image range control*. Additionally, a small tick mark is drawn in the *selected view* above the trace that indicates the that point in time as shown in the figure below.



Local Menu

Right clicking in the image area shows the *movie player local menu*.



This *menu* permits export of the image as a figure and configuration of *movie player* options. See [§Movie Player Options](#)^[47] below and [§Exporting Movie Player Figures](#)^[92] (a sub-section of [§Printing and Figure Creation](#)^[89]) for more information.

If the movie player is configured to permit certain types of image data query, additional items will be added to this menu to invoke the given query. See [§Image Queries](#)^[48] below for more information.

Image Zoom

It is sometimes desirable to view the images blown up in order to see more detail. This is accomplished by double-clicking in the image area of the *movie player*. This will cause the movie player to be placed into a sizable window. The image details will now be displayed in the title bar of the window instead of at the bottom of the movie player. This window will always remain above the other windows so that you can see it. Double clicking in the *image area* again will cause the *movie player* to be moved back to its original location in the *trace viewer*.

VCR Buttons

VCR button area is located at the top right of the *movie player*. These *buttons* are modeled after video cassette recorder buttons. There are two different modes for *VCR buttons*, stopped and running.



Running

Movie Player

VCR Buttons

The figure above shows the *VCR buttons* when the movie player is running. The central button indicates that clicking it will pause the running movie. The "+" button will make the movie run faster and the "-" button will cause the movie to run slower. The actual frame rate is displayed by the speed indicator.

If you pause the *movie player*, the *VCR buttons* change: the central button will turn into a green arrow and the + and - buttons will change to double arrows as indicated by the figure immediately below.



**Stopped
Movie Player
VCR Buttons**

Clicking the green arrow makes the movie play. The double arrows will move the movie one frame in either direction. In the event that there are gaps in the image sequence, the *movie player* jumps to the next available image.

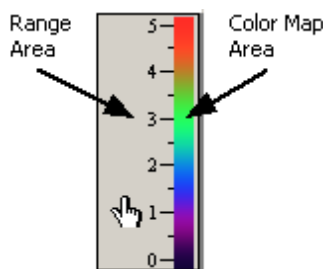
Speed Indicator



Speed Indicator

The frame rate of the movie is displayed at the top left of the movie player. The dark gray bar shown above indicates the desired frame rate while the light gray bar indicates the actual frame rate (the dark gray bar is visible around the light gray one). The number and indicates the actual frame rate. Note that the display rate is in no way correlated to the actual data rate.

Range and Color Map Control

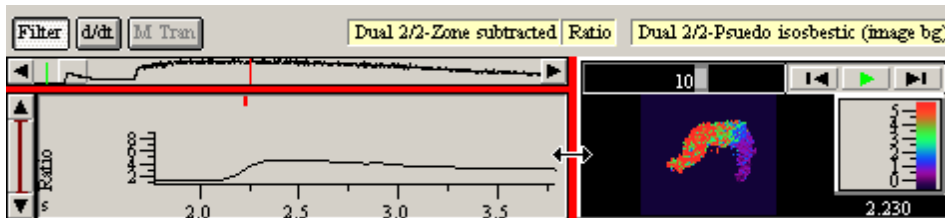


Range & Color Map Control

To the right of the image area is the unified range and color bar control, shown in the figure above. This control shows the current range of values that are being displayed and what color a given value maps to. Both the range and the color map can be edited by the user. To edit either the range or the color map, double click in the corresponding area. You will notice that the mouse cursor changes to a hand with a pointing finger when you are in one of the two areas as illustrated in the figure. For more information, see [§Editing the Color Map](#)^[45] and [§Editing the Image Range](#)^[46].

Drag Line

The red vertical line between the trace viewer and the movie player, visible in the image below, can be used to adjust the relative widths of the two regions.

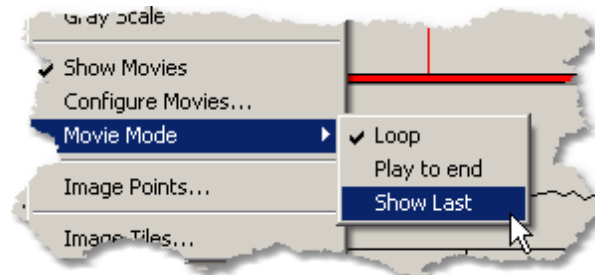


The cursor will turn into an "left/right arrow" as shown in the figure when the mouse pointer is moved over the line. Click and hold down the **left mouse button** and as the mouse is moved a highlighted version of the drag line will follow the cursor. When the mouse button is released, the position of this highlighted line will become the new left edge of the *movie player*. A minimum and a maximum width

exist for the *movie player*. The highlighted line will stop and no longer follow the mouse when the user has reached one of these bounds. Releasing the mouse button will cause the rearrangement of all *view units* to reflect this new sizing. The width of the *movie player* is stored in the [screen template](#)^[107].

Movie Modes

There are three modes that control how movie player moves from one image to the next. You can choose a different movie mode via the *Movie Mode sub-menu* under the *Images menu* as shown in the figure below. The current mode has a check next to it.



Movie Mode Menu

Loop

When the movie player reaches the end of the selected view range, it wraps around to the front. This is the default mode when opening a file as it is often interesting to loop over a small section of images again and again to see the dynamics more easily.

Play to end

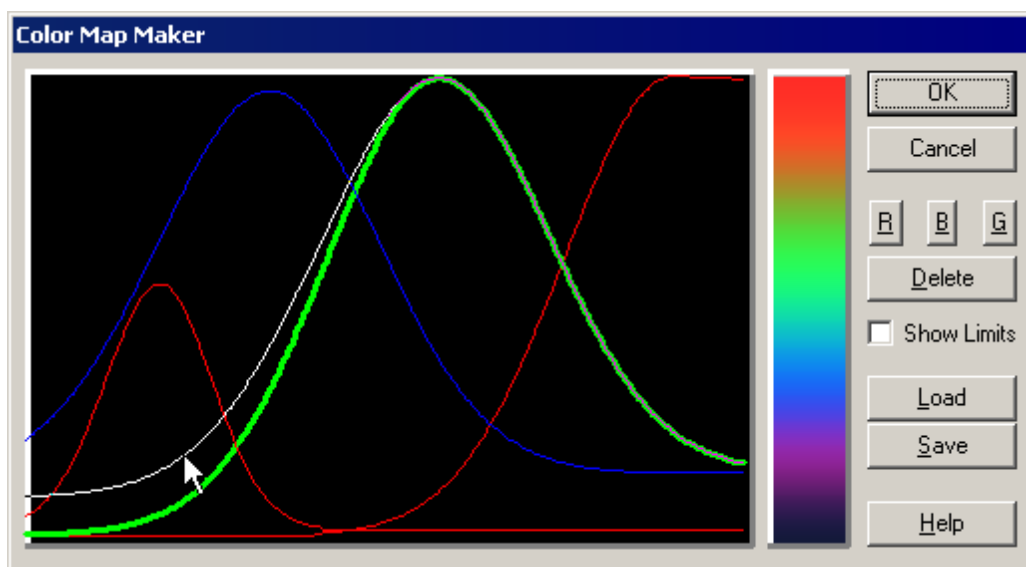
The movie player will play until it reaches the end of the selected view.

Show Last

The movie player shows the last image in the time array regardless of the selected view time range. This is the default mode when acquiring data where the user usually wants to look at the images as they are coming in and has little interest in things that have happened in the past.

6.1.1.2.2 Editing the Color Map

The actual color map which dictates the image presentation in the *movie player* can be edited. Double clicking on the color map of the *range and color bar control* brings forth the following dialog box (see the *movie player* overview in [\\$Movie Players](#)^[44] for information on the *range and color map control*).



The area to the left of the current color map is the editing area. The x axis can be regarded as the current range of displayable data values. These range values can be changed by the user as described in [\\$Editing the Image Range](#)^[46]. The y axis represents color intensities and the curves display the color mixtures that

are mapped to the data values. The curves that IonWizard uses to define colors are red, green, or blue Gaussian curves. Gaussian curves make for smooth color transitions across the palette. The curve drawn in the wider line, the central green curve above, is the active curve. The shape of the active curve can be edited with the mouse.

Editing Existing Curves

Both the **right** and **left** mouse buttons are used to edit curves. Clicking and dragging with the **left button** anywhere on the left or right arm of the curve will allow change of the baseline and the width of that arm. That is being illustrated in the figure above where the mouse pointer is moving the left arm of the green curve. The thinner white line indicates the new position of that arm. As the arm is moved, the color map will automatically update.

The left and right arms can be edited individually. This allows a greater variety of shapes of color bars to be created. In particular, it makes possible sharp ramps up and long decays off the back side. This prevents the confusion caused by having two different data values represented by the same color. If the arms need to be symmetric, hold down the **ctrl** key while editing with the left mouse button.

If the **left button** is clicked on the peak of the curve the peak will then follow the cursor while the base lines will remain the same. Clicking and dragging with the **right button** on the peak will cause a translation of the whole curve.

Adding or Deleting Curves

To add a new curve, click on the **R**, **G** or **B push button**. This will cause a new curve of the desired color to appear. If there is already a trace highlighted, the new trace will be initialized to the same position as the old trace. This affords simple construction of compound colors such as yellow or purple. Clicking on **Delete** will remove the current trace if there is one.

Show Limits

The **Show Limits check box** enables special colors for image values that are outside the current mapping range as described in the previous section. If selected, IonWizard will map overflow pixels to white and underflow pixels to black. If not selected, overflow colors will all map to whatever color is at the top of the color map, and underflow to the bottom colors.

The default is for this option to be on. It can be deceiving during image viewing to not have special colors as the images always look good regardless of how many pixels are all mapping to the same color due to over/under flows. This option is most often turned off during figure making when the image quality can be significantly degraded by spurious data values popping up as black and white points in otherwise correctly ranged images. This is particularly noticeable in ratio/ratiometric images where noise in the background of the denominator images can cause artificially high ratio (and thus ion) values.

Load and Save

The **Load** and **Save** buttons will invoke *dialog boxes* that enable the storing and retrieval of color maps on disk for quick switches. In addition, the color map is saved as part of the screen template (see [Screen Templates](#)^[107]).

6.1.1.2.2.2 Editing the Image Range

To display the images with as great a color resolution as possible, it is good to have the range of color mapping as close as possible to the range of data available in the image. The mapping range can be edited by double clicking in range area of the *range and color bar control* (see the *movie player overview* in [Movie Players](#)^[44] for information on the *range and color map control*). This will bring up the **Range Edit dialog box** shown below.

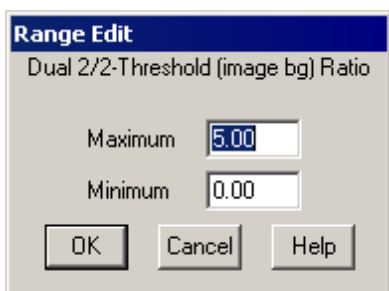


Image Range Editor

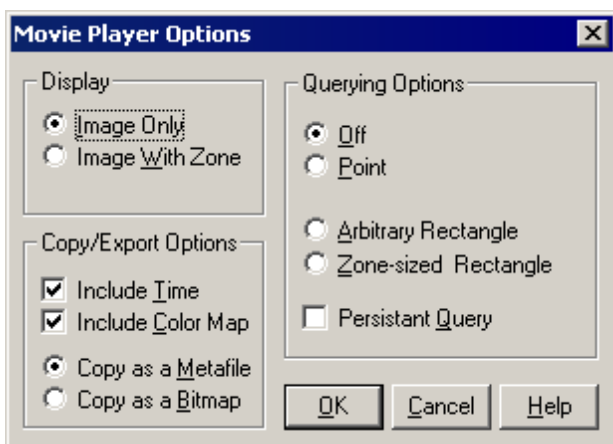
Edit the maximum and minimum ranges as desired. Selecting **OK** will force a re-mapping of the image. Note that while the range of values for the traces associated with an image give a good indication of the image range, typing in the same values will result in under and over ranges. This is because the zone plots are averages and tend to obscure spatially discrete extremes.

Each image <type : channel> combination has its own set of ranges, like *user limits* for the traces. In fact, if you wish to edit a large number of image ranges at once, you can use the *User Limits dialog box* and select *Image Arrays* as described in [§User Limits](#)^[77].

Currently, there is no mapping maintained by IonWizard between like channels. Thus changing the values for the above *Threshold (image bg) Ratio* images will have no effect on *Mask (image bg) Ratios*, if such a construction existed (for more information on the <type, channel, position> terminology (see [§Hierarchical Data](#))^[30]. IonWizard maintains the image display ranges in the [screen template](#)^[107] as part of the overall user limits.

6.1.1.2.2 Movie Player Options

Selecting *Configure Movies...* from the *Images menu* invokes the *Movie Player Options dialog box* shown below. You can also get to this *dialog box* via the *movie player local menu* as explained in [§Movie Players](#)^[43].



Movie Player Options Dialog Box

There are three control groups in this *dialog box*: *Display*, *Copy/Export Options*, and *Querying Options*.

Display

These options determine how IonWizard draws the image area. The *radio buttons* select between drawing only the image or the image overlaid with the zone position for the current trace. See the [Zones](#)^[74] section for more information on zones.

Copy/Export Options

This section configures the *movie player* figure creation options. See [§Exporting Movie Player Figures](#)^[92] (a sub-section of [§Printing and Figure Creation](#)^[89]) for more information.

The *Include Time* and *Include Color Map* check boxes determine the additional graphical elements drawn when creating a figure of the current image. IonWizard draws the time at the bottom center of the final image. The color map will be drawn to the right of the image and will include the range of values used for scaling.

If you export the figure to the Windows® Clipboard, the format indicated by the Copy as a Metafile and Copy as a Bitmap radio buttons is relevant. It is usually better to select Copy as a Metafile, unless the program that will receive the image needs bitmaps. Metafiles may be scaled to any size in most destination programs. The text and lines in the final image will scale appropriately. With bitmaps however, the text often becomes very jagged when scaled. Most graphics programs will let the user break apart metafiles and edit their individual components, such as label text or line colors. This is not possible with bitmaps.

These two options do not apply if the figure is being exported to file on the hard disk drive. The disk formats are all bitmaps.

Querying Options

The user can also do off-line pixel queries and zone averages using the *movie player*. This is explained in detail in the next section. There are three different types of data inquiry possible.

- Point** Read the value of a single pixel.
- Arbitrary Rectangle** Place any sized rectangle. IonWizard reports the average pixel intensity inside the rectangle.
- Zone-Sized Rectangle** Move a copy of the current zone about the image. You cannot change the dimensions of this rectangle, only its position. This is useful as the average value reported will have the same scaling factor (i.e., the number of pixels) as the zone traces. Thus you can do direct comparisons with the zone plot traces.

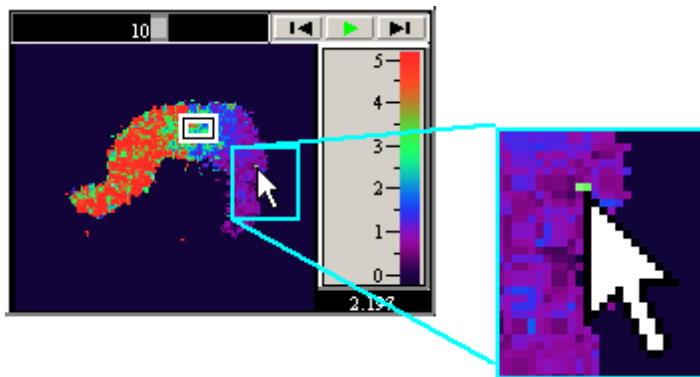
The Persistent Query check box indicates whether or not to save the same pixel or rectangle for use on multiple images. When persistence is not selected, IonWizard erases the highlight after reporting the value. If persistence is selected, IonWizard leaves the query highlight and the query can be re-run on a different image. For more information on queries, see [\\$Image Queries](#)^[48].

6.1.1.2.2 Image Queries

If a query option is set in the Movie Player Options dialog box, the **left mouse button** will allow interaction with the *movie player* image area. See [\\$Movie Players](#)^[44] and [\\$Movie Player Options](#)^[47] to familiarize yourself with these topics.

Point Queries

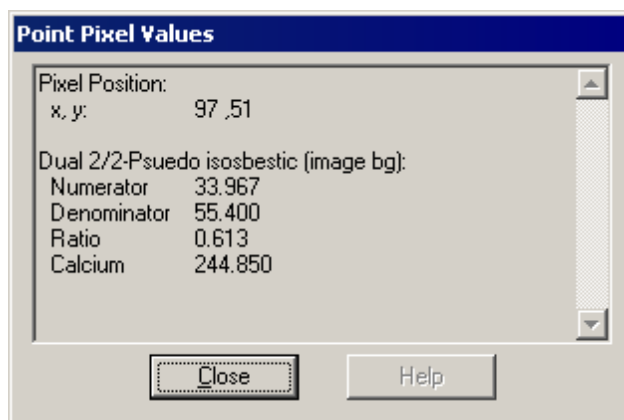
For point queries, the mouse pointer will be in the center a small pixel sized rectangle. Moving the mouse pointer around with the **left mouse button** down will move the pixel around the image area. The figure below illustrates the query point in action. In this case, the user has also selected to display the image with the overlaid zone.



Point Image Query

It takes a small amount of time for the highlighted area to appear after clicking the **left mouse button**. This is to differentiate between a query and a double click to zoom the movie player.

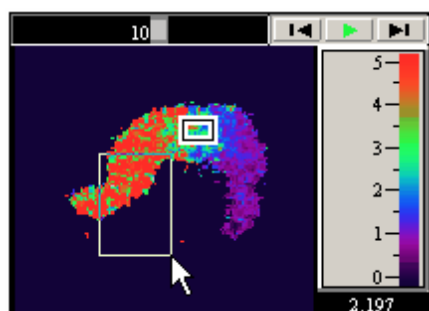
When you release the left mouse button the query results dialog box is displayed.



The layout is similar to that of the Range Averages *dialog box* (see [SRange Averages](#)^[41]). The first item is the final position of the query point. Then come the pixel values for each image channel in the given [image type](#)^[30].

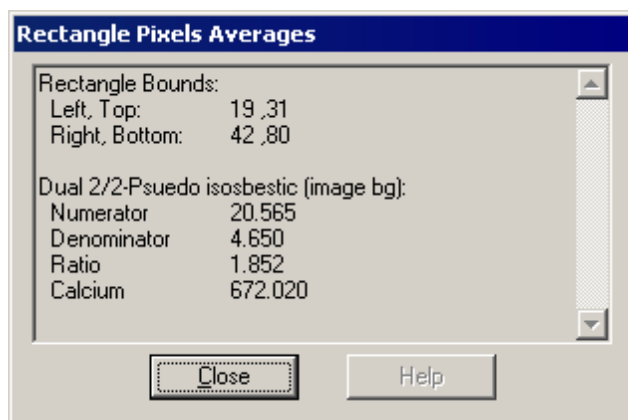
Arbitrary Rectangle Queries

For an arbitrary rectangle, click and drag with the **left mouse button** in the image area of the *movie player*. The point where the mouse button was clicked down will form one corner of the rectangle, while the release point will be the other corner. The figure below illustrates the query rectangle in action. Again, the user has elected to display the image with the overlaid zone. The query rectangle is the rectangle with the thin border, whereas the overlay is the rectangle with the double border.



Arbitrary Query Rectangle

When the mouse button is released, the results *dialog box*, shown below, will be presented.

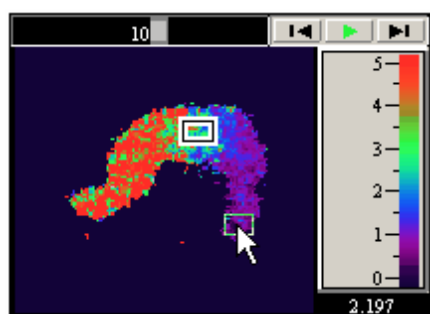


Rectangle Pixel Average Dialog Box

Again, layout is similar to that of the Range Averages *dialog box* (see [§Range Averages](#)^[41]). The first item is the final position of the query rectangle. Then come the averages for each image channel in the given image type.

Zone-Sized Rectangle Queries

For zone sized rectangle queries, the mouse pointer will be in the center of zone sized rectangle. Simply clicking with the **left mouse button** will make the rectangle appear and it will follow the mouse pointer around as long as the **left mouse button** is down. As with the point queries, it takes a small amount of time for the highlighted area to appear after clicking the **left mouse button**. The figure below illustrates the query rectangle in action. See [Zones](#)^[74] for more information on zones.



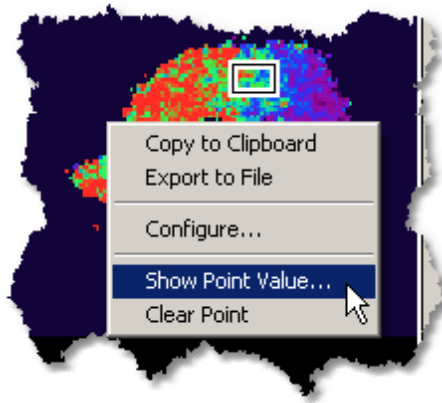
Zone Sized Query Rectangle

When the **left mouse button** is released, IonWizard will display the results of the query. The result *dialog box* is the same as that displayed for arbitrary rectangles as illustrated earlier.

Persistent Queries

If Persistent Query is selected in the Movie Player Options *dialog box*, the highlighted point or rectangle will remain after the reporting dialog box is closed. The user can now move to another image and re-query.

The Persistent Query option adds two items to the *movie player local menu*. The exact wording of these items depends on the query type. The figure below shows the local menu with a persistent point query.

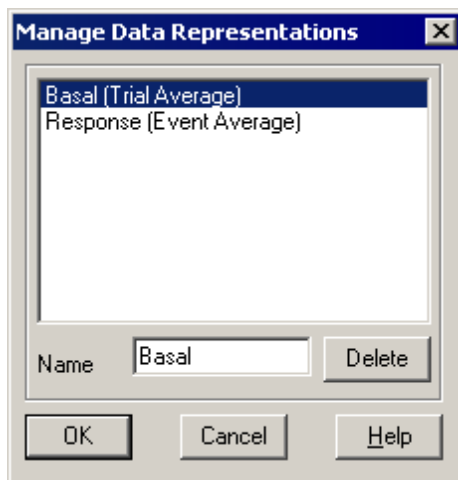


Persistent Query Local Menu Items

Select the Show xxx... menu item to run the query again. Select the Clear xxx to drop the query location.

6.1.2 Managing Data Representations

To manage [data representations](#)^[31], select Manage Data Representations... from the Operations menu. The Manage Data Representations dialog box, shown below, will appear. You can only make this selection if you have added a data representation via [trial or event averaging](#)^[83]. All data representation shown here will have their own trace bar.



Manage Data Representation Dialog Box

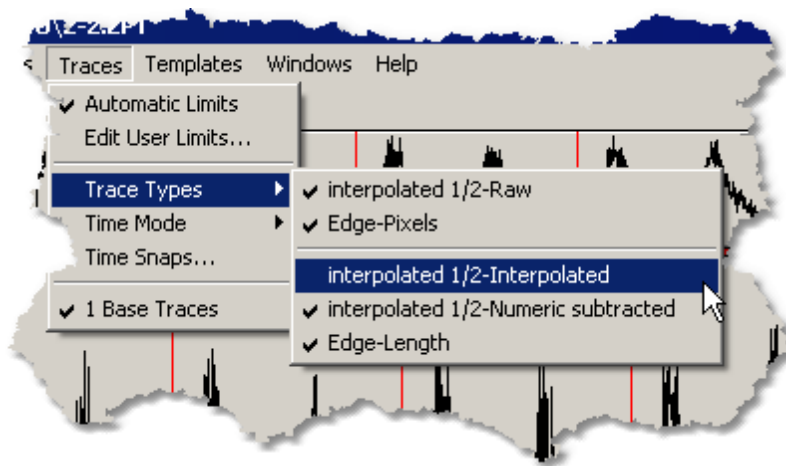
This *dialog box* will let you delete or rename a data representation. Click on any data representation in the list box to make it active. Once active, you can delete it or change its name. Keep in mind that each data representation must have a unique name.

Once you have finished making changes, click the OK button to accept the changes, or click the Cancel button to throw away the changes.

6.1.3 Trace Type Suppression

There are cases when it may be desirable to suppress the number of [trace types](#)^[30] that appear in the [yellow button menus](#)^[36]. This may be because there are simply too many traces and you would like to hide presentation of some of them for some time, or it may be because that particular trace data is not very interesting. For example, sometimes it is necessary with interpolated data traces to create an intermediate data trace that is a copy of a raw data trace but which has more data points. While this is an important operation is it not particularly interesting to look at any you may want to suppress it.

You can suppress any trace type via the Trace Types submenu under the Traces menu as illustrated below.



Trace Types Menu

Any trace type in this *submenu* that is not checked will not appear in any *yellow button menu* anywhere in IonWizard.

6.2 Time Discontinuities

IonWizard data sets comprise of time resolved traces and images. During data acquisition, there are occasions when data collection is suspended. We call these periods of suspended data acquisition time discontinuities.

Time discontinuities can be introduced into the experiment by a variety of actions. The cause of the discontinuity and the duration of the time discontinuity is recorded with the data set. The possible *time discontinuity* types are:

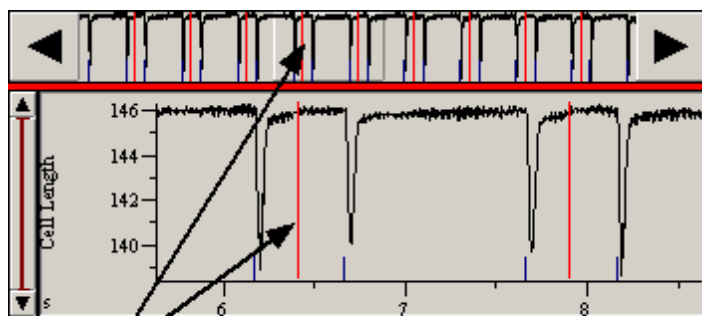
- **Pause Break** The user paused the experiment.
- **Waited for Trigger** Indicates sampling was suspended while waiting for an external synchronization trigger or keyboard input.
- **Trial Break** Marks the end of a trial. Trial sampling is an old sampling methodology from previous versions of IonWizard which permitted collection of precisely controlled segments of data with the notion of averaging them together later. This methodology has been removed as the same ensemble averaging effect can be achieved more elegantly with event averaging.
- **Offset From Anchor** ONLY IN OLD DATA FILES. An interval between the file time stamp and the beginning of data sampling. The file time stamp (called the time anchor) contains the full day, month, year, hour, minute, second that the experiment was run. In newly collected data, this stamp time coincides with the beginning of data sampling. If there is not enough information available to reconstruct expanded time for a given data set, the duration of this break will be 0.

6.2.1 Time Modes

There are two display modes for representing the time spent in a time discontinuity, *collapsed time* and *real time*.

Collapsed Time

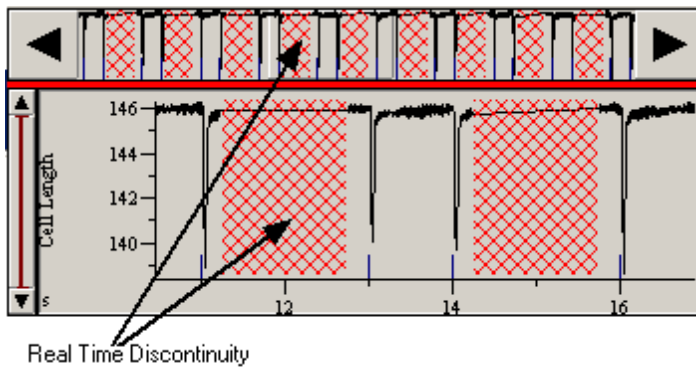
Usually the scientist is not interested in visualizing the actual amount of time spent in time discontinuities and would like for the display to maximize the space given to the trace data itself. In *collapsed time*, the time discontinuity is represented by a vertical red bar in both the full view and selected view of the trace as shown in the figure below. This bar extends the full height of the trace to emphasize the break. The bar takes up zero apparent time. Trace features on either side of this potentially large sampling break appear as if they occurred sequentially. This is the default mode of viewing *time discontinuities*.



Collapsed Time Discontinuity Display

Real Time

In *real time* mode, the *time discontinuity* is shown in an expanded form. The red bar is replaced by a red hatched rectangle whose length represents the amount of time spent with data sampling suspended. Again, this rectangle extends the full height of the trace area as shown in the figure below. Trace features on each side of the time discontinuity are shown at their actual time of occurrence.

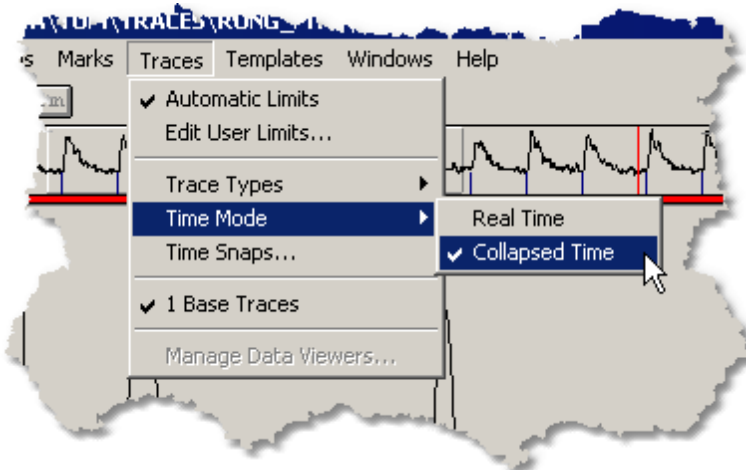


Real Time Discontinuity Display

The next section describes how to change between these two modes.

6.2.2 Setting the Time Mode

To set the time mode, go to the Time Mode sub-menu in the Traces menu as shown in the figure below. This sub-menu contains two items: Real Time and Collapsed Time. A check indicates the current time mode. Selecting the other item will switch time modes.

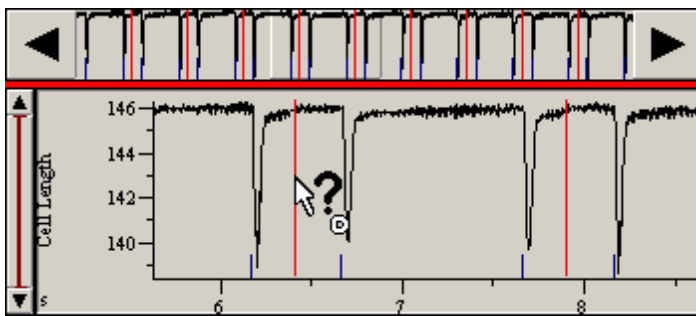


Time Mode Menu


If an old data file that did not store the needed global time information is opened, the Real Time menu item will be disabled and the user can only work in *collapsed time*. If there are no *time discontinuities* present, *collapsed time* and *real time* are synonymous, and the *real time* option is disabled.

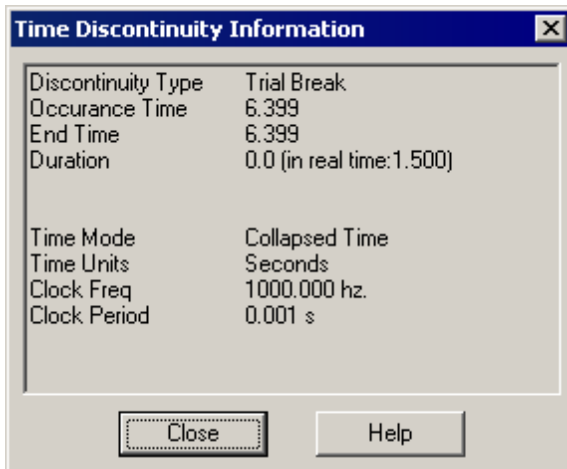
6.2.3 Querying Time Discontinuities

The user may query a *time discontinuity* for information. When the mouse cursor is over a time discontinuity in the selected view, the cursor will change to a question mark with a small D next to it as shown in the figure below.



Time Discontinuity Query Cursor

By clicking in the  with the **left mouse button** at that moment, the following *dialog box* will appear.

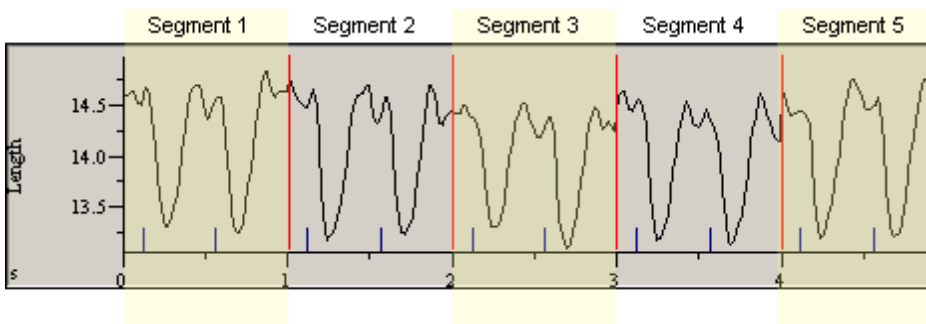


Time Discontinuity Information Dialog Box

The *dialog box* tells what kind of *time discontinuity* has been clicked on, its occurrence time, the duration and the accuracy of the clock with which it was timed.

6.2.4 Data Segments

A data segment refers to a block of data between time discontinuities. The figure below illustrates a five second experiment with four discontinuities, one every second. These four discontinuities divide the entire time course up into five data segments.



Data Segments

The key thing to understand is that the data in one segment is isolated temporally from and completely unrelated to the data in the adjacent segments. This might not be obvious sometimes when data is plotted using *collapsed time*. Nevertheless, it is nonsensical to try to perform operations that span data segments.

Indeed, often the data crossing the time discontinuity is discontinuous in the mathematical sense and can introduce mathematical problems if analysis is attempted.

Therefore, the existence of time discontinuities often introduces subtle restrictions on analysis operations. For example, it is not possible for an event average segment (see [§Ensemble Averaging](#)^[83]) to span a time

discontinuity. Likewise, a transient mark cannot span time discontinuities and must begin and end entirely in a single segment (see [§Transient Mark Editor](#)^[70]). These restrictions rarely directly impact the user, but it is helpful to understand that the restrictions exist.

6.3 Constants

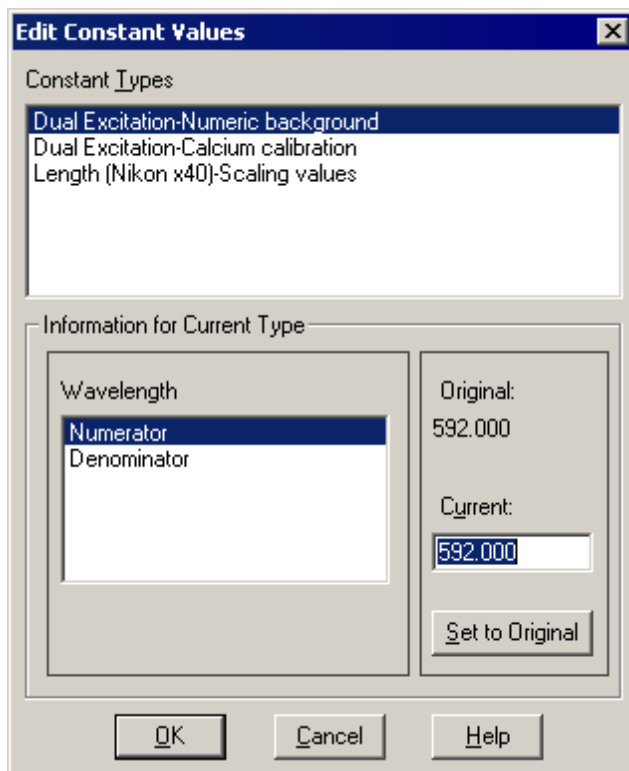
Constants is here defined as the various numeric values used in the trace and image calculations performed by IonWizard. Examples are: calcium calibration constants, background values, and linear calibration values. While these values are constant in a given setting, they may change from experiment to experiment (if they were constant in the universal notion, like pi, there would be little need to edit them).

The number of different constant types that may be present is highly dependent on the data set. Imaging data sets tend to have many more constants than non-imaging ones. Constants fit into the Data Hierarchy paradigm. See [\\$Hierarchical Data](#)^[30] for more information on this paradigm and the < type, channel, position > terminology used in the following sections.

All constants can be edited as needed by the user. There is a global editor that permits arbitrary value entry and for some image related constants there are more tailored dialog boxes that give the user immediate visual feedback to allow "fine tuning" of the values. All these dialog boxes are explained in the following sections.

6.3.1 Constant Value Editor

By selecting the Constants... item from the Operations menu, the following *dialog box* will appear. This is the Edit Constant Values dialog box which permits entry of arbitrary values for any constant.



Constants Dialog Box

The Constant Types list box contains all the type names for all values in the experiment data file. A second list box, here with the label Wavelength, contains all the constants for the given type. This list box's label changes depending on the currently selected type. Whenever the user selects a new constant type from the first list box, the lower list box is emptied and then filled with all the new constant values available for the type. IonWizard then selects the first item in the Wavelength list box and fills the Original and Current text fields with the appropriate numbers.

The Current: edit field displays the number currently used for the given constant. Here the user may type in any new, arbitrary number.

The Original: static text displays the number that was originally passed to IonWizard by the module that

created this data set. To return the current constant to its original number click the Set to Original *push button*.

Clicking the OK *push button* will cause IonWizard to accept all changes in the constant value and mark for recalculation all the traces and images that use constants. Clicking Cancel will restore any changes and dismiss the *dialog box*.

Please note that while this *dialog box* allows editing of any constants present in the experiment data file, there are separate *dialog boxes* available for the editing of the various imaging threshold constants. These *dialog boxes* gives the user visual feedback as the numbers are changed, making threshold constant value editing considerably easier (see [§ Image Threshold Editors](#)^[58] for more information).

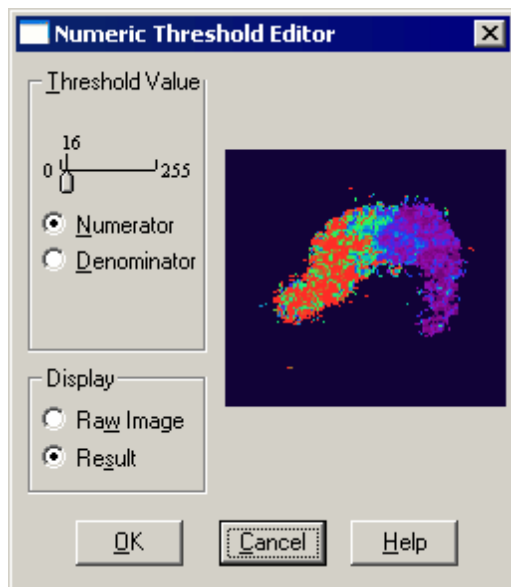
6.3.2 Image Threshold Editors

The constants used by IonWizard to threshold the ratio and ratiometric images can be edited from the Edit Data Constants *dialog box* as outlined in [§ Constant Value Editor](#)^[57]. However, this *dialog box* is not optimal for setting threshold numbers. The user really needs dynamic feedback of such changes.

IonWizard provides special *dialog boxes* for editing the constant values used in thresholding. As there are three types of thresholding: Numeric, Mask and Pseudo-Isobestic, there are three different dialog boxes. All three of these dialog boxes has a similar layout. To invoke the appropriate dialog box, select Threshold/Mask Editor... from the Images *menu*. If there is no active *movie player*, or if the image in the active *movie player* does not use thresholding, the *menu item* will be disabled.

IonWizard maintains the state of each of the three dialog boxes separately in the user's profile section^[105] of the template file. This saved information includes the display options and the size of the dialog box.

6.3.2.1 Numeric



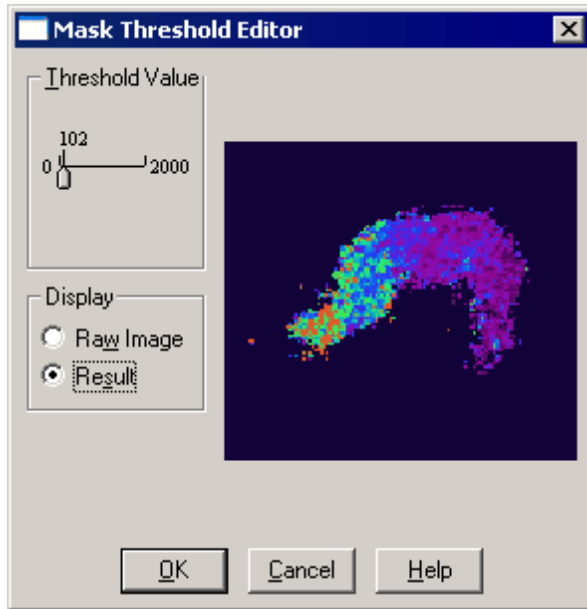
Numeric Threshold Editor Dialog Box

Numeric thresholding uses the numerator or denominator image of a given ratio or ion image as the basis for noise reduction. Any pixel in the raw image that falls below the threshold value is set to zero in the resultant ratio or ion image. The threshold value is set with the slider in the Threshold Value section of the *dialog box*. The Numerator and Denominator *radio buttons* determine which raw image the threshold value refers to. In the above example, any pixel below 16 in the numerator image of a given ratio or ion image will be set to zero.

The display section lets the user specify whether the feedback image is the resultant image (ratio or ion) or the appropriate raw image as indicated by the Numerator and Denominator *radio buttons*. If the display is set to Raw Image, the appropriate image will be shown and the areas of that image which are below the threshold value are filled in with dark gray.

The size of this dialog box and the settings are saved in the user profile section^[105].

6.3.2.2 Mask



Mask Threshold Editor Dialog Box

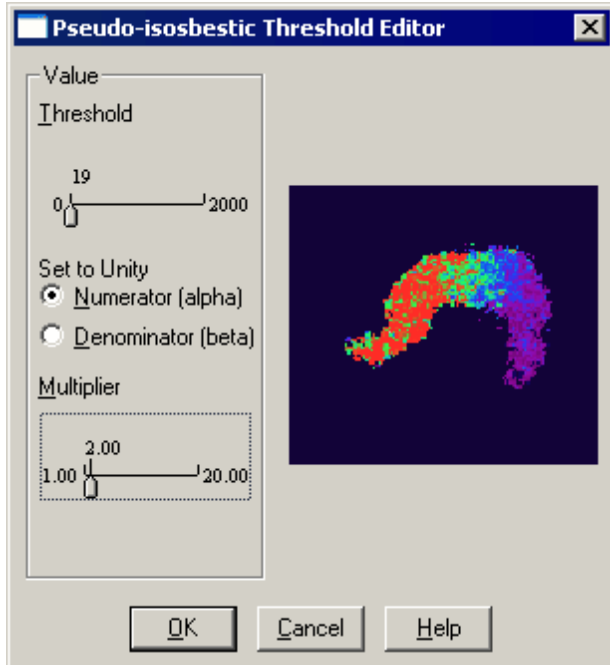
The mask noise reduction method will set to zero any pixel in the resultant image whose corresponding numerator pixel falls below the threshold value. The operation uses the first image in the numerator array the threshold all the images in the ratio or ion array.

The threshold is dynamically set by the Threshold Value slider. Any manipulation of the slider updates the display image.

The display image may be either the resultant ratio or ion image or the raw numerator image used for the masking procedure. When the display is the raw numerator image, the threshold value will be indicated by a dark gray fill of all pixels below that value.

The size of this dialog box and the settings are saved in the user [profile section](#)^[105].

6.3.2.3 Pseudo-Isosbestic



Pseudo-isosbestic Threshold Editor Dialog Box

Pseudo-isosbestic masking uses an intermediate image. This image is constructed from the raw numerator and denominator images and is designed to remain in terms of the intensity level over all levels of ion present. This type of operation prevents the blooming of the cell that often occurs with numeric thresholding while avoiding the problems the mask threshold has with moving cells. To understand what is going on in this *dialog box*, we need to do a little math.

Derivations

Each pixel in the intermediate pseudo-isosbestic image is calculated as follows:

$$p = n \cdot \alpha + d \cdot \beta$$

where

p is the pseudo-isosbestic pixel

n is a numerator pixel

d is a denominator pixel

The assumption is that this pseudo-isosbestic gives you more or less the same pixel values regardless of the input numerator and denominator images. That is, given two image pairs, $[n1, d1]$ and $[n2, d2]$, the following is true:

$$n1 \cdot \alpha + d1 \cdot \beta = n2 \cdot \alpha + d2 \cdot \beta$$

Solving for alpha and for beta, we get the following two equations:

$$\alpha = (d2 - d1) / (n1 - n2) * \beta$$

$$\beta = (n2 - n1) / (d1 - d2) * \alpha$$

or

$$\alpha = M_a * \beta$$

where

$$M_a = (d2 - d1) / (n1 - n2)$$

and

$$\beta = M_b * \alpha$$

where

$$M_b = (n_2 - n_1) / (d_1 - d_2)$$

Notice that if we choose our $[n_1, d_1]$ and $[n_2, d_2]$ data points to be at high ion and zero ion conditions, the multipliers M_a and M_b can be thought of as the ratio of the dynamic ranges of the numerator and denominator signals.

Setting the Parameters

With all that in mind we can now set up the parameters in the *dialog box*. Since alpha and beta are dependent on each other we can simply choose one of them to be unity and then calculate the other using M_a or M_b as appropriate. We make this choice such that the resulting M_a or M_b parameter is greater than one.

A simple procedure is as follows:

1. Open either a ratiometric calibration file (preferred), or a regular data file. In the selected view of the raw trace data, do a range average over a section of low ion levels and record the numerator and denominator values (i.e. $[n_1, n_2]$). See [§Range Averages](#)^[41] for information about range averages.
2. Do a second raw average in a section of high ion levels and record $[n_2, d_2]$.
3. Calculate M_a and M_b .
4. If M_a is greater than one, click the Numerator (alpha) radio button in the Set to Unity section and set the Multiplier slider to M_a . Alternately, if M_b is greater than one, click the Denominator (alpha) radio button in the Set to Unity section and set the Multiplier slider to M_b .

You will find that these parameters are pretty consistent across multiple files using the same dye - rather like the calibration constants.

Once you have determined the unity value and the multiplier, you can now use the Threshold slider as needed to suppress the background noise outside the cell.

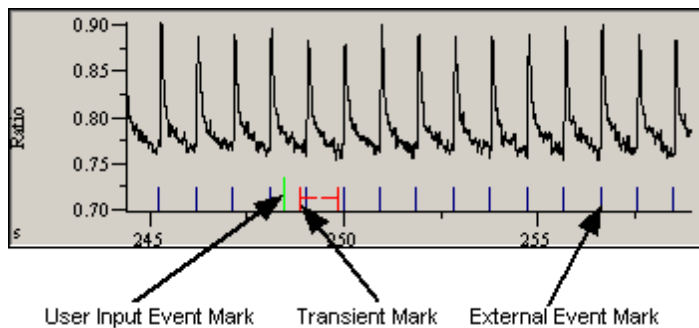
6.4 Marks

Event marks and transient marks are annotations that become part of the data set. Event marks are global tags across all traces that indicate an external hardware (i.e. from a TTL input) event or a keyboard event that occurred during acquisition. Event marks can also be added off-line via the mouse. Event marks store the time of their occurrence and provide text the user may edit indicating what event occurred. Event marks appear as a short blue or green line at the bottom of the full view and selected view traces when they are visible. The blue mark indicates an external (i.e. hardware) event whereas the green line indicates a user input mark.

Transient marks are used to define the beginning and end of a transient in the data trace. By a transient, we mean some characteristic amplitude change in the trace that is of interest. The sole purpose of transient marks is to delimit such changes for later analysis.

While transient marks have an occurrence time, there is no associated text. Instead, they have a duration which is used to indicate the length of the transient. Each data trace has its own set of transient marks. Transient marks appear as two short red lines at the bottom of the full view and selected view traces. A small set of arrows point toward each other indicating the interior of the transient.

The figure below shows both flavors of event mark and a transient mark as displayed in the *selected view*.



User Event, External Event and Transient Marks as drawn in the Selected View

The following sections explain how to add, edit, delete and use marks in varying situations.

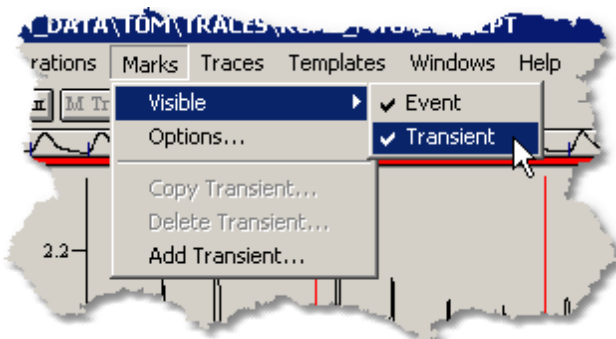


Transient marks are only useful with the optional Monotonic Transient Analysis package

6.4.1 Mark Visibility

The visibility of event marks and transient marks can be individually controlled via both a menu selection and the *Mark Options dialog box* (see [§ Mark Options](#)^[63] for more information).

The *Visible sub-menu* under the *Marks menu*, shown below, is used to control mark visibility via a *menu*.



Mark Visibility Menu

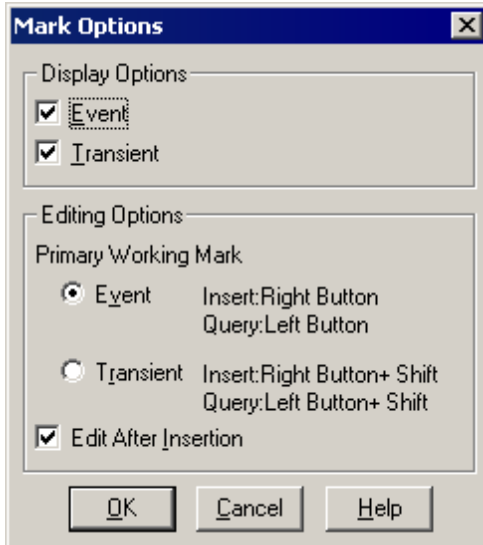
The check marks next to the *Event* and *Transient items* indicate the current visibility status each mark type.

Selecting an *item* in the Visible *sub-menu* toggles the visibility status of that particular mark.

The mark visibility state is one of the items saved in the screen templates (see [§ Screen Templates](#)^[107] for more information).

6.4.2 Mark Options

Select Options... from the Marks *menu* to invoke the Mark Options *dialog box* shown below.



Mark Options Dialog Box

Here both the visibility state and editing options can be configured.

Display Options

The Event and Transient *check boxes* indicate the current visibility status of the given mark type.

Editing Options

The Primary Working Mark option denotes which type of mark is accessed without the use of the **shift** key. The secondary mark can only be added or queried when the **shift** key is held down. The text to the right of the Event and Transient *radio buttons* indicates the mouse and keyboard sequences needed to insert and query the mark for the given primary mark setting.

The Edit After Insertion *check box* determines whether or not the mark edit dialog will appear after a mark is inserted. In general, you want to have this item checked. The one instance you would not want it is if you were manually inserting a large number of transient marks. In that case, once you had established the appropriate mark duration, it is not really necessary to see the edit dialog every time. Thus you would disable Edit After Insertion and then add the transient marks. See [Adding Marks](#)^[63].

6.4.3 Adding Marks

Event or transient marks can be added manually by using the mouse in the *selected view*. Transient marks can also be added automatically via the Add Marks... *item* under the Marks *menu*. These two methods are described in the following sections.

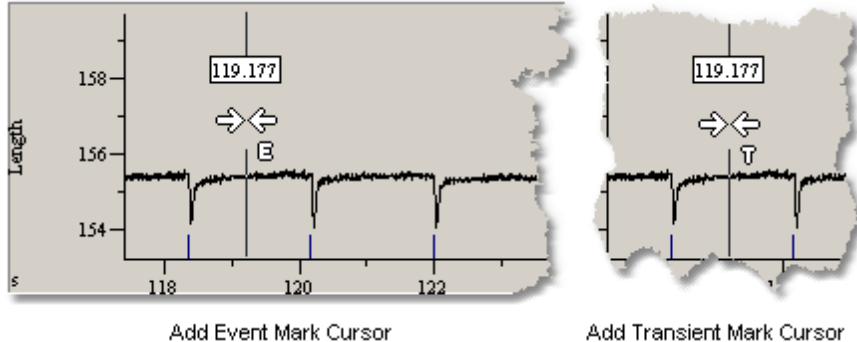
6.4.3.1 Adding Marks Manually

Marks are added manually by using the mouse in the *selected view*. If you are adding an event mark, the choice of the *trace viewer* is unimportant as event marks are global and will appear in all *trace viewers* in that *trace bar* (see [§ Trace Viewers](#)^[36] and [§ Trace Bars](#)^[33] for more information). Transient marks on the other hand are tied to a specific trace and thus you must add them in the appropriate *trace viewer*. To add

the correct mark type, transient or event, you must know the primary mark type. By default, event marks are primary and transient marks are secondary. The primary mark type can be altered via the [Mark Options dialog](#) box so if you are unsure, check there (see [§ Mark Options](#)^[63]).

Marks are added by clicking with the **right mouse button**, dragging the resulting cursor to the desired location, and releasing the **right mouse button**. If the **shift key** is being held down at the point of release, a secondary mark will be added, otherwise a primary mark will be added.

The cursor that is presented when adding the mark indicates the type of mark that will be added. The figure below shows the cursors for adding event and transient marks. This cursor is shown as long as the right mouse button is held down. Notice the white box that indicates the exact time the add mark will have. This time is updated as you move the mouse around. When you get the cursor in the position where you want to add the mark, release the **right mouse button** and the mark will be inserted.



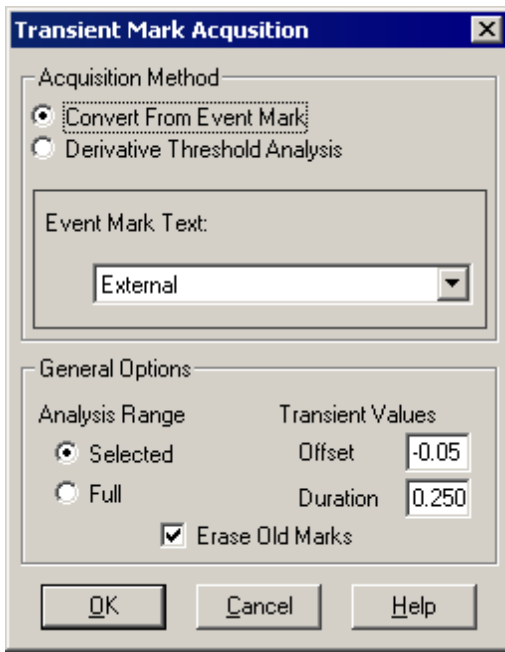
Event and Transient Mark Add Cursors

If you have the [Edit After Insertion](#) check box selected in the [Mark Options dialog](#) box, the appropriate mark editor [dialog](#) box will be presented so that you can edit the mark (see [§ Editing Marks](#)^[69] for more information). If you [Cancel](#) from the edit [dialog](#) box, the mark will be removed.

If [Edit After Insertion](#) is not selected, then the mark will simply appear and you will need to query the mark to change or delete it (see [§ Querying and Deleting Marks](#)^[68] for more information)

6.4.3.2 Transient Mark Acquisition

Frequently, a data set will contain many transients, often more than the user wants to place manually. The [Add Transient...](#) item under the [Marks](#) menu invokes the [Transient Mark Acquisition dialog](#) box, shown below. It will add transient marks to the trace in the *active trace viewer* (see [§ Trace Bars](#)^[33] for more information). IonWizard disables this menu item if there is no *active trace viewer*.



Transient Mark Acquisition Dialog Box

The Transient Mark Acquisition dialog box is split into two sections, the Acquisition Method section which determines the *base times*, and the General Options section that uses the *base times* to generate the actual transient marks. The settings of the Transient Mark Acquisition dialog box are written to the user's profile section (see [§ Profile Section](#)^[103]). IonWizard loads this stored information each time transient mark acquisition is re-run, thus allowing iterative fine tuning of the transient mark placement.

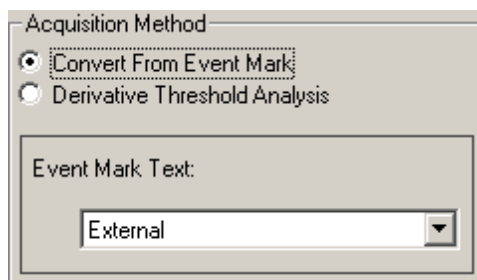
Acquisition Method

IonWizard finds the *base time* for each transient mark using the method from the Acquisition Method section of the dialog box. The *base time* is not the same as the start time of the transient mark as reported by the Transient Mark Information dialog box (see [§ Mark Information Dialog Boxes](#)^[69]). Rather the *base time* is an intermediate value used by IonWizard during automatic transient mark placement to determine the start time. Once IonWizard finds the *base time*, the offset given in the General Options section determine the final start time of the mark.

There are two methods for determining the base time of the transient marks, converting from an event mark and via a derivative threshold analysis.

Convert From Event Mark

To use existing event marks as transient mark *base times*, select the Convert From Event Mark radio button in the Acquisition Method section. That section of the *dialog box* will look like the figure below.



Acquisition Method "Convert From Event Mark"

The Event Mark Text combo-box lists the text of all available event marks. The default selection is either External or TTL, as these are the most often converted. From this list, select the text of the marks to be converted. The time of these event marks will become the *base time* of the transient marks.

Derivative Threshold Analysis

If no event marks correspond to data transients, the user may employ derivative analysis to determine the transient mark *base times*. Most data transients begin with a rapid departure from the baseline which appears as a spike in a trace's first time derivative. With derivative analysis, IonWizard looks at the first derivative and searches for these spikes as a way of identifying data transients. The time of any spike found becomes the *base time* for the transient mark. After placing a transient mark, IonWizard looks for the next spike, beginning at the end of the previously placed transient mark.

To use this process, select the Derivative Threshold Analysis radio button from the Acquisition Method section. Controls specific to derivative analysis will appear in the lower half of the section as illustrated in the figure below.

Acquisition Method

Convert From Event Mark

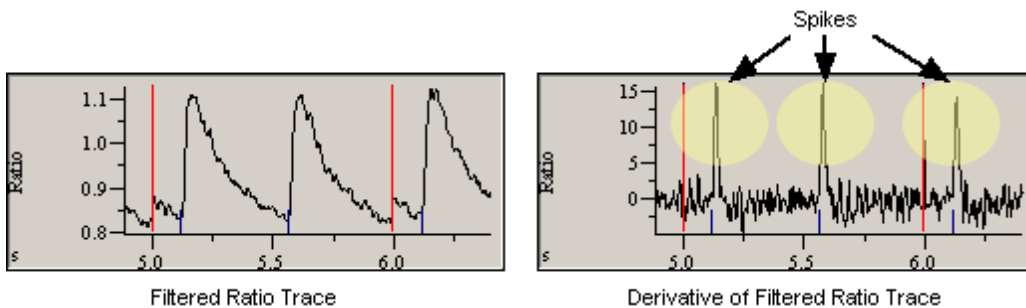
Derivative Threshold Analysis

Threshold Minimum

Direction Up Down

Acquisition Method "Derivative Threshold Analysis"

The value in the Threshold edit field is the derivative value constituting a peak in the data trace. To determine this value, click the d/dt button in the *information region* of the *trace viewer* showing the trace to which marks are being added (see [§ Trace Viewers](#)^[36]). This will display the derivative for that trace. It is sometimes useful to employ filtering on a trace to suppress noise when placing transient marks. Locate the spikes in the derivative trace that correspond to the beginning of a data transient. The figure below shows a filtered ratio trace, its derivative and the spikes in the derivative caused by the rapid departure from baseline. It is these spikes we are trying to capture with the threshold analysis.



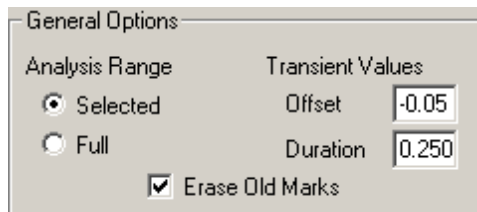
Spikes in Derivative Trace

- Threshold** The threshold is the data value in the derivative trace that constitutes a spike. Choose a threshold value as close to zero (baseline) as possible but one that excludes the baseline noise. In the example trace shown above, we would pick something like 10.
- Direction** The Up and Down radio buttons determine whether the trace derivative should be greater or less than the threshold value. Up is used for upwards going traces where the derivative must be greater than the threshold value. Down is for downward going transients where the trace derivative must be less (more negative) than the threshold value.
- Minimum/Window** In situations where the data is very noisy, employ the extra analysis enabled with the Minimum check box. Selecting Minimum enables both the Minimum and Window edit fields. The Minimum edit field indicates the smallest value that the data trace (NOT the derivative) must reach for the analysis to accept

the threshold crossing as legitimate. The *Window edit field* specifies the time period in which this value must be reached. Using the above data as a guide, assume we have a problem where the derivative value reaches 10 sometimes in the falling phase due to a lot of noise. We could block out that crossing by requiring that the data value must be at least 1.05 within the next 0.1 seconds after the threshold crossing. That would block out any spike that does not occur before the peak. These constraints will root out transients in all but the noisiest data. In such noisy data, the user may question whether further transient analysis has much hope of success.

General Options

Once the base time of the transient has been determined, the settings in the *General Options section* come into play.



Offset

The value in the *Offset edit field* is added to the *base time* to determine the actual start time of the transient mark. This value must reside within a *data segment*. If not, the value will be set to the minimum time for the *data segment* (see [§ Data Segments](#)^[55]).

Duration

Once the mark start time is determined, the desired transient mark duration, as determined by the value in the *Duration edit field*, is examined to make sure the mark end time also falls within the mark's data segment. If not, it is reduced as necessary. In general it is not essential that all transient marks have exactly the same duration. The transient mark is used only to designate a transient and the specific trace analysis tools that reference the transient marks are designed to minimize the impact of small variations in mark placement and duration on the results.

Analysis Range

The *radio buttons Selected* and *Full* determine whether the transient marks are inserted over the full time range of the trace or only over the selected time range as defined by the current *selected view*.

Erase Old Marks

If *Erase Old Marks* is checked, any existing marks for the given trace will be erased prior to inserting new ones.

Tips

There are typically three scenarios for placing transient marks. One situation is that all transients are of roughly the same duration. Simply select the duration and run transient mark acquisition on the full time range with *Erase Old Marks* selected. When using derivative threshold analysis, the acquisition can be re-run, fine tuning the various parameters until marks are in a desired position. Remember, transient marks having the same duration does not mean that the wave forms themselves are all of the same length, but rather that the longest wave form is less than the period of transient repetition. Having some amount of baseline at the end of the shorter transients does not change the subsequent transient analysis.

A second case is the presence of groups of transients, with a common duration in each group. These groups may represent the baseline, response and return stages of an experiment. Run analysis over the *selected view* with *Erase Old Marks* unchecked. Move each group into selected view and run transient mark acquisition. If *Erase Old Marks* were to be left checked, IonWizard would erase the transient marks in previous groups while processing the current group.

In both cases, it is easiest to drive the transients in some controlled manner and record the driving

pulse as an external event mark. It is more difficult to use derivative threshold analysis. This is particularly so in the second case; re-running the analysis will be difficult, as the user cannot erase the previous marks.

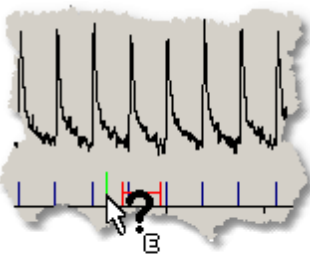
The third, and most troublesome, case is if the transient duration is of a random nature with no real grouping. Here the user will need intervene manually. The best situation has the user running transient mark acquisition on the full view using the shortest duration needed, then going back and manually extending the duration of transients that need it.

6.4.4 Querying and Deleting Marks

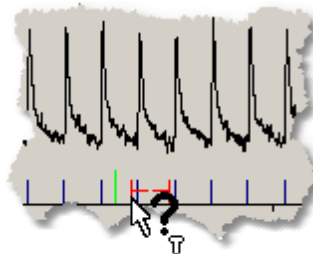
Once an event or transient mark has been added to the record, you can query it to view information about the mark, to delete the mark or to edit the mark. Marks are queried by clicking on them in the *selected view* of a *trace viewer* (see [§ Trace Viewers](#)^[36] for more information). While marks are added with the **right mouse button**, marks are queried with the **left mouse button**.

The query operation takes into account the primary and secondary mark settings. The primary mark (by default, event marks) is queried with the **left mouse button** alone whereas the secondary mark is queried with the **left mouse button** and the **shift key** (see [§ Mark Options](#)^[63] for more information about primary and secondary marks).

To query a particular mark, move the mouse over the mark in the *selected view*. When the mouse cursor goes over a mark, the cursor will switch to one of the mark query cursors as shown in the figure below. If you want to query the secondary mark type, you will need to depress the **shift key** as you are moving the mouse in order to see the correct cursor. Do not hold down the **left mouse button** as you are moving the mouse over the mark. This will execute a range average operation. Instead, simply move the mouse with no buttons depressed until you see the query cursor appear.



Query Event Mark Cursor

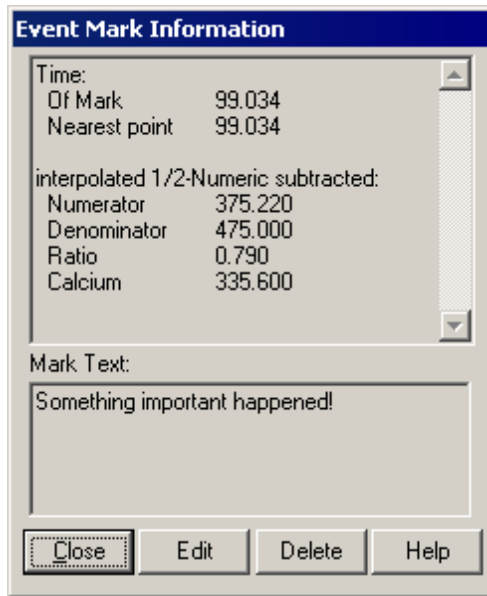


Query Transient Mark Cursor

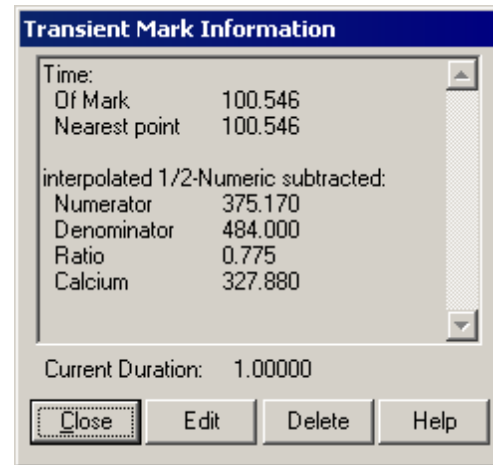
Event and Transient Mark Query Cursors

When you see the query cursor, **single click** with the **left mouse button**. This will bring up the event or transient mark information *dialog box* as appropriate. These *dialog boxes* are illustrated and explained in the [next section](#)^[69].

6.4.4.1 Mark Information Dialog Boxes



Event Mark Information Dialog Box



Transient Mark Information Dialog Box

The figures above illustrate the event or transient mark information *dialog box* that is presented when you query an event or transient mark as explained in the [previous section](#)^[68]. These two *dialog boxes* have a similar structure.

Mark Time and Trace Data Values

The top half of the *dialog box* shows the time of the mark; the time of the nearest time point for whatever trace is present in the trace viewer; the amplitude value of both that trace and the amplitude values of all other traces in that trace's hierarchy type.

Mark Information

Below the mark time and trace information is either the event mark text or the current duration of the transient mark. This is read-only information and cannot be changed in this *dialog box*.

Edit

The Edit *push button* will bring up the appropriate mark edit *dialog box* (see [§ Editing Marks](#)^[69] for more information). This will allow editing the mark text in the case of event marks, or the mark time and duration, as in the case of the transient marks. Any changes you make in the edit *dialog box* will become permanent as there is no way to cancel once you exit the edit *dialog box*.

If the mark is a read-only mark, as is the case with externally generated event marks, the Edit *button* will be disabled.

Delete

Click the Delete *push button* to remove the mark and exit the mark information *dialog box*. This will immediately delete the mark from the data set.

If the mark is a read-only mark, as is the case with externally generated event marks, the Delete *button* will be disabled.

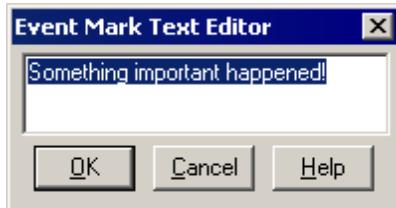
6.4.5 Editing Marks

There are two ways to edit marks. You can edit them as they are added (see [§ Adding Marks](#)^[63]), or you can edit them after the fact via the query mechanism (see [§ Querying and Deleting Marks](#)^[68]). In either

case, you will be presented with a specific edit *dialog box* as appropriate for the mark type, be it event or transient. The following two sections document the behavior of these two *dialog boxes*.

6.4.5.1 Event Mark Editor

The figure below shows the event mark editor dialog box.



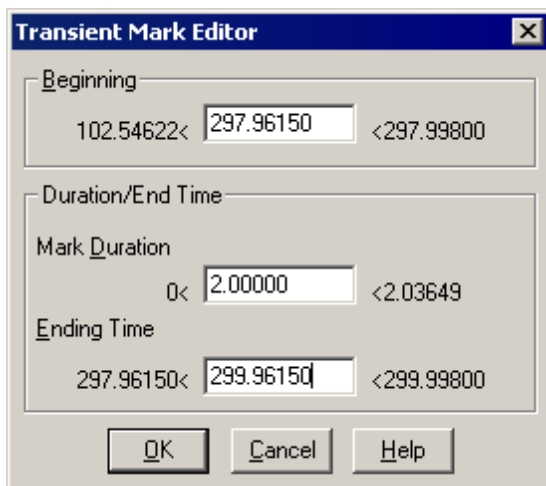
Event Mark Editor Dialog Box

Here the user can edit the text to be stored with the event mark.

If this *dialog box* has been presented as a result of adding an event mark (see [§ Adding Marks](#)^[63]), clicking the Cancel push button will delete the mark while clicking OK will add the mark with the text shown.

If this *dialog* has been presented as a result of clicking the Edit push button in the Event Mark Information *dialog box* (see [§ Querying and Deleting Marks](#)^[68]), clicking the Cancel push button will cancel the edit operation while clicking OK will change mark text.

6.4.5.2 Transient Mark Editor



Transient Mark Editor Dialog Box

The figure above shows the transient mark editor dialog box. The transient mark consists of two values, the beginning time and a duration. This dialog box is divided into two main sections, Beginning and Duration/End Time that permit editing these two values independently.

Duration/End Time section simply provides two ways of editing the duration. You can edit it directly, or specify the end time and let the computer calculate the duration for you.

There are restrictions on the values for the mark beginning and mark duration as indicated in the *dialog box*.

The restrictions stem from the fact that the entire transient mark must lie within a data segment (see [§ Data Segments](#)^[55]). Thus the maximum and minimum values for each field reflect moving the mark either completely to the beginning or completely to the end of the data segment.

The editor considers the beginning and duration to be independent and will not try to adjust one to allow modification of the other. For example, the editor will not shrink the duration to allow a larger beginning time. The maximum beginning time will be the maximum data segment time minus the duration. You would need to manually shrink the duration if for some reason you wanted to begin the mark above the current maximum.

If this *dialog box* has been presented as a result of adding a transient mark (see [§ Adding Marks](#)^[63]),

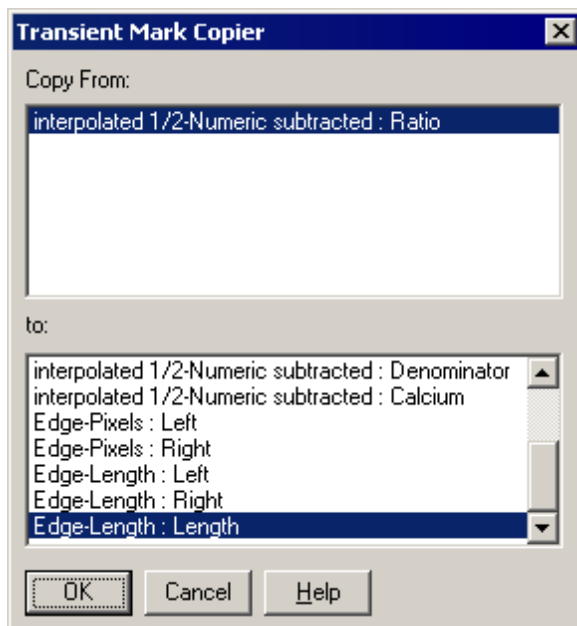
clicking the Cancel *push button* will delete the mark while clicking OK will add the mark with the parameters shown.

If this *dialog* has been presented as a result of clicking the Edit *push button* in the Transient Mark Information *dialog box* (see [§ Querying and Deleting Marks](#)^[68]), clicking the Cancel *push button* will cancel the edit operation while clicking OK will change mark parameters.

Any time you click OK in this *dialog box* to save an edit, the duration is written to the users profile section (see [§ Profile Section](#)^[105]). This value will become the default duration for any transient marks added in the future. This makes it easy to manually add transient marks to a number of similar transients. Simply edit the duration appropriately for the first one and then the remainder will automatically get the correct duration. Indeed if you plan to add marks to a large number of transients, you might consider turning off the Edit After Insertion *checkbox* in the Mark Options dialog box so that this edit dialog is not shown (see [§ Mark Options](#)^[63]).

6.4.6 Copying Transient Marks

Because transient marks are unique to each trace and the user may want identical marks on several different traces, IonWizard provides a transient mark copier. Selecting Copy Transient Marks... from the Marks *menu* will invoke the Transient Mark Copier *dialog box* shown below. This item will be grayed out if there are no transient marks in the data set.



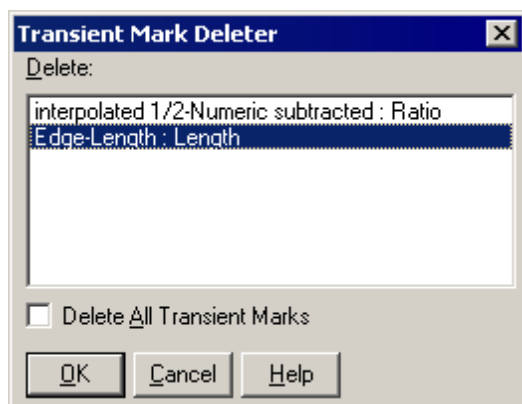
Transient Mark Copier Dialog Box

The top *list box* contains a list of all the trace that have transient marks. The selection defaults to the trace in the *active trace viewer* (see [§ Trace Bars](#)^[33] for more information).

The bottom *list box* contains all the traces that do not have transient marks. Select the appropriate traces in each list and click the OK *push button*. Cancel will dismiss the *dialog box* with no copying.

6.4.7 Deleting Transient Marks

By selecting Delete Transient Marks... from the Marks menu, the following *dialog box* appears.



Transient Mark Deleter Dialog Box

Traces which currently have transient marks will be listed in the Delete: list box. The trace in the *active trace viewer* (see [§ Trace Bars](#)^[33] for more information) will be selected if it has transient marks. Clicking OK will remove transient marks from the selected trace. If the Delete All Transient Marks check box is checked, transient marks will be deleted from all traces that currently have them.

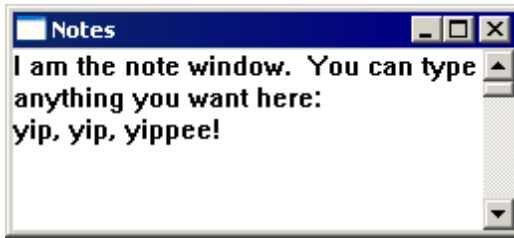
6.4.8 Other Uses for Marks

Event marks have the ability to query the value of a particular data point, a task not specifically provided by IonWizard and not entirely easy to do with the range averaging (see [§ Range Averages](#)^[41]). Simply add an event mark, and then query on it to show the mark information *dialog box*. There the time and amplitude values of the closest data point will be displayed. The mark can then be deleted directly from the mark information *dialog box*, if so desired. See [§ Querying and Deleting Marks](#)^[68] and [§ Mark Information Dialog Boxes](#)^[69] for more information.

Due to the global nature of event marks, they are also useful for determining the times between points on different traces. For example, suppose there is a calcium trace and a cell length trace. To determine the amount of lag from the onset of a calcium transient to the corresponding length transient, go to the calcium trace and insert an event mark at the beginning of the transient. For the purposes of this example name it "Ca onset". Then do the same to the length trace, using the name "Len onset". These two marks will then appear on both traces and the user can easily perform a range average between them to find the time difference. The mark text will remind the user which mark is which.

6.5 Notes

To bring up the notes window, select Add Notes from the File menu. If there are already notes attached to the data, the *menu item* will read Notes instead of Add Notes. Once selected, the *trace bars* will go to half screen and the notes window will appear.



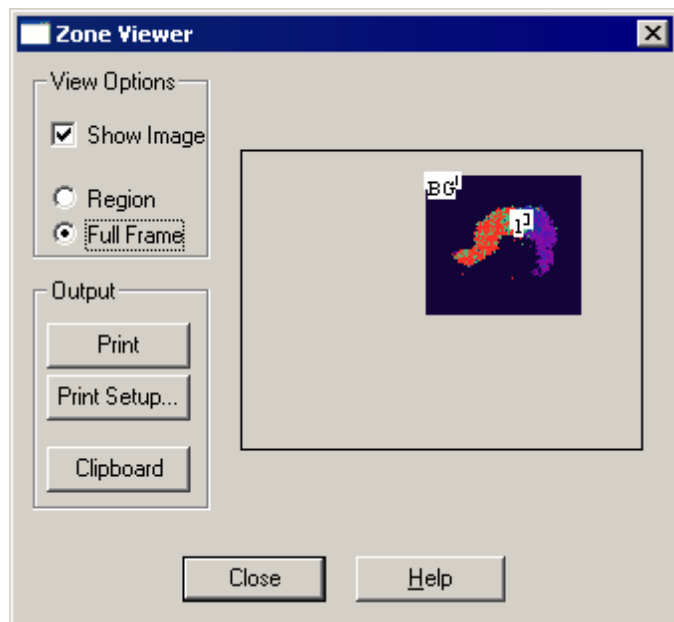
Note Window

You can type anything you want here.

Typically, the notes will be filled by default with the experiment setup information. If so, we highly recommend not deleting this information as it is very helpful in debugging. Rather, add your own notes above it.

6.6 Zones

If the data file being examined has zones, IonWizard allows the user to view the positions of those zones. Selecting the View Zones... item from the Operations menu calls up the *dialog box* illustrated below.

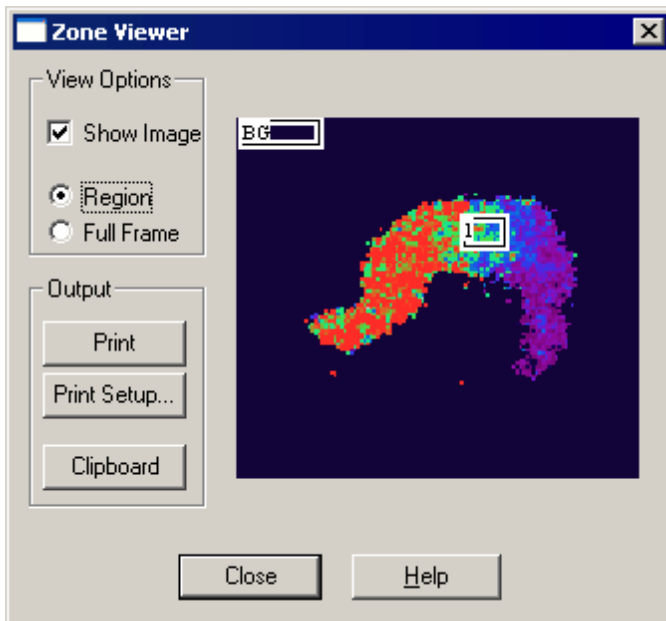


Zone Viewer Dialog Box - Full Frame View

The area on the right shows all of the zones available for the region. If images were saved with the file, the Show Image *check box* will be enabled and the user may opt to have the zones drawn over an image background. The image used will be the current image from the active *movie player*. Movie Players are discussed in the Image Analysis section of the manual. If there are no images, or Show Image is not selected, the zones will be drawn over a black background.

The zones can be drawn either relative to the full frame or relative to the region. The full frame is the maximum width and height of a camera image. Drawing relative to the full frame gives an indication of the size of the recorded image since the dimensions of a full frame image are constant for a given camera. This is illustrated in the figure above as indicated by the Full Frame *radio button*.

Drawing relative to the full frame allows the user to determine if the data is under the effect of some spatial variations in their microscope setup. For example perhaps the user has discovered that their light source goes not effectively illuminated the upper left corner of the full frame and they wish to determine if some corrective action needs to be taken with this particular data file.



Zone Viewer Dialog Box - Region View

The region is the subsection of the full frame in which images were actually recorded by the acquisition module. Drawing relative to the region has the effect of maximizing the size of the displayed zones while obscuring the size of these particular images relative to images in other data files. In the diagram above, the zones are drawn relative to the region as indicated by the Region radio button.

The three push buttons in the Output group allow the current picture to be printed or copied to the clipboard.

The Print Setup... button calls the same *dialog box* as in the File menu. In the Printer Setup *dialog box*, the user may set the printer and printer modes.

The size of this dialog box and the settings are saved in the user [profile section](#)¹⁰⁵.

6.7 Image Points

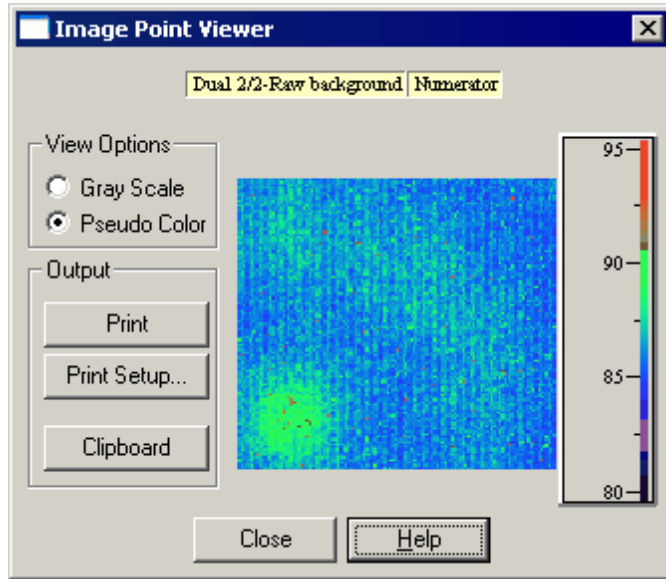


Image Point Viewer Dialog Box

To view white light or background images, call up the [Image Point Viewer dialog box](#) via the [Image Points...](#) item of the [Images menu](#). The *dialog box* displays any single image point present in the data set.

Yellow buttons at the top of the window indicate the current image and allow selection of any single images.

View Options

Radio buttons in the [View Options](#) group select whether the image is drawn using the current color map or a gray scale map. The gray scale map is useful for viewing white light images.

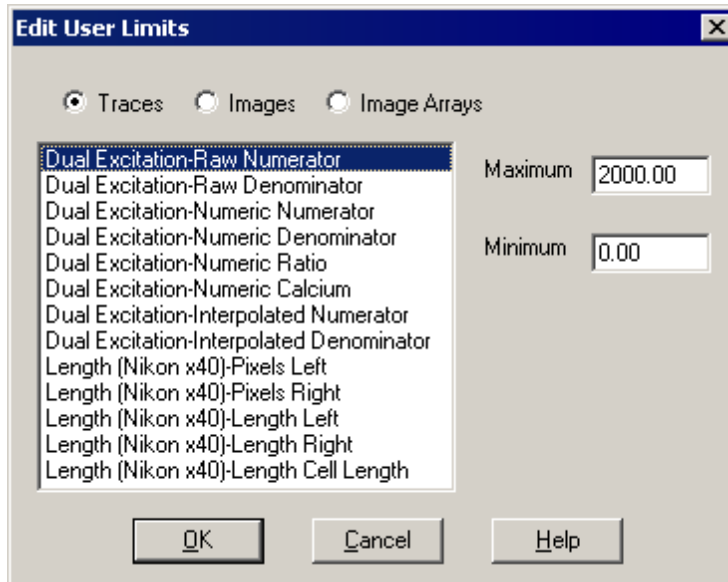
The range and *range and color map control* to the right of the image behaves identically to the one in the *movie player*. Both the color map and display range for the displayed image are edited with this control. These operations are detailed in the [Range and Color Map Control](#) sub-section of [\\$Movie Players](#)^[44].

Output

The three *push buttons* in the [Output](#) section of the *dialog box* allow the user to print the image or copy the image to the clipboard. Clicking [Print Setup](#) invokes the standard Windows print *dialog box*. There the user may select or configure a printer for use. Clicking the [Print](#) *push button* will print the image with the color map, while clicking [Clipboard](#) will copy the image and color map as a meta file to the Windows clipboard.

6.8 User Limits

In order to set the *user limits* used by the *trace viewers* for displaying traces, select the Edit User Limits... item from the Traces menu. The following dialog box will appear.



Edit User Limits Dialog Box

The three *ratio buttons*, Traces, Images and Image Arrays let you select between the three different classes of *user limits* that are tracked by IonWizard. When you select a given class, the *list box* below will be filled with descriptions of all the different data elements of that class type that are being tracked by IonWizard. In the example above, the Traces *radio button* is selected and the *list box* shows all the different trace descriptions that have been seen by the program.

Select the appropriate item from the list box and then enter the desired limits in the Maximum and Minimum *edit fields* to the right. When you are finished, click Ok to save the changes for the current session.

User limits are a part of the [screen template](#)^[107]. To save changes made to the user limits beyond the current session, [save a new screen template](#)^[108].



User Limits are matched to tasks using the task name. If you rename a task you will have to reenter the user limits.

7 Advanced Analysis

Besides the basic visualization and analysis features described already in [§Basic Data Handling](#)^[32], IonWizard provides a number of more advanced analysis features. These features include trace filtering, ensemble averaging and trace analysis plug-ins. Each of these features is described below.

7.1 Trace Analysis

The functions described in this section are present for all users of IonWizard. Users who have imaging systems have access to additional image-specific functions described in the next chapter. Note that some features described below may not be available during data acquisition when the state of the data set is not entirely known.

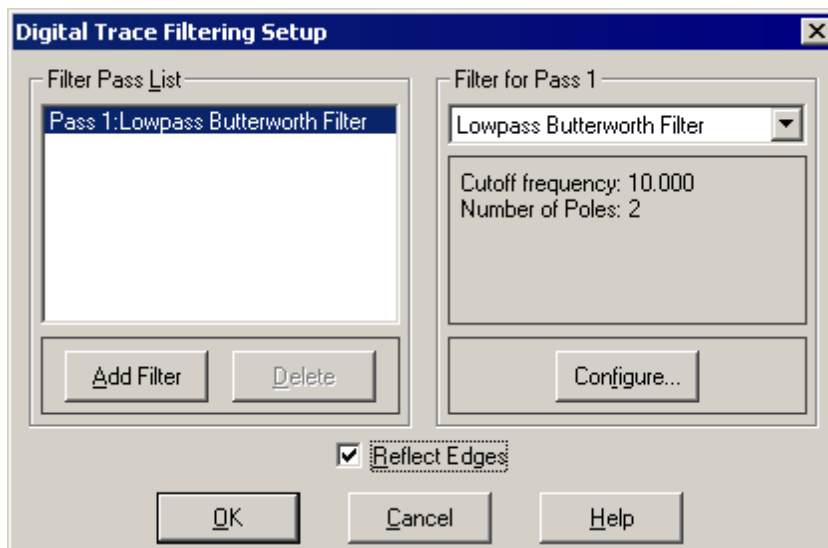
7.1.1 Trace Filtering

IonWizard has the ability to filter the raw trace data prior to any calculations being performed on it. This has been described briefly in [STrace Viewers](#)^[37] as the primary method for turning the filters on and off is via the [Filter](#) button in the information region.

The filtering functionality itself is provided by Trace Filter Plug-ins. This means that, in principal, any arbitrary processing can be done on the data prior to calculations being performed. IonWizard is shipped with three filters, a low-pass Butterworth filter, a zero phase low-pass Butterworth filter, and a Savitzky-Golay polynomial smoothing filter. These are described in detail later.

All trace filtering options are part of any analysis template you create (see [SAnalysis Templates](#)^[109]). Also, if you employ and trace filters, or edit a trace filter setup, the trace filtering data will be saved with the data file. This assures that you can get consistent filtering results with a given file over time.

7.1.1.1 Configuring the Filters



Digital Trace Filtering Setup Dialog Box

If there is an active trace viewer, and the trace displayed in that viewer can be filtered, the [Raw Filters Setup...](#) item under the [Operations](#) menu will be enabled. Selecting this item invokes the [Digital Trace Filtering Setup](#) dialog box illustrated above.

Filter Pass List

A given filter setup actually consists of a series of filter passes. IonWizard applies each filter in order: Pass 1, Pass 2, etc.; until all filters in the pass list have been applied to the trace.

The *list box* in the [Filter Pass List](#) section of the *dialog box* displays the current pass list for the filter setup. In this case, the setup consists of only one pass, the application of a low-pass Butterworth filter. To add a new pass to the setup, click the [Add Filter](#) push button. This will add a new pass and select it for editing. Clicking the [Delete](#) push button will remove the highlighted filter from the pass list.

Filter for Pass x

The controls in the [Filter For Pass](#) section allow the user to select and configure a filter for the highlighted pass in the pass list. Choose a filter for the pass from one of the filters listed in the

combo-box at the top of the section. All trace filters available on the system are listed there. The current configuration for the selected filter are displayed immediately below the *combo-box*. To change the filter parameters, click the Configure... *button*. A *dialog box* will be shown to edit the filter options. The layout and function of this *dialog box* is filter dependent. It will display its own separate help information.

Reflect Edges

The Reflect Edges *check box* indicates whether or not the filter will correct for edge effects. Typically, digital filters will use what is called a kernel operation to do their filtering. In a kernel operation, each point in the trace is replaced by the result of an operation carried out on some number of surrounding points. For example, a point may be replaced by the average of itself and three neighboring points on either side. Clearly, the first and last three points in a trace cannot have such an operation applied to them. In general, a number of points equal to half the kernel width will be effected on each end of the trace. The question becomes what should be done with these points that cannot be filtered? One solution is to make these points have the same value as the nearest valid point. In the above averaging example this would mean that the first three points would have the value of the fourth point, while the last three points would have the value of the fourth to last point. This tends to make the edges of the trace look 'wrong' as it is clear that the data was not perfectly unchanging in those regions. This is particularly troublesome when the total length of the trace is short.

Another solution to this problem is to effectively lengthen the trace by reflecting values around the first and last points. Then the first and last points of the real data array will have values around them so the kernel may be applied. This is effective only if the data near the edges is not changing. Any rising or falling trend in the data near the ends will cause the edges to be no more an accurate representation of the real signal than simply copying values as suggested before. In fact it may be slightly more dangerous in these situations since it appears that something really was going on whereas there would not be any such confusion with a section of copied values.

Thus, if the data traces are at a constant value near the beginning and end of the trace, selecting to reflect the edges will prevent the flat line of points on the ends. If the data is changing near the edges, selecting not to reflect the edges will cause the filter to copy edge points hereby preventing the display of nonexistent signals.

Filters are applied on a data segment by data segment basis (see). This means the data should be flat both before and after each time discontinuity in order to apply the edge reflection technique.

OK and Cancel

Clicking the OK *push button* will accept any changes made and cause a recalculation of the filtered traces, while selecting the Cancel *push button* will dismiss the *dialog box* with no changes.

7.1.2 Trace Analysis Plug-ins

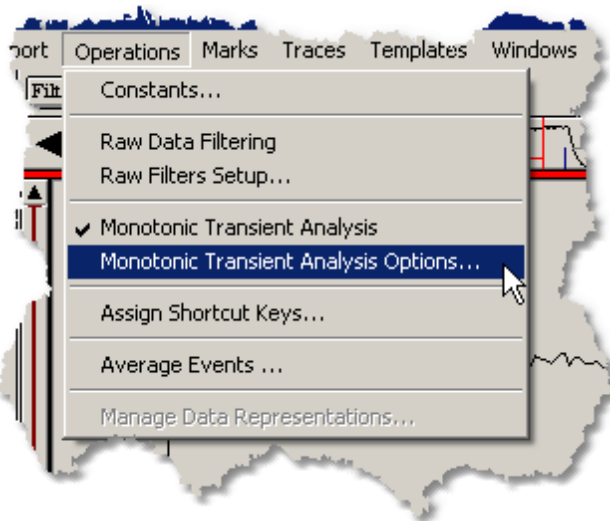
IonWizard provides a plug-in interface for trace analysis modules. This permits arbitrary extension of IonWizard's trace analysis capabilities. At the moment, only one plug-in is available, the Monotonic Transient Analysis plug-in. This is described in detail below. Here will describe the places in IonWizard where trace analysis plug-ins can interact with the user.

Menus

Trace analysis plug-ins can inject items into both the Operations *menu* and the Export *menu*. This lets the user configure the trace analysis, apply the analysis and export the results.

Operations Menu

Each trace analysis plug-in will cause two *menu items* to be added to the Operations *menu*. The figure below illustrates those items in the context of the Monotonic Transient Analysis plug-in.

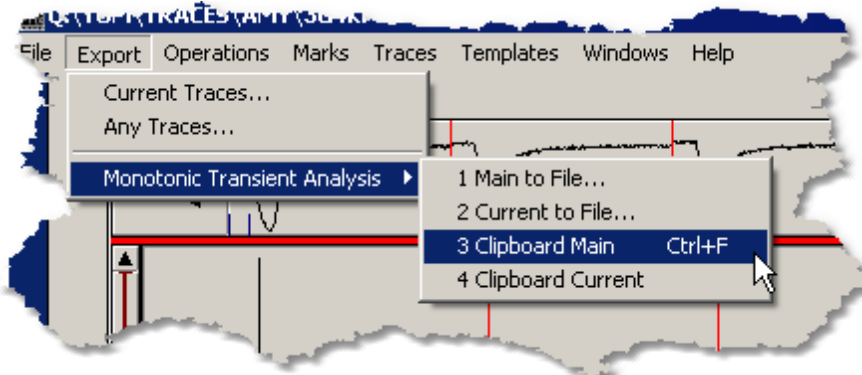


The first item is simply the name of the plug-in, in this example, Monotonic Transient Analysis. This item invokes the given analysis on the trace in the *active trace viewer*. If there is no *active trace viewer*, or that *trace viewer* does not have any transient marks, this *item* will be disabled. A check mark indicates that the analysis is showing.

The second *menu item* will be the name of the plug-in plus "Options...", Monotonic Transient Analysis Options... above. This item lets you configure whatever options are available for the analysis. These dialog boxes are highly plug-in dependent and are documented fully in the specific plug-in documentation.

Export Menu

Each trace analysis plug-in will cause one *pop-up menu* to be added to the Export menu. The figure below illustrates those items in the context of the Monotonic Transient Analysis plug-in.



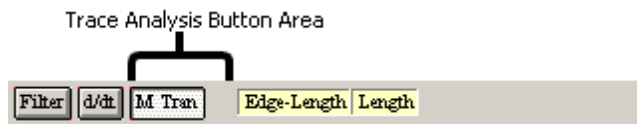
The name of the *pop-up menu* will be the same as the name of the plug-in, in this case, Monotonic Transient Analysis. The actual number and names of the individual *items* in the *pop-up menu* is dependent on the plug-in and will be documented in detail in that specific documentation.

Note that you can assign keyboard short cuts to the commands in this menu via the shortcut key manager (see [§Shortcut Key Manager](#)^[28]).

Information Region

For each trace analysis plug-in, IonWizard inserts a button into the *information region* of each *view unit*. These buttons will be on the left, in a sub-section the *trace viewer* control area (see [§View Units](#)^[36] for more information). They permit the user to employ the analysis from that plug-in on the given trace.

This is identical to selecting to employ the analysis from the Operations menu as described above. The figure below shows the *information region* and the position of the trace analysis *buttons*.



Information Region With Trace Analysis Plug-in Buttons

Here we see the *push button* for the Monotonic Trace Analysis plug-in, labeled M Tran. The text in the button comes from the plug-in itself.

Selected View

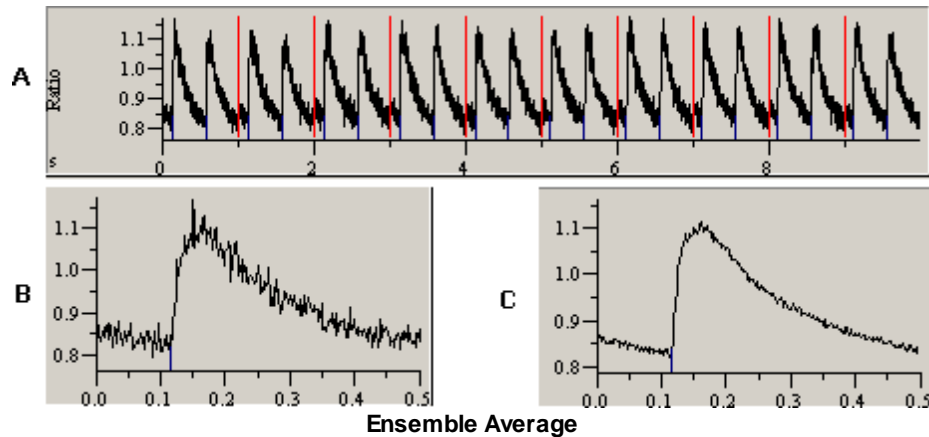
Each trace analysis plug-in is permitted to insert controls and graphics into the selected view portion of a trace viewer when the analysis is being run. This behaviour is highly dependent on the specific plug-in and is described in detail in the documentation for that particular plug-in.

Analysis Templates

The settings for each trace analysis plug-in in the system are saved in each [analysis template](#)^[109] you create. Furthermore, if you apply any trace analysis the settings will be saved with the data file. This assures that you can re-run the analysis at a later date and get back the same results.

7.2 Ensemble Averaging

IonWizard has the ability to ensemble average identical transients together for the purpose of noise reduction as illustrated in the figure below.

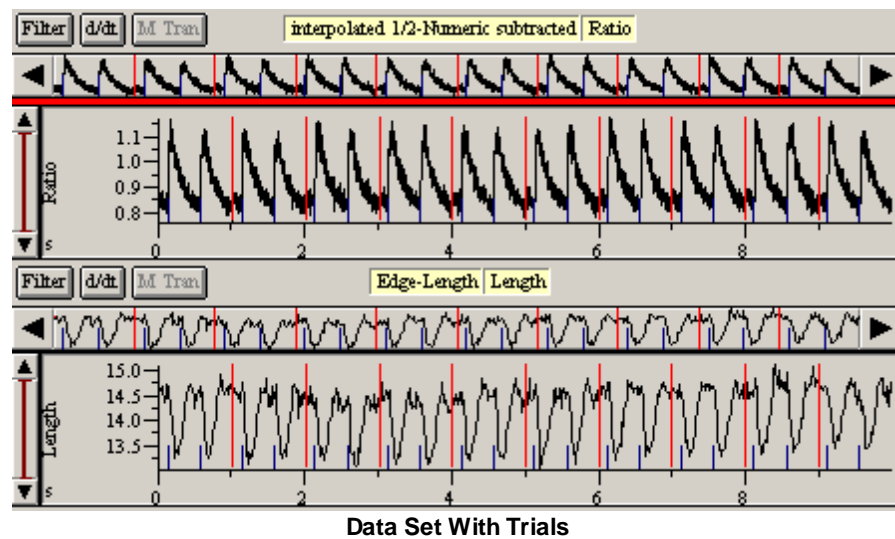


Graph **A** shows a trace of 20 identical calcium ratio transients. Graph **B** shows an enlarged view of one of those transients. Graph **C** shows the result of ensemble averaging all 20 transients together. The reduction in noise is significant. Furthermore, unlike digital filtering, there is no associated phase shift or distortion of the transient. Thus this is the preferred technique of noise reduction.

One big drawback of ensemble averaging is that it requires timing markers in the data set that allow IonWizard to precisely register each individual transient prior to averaging them together. Practically speaking, this means that the preparation must be capable of being electrically stimulated and the experiment must be set up correctly to record the necessary timing markers. IonWizard supports two methods of averaging: event averaging and trial averaging.

Event averaging uses an external event mark as the time marker for each transient. It is assumed that this event mark is being generated by a hardware signal coming from the stimulator that is driving the preparation.

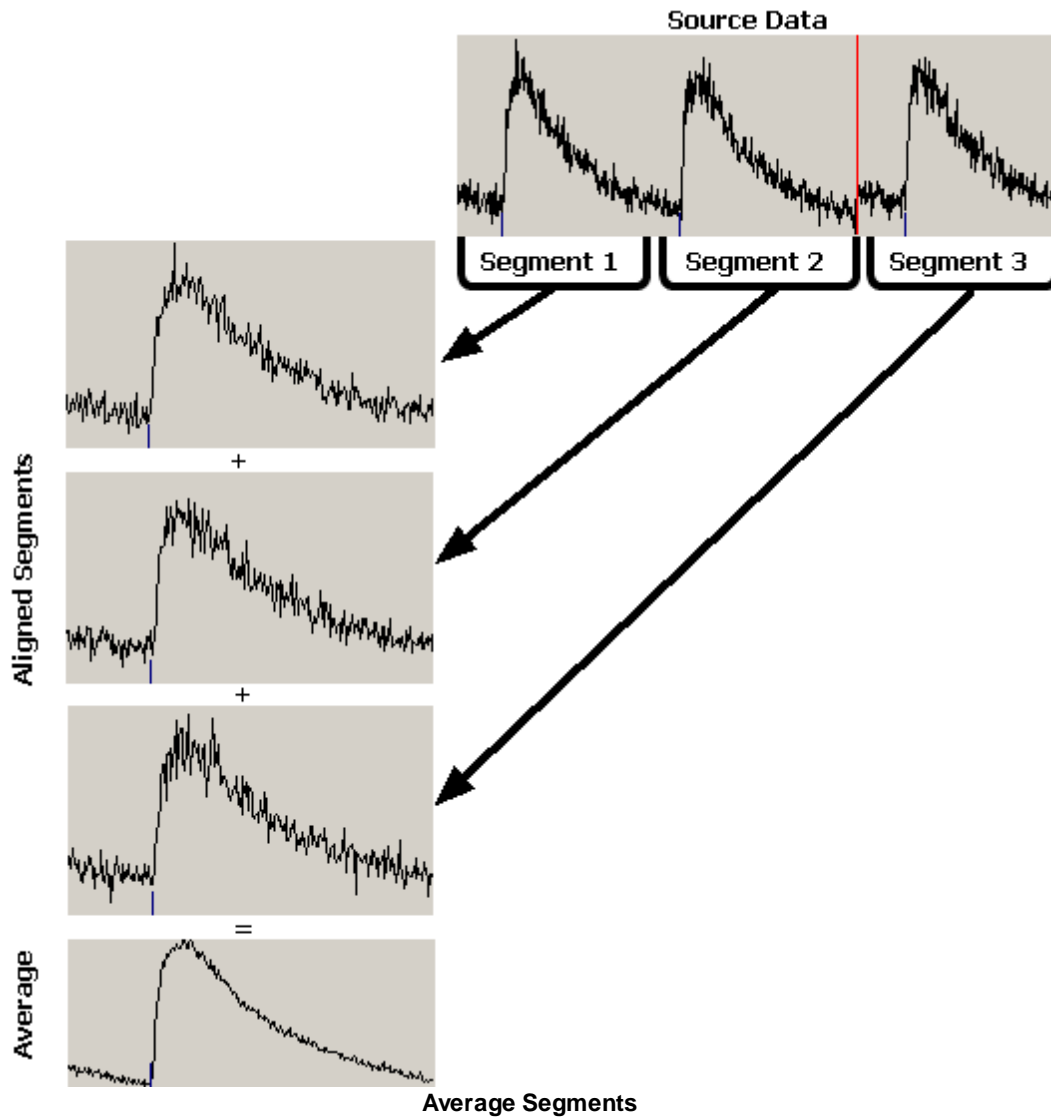
Trial averaging requires significantly more acquisition support than event averaging. Trial averaging is enabled in IonWizard by the existence of trial time discontinuities (see [§Time Discontinuities](#)^[53]). The acquisition module will assure that the time between trial discontinuities is identical. That is, all data segments in an experiment with trial averages will be of exactly the same length (see [§Data Segments](#)^[55]).



The figure above illustrates a data set collected with trials. This mode of acquisition is no longer supported by IonOptix acquisition modules and trial averaging is present in IonWizard to support old data.

7.2.1 Average Segments

Before moving on, we will clarify exactly what is happening when an ensemble average is created. An ensemble average is created by taking sections of the source data trace and averaging them together to form a second data trace. These sections of data in the source trace are called *average segments*.



The figure above shows a source data trace with three transients. This trace is divided into three average segments, one for each transient. To create the ensemble average, those three segments of data are pulled out of the source data trace and are arranged such that the first data points of the transient are aligned as shown on the left of the figure. After alignment, the segments are added together point by point starting with the leftmost data point and proceeding for the number of points in the average segments. Obviously, IonWizard must assure that all average segments have the same number of data points.

The nature of the average segments themselves differ between event and trial averages. In event averages, the segment is defined by applying an offset and duration to the time of an event mark. This is very similar to how transient marks are added from event marks (see [§Transient Mark Acquisition](#)^[64]).

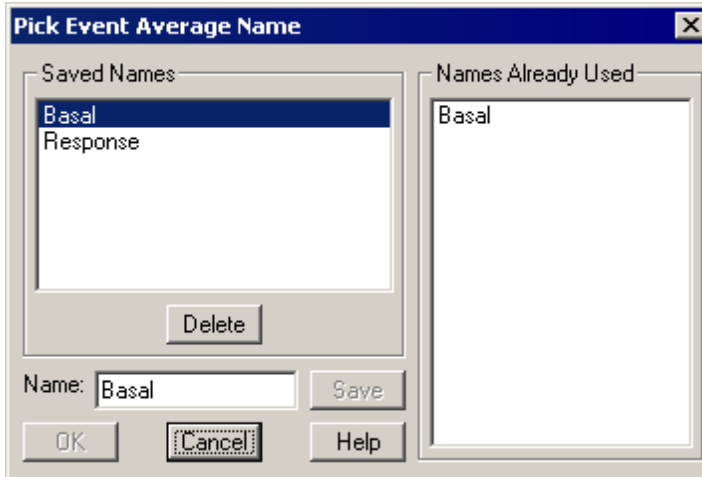
In trial averages, the segment is defined by the trial discontinuities.

The specifics of the average segments for each type of ensemble average will be explained in detail in later sections.

7.2.2 Creating an Ensemble Average

To create an ensemble average, select [Average Trials...](#) or [Average Events...](#) as desired from the [Operations menu](#). If a particular type of average is not supported for a given data set, the corresponding *menu item* will be disabled. Event averages require the existence of external event marks whereas trial averages require the existence of trial discontinuities.

Selecting either *item* will present the following *dialog box* (with the title adjusted appropriately).



Average Name Dialog Box

Here the user selects the name of the average to be created. This name is used to differentiate between multiple averages. There are numerous reasons one may need multiple averages. The user may want to average a different groups of transients for analog traces than are averaged for fluorescence traces, for example. Alternately, the data may be sampled such that different blocks of transients have different meanings. For example, the first twenty minutes of data might be the baseline condition, the next twenty the response condition, etc. In this case, you would want to create different averages for the transients in each of these experimental regimes.

The [Name edit field](#) displays what will become the name for the new average when the user clicks the [OK push button](#). The default entry for this *edit field* is the first item in the [Saved Names list box](#). Clicking on any item in the [Saved Names list box](#) will enter it into the [Name edit field](#).

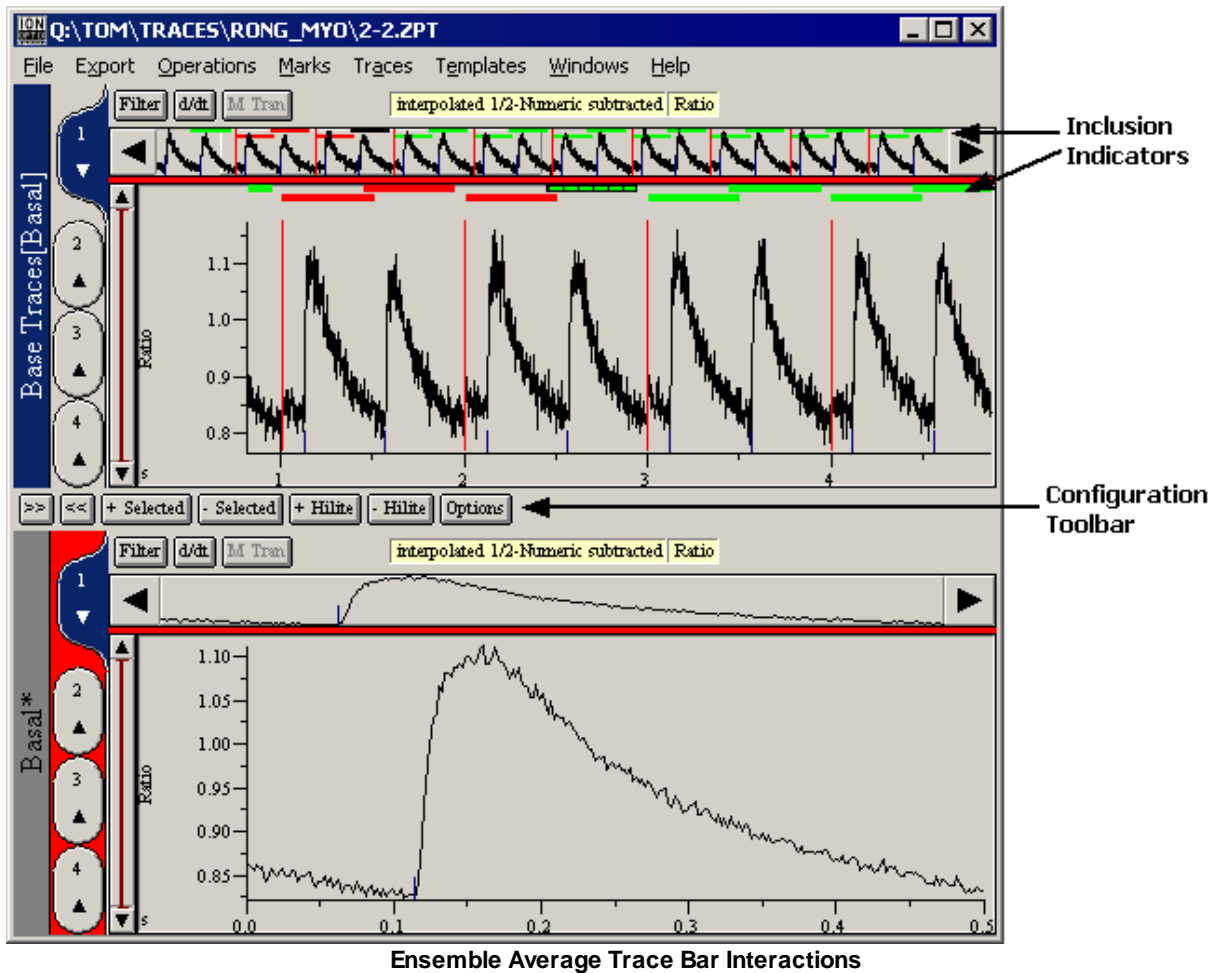
If you type a name into the [Name field](#) that does not exist in [Saved Names](#), the [Save push button](#) will be enabled. Click it to add the new name to [Save Names](#). To delete a name from the [Saved Names list](#), highlight the name and click the [Delete push button](#). Adding or deleting names from the [Saved Names list](#) does not effect the data set in any way. This list is saved in the user's [profile section](#)^[105] and exists solely as a convenience.

You cannot give the new average the same name as any existing average of that type (event or trial). The [Names Already Used list box](#) shows the names of all existing averages.

Once a name is selected, click [OK](#) and IonWizard will create a new average [data representation](#)^[31] and new [trace bar](#)^[33] to display it. They can then be managed in the [Manage Data Representations](#)^[51] *dialog*. By default, only the trials or events within the *selected view* of the [Base Traces trace bar](#) will be selected in this average. The next section talks more about the interactions between *trace bars* and configuring the averages.

7.2.3 Trace Bar Interactions

When IonWizard creates an event or trial average, it creates a new data representation and *trace bar* for that average, and then shows that *trace bar* as illustrated below (see also [Trace Bars](#)^[33]).



Ensemble Average Trace Bar Interactions

When the Base Traces trace bar is visible alongside a trace bar showing an ensemble average, the program is in a mode that permits configuring the average. In this mode, there is communication between the two trace bars; the average trace bar injects visual elements, the inclusion indicators, into the Base Traces trace bar; and the average trace bar shows its configuration toolbar.

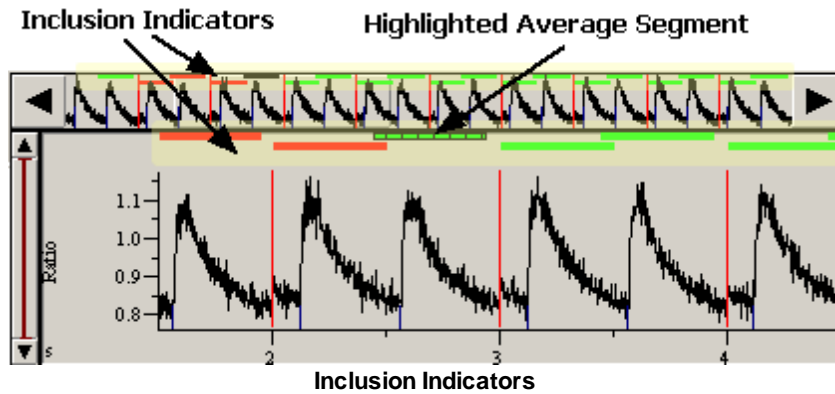
Since it is possible to have multiple ensemble averages, it is important to know which average is being configured. This is shown by changes in the titles of the trace bars. Notice in the figure above that the Basal trace bar has a star character (*) after Basal. Also, the Base Traces trace bar has [Basal] appended to its title. These two visual changes mean that the Basal trace bar is being configured.

Communication

It is very helpful to be able to see both the raw (i.e. the Base Traces) and averaged view of the same trace when configuring an average. Thus when in configuration mode, there is linkage between trace viewers in the two trace bars: changing the trace in either the average or the Base Traces trace bar will cause the other to change also.

Inclusion Indicators

As explained earlier, each average consists of a number of average segments. It is often necessary to reject some number of average segments present in the source data trace from the final average. Inclusion indicators are used to display which average segments are included and which are not. The inclusion indicator also shows the location in time of each average segment.



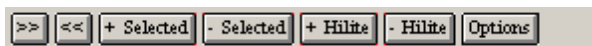
Inclusion indicators are present in both the *full view* and the *selected view* of each trace viewer as illustrated above. Each green or red bar indicates a single average segment. Any segment drawn in green is included in the current average while any bars drawn in red are excluded from the current average.

One segment will always be drawn with an overlaid vertical hatch pattern. This is the highlighted segment. Moving the mouse pointer over a segment in the *selected view* will cause the highlight to jump to that segment. The highlighted segment is explained in detail in later sections.

Configuration Toolbar

Each ensemble average has its own configuration toolbar. The functionality of this is specific to each average type and is explained below.

7.2.4 Event Average Configuration



Event Average Toolbar

The Event Average toolbar is used to configure an event average. There are four groups of buttons in this toolbar.

>> and <<

These two buttons move the highlight from one [average segment](#) ^[84] to the next. If the highlighted average segment moves outside the *selected view* of the source *trace bar*, the *selected view* will move up or down as appropriate (see [\\$Trace Viewer](#) ^[39] and [\\$Trace Bars](#) ^[33]). This permits systematic examination of every average segment and by using the [+ Hilite](#) and [- Hilite](#) buttons, the user can add or remove segments as needed.

+ Selected and - Selected

These buttons will add or remove every segment visible in the *selected view* of the source trace bar.

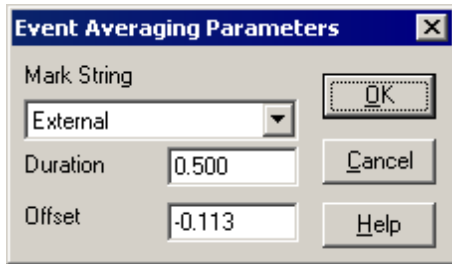
+ Hilite and - Hilite

These two buttons add or remove the currently highlighted average segment from the event average.

Options

The [Options push button](#) invokes the [Event Average Options dialog box](#). This *dialog box*, explained in detail in the next section, permits adjusting the placement of the average segment.

7.2.4.1 Event Averaging Parameters



Event Average Parameters Dialog Box

Clicking the Options push button in the [event average toolbar](#)^[87] invokes the [Event Averaging Parameters dialog box](#) shown above. The settings here determine mark and the exact placement of the average segment (see [Average Segments](#)^[84]) relative to that mark for the average.

Mark String

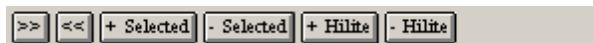
The [Mark String combo box](#) is used to select the text for the external event mark to use for this average. Generally there is only one type of external event mark and thus this selection is not needed.

Duration and Offset

Given an event mark string, IonWizard uses the time of each event mark plus the [Duration](#) and [Offset](#) parameters to create the average segments. The length of the average segment is given by the [Duration](#) parameter. The beginning of the segment relative to the event mark is determined by taking the time of the mark and adding the [Offset](#) parameter. Negative values of [Offset](#) will cause the segment to start before the mark while positive values will cause the segment to start after the mark.

Any average segment created with this procedure that falls outside of the *data segment* for the given mark is rejected (see [Data Segments](#)^[55]).

7.2.5 Trial Average Configuration



Trial Average Toolbar

The Trial Average toolbar is used to configure a trail average. There are three groups of buttons in this toolbar.

>> and <<

These two buttons move the highlight from one [average segment](#)^[84] to the next. If the highlighted average segment moves outside the *selected view* of the source *trace bar*, the *selected view* will move up or down as appropriate (see [Trace Viewer](#)^[39] and [Trace Bars](#)^[33]). This permits systematic examination of every average segment and by using the [+ Hilite](#) and [- Hilite](#) buttons, the user can add or remove segments as needed.

+ Selected and - Selected

These buttons will add or remove every segment visible in the *selected view* of the source *trace bar*.

+ Hilite and - Hilite

These two buttons add or remove the currently highlighted average segment from the trial average.

8 Printing and Figure Creation

IonWizard provides a number of mechanisms to create and output figures of your data for inclusion in presentations and manuscripts. IonWizard also provides several mechanisms to export the actual data rather than simply figures of the data. This is considered a separate topic and is discussed in later chapters.

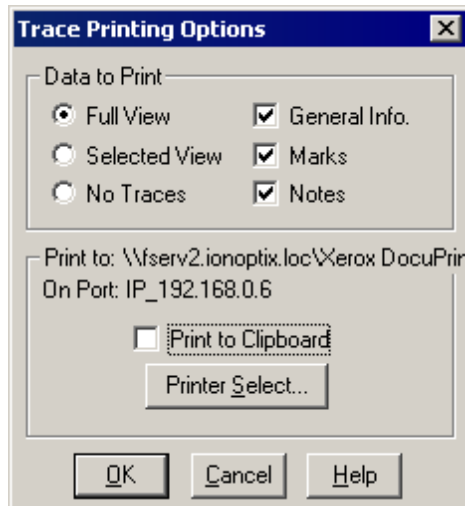
In the following sections we will discuss specific functions to create figures of trace arrays and image arrays. Also, we will briefly recapitulate some information already covered in earlier sections related to figure making.

8.1 Trace Arrays

Generating graphical output of trace plots and other experiment information is important for creating final figures for presentations. IonWizard can direct graphical output to either a printer or to the clipboard. There are two entry points into the trace printing facility. [Configurable Trace Printing](#)^[90] lets the user configure all the print parameters including selection of the printer or clipboard as the output destination. [Fast Printing](#)^[91] lets the user rapidly execute the last print job. Both of these options are described below.

8.1.1 Configurable Trace Printing

Selecting the [Print...](#) item from the [File menu](#) reveals the following *dialog box*.



Trace Printing Dialog Box

This dialog box provides the user with options as to what can be printed and where the output goes. The printing always uses as a source all visible *trace viewers* in the [active trace bar](#)^[34]. The [Data to Print](#) and [Print to:](#) sections permit configuration of the print job.

Once the print job is configured, click the [Ok push button](#) to start the job. This will also save all the configuration options in the current users [profile section](#)^[105]. It will also determine the parameters for any subsequent fast print job (see [§ Fast Printing](#)^[91] for more information).

Data to Print

The three *radio buttons* in the [Data to Print](#) section determine the trace data content of the printed figure.

Full View The full view time range of the traces is printed without regard to the current selected view settings.

Selected View Only the selected view time range is printed.

No Traces No traces are printed at all. Only the extra information is printed.

The three *check boxes* determine what, if any, extra information is printed after the traces.

General Info. The general information includes the experiment name; the date and time of collection; and the three strings cell type, tape and other input as entered by the user in the experiment setup.

Marks All event mark times and texts.

Notes The experiment notes.

Print to:

The traces and associated information can be directed to either the current printer (as described in [§ Selecting a Printer to Use](#)^[25] above), or the the clipboard. If the [Print to Clipboard](#) check box is

selected, the destination is the clipboard, otherwise it is the current printer.

If the destination is the clipboard, a scalable vector graphic object is created (known in Windows parlance as a metafile). This object can be pasted into programs like Microsoft Word, Corel Draw or Microsoft PowerPoint. There, the object can be stretched and manipulated as a group of graphical entities or it can be ungrouped and the individual entities manipulated. The degree to which the object can be manipulated depends greatly on the abilities of the destination program.

If the destination is the printer, a print job will be sent to the current printer. You can change the current printer by clicking the *Printer Select...* push button. Any changes made there are global for the current user and will impact all future printing operations.

8.1.2 Fast Printing

The fast printing option sends a print job directly to the configured destination without any further user intervention. The configuration options used are those set by the user during the last configurable printing session (see [§ Configurable Trace Printing](#)^[90] for more information).

Fast printing is executed from the *File menu*. The exact text of the *menu item* changes depending on the configuration options in use and given in the following table:

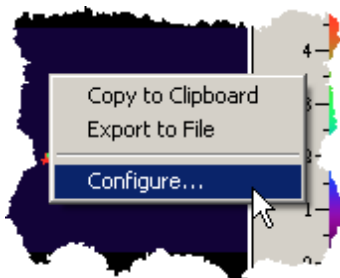
Configurable Printing Trace Option	Fast Printing Menu Item Text
Full View	<u>Print Full Views</u>
Selected View	<u>Print Selected Views</u>
No Traces	<u>Print Experiment Information</u>

8.2 Image Arrays

There are two ways to create figures of image array data. You can create figures of single images directly from the movie player, or you can create montages of images via [Image Tiles](#)^[92].

8.2.1 Movie Player Figures

The *movie player* permits creating figures of specific time points within the image array via its local menu. The *local menu* is accessed by clicking with the right mouse button in the image area (see [\\$Movie Players](#)^[42] for more information). The *local menu* is shown in the figure below.



Movie Player Local Menu

Selecting [Copy to Clipboard](#) will copy the image into the clipboard for pasting into other programs. The configuration settings, previously discussed in [\\$Movie Player Options](#)^[47], determine the image format.

Selecting [Export to File](#) will bring show the standard Windows® Save dialog box. There you can input the filename and path for the export. All exports to file are bitmaps but, unlike clipboarded bitmaps which are always DIBs, these images can be in a variety of formats. IonWizard currently supports the following formats:

- MS Windows Bitmap (*.bmp)
- MS Windows Bitmap (*.dib) - this is the same as the clipboard bitmap.
- CompuServe GIF (*.gif)
- Joint Photographic Experts Group(*.jpg)
- Photo CD (*.pcd)
- ZSoft Paintbrush (*.pcx)
- Portable Document Format (*.pdf)
- Adobe PostScript (*.ps)
- Adobe Photoshop bitmap (*.psd)
- Truevision Targa(*.tga)
- Tagged Image File Format (*.tif)

8.2.2 Image Tiles

Image tiles are arrays of images used as components of image figures. The image tile functions permit the user to create these arrays quickly and easily, while allowing as many options as necessary to minimize the need for further processing after the tile has been moved into the final document. Once made, the image tiles may be transferred to other applications via the clipboard or disk-based files.

To produce an image tile, select [Create Image Tile...](#) from the *Images menu*. The trace bar will go to half screen and the image tile window, shown below, will appear. The *movie player* will be paused if it is not already. The image tile contains images of the same type as displayed in the *active movie player*. If there is no *active movie player*, the [Create Image Tile...](#) *item* will be disabled.

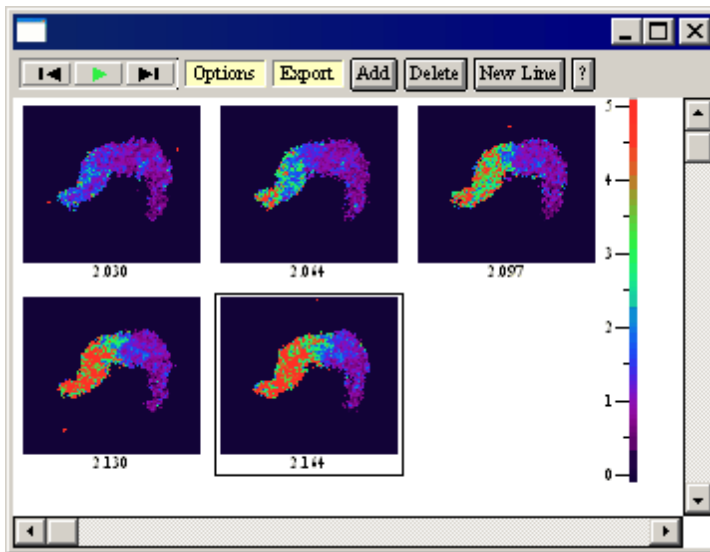


Image Tile Window

Populating the Tile

There is an invisible grid covering the window. The grid size is the size of the images plus a border to allow room for the images times, if those are displayed. Any click with the **left mouse button** in the window will cause a border to form around the nearest grid cell. This outlined cell is the destination cell. The image shown in this cell represents the image that will be placed in the cell when the **Add push button** is clicked. If there is already an image in the destination cell, it will be hidden until the destination cell is moved. Any image in the destination cell will be overwritten by new images.

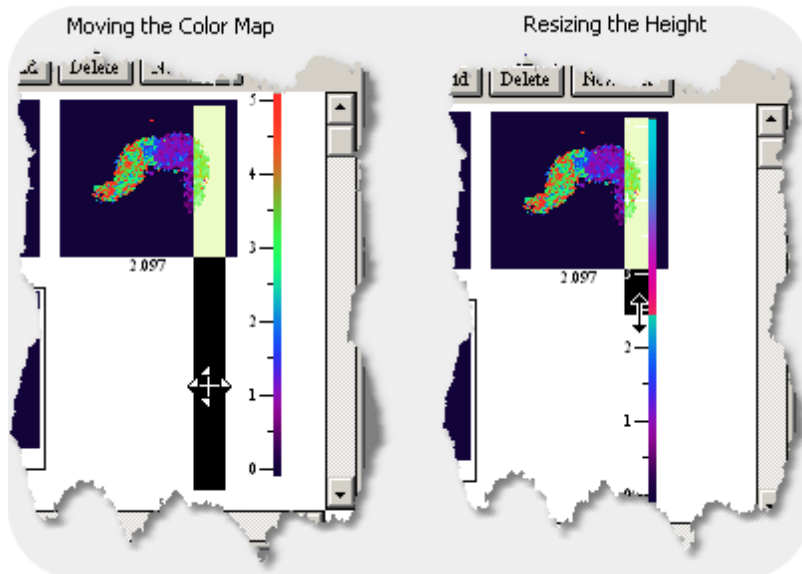
The *VCR control* at the top of the window is used to change the image in the destination cell (see [§Movie Players](#)^[42] for more information on *VCR controls*). Pushing the play button of the *VCR control* will cause the image in the active cell to play. It will also cause the visible *movie players* to play. This *VCR control* is linked to the *movie players* and vice versa. There must be one *movie player* visible in order for the active cell images to change via *VCR control* interaction.

After an image is added, the destination cell will automatically jump to the next cell in the grid. Additionally, the *movie player* will be incremented to show the next time point. The movies must be paused for images to be added to the image tile. If the destination cell movie is running, the **Add button** will be disabled.

The **New Line push button** will jump the active cell to the first cell in the next row. This is equivalent to clicking on the cell using the left mouse button.

Color Map

The color map is a floating object that can be moved around as needed. All editing is done with the mouse using the left mouse button. Clicking and dragging on the center of the map will move it. You can also grab the edges and corners to resize it. The mouse cursor will change to indicate the possible adjustment.



Repositioning the Color Map

In the figure above we show first moving the color map to a new location and then reducing its size.

Options

There are several options available with respect to the image tile. These options are accessed through the Options yellow button menu as shown below.



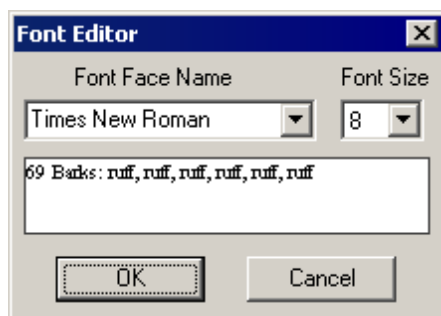
Image Tile Options Menu

Show Colormap and Time

Show Times adds the current time below each image in the tile. Show Colormap adds a movable, scalable, color map and range display to the tile as discussed in the previous section.

Font

Any font may be chosen for the image times and the color map axis. Selecting Font... from the Options yellow button menu will invoke the Font Editor dialog box seen below.



Font Editor Dialog Box

Choose the type face and the point size of the font. Clicking the OK button will cause the selected font to be used for subsequent image tile drawing. Clicking Cancel will dismiss the *dialog* with no changes.

Display Zoom

You can shrink or expand the display by selecting the Display Zoom... item. This will bring up the dialog box shown below.



The display zoom does not impact the size of the exported figure, only the image tile screen display size.

Export

Once the image tile is constructed, it may be exported to the clipboard or a file by using the Export yellow button menu as shown below.

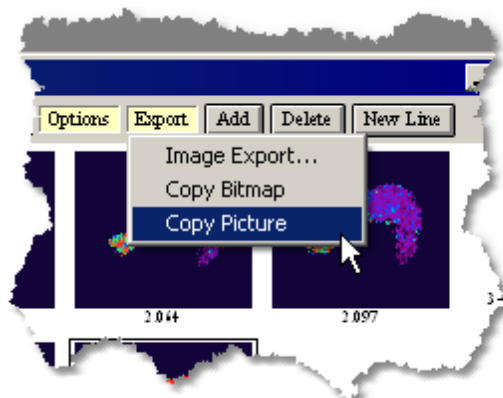


Image Tile Export Menu

The same three options are available as are available for the movie player export. You can export a picture (metafile) or a bitmap (DIB) to the clipboard; or you can export a number of different bitmap formats to a file. See [\\$Movie Player Figures](#) [92] for information on the specific file formats supported.

8.3 Image Points and Zones

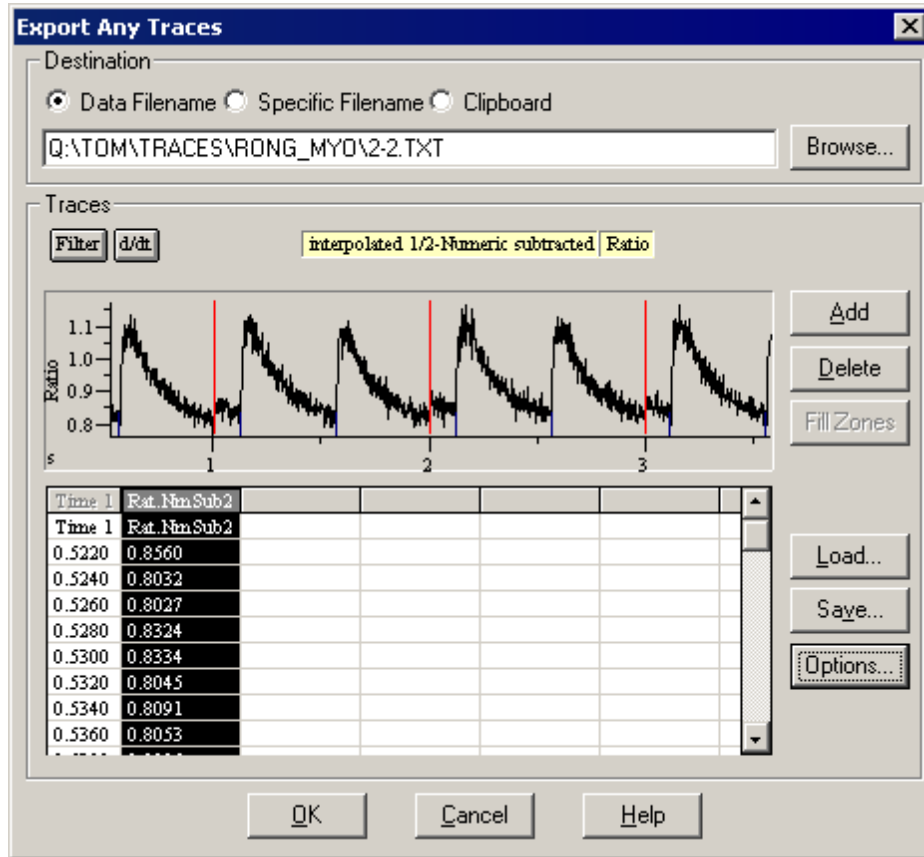
You can print or copy to the clipboard figures of image points (i.e. background images) and zones. These functions were already described in detail in the documentation for the respective viewers. See [\\$ImagePoints](#)^[76] and [\\$Zones](#)^[74] for more information.

9 Data Export

IonWizard supports data export of both trace array and image array data. This allows the user to conduct specific analysis using third party analysis tools.

9.1 Trace Exporting

IonWizard can export any trace data as a text file for import into other programs. There are two export types fast and custom. Both the fast and custom trace exports operate on the data representation in the *active trace bar* (see [§Data Representation](#)^[31] and [§Trace Bars](#)^[33] for more information). Custom export permits export of any trace whereas the fast export exports only the traces in the visible *trace viewers* in the *active trace bar*. For the fast export select Current Traces... from the Export menu. For the custom export, select Any Traces... from the Export menu.



Trace Export Dialog Box

Above is an illustration of the trace export *dialog box* as it would look if we ran a custom export. The fast export dialog is exactly the same but with different initial setup behavior and with some options disabled. Since the fast export supports a subset of the custom functionality, we will discuss the custom export *dialog box* below with notes indicating points where the fast *dialog box* behavior differs.

Setup

The initial setup state of the *dialog box* differs between custom and fast export. For a custom export, the last saved export configuration file is loaded. This file contains the traces to export and the export options needed. Such files are setup and saved by the user ahead of time and would likely represent the trace data desired for some user specific operation. Perhaps the user needs to use a spreadsheet to perform a specialized sort of analysis not supported by IonWizard, for example a Fourier transform. The user would save an export configuration file containing all the traces needed to do that analysis. Each time the user runs a complex export, IonWizard loads the configuration file and selects the required traces from the current data set for export. This, in essence, allows the user to define export behavior to suit their need; the user could mentally equate "complex export" with "export for Fourier transform".

In a fast export, the traces visible in the *active trace bar* are exported with settings stored in the current template. In this way the user may export data for the traces currently being viewed with a minimum of hassle.

For both types, the range of times that is being exported corresponds to the *selected viewtime* range in the given *trace bar*.

Destination

The Destination section at the top of the *dialog box* determines where IonWizard writes the export data. If the Clipboard check box is selected, IonWizard directs the export to the Windows® Clipboard, otherwise it writes the export to a file.

If Data Filename is selected, the filename will default to the full path and filename of the data file with the normal data file extension (ZPT, etc) replaced with TXT. If Specific Filename is selected, the filename will default to the last specific filename you entered. In either case you can modify the filename manually as needed.

For both custom and fast exports the information in the Destination section is saved in the users [profile section](#) [105] and thus is remembered from export to export.

Traces

The screenshot shows the 'Traces' dialog box. At the top, there is a 'Filter' button set to 'd/dt' and a yellow button labeled 'interpolated 1/2-Numeric subtracted Ratio'. Below this is a graph showing a periodic signal with a y-axis labeled 'Ratio' ranging from 0.8 to 1.1 and an x-axis labeled 's' ranging from 0 to 8. To the right of the graph are three buttons: 'Add', 'Delete', and 'Fill Zones'. Below the graph is a table with the following data:

Time 1	Rat. Nbr Sub2	Time 2	len. Len1
0.0000	0.8654	0.0000	14.5900
0.0020	0.8452	0.0150	14.6300
0.0040	0.8293	0.0320	14.5800
0.0060	0.8337	0.0490	14.6700
0.0080	0.8436	0.0660	14.3700
0.0100	0.8594	0.0820	14.4900
0.0120	0.8395	0.0990	14.7100
0.0140	0.8614	0.1160	14.6600

To the right of the table are three buttons: 'Load...', 'Save...', and 'Options...'. Labels 'Export Preview' and 'Options and Configurations' point to the table and buttons respectively.

The Traces group of controls provides an interface by which the user selects the contents of the export. This section has three main sub-sections as shown in the figure above.

Export Preview

The export preview shows, as closely as possible, the form of the final export, displaying every row of data in the export. Column titles in the output table drawn in black indicate the user may select that column by clicking with the **left mouse button**. Columns that cannot be selected, such as the Time 1 column, are drawn in gray.

Column Trace Controls

When a new column is highlighted, IonWizard loads the trace sample and *yellow buttons* in the column trace control area with the current trace for that column. By manipulating the *yellow buttons*, the user can change which trace IonWizard exports in the indicated column. These *yellow buttons* will only allow selection of traces that are marked for viewing via the Trace Types menu (see [§Trace Type Suppression](#) [51] for more information).

By using the *d/dt* and *Filter push-buttons* to the left of the *yellow buttons* the user may specify that the export column contain the traces derivative or that the trace be filtered. Each export column of may

have independent settings for the Filter and d/dt operations.



All column trace selection, column addition and column deletion is disabled for a fast export. The export column trace content is entirely defined by the traces in the trace bar.

If the new trace has a different time base than the trace in the previous column, IonWizard creates a new time block by inserting a new time column and then the desired trace. As long as subsequent traces use the same time base, no new time columns will be added. A time block is thus defined as a time data column followed by a series of amplitude data columns. Each of these amplitude columns is plotted against the times in the first column. To minimize the number of time blocks, and hence export columns, keep traces of like time base together.

IonWizard also supports exporting other data such as marks and pauses. These features are controlled by the [Trace Export Options](#) ^[100] dialog box which is explained below.

Options and Configurations

The Options... push button will bring up the [Trace Export Options](#) ^[100] dialog box where the user can make many settings such as the label location and the number of decimal places. This dialog box is explained below.

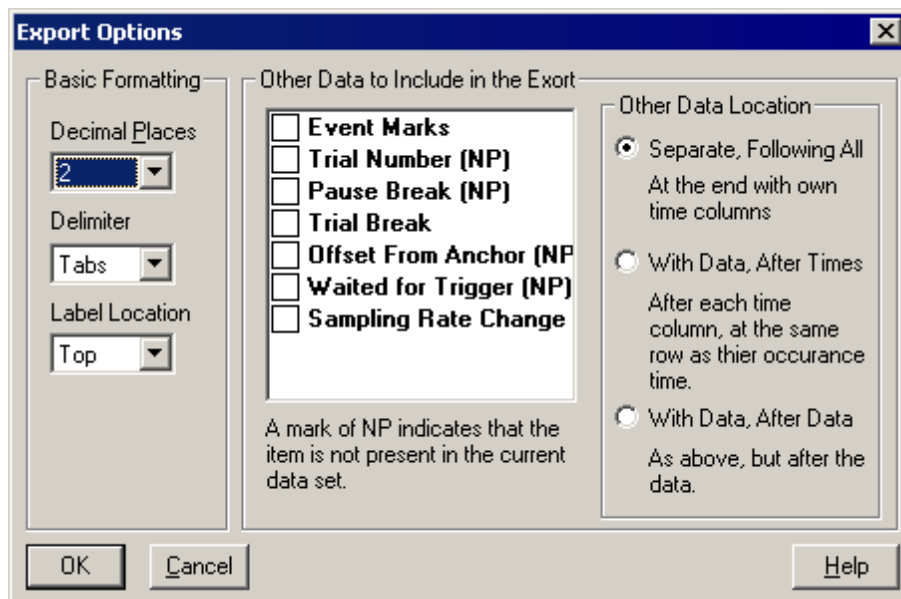
Once the traces and export options have been selected, the user may wish to save the configuration. Clicking the Save push button will invoke the Windows® Save dialog box allowing the selection of a path and filename for the configuration file. This configuration may be loaded again at any time by clicking on the Load push button and selecting the appropriate file. The configuration file contains all the traces selected, their filter and derivative status, and all the options selected in the [Export Options](#) dialog box.

As stated before, the *yellow buttons* in this dialog box obey user choices made under the [Trace Types](#) sub-menu of the [Traces](#) menu and display only selected types of trace data. If a configuration file is loaded that contains trace types currently not selected for viewing, these columns will be dropped. If this occurs, IonWizard will notify the user that some columns were rejected.



Configuration loading and saving is not available for fast exports. All options are instead saved in the users [profile section](#) ^[103].

9.1.1 Trace Export Options



Trace Export Options Dialog Box

The [Export Options](#) dialog box allows configuration of the trace export.

Basic Formatting

The basic formatting options control how the actual export file is written.

- Decimal Places** select anywhere from zero to six decimal places of accuracy for the numeric values in the export table.
- Delimiter** select delimiter used between columns in exported file: tabs or commas.
- Label Location** select where the column labels are located. Column labels may be located at the top of the column, at the bottom of the column, or the user can choose to have no labels at all.

Other Data

Any of the items in the checked list box may be selected for export along with the numeric data. A check indicates that a given line is currently included for exporting. To change the status of an item's check, simply double click on that item with the left mouse button. There are three types of extra output:

- Event Marks** The text of both user and external Event marks will be included.
- Time Discontinuities** There will be one line for each type of time discontinuity present. For example, pauses and sampling rate changes may be individually exported.
- Trial Numbers** If there are trial discontinuities, IonWizard can export the trial number next to each row of numeric data.

Additionally, the user has three options as to where in the export file this extra output will appear.

Separate, Following All option is not valid when exporting trial numbers as trial numbers are intrinsically 'With Data' since the trial numbers are exported after every data point. Choosing this method of export provides that marks and time discontinuities will be exported in the very last columns of the export table. Both the mark and time discontinuity columns will have their own time column.

With Data, After Times Indicates that the while the marks and discontinuities will remain in separate columns, no extra time columns will be added. Instead, this data will appear at the beginning of each time block, immediately after the time column. The text labels that constitute the extra export will appear next to the time at which they occurred.

With Data, After Data This is the same the With Data, After Times option, except that the additional columns appear at the end of each time block, following the amplitude export columns.

9.2 Image Exporting

IonWizard supports exporting image array data via a proprietary binary format. You will need to write a program or a script to import this data into your third party analysis tool. To export the image array in the *movie player* in the active *view unit*, select Images... from the Export menu (see [\\$Movie Players](#)^[42] and [\\$View Units](#)^[36] for more information). This will bring up the Image Export dialog box shown below. If there is not currently *active movie player*, the *menu item* will be disabled.

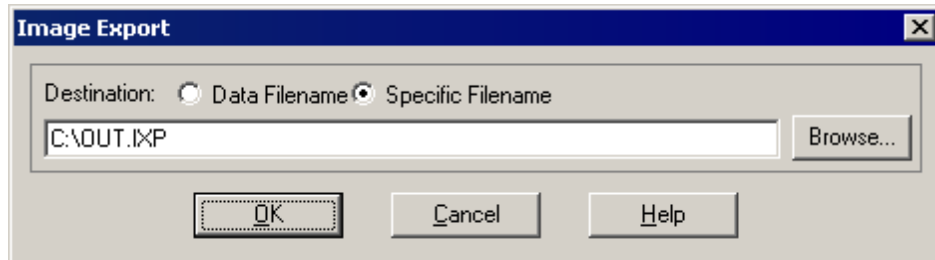


Image Export Dialog Box

The Destination section at the top of the *dialog box* determines where IonWizard writes the export data. If Data Filename is selected, the filename will default to the full path and filename of the data file with the normal data file extension (ZPT, etc) replaced with TXT. If Specific Filename is selected, the filename will default to the last specific filename you entered. In either case you can modify the filename manually as needed. Information in the Destination section is saved in the users [profile section](#)^[105] and thus is remembered from export to export.

If you click OK, IonWizard will all images in the *selected view* time range for the active *view unit* (see [\\$Trace Viewers](#)^[39] for more information on the *selected view*). The layout of this binary file is as follows.

Header

The file begins with a fixed size header.

File Offset	Element	Size (bytes)	Type	Description
0	Magic Number	2	unsigned	ID for this file, must be 0xF01C
2	Version Number	2	unsigned	File version number, must be 1.
4	Num_Images	4	unsigned	Number of images after header.
8	XDim	2	unsigned	Width of images.
10	YDim	2	unsigned	Height of images.
12				Beginning of images

Images

Starting at offset 12 in the file we have the data for Num_Images images. Each image has the following layout. The offsets are obviously not file absolute but rather from the beginning of each image data block.

File Offset	Element	Size (bytes)	Type	Description
0	Time	8	double	Time in seconds for the image (taking into account the current time mode ^[53]).

8	Pixel Bit Size	1	unsigned	Bits per pixel value: 8 or 16.
9	Divisor	1 or 2	unsigned	Divisor for the pixel data. Actual data values will be raw data/divisor
10 or 11	Raw Pixel Data	1 or 2 * XDim* YDim	unsigned	Raw image data.

All values given above can vary from image to image. In particular, the pixel bit size can change which will naturally impact memory allocations and the amount you need to read from the file.

10 Templates

One of the central features of IonWizard is the ability to tailor the data presentation to the preference of individual users. This is accomplished by creating data configuration templates specific to a user's task. These templates are complete descriptions of the various trace presentation and data conditioning choices.

Once IonWizard is optimized for a particular analysis task, the user can create a template which saves these settings and becomes associated with the user's name. In fact, each user can have multiple templates in their template list to allow quick alternation between analysis or acquisition "environments".

There are two classes of templates, [screen templates](#)^[107] and [analysis templates](#)^[109] which are described in sections below.

In addition to templates, IonWizard stores a [profile section](#)^[105] for each user, also described below.

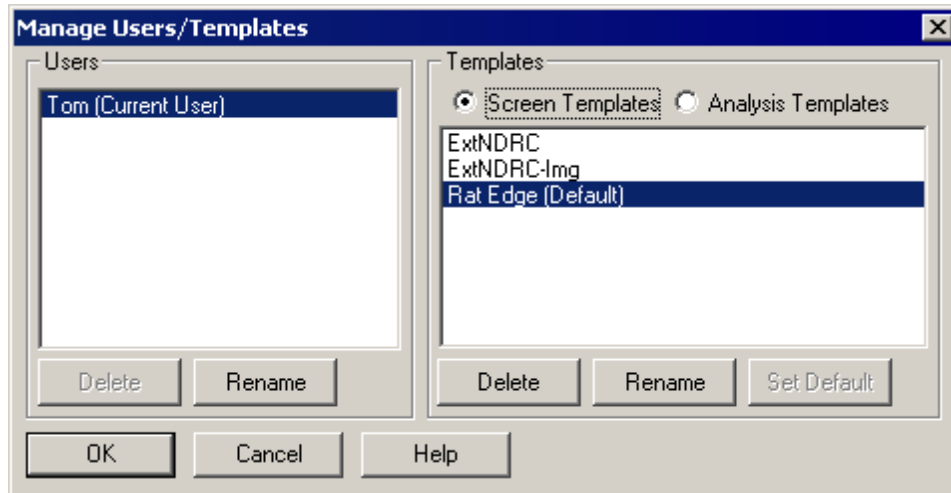
10.1 Profile Section

Profiles are used to store information about the state of a program such as the placement of windows, or the last settings in dialog boxes, after the program has exited. This information is used to restore the program to that state when it is run again at a later time. The user's profile section is not part of the named template list, nor does the user have direct control over the information stored there. When the user has completed a task, IonWizard writes information about the exiting state of that task to the profile section. If that task is done again in the future, the settings previously used will be restored. In this way, the user effectively sets the default options for a given task by their own actions. Following is a partial list of things stored in the profile section:

- the size of any resizable *dialog boxes*,
- options in the [Transient Mark Acquisition](#)^[64] *dialog box*,
- the [duration](#)^[70] of last added transient mark,
- the [current printer](#)^[25],
- the current [trace printing options](#)^[90],
- the export options for fast trace export and destination options for all [trace exports](#)^[98] and
- the destination options for [image exports](#)^[102].

10.2 Managing Templates and Users

To manage users or their templates, select Manage... from the Template menu. This brings up the Manage Users/Templates dialog box as shown below.



Manage Users/Templates dialog box

Users

The Users section on the left lists all the users which have saved settings. By selecting a user from the list box, their templates are loaded into the Templates section on the right.

The Delete and Rename push buttons in the Users section allow you to delete a user or to rename a user. If you delete a user, you will also delete all of their templates and their profile section.

Templates

The Templates section lists the templates for the user currently selected in the Users list box on the left. Use the Screen Templates and Analysis Templates radio buttons to select which template set is being edited.

The Delete, Rename, or Set Default push buttons can then be used as appropriate to modify the template list.

Note that when analysis templates are being edited, the Set Default option is disabled. This is because there is no concept of the default analysis template. Analysis templates are applied only by explicit user command.

10.3 Screen Templates

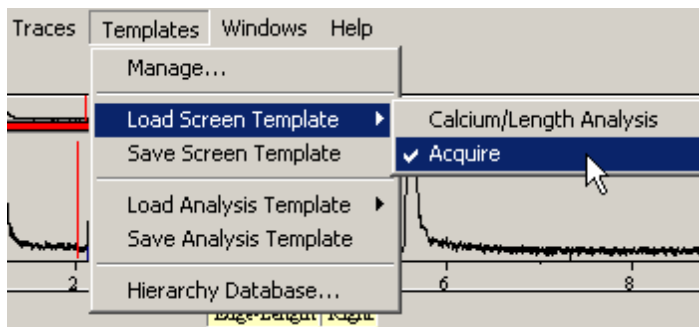
Screen templates gather together a variety of settings that apply to the way that things are presented to the user. These settings do not include anything that impacts on the actual calculation of the data values, such as calibration constants, filtering, and so-forth. A partial list of items saved in screen templates includes:

- Trace viewer options including:
 - trace selection,
 - visibility state,
 - filtering/derivative settings,
 - full view height and
 - full view snaps;
- Movie Player Options including:
 - movie player width and
 - Image color maps;
- User limits for traces and images;
- User or automatic limit selection;
- [Mark visibility](#)^[62] and [primary mark settings](#)^[63];
- Excluded traces.

10.3.1 Loading a Screen Template

To load an existing screen template select the Load Screen Template menu item from the Templates menu.

This will cause a list of all templates to appear with a check mark identifying the current (i.e. the last applied) template as shown below.



Loading a new Screen Template

Selecting any one of them will load that template and redefine the visual settings applied to the data currently being examined. This template will now become the current template and will be applied to the visual aspects of all files subsequently opened or created.

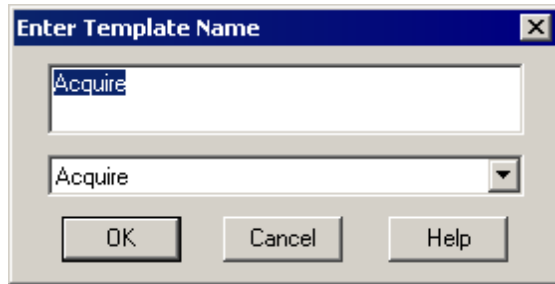
Note that screen templates are used to return the visual state to a preset configuration. They are not persistent in the sense that they do not enforce that configuration after they have been loaded. Thus after loading a screen template, you are free to change any settings you wish.



The traces displayed in a screen template are matched to tasks using the task name. If you load a template and the task name can not be found, either because it has been renamed or because it is not part of the current experiment, the "default" trace will be displayed.

10.3.2 Making\Saving a Screen Template

To save a screen template or create a new screen template, select Save Screen Template from the Template menu. This will invoke the save template *dialog box* as shown below.



Save Screen Template dialog box

The name in the *edit field* at the top of the *dialog box* will become the name of the new template. If that name is the name of an existing template, then that template will be updated. Otherwise, a new template will be created. The name of the current template is entered by default. Any template name selected from the *combo-box* will be loaded into the *edit field*.

To create a new template, type a unique name into the *edit field*. A template name may reflect the data that it was designed around, or the current task at hand. Recall that if the name in the *edit field* matches a name in the *combo box*, that template will be updated.

Click OK to update the template list. This template, whether created or updated, will become the current template used for subsequently opened or created data.

10.4 Analysis Templates

Analysis templates are a new feature for IonWizard 6 and reflect a change in the way we handle data. In older versions of IonWizard, data analysis settings such as trace filtering options or transient analysis options were considered "visual" settings not unlike the user range settings or trace colors. It was realized that that view was a mistake because it broke one of our fundamental rules, namely you should always be able to open a file and get back the exact same data values as the last time you opened it. Treating analysis settings as visual elements and relying on the screen template mechanism made it basically impossible to restore a data file that had any significant filtering applied. How would the user know for sure that the screen template (and thus filtering values) had not been changed since the last time the file was opened? The user could write such information down somewhere, but that is a fundamentally error prone (an annoying) solution.

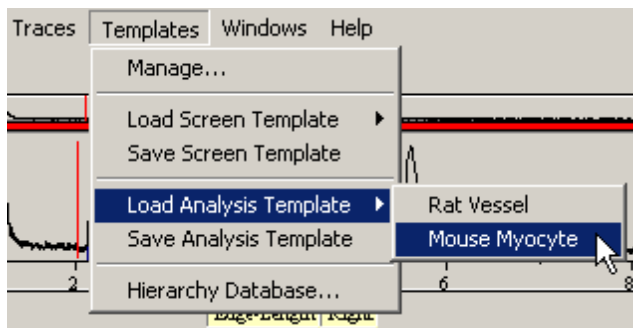
A better solution is to treat analysis options rather like calibration constants and save them with the file. This way, the data always returns to the previous state when a file is opened. Therefore, in IonWizard 6, filtering and trace analysis options are no longer a part of the screen template and instead are saved with the file.

To ease analysis, we introduced the concept of analysis templates. These are similar to screen templates in that they allow the user to capture all the analysis settings in use at a some moment and give them a name so that they can be applied in one fell swoop to any other file.

However, they differ from screen templates in that there is no concept of a "current" analysis template that is automatically applied when a file is opened. Instead, the file always uses whatever analysis options it was last saved with. This prevents silent changes to the data file from happening solely from the act of opening a file.

10.4.1 Loading an Analysis Template

To load an existing analysis template select the Load Analysis Template menu item from the Templates menu. This will cause a list of all analysis templates to appear as shown below.



Loading a new Screen Template

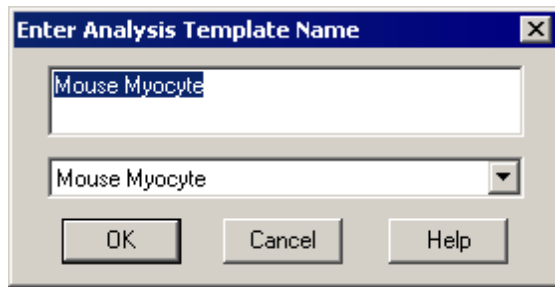
Selecting any one of them will load that template and redefine the analysis settings applied to the data currently being examined. However, unlike screen templates, analysis templates are never automatically applied to files as they are opened. This is because the analysis settings are considered part of the data set and are therefore not changed without explicit user action.

Note that analysis templates are used to return the analysis state to a preset configuration. They are not persistent in the sense that they do not enforce that configuration after they have been loaded. Thus after loading an analysis template, you are free to change any settings you wish.

Loading an analysis template will mark the data file as modified and you will be prompted to save changes on exit.

10.4.2 Making\Saving an Analysis Template

To save an analysis template or create a new analysis template, select Save Analysis Template from the Template menu. This will invoke the save template *dialog box* as shown below.



Save Analysis Template dialog box

The name in the *edit field* at the top of the *dialog box* will become the name of the new template. If that name is the name of an existing template, then that template will be updated. Otherwise, a new template will be created. Any template name selected from the *combo-box* will be loaded into the *edit field*.

To create a new template, type a unique name into the *edit field*. A template name may reflect the data that it was designed around, or the current task at hand. Recall that if the name in the *edit field* matches a name in the *combo box*, that template will be updated.

Click OK to update the analysis template list.

10.5 Hierarchy Database

The hierarchy database is another new feature for IonWizard 6. This is designed to solve a long standing problem with the identification of traces and images in IonWizard.

The problem quite simply is how does one differentiate in a meaningful way between two different traces? In the past we simply used the collection method as an identifier. We call these identifiers *type tags*. Collection method type tags might include METHOD_DUAL_EXCITATION, METHOD_DUAL_EMISSION, METHOD_ANALOG, and so-forth. These type tags are used to identify data traces (and images) for purposes such as saving screen templates where the user desires particular traces in the trace viewers, or where the user wants specific user ranges for specific traces.

Unfortunately, the collection method type tags approach falls on its face whenever there is a data file where more than one recording source used a given collection method. The most common culprit being multiple analog recording sources. Often people will collect multiple analog traces, for example temperature and pressure. In some cases, people are collecting only analog data. With the collection method type tags approach, one could not save a template with, for example, pressure in trace viewer one and temperature in trace viewer two. When they opened a such a file with their saved template, all trace viewers would show up with either pressure or temperature because they both were tagged with METHOD_ANALOG.

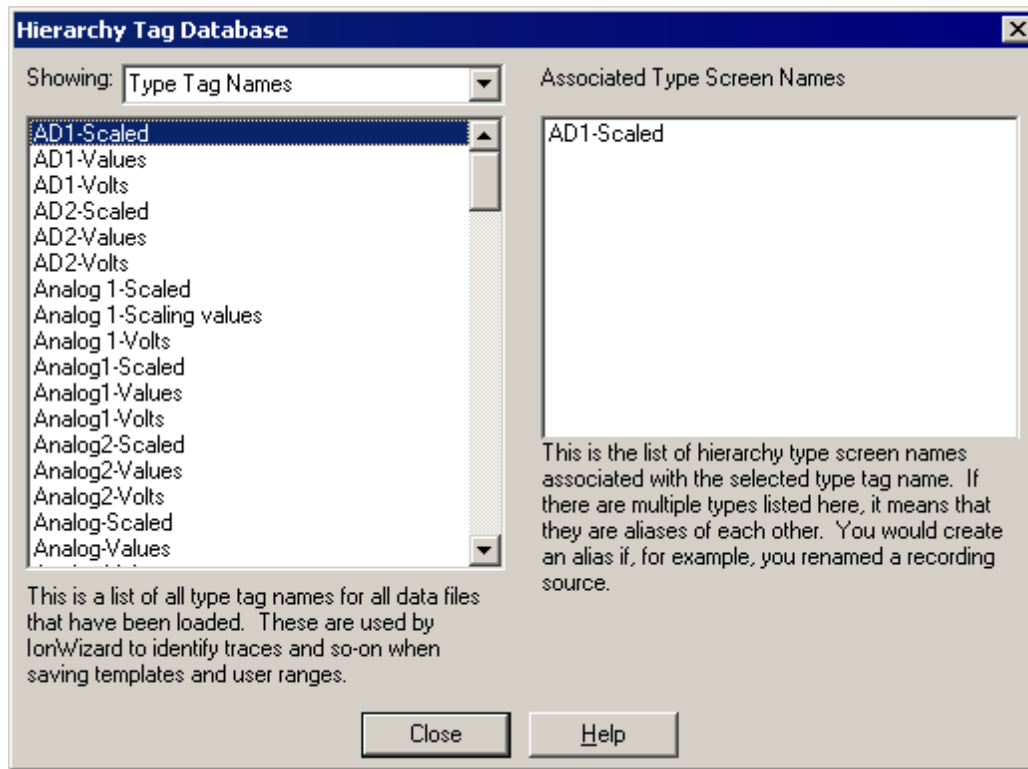
To solve this problem we have now introduced the idea of a hierarchy database. In this system, we use the strings entered by the user when collecting data as the type tags. Thus if you set up your data acquisition to collect from a dual excitation recording source called Fura2 Ca 40x, Fura2 Ca 40x becomes a trace type tag. IonWizard maintains a database of all the type tags it has seen in the template file. In this way, an arbitrarily large number of types can be accommodated simply by the act of collecting data and opening files.

Unfortunately, this extreme flexibility is also a weakness. Since the string entered by the user entirely defines a data type tag, you can very easily create aliases of a single data type by slightly editing the string. Say for example you created a number of files using a Fura2 Ca recording source using a 40x objective. When you started using a 20x objective, you realized you needed a second recording source because the calibration constants change as a result of the new optical path. Thus you add a Fura2 Ca 20x recording source and rename your old recording source to Fura2 Ca 40x. This act will result in three different trace type tags in the hierarchy database, Fura2 Ca, Fura2 Ca 20x, and Fura2 Ca 40x. The difference between the 20x type and the others may well be ok as we could expect the user ranges for the 20x and 40x raw data traces to be different based in the higher numerical aperture of the 40x objective. However, there is no difference at all between the Fura2 Ca and the Fura2 Ca 40x traces. They are aliases of each other. But without some sort of alias mechanism, the program will maintain them as different types and thus keep different user ranges and so-on.

To handle this situation, the hierarchy database is actually a map. It takes a name read directly from a file or a new experiment, the type screen name, as the input to the map and then produces a second name, the type tag name, based on the map relationship. In an environment with no aliases, the screen name and the tag name is the same. However, if an alias is created, we can map the new type screen name alias to the old type tag name. This allows us, for example to map the new screen name Fura2 Ca 40x to the existing tag name Fura2 Ca. These maps are viewed and edited with the [Hierarchy Database Editor](#)^[11].

10.5.1 Hierarchy Database Editor

To view the hierarchy database, select [Hierarchy Database...](#) from the [Templates](#) menu. This brings up the dialog box shown below.



Hierarchy Tag Database editor

The left *list box* can show either all the type screen names or all the type tag names (see the [previous section](#)^[11] for definitions) in the tag database. The right hand list shows the associated tag or screen name as appropriate. Use the *Showing: combo box* to switch between being able to select from a list of screen names to view the associated tag name, or from being able to select from a list of tag names to view the associated screen name(s).

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