

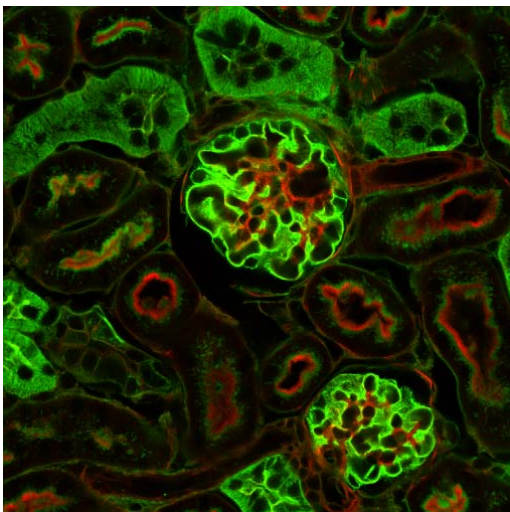
Image-Pro Plus[®] Product Note

Managing Image Sets

Introduction

Microscopy applications are becoming increasingly demanding. As software and hardware automation become more affordable and easily accessible, researchers are looking to perform more elaborate imaging procedures, resulting in potentially complex image sets. And as a result, applications requiring through-focus stacks, time-lapse, multiple wavelengths and multiple stage positions need a specialized means for handling image data.

Image-Pro Plus version 5.0 and later contain a trio of tools that allows you to manage, manipulate and process image data in an easy to understand format. These tools are collectively known as the Set Manager and consist of tools for creating image sets, extracting image variables and providing information about each of the image sets being analysed. Multiple variables such as wavelengths, time-points and stage positions may be extracted, and then analysed separately from the other variables. Extracted data may be saved independently, merged with other image data from the same set, or even merged with image data from other sets. Image stacks acquired from imaging devices such as confocal microscopes may also be imported and analyzed through the Set Manager tools.



Bovine Kidney Tissue. Glomerulus- Red (Alexa 568). Proximal and Distal Tubules- Green (Alexa 488). Courtesy of Brian Matsumoto, University of California- Santa Barbara.

The Set Manager tools are especially useful with Media Cybernetics automation modules like Scope-Pro and AFA[™]. These modules are capable of generating a large amount of image data, and the Set Manager greatly simplifies its management. These tools are also extremely useful in the management of image data acquired through third party hardware and software.

Applications and Examples

The three components of the Set Manager include Set Manager, Set Navigation and Set Info. They are found in the **Sequence** drop-down list. Comprehensive details for the commands may be found in **Help|Set Manager**.

In most cases, the acquired image data will be presented to Image-Pro Plus as a single stack of image planes. The stack will contain different parameters such as multiple fluorescent wavelengths, different focal depths for through-focus acquisitions, etc. Deciphering this information and how to logically put it all together can lead to confusion. So the Set Manager tools are really useful for putting all the different parameters together in a straightforward manner.

Here are the three tools that help you manage the image data you are generating-

Set Manager gives you the opportunity to tell Image-Pro Plus about image set information. This is especially useful if the set is being imported from a third party application. If multiple wavelengths compose part of the set, you will also have the opportunity to name each channel to reflect the fluorochrome used. If the set was acquired through the use of the modules that make up the Advanced Microscopy Suite, information about the set will automatically be included with the image stack. For example, an image set may be comprised of variables such as wavelength, through-focus (z) position and stage (x/y) position. You can give Set Manager more information about the set by breaking it down into its sub-components. First, a component that identifies the wavelengths/fluorochromes being

used. Second, a component that identifies the number of z- planes acquired for each wavelength. And finally, the location on the slide from which the z-planes were acquired.

Set Navigation is quite useful for extracting information about one or more of the components identified in the Set Manager. A Navigation window will appear that allows you to view individual aspects of the set (i.e. wavelengths, image planes from a specified location within the image stack, a particular x/y position that has been captured, etc.). Use of the Set Manager lets you extract information from the acquired data set and view it separately. In this fashion, you can view events occurring in a particular dimension or combination of dimensions (for example, time points in a particular wavelength or position) without any other set information present.

Set Info provides information about the set that is currently active in the workspace. Dimension information such as x, y, and z positions, z-plane position, time points and wavelength can be displayed here. In addition information about the objective lens (numerical aperture, refractive index of immersion media, magnification, etc.) can be displayed in this view as well.

Implementation

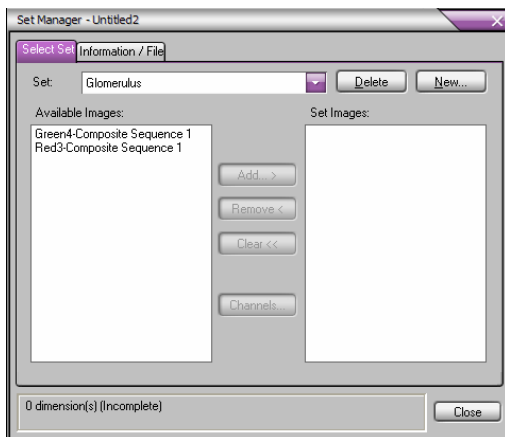
Sequence|Set Manager...

Sequence|Set Navigation...

Sequence|Set Info...

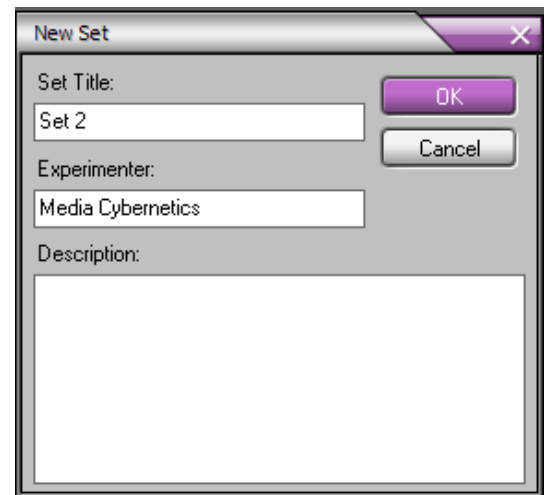
Set Manager

- Click Sequence|Set Manager... The Set Manager dialog box appears, and the 'Select Set' tab will be active:

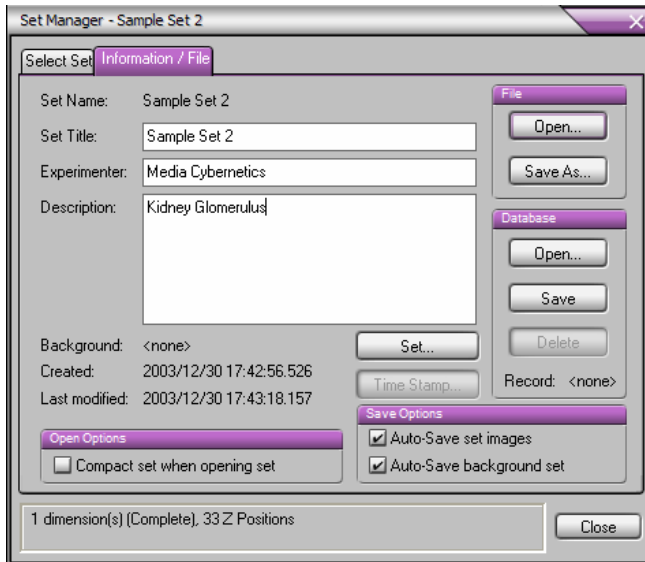


The Set Manager Dialog Box

- If an image created using the Advanced Microscopy Suite is open in the workspace, it will appear in the **Available Images** list.
- If an image set was not created using the Advanced Microscopy Suite, you will need to add it to the set.
 - Click the 'Select Set' tab to create new image data sets.
 - Click 'New' to create a new image set. The New Set Dialog Box appears:



- Type in a name for the set, the name of the person creating the set and any information that may describe the newly created set.
- Click 'OK' to create the new set information.
- Each available image you add to the set will then become part of the Set Images.
- Click the 'Information/File' tab. Text information generated when creating the new set will appear in the appropriate fields:



The Information/File Tab

The 'File' Group allows you to save and recall image sets to and from files.

- Click '**Open...**' to open a previously stored image set from a file.
- Click '**Save As...**' to save the image set to a file.

The 'Database' Group allows you to save and recall image sets to and from databases, including IQBase.

- Click '**Open...**' to open a previously saved set from a database.
- Click '**Save**' to save the set to a database.
- Click '**Delete**' to remove a selected set from a database.

The 'Save Options' Group gives you the opportunity to automatically save image set data.

- Check 'Auto-Save set images' to automatically save a set once it is created.
- Check 'Auto-save background set' to save any background set images associated with an image set.

The 'Open Options' Group manages image planes that may be stored individually, but later compiled with other individual planes to create an image set.

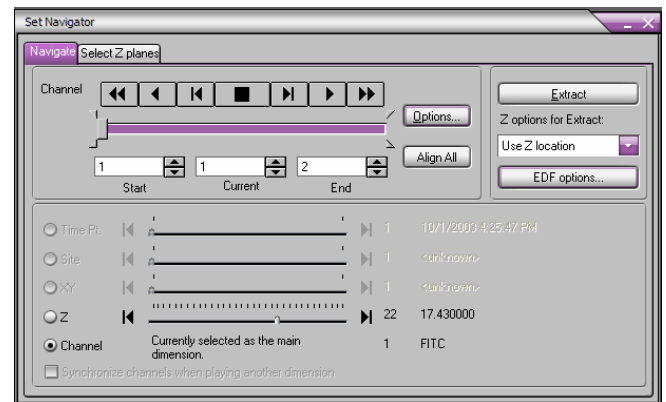
- Click 'Compact set when opening set' to merge individual planes from the 'Set Images:' list into an image stack. The stack data may then be managed using the **Set Navigator**.

Other options are also available to help you manage your set data.

- Click '**Set...**' to specify from which set to apply background images (if necessary).
- Click '**Time Stamp...**' to place a time stamp on the images that comprise the set.

Set Navigation

- Click Sequence|Set Navigation... The Set Navigator appears:



The Set Navigator

The Set Navigator contains five separate 'dimensions' each of which can be selected as the active dimension. These include:

- Time Points
- Well locations/sites (for microtiter plates)
- X/Y positions (for sets containing images acquired from multiple positions on the slide)
- Z-position (for sets containing z-stacks)
- Channels (which represent the different fluorescent wavelengths collected during an acquisition)

When you click the radio button next to the dimension of interest, it becomes the 'active' dimension. You can then move through this dimension by using the Sequence Toolbar (the sequence player at the top of the box).

Any other dimension may also be navigated by using the slider associated with that dimension. Any dimension that has not been specified during the acquisition process will not be active within the Set Manager.

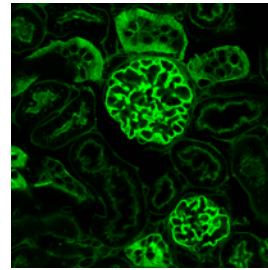
Click the 'Navigate' tab. The sequence toolbar will be active with the current dimension (z-plane, x/y position, etc.).

- Click the arrow buttons to move through the currently selected dimension.
- Click 'Extract' to create a new image stack representing only the selected dimension. If the image set contains z-plane data, the 'Z-options for extract' drop-down list will be active.
 - 'Use selected planes' will extract the planes in the currently selected dimension (a dimension other than z).
 - 'Use z-location' will extract z-plane information for the same z-position in any other color/wavelength present in the image stack.
 - 'Use best focus plane' will return the most "in-focus" plane for the currently selected dimension.
 - Use 'Composite best-focus' will create a composite image of the planes in the currently selected dimension.
- Click 'Options' to adjust the playback of the selected dimension. You can speed up the playback, slow it down, auto-reverse, and 'wrap-around' the playback to the start.
- Click 'EDF Options...' to activate the Extended Depth of Field function. More details for this function may be found in the EDF Product Note and white paper found on our website- www.mediacy.com.

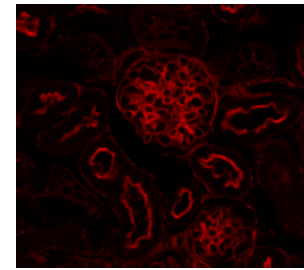
An Example

A typical set will contain channel information representing two or more fluorochromes and z-plane information representing an image stack. Acquisitions through confocal microscopes are perfect examples of how a set of this type might be presented to the Set Navigator.

Let's say the image set contains two separate stacks of data. Each stack represents 33 image planes and a single fluorochrome. The stacks will be imported into Image-Pro Plus as a single stack- the first 33 image planes will represent fluorochrome #1 and the next 33 planes as fluorochrome #2. A total of 66 images planes are present:

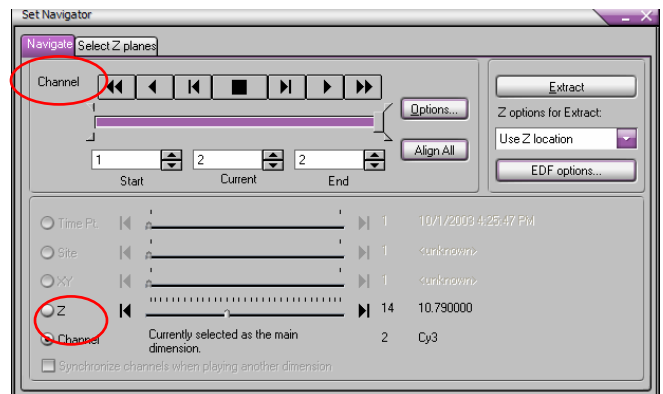


Planes 1-33- Green

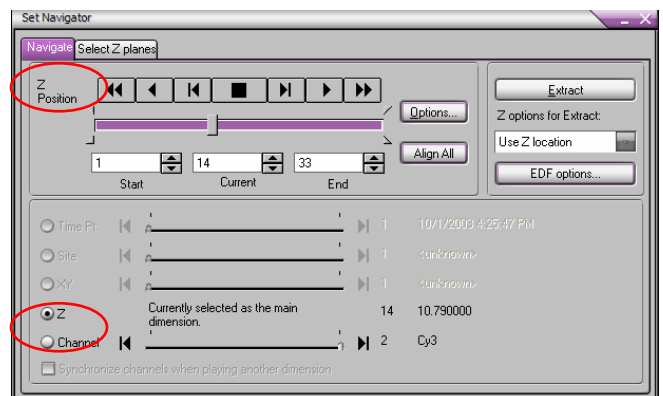


Planes 34-66- Red

- Click the 'Channel' radio button, to independently view either fluorochrome. Use the Sequence toolbar to flip back and forth between the locations in the stack that represent the two fluorochromes:

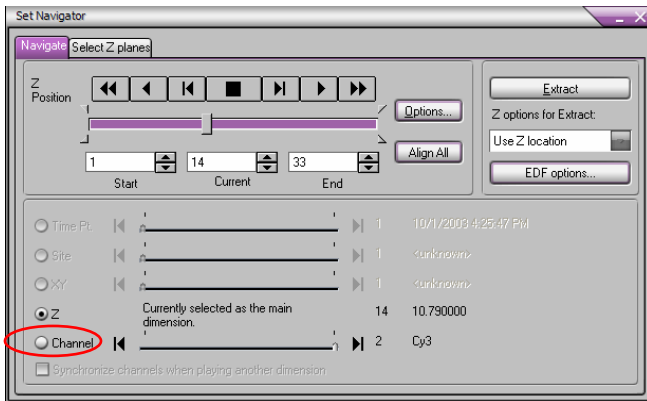


Click the 'Z-stack' radio button to play through the stack using the sequence toolbar:



Even though one dimension may be active over others, it is still possible to view information in other dimensions. For example, you may be looking at the image stack and see something of interest in a particular plane of interest. While still maintaining the plane of interest in the workspace, you can switch views to the other fluorescent channel and look at the equivalent image plane.

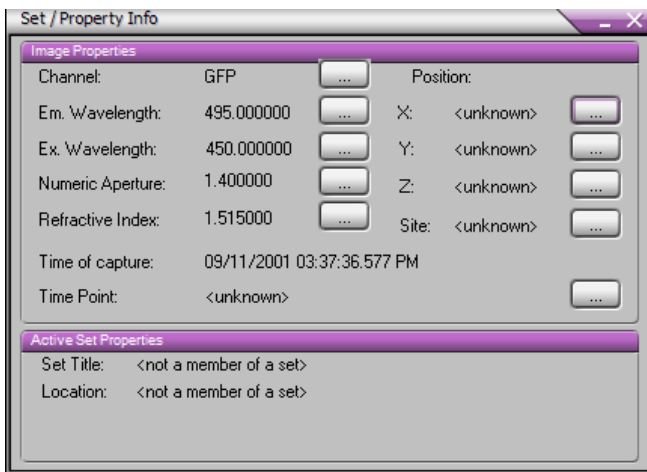
- To view dimensions other than the active one, just adjust slider next to the available dimension of interest.



- To separate the larger stack into individual dimensions, select the dimension of interest and click 'Extract'. A new stack representing only the selected dimension will be created.

Set Information

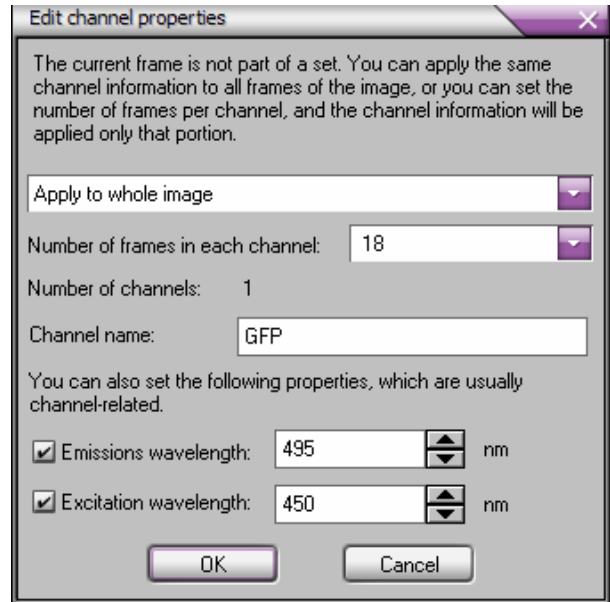
Click **Sequence|Set Info...** The Set/Property Information box appears:



The Set Information Information Dialog Box.

The attributes associated with the image and its acquisition environment may be examined and modified in this dialog box.

- Click the '...' button adjacent to the attribute that requires editing. A dialog box will appear. The exact contents of the box will be slightly different depending upon which attribute you are editing:



The 'Channel' Property information window.

You may apply properties a number of ways. Table 1 details how the attributes may be applied.

Property	Apply to Current Channel	Apply to Whole Image	Apply to Only the Active Frame	Apply to Entire Set
Channel	•	•	•	
Emission Wavelength	•	•	•	•
Excitation Wavelength	•	•	•	•
Numerical Aperture	•	•	•	•
Refractive Index	•	•	•	•
Time Point	•	•	•	•
X,Y,Z Positions	•	•	•	•

Table 1. Assigning Image Properties to Sets

Explaining Image Properties

Apply to Current Channel- Applies the specified value to the channel that is currently active in the Set Manager.

Apply to Whole Image- Applies the specified value to all the planes in the active workspace. A workspace is an acquired set of images that can included multiple image parameters such as wavelength, x,y and z positions, objective lens information and so on.

Apply to Only the Active Frame- Applies the specified value to only the active frame.

Apply to Entire Set- Applies the specified value to any image set that has been assigned to the set. Sets are created using the Set Manager. Information about

creating sets may be found in the **Set Manager** heading, above.

See Also

Media Cybernetics Product Note- Extended Depth of Field

Media Cybernetics White Paper- Extended Depth of Field

Related Products

IQBase™ Relational Database for Image Informatics

Scope-Pro® Plugin for Image-Pro Plus and Image-Pro Discovery

Advanced Fluorescence Acquisition (AFA) for Image-Pro Plus and Image-Pro Discovery

Image-ProPlus MDA and AMS (Multidimensional Analysis and Advanced Microscopy Suite)

How to Order

For more information on Image-Pro Plus, and to locate a Media Cybernetics' reseller in your area, visit our website at www.mediacy.com.

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