SPOT | Advanced Plus

SPOT™
Software
version 4.7
Guide

Advanced Plus Supplement

Advanced Plus Features

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Intensity Display Scaling

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Preservation Protection

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V4.7.0

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About This Manual

Introduction

This software user guide is designed as a supplement for the SPOT Software User Guide for version 4.7 and contains instructions for using the *4.7 Advanced Plus* version of SPOT software with the following camera lines: Idea, Insight, RT, Flex, Xplorer, Pursuit, and Boost. Some features described in this manual apply only to specific camera lines, or to specific types of cameras within those lines. In these cases, the camera line or type of camera is listed in parentheses after the feature name.

A PDF (portable document format) version of this document has been included on the CD that contains the SPOT software. Refer to your installation manual for installation procedures and technical specifications specific to your particular camera line.

Document Conventions

The following is a list of the textual conventions used in this manual:

Notes

> *Note: This is an example of a note. Notes appear within the text to indicate information for further consideration or reference.*

Cautions

> **CAUTION!**
> **This is an example of a cautionary note. Cautionary notes appear to indicate that you should carefully consider the implications of an action.**

Warnings

> **⚫☀WARNING⚫☀**
>
> THIS IS AN EXAMPLE OF A WARNING. WARNINGS INDICATE THAT THE ACTION YOU ARE TAKING COULD EITHER CAUSE INJURY TO YOURSELF OR COULD HARM YOUR SYSTEM

Organization of the Manual

The *Spot 4.7 Advanced Plus Supplemental User Guide* is structured as shown in the table below. In addition to the chapter names and descriptions, the table indicates whether the chapter applies to the Basic program mode, the Advanced program mode, or both:

| Chapter	Description
About This Manual	Describes document conventions, manual structure.
Ch. 1 – Program Features	Describes SPOT Advanced Plus Features in addition to SPOT Advanced program features in order of their menu placement.
Ch. 2 – Boost Features	Describes features available for Boost cameras.

Chapter 1 - Program Features

This chapter outlines the features which are unique to the SPOT Advanced Plus software package as they appear in the menu listings.

File Menu

Duplicate Image

Creates a duplicate image so that a copy is available to further manipulate, or you can create a duplicate of the original image after manipulation. If changes have been made to the display of the image, you will have the option to either duplicate the raw, actual image data or duplicate the displayed image data. You can also set the bit depth of the newly created image and, if any annotations, calibrations, or measurements have been added to the image, you can choose to Merge all of these objects to the image. The option to Merge Objects in layers is only available when selecting As Displayed.
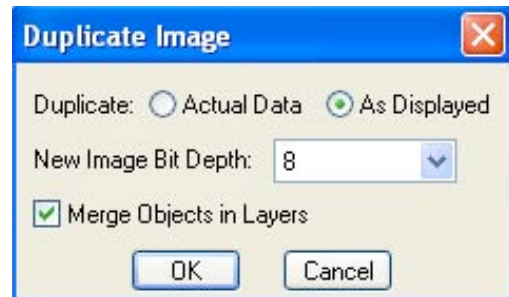
Create Extended Focus Image

This feature allows you to create one single best image from a series of images where the focus, contrast, or intensity may not be even across a single plane of focus. You can use either the Entire Image Area or a Selected Area from which to create the Extended Focus Image.

If the Entire Image Area is selected, the entire image data will be used with the selected Method for creating the Extended Focus Image.

To use a Selected Area, make this selection in the Create Extended Focus Image dialog box and then use your cursor to click drag a rectangular selection on one of the open images. Only the pixel data within this area will be used for creating the new Extended Focus Image. The entire original image area will still be displayed while the Selected Area will reflect the method chosen for creating the Extended Focus Image. There are three Methods available for creating an Extended Focus Image:

- Best Local Contrast-The best contrast at each point, or neighborhood, is analyzed throughout all indicated images and the best contrast image data is used to create one final image. The user has the ability to set the size of the neighborhood for this process. A grid of pixels either 3X3, 5X5, or 7X7 in dimension can be used.

- Best Local Image-The single image, from all indicated images, with the best overall contrast will be used to create a new image file.

- Maximum Projection-The brightest pixel data at each pixel position throughout all indicated images will be used to create one final image.
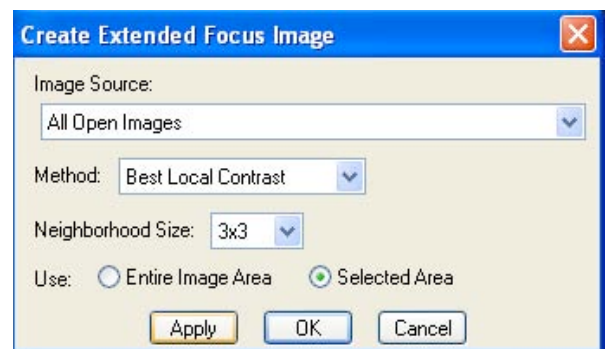
Export Pixel Values

Exports and saves the image data to either a binary or text format file. If only a selected region of the image is desired, this area can be selected prior to accessing this feature and using the 'Selection' option. If a selection is not indicated on the image prior to accessing the Export Pixel Values feature, the option for 'Entire Image' or 'Selected Area' will be unavailable and grayed out.
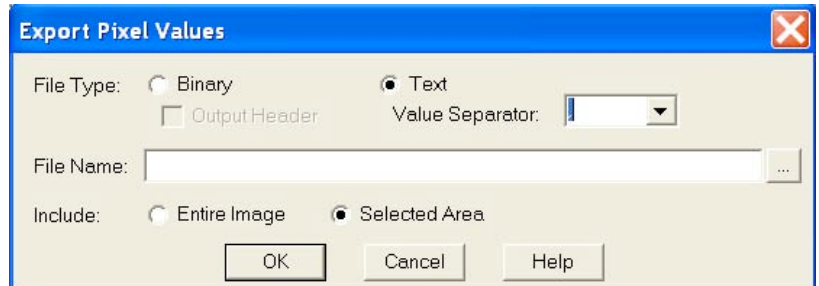
Edit Menu

Preserve Pixel Intensity Data

When this feature is activated, the actual image data will be preserved throughout image manipulations. Preserve Pixel Intensity Data can be set in the Image Setup to ensure data integrity for all captured images. Also, under the Edit menu, you can make the selection to activate or de-activate the Preserve Pixel Intensity Data feature.

When turned on, certain functionality will be disabled in the software so that the raw pixel data can not be altered. If "Preserve Pixel Intensity Data" has been selected for an image and you de-activate this feature from the Edit menu, you will be asked to verify this choice. Once Preserve Pixel Intensity Data is turned off, any changes made to the way the image is displayed will permanently change the pixel data which is saved.

Set Intensity Display Scaling

This feature is accessible from the menu command or by clicking on the icon ➡ on the Image Display Settings Panel (see View menu section).

Any changes made to these settings will only change the way an image is displayed as long as Preserve Pixel Intensity Data is activated for the image.
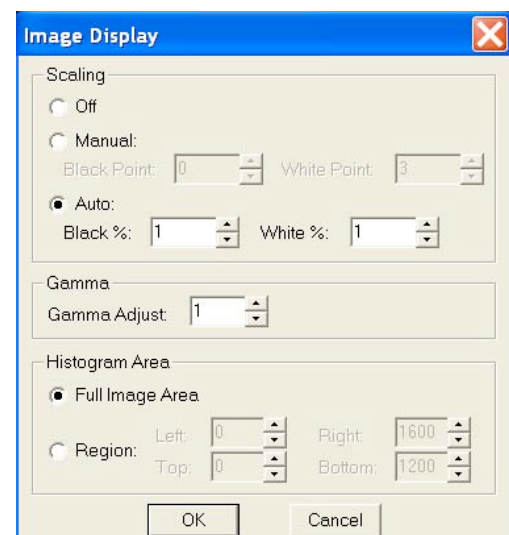
- Scaling-

    Off-Default selection to apply no change to the stretching of the black and white points of the image display.

    Manual-Enter a set value to which you would like the Black and/or White points to be reset for image display.

    Auto-Enter a value in the form of a percentage to adjust the black and white points for image display. The value indicated will be the percentage of pixels that will become Black/White.

- Gamma-Gamma Adjust smoothly lightens darker areas of an image without burning out bright areas or lightening black areas. This is especially useful for editing images with a wide range of brightness values (dynamic range). Values above 1 will brighten and values below 1 will darken the image display.

- Histogram Area-Adjustments to this selection will change the information that is displayed in the histogram as well as define the area that will be used for calculating black/white values as

indicated in the Scaling section. You can either choose to use the Full Image Area or select a Region. If you choose a Region, you can set the pixel values to define a rectangular area, or if a previous selection (click and drag) was made from the Select/Show Histogram Area, ▣ these pixel values will be displayed here.

The values that are set for the Intensity Display Scaling can be saved to an image setup via the Set Image Display Values (See Camera Menu section).

View Menu

Show Image Display Settings Panel

The panel below is the Image Display Settings Panel which can be toggled on and off from the View menu. If this feature is activated, the panel will be displayed within the image window. The Image Display Panel feature can be turned on or off as a Preference under the Setup Menu.
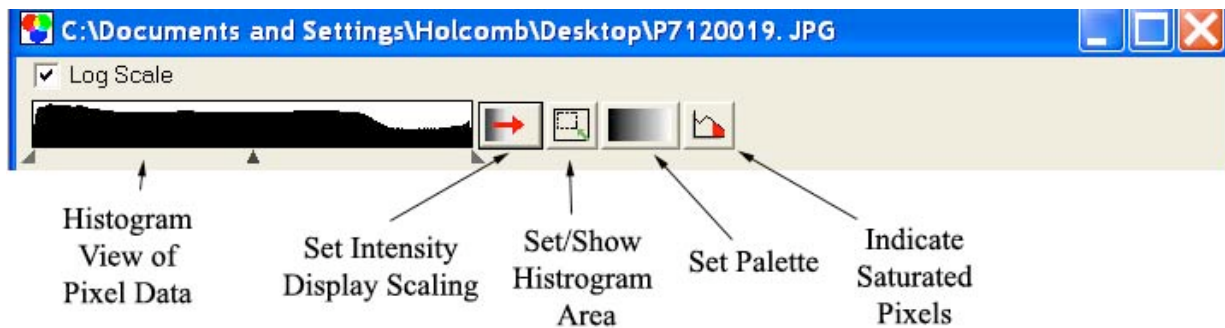
This feature allows easy access for many tools for adjusting the way your image is displayed. Certain tools will not be available for color images (i.e. Set Palette and Indicate Saturated Pixels).

Histogram View of Pixel Data: A graphical representation of image pixel data which allows for quick adjustments by grabbing on to the end pointers (to scale the white and black points, or the center pointer (to adjust the Gamma).

Set Intensity Display Scaling: This button will bring up a dialog box to enter values for Scaling the image, adjusting the Gamma, or to set the area of the image which is represented in the Histogram. This dialog box is also available from the Edit menu.

Set/Show Histogram Area: If this toggle button is depressed, the area of the image which is graphically represented in the histogram will be outlined in turquoise. If the entire image is being used for the histogram display, the turquoise outline will not be viewable. The menu listing can also be used to show/hide the histogram selection area.

Set Palette: Use this button for quick access to existing color Palettes or create a New Palette.

Indicate Saturated Pixels: This button allows for easy toggling of viewing saturated pixels.

Show Image Display Histogram Area

Use this feature to toggle the display (with a cyan rectangle) of a selected region that is used for the Histogram (see Show Image Display Settings Panel).

Camera Menu

Save Image Display Values

With an image open in the SPOT application, the option to Save Image Display Values will be available. If you like the values that you have used on an image for Scaling, Gamma, Palette selection, etc, you can use those values from the image and save them to the Image Setup of your choice.
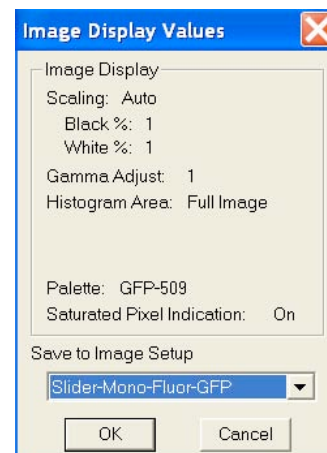
Setup Menu

Peripheral Devices – (Peripheral Device Controller)
What is the Peripheral Device Controller?

The peripheral device controller allows the SPOT program to communicate with external devices. This provides a means for directing the external device to obey simple commands. As most external devices (filter wheels, shutters, etc.) will have their own unique set of commands that they obey, you will need to access the reference documentation of the external device in order to make use of this function in SPOT software.

Why and when would I use this feature?

One of the powerful uses of the peripheral device controller is to automatically change the condition of the external device while you carry out an experimental process. This is most powerful when the peripheral device control commands are placed in a Macro (see chapter 13 – Macro Scripting to learn or review the use of the Macro feature).

☙⁂**WARNING**☙⁂

Note: External Devices are typically electromechanical devices that may have their own hazards of use. Before you attempt to use any external device alone or in conjunction with SPOT software, refer and abide by all instructions and warnings that accompany that external device.

Particularly note the potential hazards associated with the use of that device and do not attempt to control that device without being able to view its operation from the control location, including the computer running the SPOT software.

Additionally, note the limitations of the device you are controlling. Do not set the SPOT software to exceed those limitations as doing so could result in damage to the external device or a hazardous situation to the user.

Developing Peripheral Device Commands

Once you have the external device's documentation and then locate its command list, you are ready to begin.
Access the peripheral device set-up editor by selecting it from the Setup dropdown menu on the menu bar.

The Peripheral Devices window will appear and will initially be empty. Select the Add button to begin.
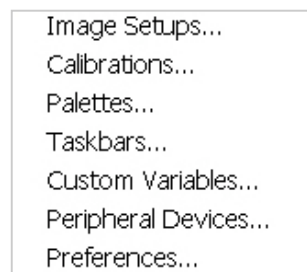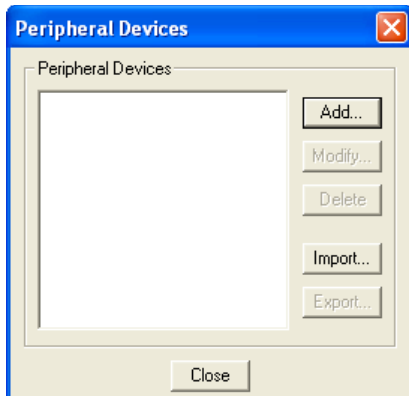
Naming the External Device - Start by giving the device you are controlling a name that you will recognize. For example, if you are developing commands for a Sutter filter wheel, you might want to name the device as such. Be as general or specific as you need to be depending upon the number and type of external devices you could connect to your computer.
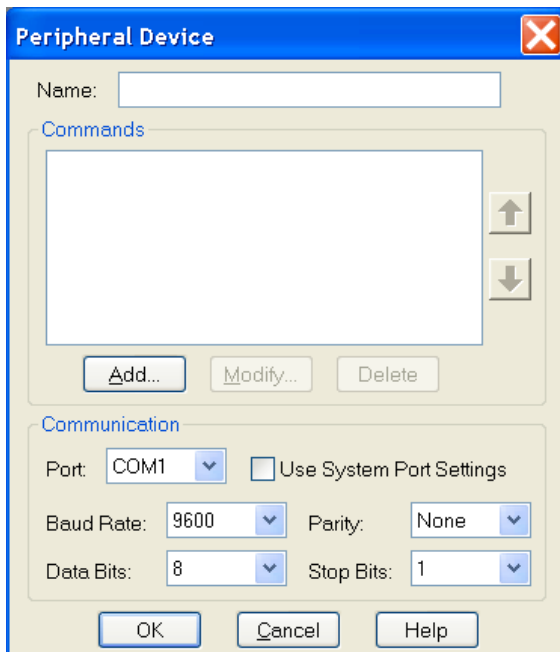
Specifying the Device's Communication Settings - Before you push the add button, you may specify the communication information of your external device. To begin, specify the output port that the communication cable is connected from at your computer (The other end will be attached to the external device controller). Select this from the dropdown menu in the PORT box.

You will often find the other communication information in the external device reference manual, in the same section as the external command list or in the specification section. As an example, the following excerpt is from the Uniblitz VMM-D1 Shutter's User Manual.

RS-232C (DB-9 female)	**Baud rate 9600, 8 Data bits, 1 Stop bit, No parity,** No flow control, command acquisition time 0.94 msec; 6 commands available; 1 global, 8 local address locations for commands (see "ADDRESS Select" specification for switch settings of local address locations).

Note that the specification tells you the type of communication mode (RS232C) and gives you communication specifications including Baud rate, the number of data bits, the number of stop bits, whether there is a parity bit or not and what type it is.

Select the matching information from one of the drop down menus for each Baud Rate, Parity, Data Bits, and Stop Bits.

Alternatively, you can select the Use System Port Settings checkbox. The boxes for the technical information noted above will become inactive. The communication will default to the settings specified for that port on your computer.

Note: The communication information must match between that specified and that of the external device. Otherwise, information exchange may be flawed and the external device controller may not understand the external device commands.

Push the add button to add a command name to your library.

Naming the External Device Command - You will want to name the peripheral device command. You can name it whatever you choose without regard for the function that the command will send to the external device. Logically, however, you probably want it to be closely related to the function (i.e. "Open the shutter" might be better than "Bob's eye-opening experience") especially if other users may be accessing these commands.

Entering the Desired Command - Once you have named the command, you must enter the corresponding command string in that box that will be understood by the external control device.

The following special notation can be used in the command string:

$ .... "Escape" character

^A-^Z .... Control A through control Z

\$ .... Dollar sign

\\ .... Backslash

\^ .... "^" character

\b10101010 ...... Binary value "10101010" expressed in true binary

\dyy .... value "yy" expressed in decimal

\xyy .... value "yy" expressed in hexadecimal

For example, the following command options would be available for the Uniblitz VMM-D1 shutter. Note that the Peripheral Device Command feature will not support the Octal command codes listed in the following table.

RS232 Global Address Codes					

	Commands	Decimal	HEX	Octal	Binary	ASCII
	Open	64	40	100	01000000	@
	Close	65	41	101	01000001	A
	Trigger	66	42	102	01000010	B
	Reset	67	43	103	01000011	C
	Open B	68	44	104	01000100	D (sets AUX Pin-1 low)
	Close B	69	45	105	01000101	E (returns AUX Pin-1

If you wanted to send the "Open" command in hexadecimal, you would type the following:

✎   *Note: You no longer need to use three alphanumeric characters to describe a decimal command. For example, the previous command \d05 can now be specified as \d5.*

Setting a Response Command - You may elect to receive a response when the SPOT program has sent the command, and the external device has completed that command. Click on the Response String checkbox and type in the appropriate response command. This response command string will also be found, if available, in the external device documentation.

Using Variables - In the command string, you may insert variables. The variables will be expanded at the time that the command is sent to the device. If you insert a "RunTimeText" variable, the user will be prompted, with your supplied prompt text, at the time the command is sent, and the user's response will be inserted into the command string at the point where the variable appears. The "TextVar" and "NumVar" variables may be useful when the command is used from within a macro (see Chapter 13 – Macro Scripting).

Using Timeouts - You may also select a time limit between the time that the command is sent by SPOT and the time it takes for the command to be received and the action of the function is completed. This can be handy in letting you know that there is a communication problem between the computer and the external device, when you expect an action to take place within a prescribed length of time.
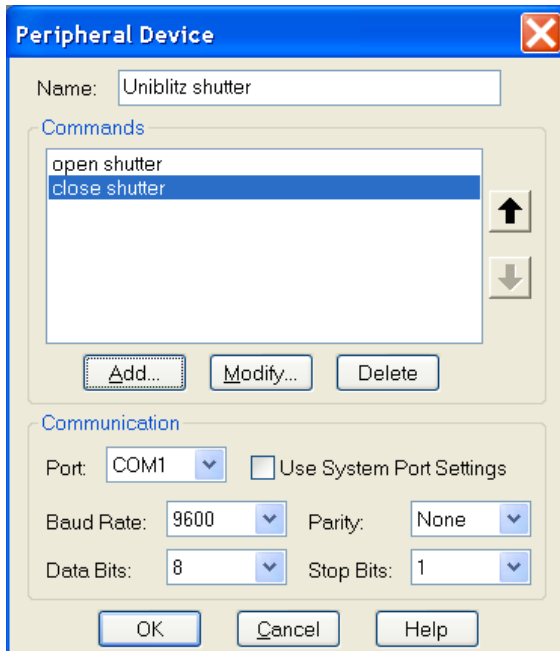
Testing the Peripheral Device Command - You may test the command immediately by selecting the Test button. If you have selected timeout duration, a response window will let you know if the command has not been received and carried out within the prescribed length of time.

✎   *Note:* Make sure your external device is connected properly to the computer and that it is turned on

before considering other potential communication problems.

Modifying and Deleting the Peripheral Device Command – Once you have created a command, you can modify its parameters or delete it. Select the command from the Peripheral Device Window and push the modify or delete button.
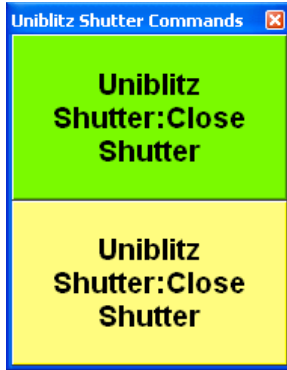

Using the Peripheral Devices Commands
From the Dropdown menu - Once you have created at least one peripheral device, a "Peripheral Devices" menu will appear in the application. You can send any of the commands you defined by selecting it from the appropriate submenu.
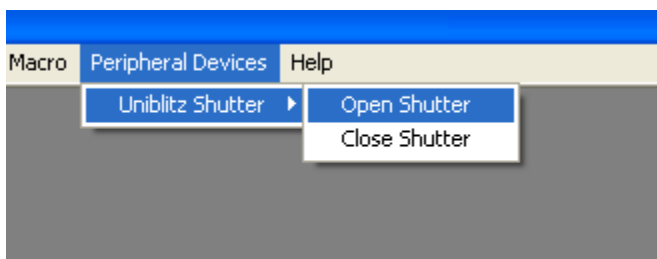
In a Taskbar - You can also add any of your peripheral device commands to your floating taskbar in the same way that you can add any other menu command (see screenshot below). Once placed on a taskbar, simply click on the Peripheral Device command and it will be carried out (For a review of taskbar development, see Chapter 12- Taskbars).

In a Macro - Finally, you can use any of these peripheral device commands as part of a Macro program that you develop. On playback of the Macro, the peripheral device commands will be carried out in the sequence and order specified in the Macro program (see Chapter 13 for a review of developing Macros). You can move your peripheral device commands to and from another computer. To import peripheral device commands, follow these steps:

1. Choose Peripheral Devices from the Setup menu.

2. Click on Import.

3. Select the peripheral device export that you would like to import. This will be a file of type SPOT Export (spx).

4. Choose Open. The peripheral device export will appear in the Import menu.

5. Select the peripheral device export and choose Import. The peripheral device commands will now be ready for you to use.

To export peripheral device commands, follow these steps:

1. Choose Peripheral Devices from the Setup menu.

2. Select the peripheral devices you would like to export. To select multiple peripheral devices, hold down the Control button while selecting them.

3. Click on Export.

4. Select a File name and location and choose Save.

Macro Menu

New Features Available as Macro Commands

If you use Macros to simplify and stream line your work, you will notice that most of the SPOT Advanced Plus features (detailed at the beginning of this chapter) are also available as macro commands. These new commands include:

> Duplicate Image, Create Extended Focus Image, Export Pixel Values, Set Intensity Display Scaling, and Send Peripheral Device Command.

Chapter 2 - Boost Camera Features

The Boost cameras offer additional features which can be found under the Image Setup section of the software. Features which are also available in the SPOT Advanced program are detailed in SPOT Software User Guide for version 4.7. Boost camera and Advanced Plus specific features are the only features documented here.

Readout Circuit

This feature is only available on certain BT2000 models. Choices can be made between 14- and 16-bit readout circuits as well as whether or not the Electron Multiplying or Conventional circuit should be used.
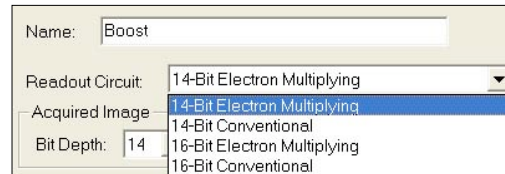
Exposure

Pre-Amp Gain

The main purpose of the Pre-Amp Gain is for controlling the dynamic range capabilities of the camera.

For nearly all low light applications, try the Pre-Amp Gain at 1.
Used in conjunction with binning, a Pre-Amp Gain setting of less then 1 will maximize the dynamic range capabilities of the camera.

Combining binning with a lower Pre-Amp Gain setting can help to maximize the visualization of bright and dark areas (dynamic range) of an image as these settings allow for a deeper well to be read out by the A/D Converter while maximizing the spread of the intensity information which is fed to the converter.
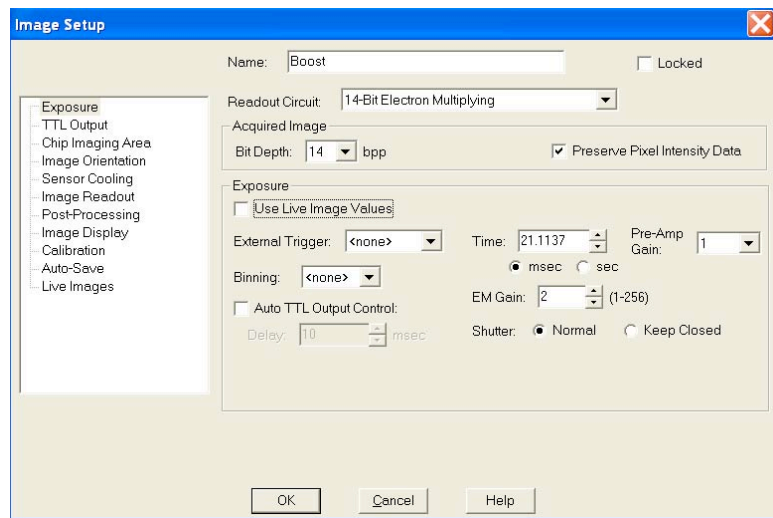
EM Gain

While the Pre-Amp Gain setting is used to maximize the dynamic range of the camera, the EM Gain is primarily used to enhance the signal to noise ratio and increase sensitivity, i.e. detect fewer photons. It is advised to start with a low EM Gain setting and low light and increase the EM Gain as needed. If the EM Gain is set too high, there is a risk of overwhelming the A/D Converter, thus resulting in unrealistic darkening of bright areas.

Shutter

There are two options for controlling the shutter; most often, the Normal setting will be used.

- Normal-Default selection for normal operation of camera.

- Keep Closed-Internal shutter will remain closed, even during an image capture. This feature may be useful when images of dark current are desired.

Sensor Cooling

Sensor Cooler

This window allows you to regulate the temperature of the CCD in your camera. Since the dark current noise increases as the temperature of the CCD rises, keeping the temperature of the CCD constant will ensure that each image taken has similar dark current characteristics.

Temperature regulation also increases the accuracy of the background subtraction. The temperature should be the same when creating background subtraction files as when using them. Please refer to the section "Get Background Subtraction" in this chapter for more details.

To begin regulating, check the Regulate Temperature box, and input the desired temperature in Celsius. The thermoelectric cooler will immediately begin cooling (or heating) the CCD to the target temperature. The temperature of the CCD is displayed on the status bar across the bottom of your screen. The temperature is read out and updated on the screen every 30 seconds by default. If you wish to view the changes in temperature more regularly, you can change this in the Temperature Readout section of the Preferences in the Setup menu.

The set point for the regulated temperature should be at least 3 degrees warmer than the maximum cooling capacity of the camera. The maximum cooling capacity of the camera is influenced by the room temperature. For example, if the ambient temperature of the room is 20˚C, the maximum cooling temperature of an Xplorer camera should be between -45˚C and -49˚C. The value for the Regulate Temperature box should be no more than -42˚C (-45˚C + 3˚C = -42˚C). If the room temperature is around 25˚C, the maximum cooling temperature will be closer to -40˚C to -45˚C, and thus the maximum (lowest temperature) value that should be used is around -37˚C.

To optimize the Regulated Temperature option, it is recommended to perform a small experiment where the Regulated Temperature is turned off and the camera is allowed to run to its maximum cooling possible in the maximum expected room temperature. The SPOT software will display the temperature of the camera in the status bar at the bottom of the application; note this value, add 3˚C, and use the resulting value for the Regulated Temperature.

If the temperature of the camera is not within the range set by the Regulated Temperature and the Allowable Variance, an asterisk will be displayed next to the temperature display in the bottom of the SPOT application.
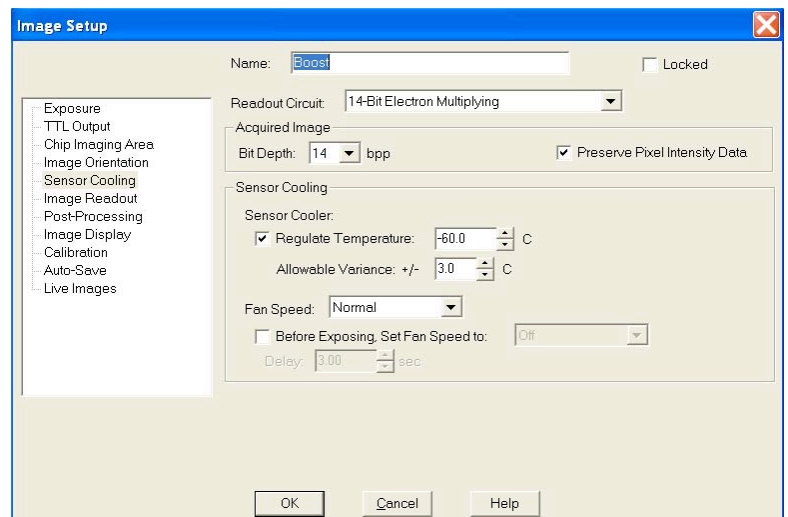
Fan Speed

There are 3 options for Fan Speed:
- Normal
- Low
- Off-If you select the Fan to be set to Off for extended periods of time, the camera will sound an alarm to remind you to turn the Fan back on to assist in keeping the CCD cooled.

The selection made for Fan Speed will dictate the speed of the Fan while the camera is running, while idle, or during image capture.

If further control of the Fan Speed is desired, use the additional check box for "Before Exposing, Set Fan Speed to:". Once this box is checked, you will have the option to set the Fan Speed to the next lower Fan Speed(s), as well as set the time you would like the camera to wait prior to starting an exposure once the Fan Speed is adjusted. If vibrations are of concern, adjusting the Fan Speed prior to exposing may be of benefit.

> ✎ *Note: We recommend using the water cooler accessory if it is desired that the Fan is turned off (as would be necessary for high-speed sequential imaging) or if you need long exposures with the Fan off.*

�WARNING�

IF THE FAN HAS BEEN TURNED OFF AND THE ALARM IS SOUNDING, THE CAMERA'S COOLING SYSTEM IS OVERHEATING AND NOT MAINTAINING THE TEMPERATURE INDICATED IN THE IMAGE SETUP. LEAVING THE CAMERA IN THIS CONDITION MAY CAUSE DAMAGE TO THE CAMERA.

Image Readout

Horizontal Readout Frequency

The options available will depend on the Readout Circuit (BT2000 only) choice indicated and the model of Boost camera. Choices for Horizontal Readout Frequency may include 1, 3, 5, 10, 13, 27 and 35 MHz.

Vertical Shift Period

The Vertical Shift Period is used to speed up or slow down how fast the electrons are shifted on the CCD for readout. Shorter periods can help with frame rate and reduce electron smear that may appear. The settings available will depend on the model of the Boost camera and may include options from 0.1 to 6.5 µsec.
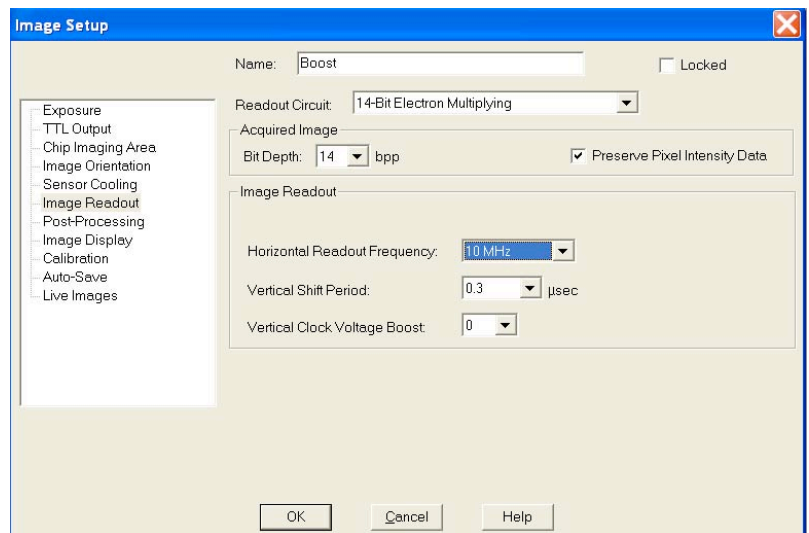
Applications for Long Vertical Shift Periods:
- Extremely low light level conditions. In these long exposures, the likelihood of smear is reduced, so the longer shift period is typically acceptable.
- Setups where a region of interest is specified and it is small and close to the bottom of the image.

Applications for Short Vertical Shift Periods:
- High Light Level Conditions. In these situations, many electrons are bombarding the CCD, and may produce smear while the information is being read off of the CCD. Shortening the Vertical Shift Period can help minimize this smear.
- Setups where the region of interest is large and/or relatively close to the top of the image.

Vertical Clock Voltage Boost

If a relatively short Vertical Shift Period has been set, you may need to increase the Vertical Clock Voltage Boost to help move the electrons from pixel to pixel for proper readout. The options available are 0, 1, 2, 3, and 4. The following are general guidelines for setting the level of the Vertical Clock Voltage Boost:

- Low light level conditions require a low vertical clocking voltage to keep "clock induced charge" low.
- High light levels require a high vertical clocking voltage to better transfer the charge and avoid blooming.
- Very short vertical shift times require a high vertical clocking voltage for better charge transfer efficiency to avoid smearing.

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