Version 7.0

Camera Guide: PVCAM Digital Cameras

Imaging Research Inc.

Contents

Introduction

A variety of digital cameras from Photometrics® and Princeton Instruments® (collectively known as Roper Scientific®) can be controlled by a software interface known as PVCAM® (Photometrics Virtual Camera Access Method). Examples include the CoolSNAP, Cascade®, Quantix®, SenSys®, and MicroMAX. These cameras all come with their own PCI controller card and all camera functions can be controlled by the **MCID™ Basic** imaging system utilizing this PVCAM interface.

This chapter describes the installation, use and adjustment of monochrome PVCAM cameras. Most of the functions and features described here are exclusive to PVCAM based cameras. **MCID Basic**, however, provides many other features related to camera-based image acquisition in general (e.g., frame averaging for noise reduction, real-time image alignment). These are described in the *MCID Basic Reference Manual (Chapter 2: Acquiring Images)*.

Installing PVCAM Cameras

The basic procedure for installing a PVCAM-based camera is as follows:

1. Install the appropriate PVCAM driver from the **MCID Basic 7.0** CD-ROM.
2. Install the controller card and connect the camera components.
3. “Install” the camera in **MCID Basic**.

If you purchased your camera from Imaging Research as part of a “turnkey” system (i.e., with a computer), the camera, controller card and driver were installed prior to shipping. You need only connect the camera components together.

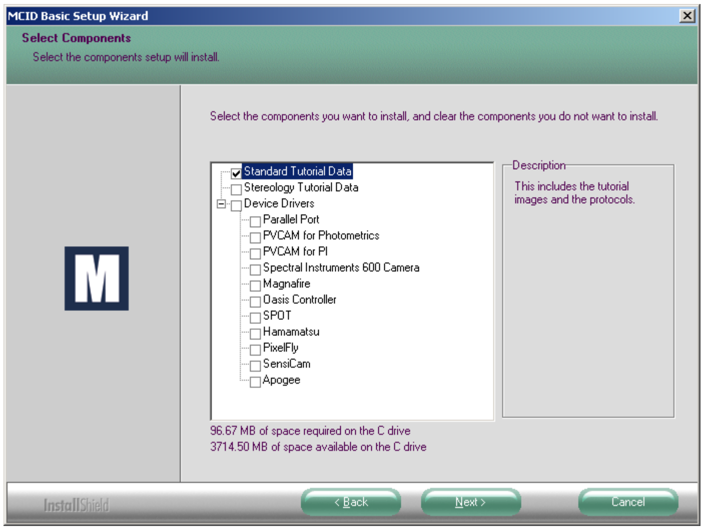
Installing PVCAM Drivers

Drivers for all PVCAM models are contained on the **MCID Basic 7.0** installation CD-ROM. If a driver has not been installed, do the following:

IF YOU ARE INSTALLING MCID BASIC 7.0 FOR THE FIRST TIME:

1. Log on to Windows® as an *Administrator* or as a User with administrative privileges.
2. Insert the **MCID Basic 7.0** installation CD into the CD-ROM drive.
3. Press **[Install MCID Basic]** and follow the instructions that appear.
4. When the *Select Components* installation dialog box appears (Figure 1), select the appropriate PVCAM device driver (see below).

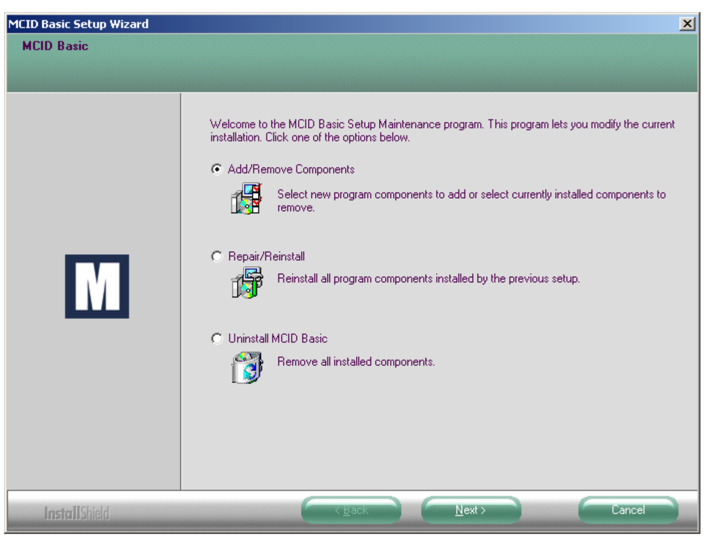
Figure 1: *Install PVCAM drivers from the Select Components installation dialog box.*



IF MCID BASIC 7.0 IS ALREADY INSTALLED:

1. Log on to Windows as an *Administrator* or as a User with administrative privileges.
2. Open the Windows *Start* > *Settings* > *Control panel* folder.
3. Open *Add/Remove programs*.
4. Select **MCID Basic 7.0** from the list of applications and press the **[Change/ Remove]** button. This launches the *MCID Basic Setup Wizard* (Figure 2).
5. Select the *Add/Remove Components* option.
6. When the *Select Components* installation dialog box appears (Figure 1), select the appropriate PVCAM device driver (see below).

Figure 2: *The MCID Basic Setup Wizard.*



Which Driver?

Install the **PVCAM for Photometrics** device driver if you are using any of the following cameras:

- CoolSNAP
- CooSNAPcf
- CooSNAPfx
- CoolSNAPHQ
- Cascade
- SenSys
- Quantix

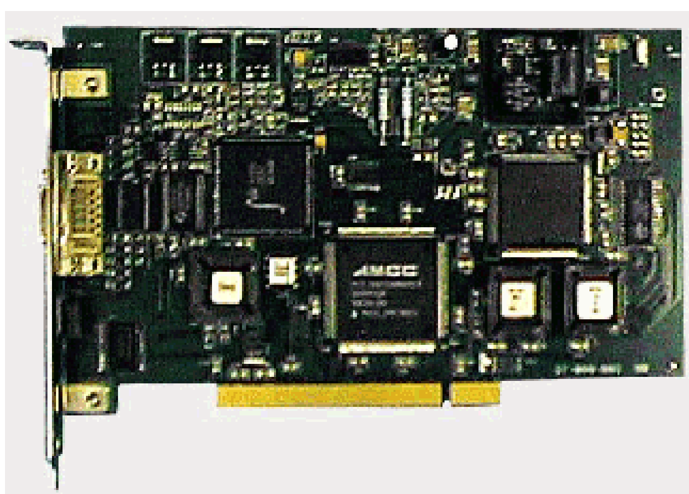
Install the **PVCAM for PI** if you are using the Princeton Instruments MicroMAX.

Installing and Connecting Camera Components

The CoolSNAP and CoolSNAPcf cameras are supplied with a camera head, a PCI controller card, and a digital interface cable. Plug the PCI controller card into a vacant PCI slot in your computer. Connect one end of the digital interface cable into the controller card and the other end into the **Data** socket on the rear of the camera head.

Most other PVCAM models (e.g., CoolSNAPfx, CoolSNAPHQ, Quantix) are supplied with an external power supply/control box in addition to the controller card and camera head. Cables are included to connect the camera to the PCI controller card and the power supply/control box. They are supplied to match the appropriate camera make and model.

Figure 3: *A PVCAM PCI controller card.*



Installing in MCID Basic

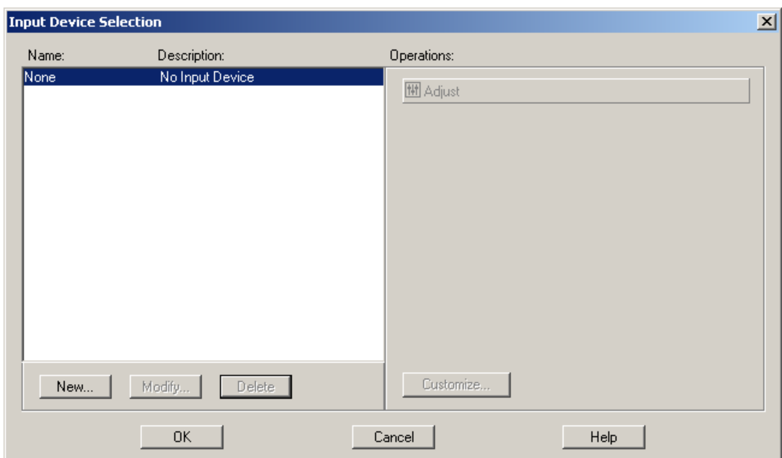
Once you have installed the camera driver and have connected the camera components together, you must start **MCID Basic** and add the camera to its list of input devices. This procedure tells **MCID Basic** that you have connected a camera to the system and tells it what kind of signal to expect from it.

The majority of PVCAM based cameras supported by **MCID Basic** are 12-bit devices (yielding 4096 gray levels), while some CCD chips are also available as 16-bit devices (yielding 65,535 gray levels). The camera should be installed at the appropriate bit density (12-bit or 16-bit). The camera will still work if installed at a lower bit density (e.g., installing a 12-bit camera as a 10-bit one), but you will not be utilizing the CCD to its full potential at the lower bit density.

Procedure

The **MCID Basic** interface to cameras is controlled through the *Settings > Input select* menu command. The *Input Device Selection* dialog box (Figure 4) contains a list of every camera that you have already installed. You can select, add, or delete any camera input from the list. You can also edit the definition of a specific input, and assign specific operations and settings to it. See *Chapter 2: Acquiring Images* in the online *Reference Manual* for details.

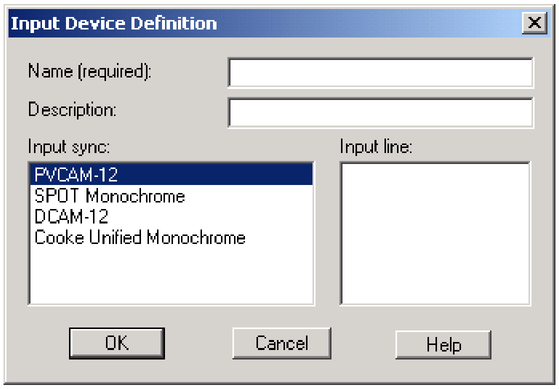
Figure 4: *The Input Device Selection dialog box lists all cameras installed in MCID Basic. It is also used to add new cameras to the list.*



1. Open the *Settings* menu and select *Display format*. Set the *Image Type* to the appropriate bit density (e.g., 12 bit).
2. Open the *Settings* menu again and select *Input select*. The *Input Device Selection* dialog box appears (Figure 4), which lists every camera that is currently installed in **MCID Basic**.
3. Click the **[New]** button.
4. If another camera has been installed already, a *Create Input Device* dialog box will appear (Figure 5). Select the **New input device** option and click **[OK]**. If no other cameras have been installed, the *Input Device Definition* dialog box appears (Figure 6).
5. Select the **PVCAM** input sync (e.g., **PVCAM-12**).
6. Enter a unique **Name** and **Description** in the appropriate entry fields (e.g., “CoolSNAP” and “12 bit monochrome”).
7. Press **[OK]** to exit the dialog box.
8. The *PVCAM Camera Settings* dialog box appears next (Figure 7).

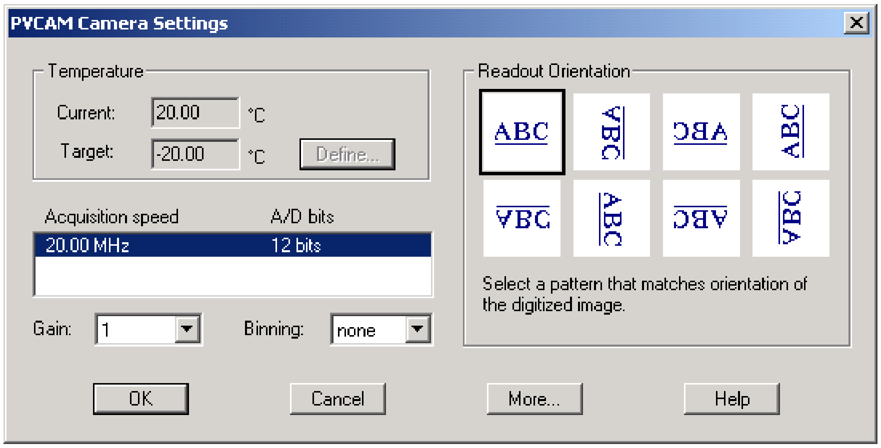
Figure 5: *The Create Input Device dialog allows you to install a new camera from scratch, or to copy all of the settings associated with an existing device.*

Figure 6: *The Input Device Definition dialog box is used to describe a camera and its Input sync.*



The *PVCAM Camera Settings* dialog box contains additional settings available to all PVCAM based cameras. Some of the settings are camera specific such as temperature control and acquisition speed, while all cameras have the ability to select gain, binning factor and readout orientation.

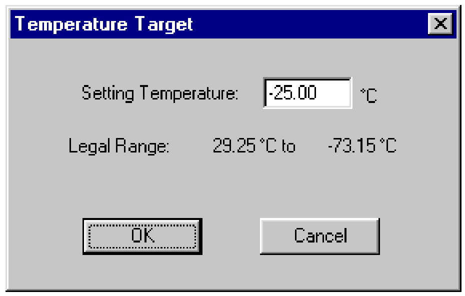
Figure 7: *The PVCAM Camera Settings dialog box.*



Temperature

PVCAM cameras such as the CoolSNAPfx, MicroMAX and Quantix are cooled from -15°C to -35°C and the target cooling temperature is adjustable. For these PVCAM based cameras click on the **[Define]** button to access the *Temperature Target* dialog box (Figure 8). Other PVCAM based cameras, such as the SenSys, are also cooled but only to 10°C and the cooling temperature cannot be adjusted via the *PVCAM Settings* dialog box.

Figure 8: *Adjusting camera cooling from within the Temperature Target dialog box.*



Acquisition Speed

The acquisition or readout speed determines how fast the captured image can be transferred from the CCD chip to the computer. A high acquisition speed of 1 MHz simply means that a million pixels can be transferred from the CCD chip, per second and a regular high resolution image would take approximately 1.5 seconds to appear on the image monitor once you have finished digitizing. A higher acquisition speed is always associated with more electrical noise than a slower speed and if a very low background is important, then a slower acquisition speed should be chosen.

Gain

The analog processor card in the camera control unit can be set at different gain. For bright signals, use a gain of “1” so that the camera electronics are set to have a reasonable signal-to-noise ratio (SNR) over a broad dynamic range. For dim signals, we usually set the gain at higher gain levels. This gives a larger gray level value and slightly better SNR for dim specimens, though dynamic range is compromised relative to the “1” gain setting. In general, dynamic range is not a major issue with very dim signals, while brighter signals may be easier to work with at “1”.

Binning

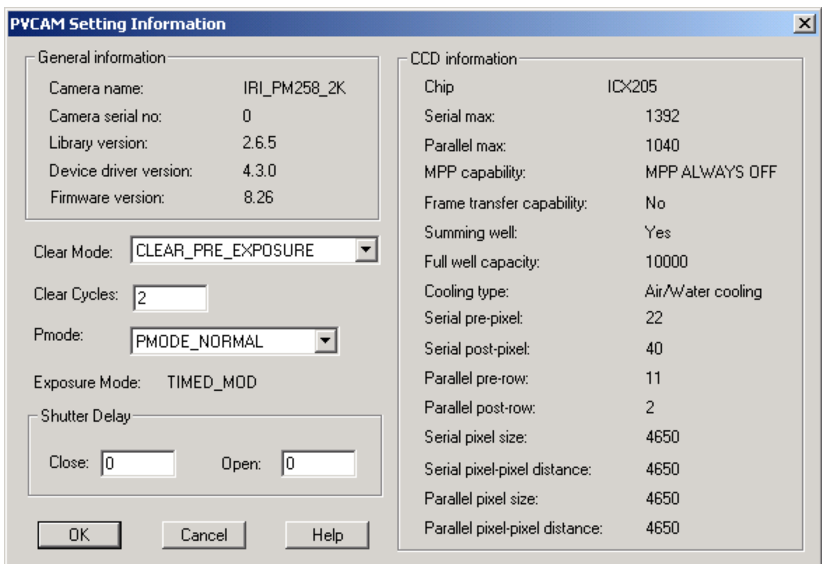
Binning sums groups of individual pixels into a smaller number of superpixels. By binning pixels we increase sensitivity and dynamic range, while sacrificing resolution. Binning results in shorter integration and readout periods. In general, we prefer to use the highest binning factor that gives adequate resolution for the specimen. Too high a binning factor can result in specimen details merging into each other. Too low a binning factor yields high resolution, but much lower sensitivity.

Readout Direction

We have selected the default readout direction to take the best advantage of the CCD. The number of pixels available is dependent on the camera and CCD chip. The default readout direction assigns the wider part of the CCD to the horizontal dimension. Selecting other readout directions will rotate the image orientation on the display monitor.

Clicking on the **[More]** button will display the PVCAM setting information dialog box (Figure 9). This dialog box lists the various PVCAM based camera settings and in the example in Figure 9, displays information for the SenSys camera.

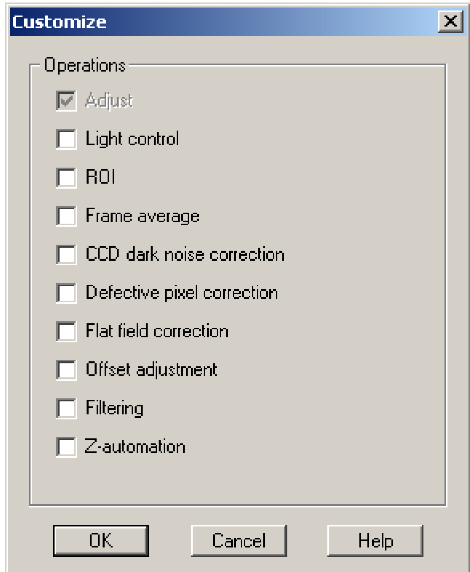
Figure 9: *The PVCAM Setting Information dialog box.*



9. A *Customize* dialog box appears next (Figure 10), which allows you to assign various input device operations to this camera (e.g., frame averaging controls). You can select them now or assign them later (see *Chapter 2: Acquiring Images* in the online *Reference Manual* for details). Click **[OK]** to exit the dialog.

The camera is now installed in the list of input devices. When you exit the *Input Device Selection* dialog box, the camera (and all of the settings and operations associated with it) becomes the default input device.

Figure 10: *The Customize dialog box is used to link controls for various camera operations to the camera.*



Acquiring Images

The basic procedure for acquiring images with any PVCAM based camera is as follows:

1. Select the desired **Display format** (12-bit or 16-bit).
2. Select the camera from the list of input devices, if necessary.
3. Press the **Digitize** icon to display a "live" image.
4. Make any necessary adjustments to the live image (e.g., focus or exposure time).
5. Press the **<Return>** key to complete digitization.

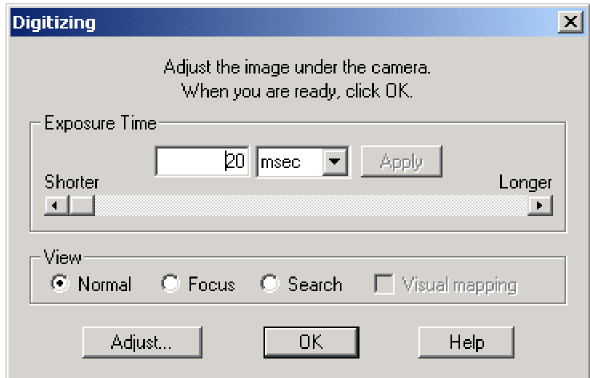
The image is now "frozen", and any **MCID Basic** functions can be applied to it (e.g., the image can be processed, calibrated, sampled, or saved to disk as an image file).

Digitizing

Click the **Digitize** icon (located in the **Application** toolbar) to initiate digitization or press **<Ctrl - D>**. A dialog box appears to indicate that **MCID Basic** is digitizing continuously and a live image appears on the image monitor.

Light is accumulated as long as the shutter is open. Longer exposure yields higher sensitivity. The camera exposure is controlled by moving the **Exposure time** slider (Figure 11). For most purposes, an exposure of about 100 to 200msec is fine. Adjust lighting to give proper illumination. In low light situations, the exposure time can be increased to periods of up to about 5 sec with a gradual increase in background. With cooled PVCAM cameras exposures of 20 sec and greater are possible with minimal increase in background.

Figure 11: *Clicking the Digitize icon initiates the digitization procedure and displays a dialog box. Move the slider along the Exposure Time bar to control the length of time the shutter remains open.*



Unlike video cameras, which show a “live” digitizing image, digital cameras tend to have a slower frame rate and the image will appear jerky. With longer exposure times it may take a few seconds before the new frame is displayed. The **View** section of the *Digitizing* dialog box offers some options to help in positioning and focusing of images being acquired with digital cameras.

Search

Search view displays a compressed view of the final image, but at a much faster refresh rate than would occur at the full resolution. This view is centered in the middle of the Image View and is especially useful for locating and/or positioning features of interest. The image is displayed in full resolution when digitization is terminated.

Focus

Focus view displays the central portion of the field at full resolution. As there are fewer pixels to read out, the refresh rate is much faster than would occur if the entire field of view was displayed. This helps with focusing the image during longer exposures. The image is displayed in full resolution when digitization is terminated.

Normal

The **Normal** view will allow you to see the whole image during the digitizing process, as it will appear when you finish digitizing. Notice that the frame rate has slowed down to reflect the exposure time combined with the appropriate camera readout time.

Adjusting the Camera Response

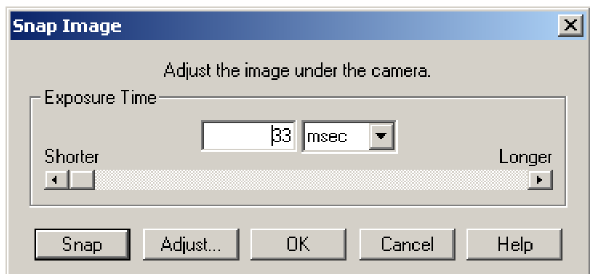
MCID Basic allows digital control over a number of PVCAM camera settings. To access these controls, press the **[Adjust]** button while digitization is occurring. **MCID Basic** will display a dialog box for camera adjustment (Figure 7).

Snap Shots

In the previous section, clicking on the **Digitize** icon displayed a dialog box where you could make various adjustments to the live image before actually capturing it and displaying the result. The **Snap Shot** icon skips the display of the dialog box and simply snaps and captures a new image. This function can be very useful if you are working with dim specimens and you have already chosen the correct exposure time. Clicking the **Snap Shot** icon will automatically snap a fresh image.

Holding down the **<Ctrl>** key while clicking on the **Snap Shot** icon will allow you to access an additional set of controls for the **Snap Shot** icon.

Figure 12: *The Snap Image dialog box.*



The *Snap Image* dialog box allows you to adjust the camera exposure time as well as access the **[Adjust]** functions. The only difference between this and the *Digitize* dialog box is that there is no display of a live image during any of these procedures. To see the effects of any adjustments you must click on the **[Snap]** button.

Maintenance and Troubleshooting

Cleaning the Camera

PVCAM based cameras contain a solid state sensing element (a chip) covered by a thin glass window. The glass window attracts dirt like a magnet. Dirt will appear as dark blots on the image. To determine if dirt is on the chip window, move the camera a bit, while looking at an actively digitizing image. Dirt that does not move is on the chip. To avoid gathering dirt, we recommend that you remove the lens or microscope video adapter as little as possible.

To clean the CCD chip window, we must first expose it. PVCAM based cameras that have an electronic shutter, like the CoolSNAPfx and MicroMAX, have no mechanical shutter blocking your view of the CCD chip. With PVCAM based cameras, which have a mechanical shutter like the SenSys and Quantix, considerable care is necessary. You could damage the shutter if it were to close while a cleaning instrument was inside the camera. To open the shutter, set the digitization control to maximum integration time. Watch the camera for a few minutes. Does the shutter stay open? Time the length of shutter opening. Now, digitize again to open the shutter and proceed to clean the chip window. With the SenSys and Quantix cameras, make sure that you take the cleaner out of the camera well before the shutter is due to close.

The first step in cleaning is to try blowing off dust with clean compressed air, of the type sold for cleaning camera lenses. Use canned air, not air from a lab tap, which often contains oil. Reassemble the camera and digitize an image to see if the dust is gone. If it is not, moisten a cotton swab or piece of lens paper with a glass or lens cleaner. Do not use alcohol or other solvents on optical surfaces (the optical coating and cements can be damaged by such solvents). Remove the lens and gently wipe the glass with the swab or paper. Make sure that the swab or paper has not become dry. Then blow the chip dry with compressed air. Replace the lens and digitize an image to inspect for dust. You may have to repeat the cleaning process a few times to remove all dust.

You may also clean the chip window with “Prophot” cleaners, available from many camera stores. Do not use tissue paper, which often contains impurities. Do not use dry lens paper or swabs to clean the glass over the chip, either. Dry rubbing may produce static charges.

Cleaning the camera is one of those unpleasant and thankless tasks that everyone detests. The best way to avoid cleaning is to keep the camera sealed, so that dust does not enter.

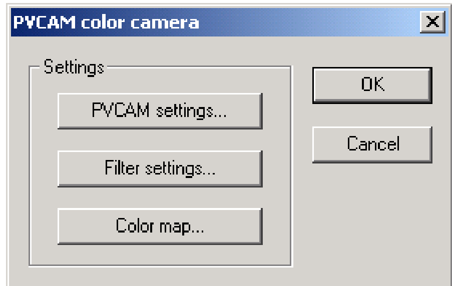
Making 24 Bit Color Images

Together with a filter wheel or liquid crystal color filter assembly, PVCAM based cameras can be configured to acquire true color images. Most high-resolution color cameras are really just a 10 or 12 bit monochrome camera with either a filter wheel or liquid crystal tunable filter placed in front of the CCD chip. During the digitization and subsequent acquisition stage three separate images are acquired, one with a red filter, another with a green filter and the third with a blue filter (RGB). These three images are then combined to create a high-resolution color image. These cameras are usually available with either a built in filter wheel or a movable slider that can be removed and the camera used as a regular monochrome camera. Color filter support within **MCID Basic** goes one step further. **MCID Basic** software can control a variety of different filter wheels and these can either be placed in the light path or in front of the CCD chip of the camera. The main advantage of placing a filter wheel in the light path becomes apparent when considering color fluorescence imaging. Existing, high quality fluorescence excitation filters can be used and there are no additional filters required in front of the CCD chip, resulting in overall shorter exposures and better quality images.

Enabling Support

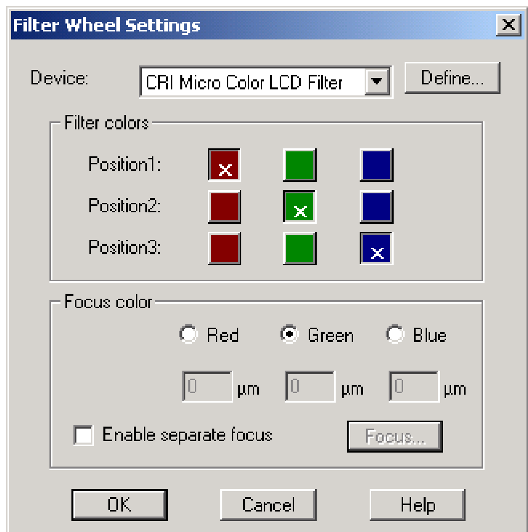
To access the color support for your PVCAM based camera you first need to install the camera within **MCID Basic** as a 24-bit color camera. Go to *Settings > Display format* and change the **Image Type** to **24 bit color**. Then go to *Settings > Input select* and go through the procedure of installing a camera in **MCID Basic**. For details see the Installing in MCID Basic section at the beginning of this chapter. Install the camera with the **PVCAM Color** input sync. Once installed the PVCAM based camera needs to be configured with the appropriate filter wheel (Figure 13).

Figure 13: *The PVCAM Color Camera dialog box.*



To configure the camera with the appropriate filter wheel click on the **[Filter settings]** bar. This will display the *Filter Wheel Settings* dialog box (Figure 14).

Figure 14: *Selecting the appropriate filter wheel from the Filter Wheel Settings dialog box.*



Choose the appropriate filter from the **Device** drop down menu option. Make sure the filter wheel/module is correctly connected to the computer by clicking on the **[Define]** button. This will bring up a device-specific dialog box for connection settings (e.g., serial or parallel port characteristics).

Once the communication parameters are correctly set up you need to assign the positions of the filters. Use the color-coded squares to denote the locations of your red, green and blue filters. In the above example (Figure 14) there are only three positions available. Filter wheels with more than three positions can have the red, green and blue filters installed at any of the filter locations.

Color and Focus Control

For users who have a motorized Z-axis there is the additional ability to control individual focus positions for the three separate colors. To turn this option on, place a checkmark in the Enable separate focus checkbox and then click on the **[Focus]** button. If your Z-axis control dialog box is not active it will be switched on and you will be asked to adjust the focus for each of the three separate colors. Once you have completed all three color focus adjustments the differences in the Z positions of the three color focal planes will be displayed in the appropriate boxes of the **Focus Color** section of the *Filter Wheel Settings* dialog box, (Figure 14). If you subsequently change the focus for the main focus color then the relative Z-axis positions for the other two colors will be maintained. For example, if the green focus Z-axis plane is initially at 10 sum and the blue is at 5um, a change in the green position to 15um will automatically set the blue Z-axis focal plane to 10um, keeping the original 5um interval constant.

Adjusting Colors

Clicking on the **Color map** bar on the *PVCAM Color Settings* dialog box (Figure 13) will display the *Camera Color Settings* dialog box (Figure 15).

Gain

Increases or decreases the overall image gain relative to the level of input illumination. Move the slider controls to adjust.

Offset

Move sliders to adjust the size of the no-light (black level) signal.

Gamma

A gamma setting of 1.0 sets a linear camera response to input illumination. Numbers greater than 1.0 make the camera less sensitive to low illumination levels and gamma settings less than 1.0 makes the camera more sensitive to low illumination levels.

Auto gain

Placing a check mark in the **Auto gain** box will have the effect of carrying out an automatic gain adjustment each time an image is acquired.

Auto white

Allows automatic white balance adjustment (see instructions below). If disabled, white balance can be achieved by manually adjusting the **Red Green** and **Blue mapping** controls.

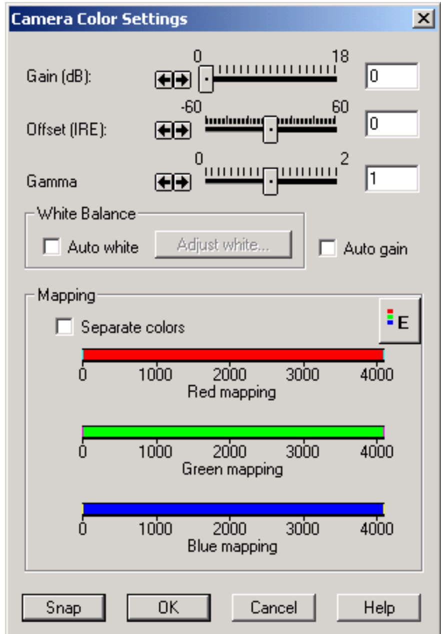
Adjust white

Perform automatic white balance adjustment. The procedure is as follows:

1. Place a checkmark in the **Auto white** checkbox.
2. Click on the **[Adjust white]** button.
3. A message box appears asking you to block all light to the camera. Press **[OK]** when this has been done.
4. Next, place a white object in front of the camera. If the camera is attached to a microscope, move to a blank field of view. Press **[OK]** when done.

The camera will automatically adjust the **Red** and **Blue mapping** levels relative to the **Green** level to set the white balance.

Figure 15: *Various options for making adjustments to the color image.*

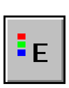


Mapping

The PVCAM cameras are inherently single chip 12 bit monochrome cameras. To create a color image the camera is used together with a filter wheel within the 24 bit color display format. In effect three 12 bit monochrome images are created, one for each of the three (red, green and blue) color filters. Unfortunately, **MCID Basic** is unable to handle or display 36 bit color images (3 x 12 bit images) so this 36 bit color image has to be mapped in some way to fit into a 24 bit color image on the display monitor. The mapping adjustments allow the user to have some control over how this mapping is to be carried out. By setting each of the sliders to their maximum range, the 12 bit red, green and blue image data (4096 levels for each color) are mapped to their corresponding 8 bits. The full 4096 levels are squashed into 256 levels. For dim fluorescent specimens the mapping can be set to the lower 8 bits (256 levels) for each color. This makes the camera more sensitive as there is a one to one relationship when mapping the actual levels.

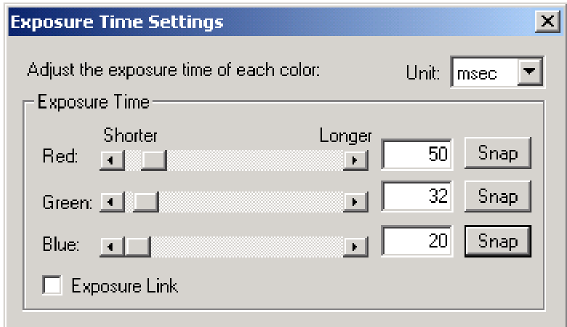
Placing a check mark in the **Separate colors** box allows the user to adjust each mapping color independently. Clicking on the **[Snap]** button allows you to refresh the digitized image.

Adjusting Exposure Times



By clicking on the **Exposure** control icon located to the side of the *Color Camera Settings* dialog box you can access the individual *Exposure Time Settings* dialog box, (Figure 16).

Figure 16: *The Individual Color Exposure Time Settings dialog box.*



This additional control allows you to setup and adjust a different exposure for each separate color. For example, if your fluorescence signal is very strong in the blue, but fairly weak in the red, you can decrease the camera exposure time in the blue, while increasing the camera exposure time in the red. Clicking on the **[Snap]** button next to each color exposure slider will display the appropriate image for that color. These exposure controls are set up to work together with the color mapping features of the *Color Camera Settings* dialog box. They are also proportional to the main focusing color chosen from the **Focus color** section of the *Filter Wheel Settings* dialog box. (Figure 14).

Adjusting the main image exposure via the *Digitize* dialog box (Figure 11) will change the main focusing color exposure and adjust the two secondary color exposures proportionally. When you have finished making all your exposure and color adjustments, press the **[OK]** button to exit the *Color Camera Settings* dialog box.

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