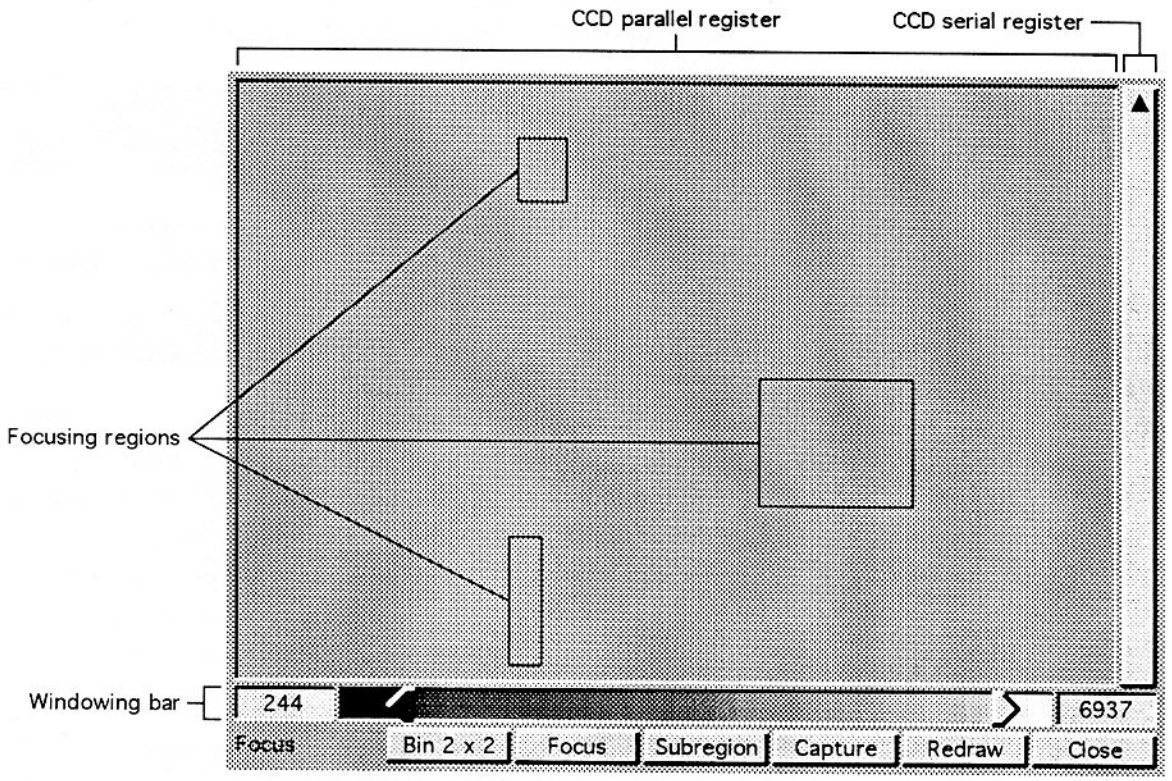
Focusing

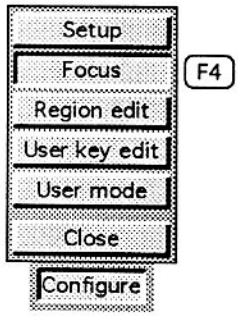
With Image200 configured to match your hardware setup, you should make sure the CCD camera head is properly aligned and focused. The Focus screen



provides rapid readout and display of up to four regions on the CCD. As the display is updated, you can manually adjust the CCD camera head and lens.

This chapter also introduces two operations used elsewhere in Image200: defining regions using the mouse and adjusting the gray-scale windowing of a displayed image.

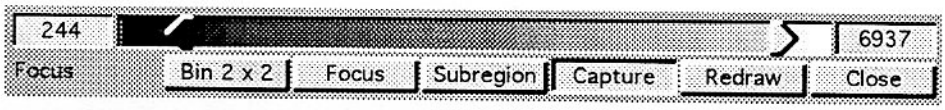
To switch to the Focus screen, choose **Focus** from the Configure menu, or press function key F4. The Main screen is replaced by the Focus screen.



Acquire a Sample Image

A sample CCD image is needed to determine the best focusing regions. The **Capture** command acquires a sample exposure using the integration parameters from the Setup dialog box. This image is stored only in a temporary buffer in memory and is discarded when you leave the Focus screen.

Click the **Capture** button to acquire a sample image from the CCD.



If **Exposure time verification** is On (in the Setup dialog box), an entry box appears to let you confirm the exposure time or enter a new one.

Confirm or change the exposure time and press ENTER to proceed, or press ESC to abort the exposure and leave the current time unchanged.

After a short delay, a gray-scale CCD image is drawn in the parallel register display area on the screen and the windowing bar appears.

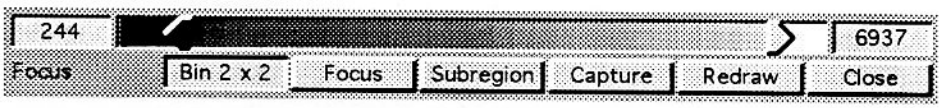
The sample image may reveal some small problems related to your system configuration and the integration parameters set in the Setup dialog box. Simple adjustments now will keep these problems from interfering with later work.

Adjusting Memory Use

Image200 may not display an image at all, but instead a message explaining that there is not enough memory available to acquire a full CCD image. The minimum system requirements, listed in the chapter *Installing Image200*, will allow you to acquire a full image. Check that you meet the requirements and that the necessary amount of EMS memory is not in use by other software.

If you cannot increase the available memory, you will need to bin to acquire a full image. As explained in the *AT200 CCD Camera System Hardware Reference Manual*, binning is the technique of combining charge from adjacent CCD pixels before digitization. Binned pixels have a higher signal-to-noise ratio than the equivalent single pixels, but lower spatial resolution. The lower resolution means that the image data will require a smaller buffer, and camera readout will be faster.

To increase the binning factor for the sample image, click the **Bin** button.



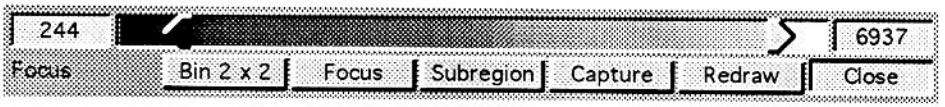
It cycles through four settings, allowing binning factors of 1 x 1, 2 x 2, 3 x 3, and 4 x 4. When you have set the binning, click **Capture** again.

Binning will increase pixel intensities in the image, so adjustments to brightness might also be in order.

Adjusting Orientation

The sample image may not be correctly oriented. To fix this problem, you can rotate the CCD camera head relative to your subject, or change Image200's display orientation. (If the sample image shows a mirror image of your subject, you will need to change the display orientation.)

To change the display orientation, click **Close** to return to the Main screen.



From the Main screen, return to the Hardware Options dialog box and change the setting of the display orientation button, as discussed in the chapter *Configuring Image200*.

The Focus screen saves nothing, so when you re-enter the Focus screen you will need to acquire a new sample image and repeat any adjustments you have made.

Adjusting Brightness

The sample image may be too bright or too dark. Unless the image appears mostly white or black, you may be able to adjust the image contrast to resolve the necessary details. Adjusting contrast is discussed in the next section.

If the sample image is *uniformly* too bright or too dark, there are several approaches to solving the problem. The preferred result is the brightest image possible without allowing the CCD to reach saturation during exposure.

If the sample image is too bright and you cannot discern details, you can:

- Use a shorter exposure time
- Increase the *f*-stop of the camera lens, for a smaller lens opening

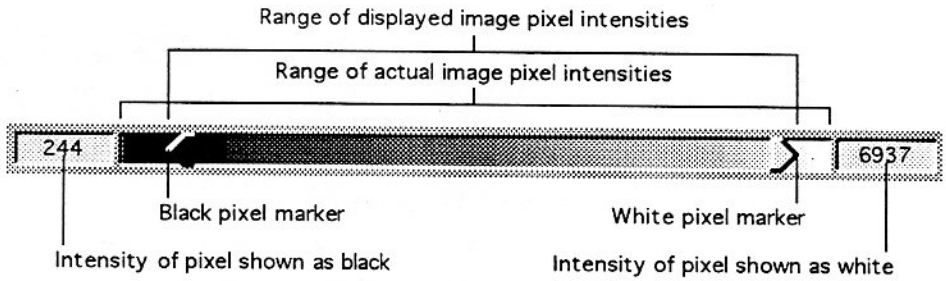
If the sample image is too dim, you can:

- Use a longer exposure time (at the expense of additional dark current)
- Decrease the *f*-stop of the lens (at the expense of depth of field)
- Increase the gain factor to 4X, using the **Gain** button in the Setup dialog box (at the expense of dynamic range)

Adjusting Contrast

For precise focusing and alignment, you must be able to resolve fine details. Photometrics CCD cameras can acquire up to 16 bits of information. Unfortunately, VGA hardware can display only six bits of gray-scale information.

Image200 addresses this limitation with the Windowing bar.



The Windowing bar lets you adjust display contrast by changing the mapping of CCD pixel data into grays on the display. The Windowing bar appears on the Focus screen, the Region Edit screen, and the Main screen.

The Windowing Bar

The center of the Windowing bar is graded from black to white. The full width of the bar represents the full range of pixel intensities in the sample image.

Two markers are drawn on the bar. Only the pixels with values which fall between these markers are shown on the screen. The marker pointing left shows the pixel intensity value which is displayed as black on the screen; the marker pointing right shows the intensity value which is displayed as white on the screen. The exact values of each marker are shown in the boxes at either end of the bar.

Pixels with intensities between the marked limits are displayed as shades of gray; pixels with intensities beyond the limits are displayed as black or white. If the markers point towards each other, the white-pixel intensity is less than the black-pixel intensity and the image is inverted black-to-white.

Moving Markers

You can move either marker by dragging it with the mouse. To drag, position the crosshair over the marker, press any mouse button, and *hold the button down* as you move the mouse. When the marker is in the correct position, release the mouse button.

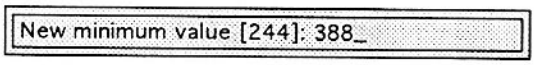
The exact marker value is determined by the tip of the marker. The value ion the display box updates as you drag.

Entering Values

You can also adjust the markers by entering exact pixel values, including values beyond the actual minimum or maximum of the sample image.

If you click in either display box

an entry box appears, allowing you to edit the current value.



Resetting Markers

To reset a marker to the actual minimum or maximum pixel intensity value of the sample image, drag the marker back to the end of the bar.

The minimum and maximum are determined by a sparse sample of the pixel data and may not represent the absolute limits of the data.

Applying Changes

When you move a marker in the Windowing bar, the changes are not immediately reflected in the displayed image.

To see the result of your changes, click the **Redraw** button.

The image is redrawn with the new windowing.

Regions

Focusing regions are subarrays on the CCD, as explained in the *AT200 CCD Camera System Hardware Reference Manual*. Because fewer pixels are digitized for subarray readout, new images can be acquired at the fast repetition rate required for focusing.

The sample image shown in the parallel register display area is scaled up or down to fit the screen, but aspect ratio is preserved. Thus, the sample image represents the entire CCD image plane. It provides a visual basis for positioning the focusing regions.

The Region Definition

The focusing region definition contains information about the focusing regions: their positions, their sizes, and their display settings. When you start the focusing loop, Image200 uses the region definition to read out and display the required subarrays as quickly as possible.

You can add, delete, or adjust regions using the Subregion menu. The region definition may contain up to four regions, but it must contain at least one.

Drawing Regions

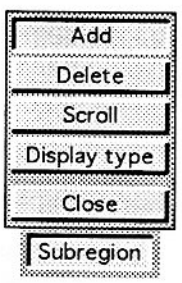
The position and size of a region are determined by a rectangle you draw on the screen. Regions can be as small or as large as you like. They can even be the size of the whole CCD.

To draw a region rectangle, position the crosshair pointer at one corner of a region you would like to focus on and drag towards the opposite corner of the region. As you drag, a magenta rectangle follows you on the screen. When the rectangle surrounds the intended region, release the mouse button.

If you make a mistake, just draw another region. The first one disappears. When you're satisfied with the region, you can add it to the focusing definition.

Defining Regions

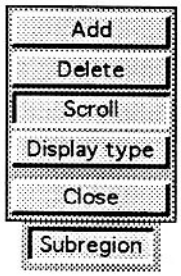
To add the region to the definition, click **Subregion** to bring up the Subregion menu, then click **Add**.



The magenta rectangle turns red.

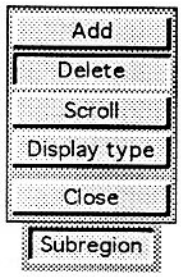
Defined regions remain on the screen as you draw new regions. You can define up to four regions using the **Add** button. (Regions cannot be defined to overlap.) Each region turns red as you add it, while previously defined regions are shown in blue.

The red region is the current region; the **Display type** and **Delete** commands will apply to this region. The **Scroll** button



lets you pick a new current region by cycling through the defined regions.

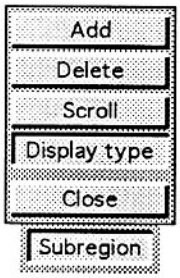
To remove the current region from the focusing definition, click **Delete.**



Display Type

Each region can be displayed as either an image or a line trace during the focusing loop. Line trace regions show plots of pixel intensities which have been summed in the vertical direction on the screen (which may be either serial or parallel on the CCD). Line traces allow you to isolate bright points as peaks in the trace.

The setting for the current region is shown on the **Display type** button. To change the display type of a defined region, click the button.



New regions start out with an image display type.

To draw another region, click **Close** to remove the Subregion menu.

The Focusing Loop

To start the focusing loop, click **Focus**.

After you verify the exposure time (if **Exposure time verification** is On), Image200 takes two exposures before displaying the focusing regions; the first exposure is not displayed. Image200 continues to take exposures and display the focusing regions as you adjust the alignment and focus of the camera head.

The regions are arranged on the screen to preserve aspect ratio and relative horizontal positions, while showing each region at a large size. For regions displayed as line traces, a Y axis of pixel intensity values is provided as a reference.

Adjusting Display

Image200 uses the first exposure to determine binning and display scaling for the focusing regions.

- Line-trace regions are binned as much as possible on the CCD, then summed in the computer to create a single trace
- Image regions are binned according to the setting of the **Bin** button.

Image regions are windowed according to the settings on the Windowing bar for the sample image.

As you adjust the camera head, the original display settings may become inappropriate for your focusing regions. Image200 can automatically calculate the best windowing and binning values for the focus display, but the calculations take too long to perform them for every exposure.

To calculate new values without stopping the focus loop, press the "A" key (for Auto-window). Each image region is independently re-windowed to show the greatest contrast within the region.

Aligning Regions

The focusing loop can be used to check the alignment of your CCD camera head to a known feature in the focusing image. To help you precisely align features as you turn the camera head, an alignment guide is shown during the focusing loop whenever two or more regions are defined to be directly above or below each other.

You can reposition the guide by dragging it. First, hold down any mouse button to pause the focusing loop; the crosshair appears. *With the button held down*, move the crosshair until it touches the alignment guide, then drag the guide to its new position. When you release the mouse button, the focusing loop resumes as before.

Stopping the Loop

To stop the focusing loop, press the ESC key.

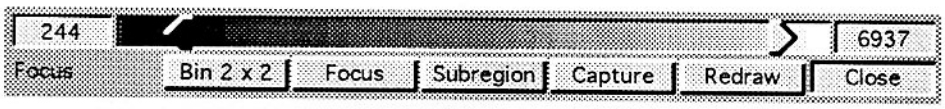
You can change the region definition by clicking the **Subregion** button. Image200 clears the focusing display and show the original sample image. If there wasn't enough memory to preserve the sample image, the display area will be a gray blank.

When the Subregion menu appears, you can delete regions, change their display type, or add new ones.

If you capture a new sample image, *the old focusing region definition is lost.*

To restart the focusing loop, click the **Focus** button again. New binning and windowing values are calculated and the loop resumes.

When you are done with the Focus screen, click **Close**.



The Focus screen disappears and you return to the Main screen. The sample image and the focusing region definition are not preserved.