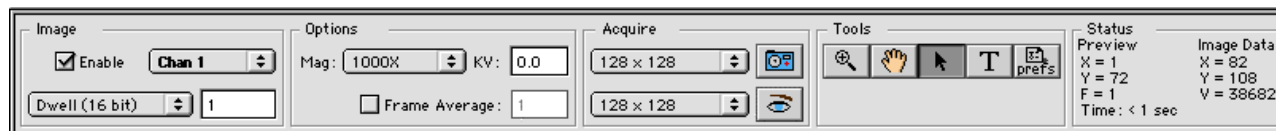


SEM Image Mode

The SEM Image Toolbar

NOTE: Before any images can be acquired with Revolution, several steps must be completed, namely, the physical connection of the 4pi system to the microscope. The system comes complete with an interface box and cable. The interface box must be connected to the SEII computer card with the included ribbon cable. The interface box must be connected to the microscope with the included cable, and turned on. The microscope must be on and in working order. That is, **the microscope must be able to acquire an image to a CRT monitor.** In addition, the FPXDriver and hasp software must be installed. *Review and perform the [Hardware and Software Installation procedures before proceeding!](#)*

SEM Image display and acquisition functions are controlled from the **SEM Image Toolbar**. If the toolbar is not visible at the top of your screen, selecting **Mode → SEM Image** from the top menu will make it appear:








The tool bar is divided into five sections:

Image. Used to enable up to 4 input channels for simultaneous acquisition. Also used to set the acquisition mode (single, average, dwell), and the value of the dwell. For a complete description of these controls, see the [SEM Image Preferences](#).

Options. Used to match Revolution's magnification to the magnification set at the microscope (the software cannot directly read this information). Refer to the [Micron Marker Preferences](#) for detailed information. Also used to specify the beam energy in kVolts (informational only, Revolution cannot control or read the beam energy from the microscope). Also used to control whether frame averaging is turned on, and how many frames are acquired. See the [Scan Generate Preferences](#) for more information about frame averaging.

Acquire. Used to set or define image resolution for either or both of the acquire and preview modes. The buttons initiate the actual start of acquisition or the on-screen preview. The resolutions of the two modes can be set or changed independently, directly from the toolbar (refer to the [Scan Generate Preferences](#) for more information about setting and changing the resolutions). The actual acquisition (preview) is controlled by the "camera" ("eye") buttons, and is described in more detail below.


Tools. Used to select tools for image manipulation. In order from left to right, these are:

-  Switch to magnify-cursor. Used to zoom in or zoom out of images. Use the **option** key (Macintosh) or **alt** key (Windows) to toggle in/out control of the zoom.
-  Switch to hand-cursor. Used to drag image around in zoomed mode.
-  Switch to pointer-cursor. Used for selecting any item with a valid handle in an image.
-  Switch to text-cursor. Used to create or edit text annotations in an image.
-  [Opens the preference panels](#)

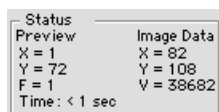
Status. Lists useful information about the current operational state.

Previewing Images

If the hardware has been [properly installed and connected](#), click on the preview button to open a preview image on the computer screen. The number of SEM images which appear on screen, as well as many other characteristics of the image acquisition, depends on the settings in the [SEM Image Prefs](#), which can also be used to change the image characteristics in real-time.

To launch a preview window, click on the preview button → 

When the preview image window appears on screen, the **status** section of the toolbar will inform the user of the current x- and y-pixel positions in the scan, the current frame, and the remaining time for the frame to complete (for faster setups, such as small images with small dwells, the remaining time may be pinned to < 1 second, and the x-pixel position may not update).



Holding the cursor over any particular pixel in the image will cause the image datum at that pixel to appear in the status section as well: x- and y-pixel position, and gray-scale value at that pixel.

Starting a preview puts the 4pi system in direct control of the microscope's scanning circuitry. At the moment control is actuated, the user should hear a click from the 4pi Scanning Interface Unit (the click is the relay set being energized) and the microscope's **CRT screen should be blanked**.

If the microscope's CRT screen is not blanked, it is an indication of an incorrect connection or other fault. Immediately click the preview button again to relinquish control back to the microscope. Double-check for incorrect installation.

If blanking problems persist, contact 4pi Analysis before proceeding!

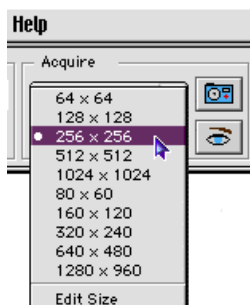
First Run

When the preview is first displayed on the computer screen, a number of controls may not be set correctly. Before accurate images can be acquired, several procedures should be followed. These procedures can be repeated at any time, but should not be required more than once:

- Adjust Resolution and Aspect Ratio
- Adjust Contrast and Brightness
- "Square" the pixels
- Initialize Scan Parameters and Remove Artifacts

First Run - Adjusting the Image Resolution (Size) and Aspect Ratio

This step is **not mandatory** for the operation of the software, but it will reduce much of the confusion associated with image acquisition using the 4pi system. We recommend that the aspect ratio in Revolution match that of the microscope's CRT. The default aspect ratio in Revolution is 1:1 (square) with a resolution of 256×256 pixels; however, a number of microscopes have non-square aspect ratios. A common value is 4:3 (horizontally rectangular), but there are notable exceptions (Hitachi, 3:4).



Click the menu to the left of the **preview** button to see the available resolutions.

Revolution default settings include 5 square (aspect ratio 1:1) and 5 horizontally rectangular (aspect ratio 4:3) resolutions.

The aspect ratio and/or image resolution is changed by simply selecting from the menu. New aspect ratios and image resolutions can be created (or deleted) by selecting **Edit Size** in the menu. For complete details, refer to the **Image Size Options** in the [Scan Generate Prefs](#).

First Run - Adjusting the Brightness and Contrast

The [line profile](#) overlay represents the microscope video signal and is a good tool to use for setting video levels. If set to show every line, it is a real-time picture of sequential linescans, and may jump around quite a bit because of the sample morphology. The SEM Gain and Offset potentiometers on the Scanning Interface Unit directly control the magnitude (contrast) and vertical offset (brightness) of this video signal. The top and bottom of this window define the full-scale ADC values. The user should use the microscope **Contrast** and **Brightness** controls to set the CRT image to the desired level; the Scanning Interface Unit can then be adjusted to set the signal shown in the linescan box to the desired level. Channel ADC A is adjusted with the SEM Gain and Offset potentiometers on the **front panel** of the Scanning Interface Unit. The gain and offset for ADC B, ADC C, and ADC D can be set with potentiometers **inside** the Scanning Interface Unit. Contact 4pi if you need access to the internal potentiometers. *Note: while making this adjustment, the [Display preference](#) should be set to Full Range.*

First Run - Squaring the Pixels

After selecting the desired aspect ratio, the pixels must be set square. In principle, this requirement is met when the image acquired on the computer screen matches the master image on the CRT or polaroid. In practice, the user must make sure that the CRT or polaroid are also displaying square pixels!

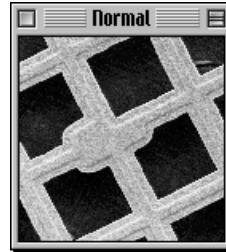
To square the pixels, adjust the size and location of the image that appears in the preview window until it matches the S(T)EM CRT or polaroid image, using the Scanning Interface Unit's front-panel X-Scan and Y-Scan Gain and Offset pots. Use the X-Scan Offset pot to shift the image left and right. Use the Y-Scan Offset pot to shift the image up and down. Likewise, use the X-Scan and Y-Scan gain pots to adjust image compression in the horizontal and vertical directions. This is an iterative process, because the Gain and Offset controls will appear to affect one other. Furthermore, the microscope's CRT is blanked when the preview mode is active; thus, alternating between preview mode and normal microscope operation is required in order to compare the result to the microscope CRT and make adjustments. When finished, the pixels will be square regardless of the image resolution defined in the software (as long as the size conforms to the defined aspect ratio) and no further adjustment should be necessary. **To perform this procedure correctly, one should use a calibration standard.**

More information on squaring the pixels can be found below in the discussion about **horizontal or vertical distortion**.

First Run - Initial Scan Adjustment

Even after the correct aspect ratio has been selected, a number of artifacts may appear with respect to the **final correct** rendition of the microscope CRT image (shown below at right).

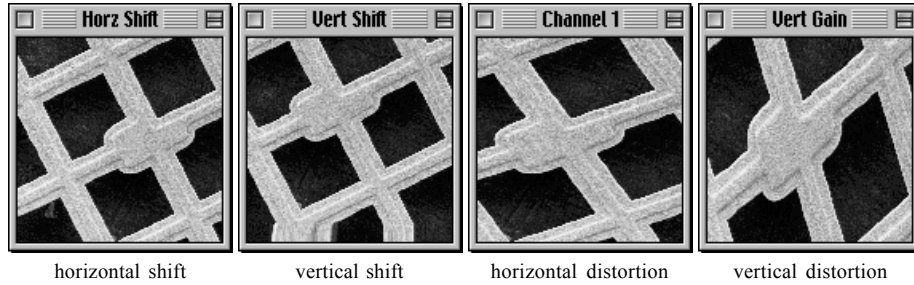
- horizontal or vertical shift
- horizontal or vertical distortion
- horizontal or vertical overscanning
- horizontal or vertical mirror image
- inverted video
- rotated image
- 60/50 Hz noise
- retrace delay distortion
- nonlinear compression artifact



normal and correct

A description of each of these artifacts is shown below, with remedies. To make adjustments, the user must continually compare the microscope's CRT image with the acquired computer image. This requires that the preview window be dismissed and restarted repeatedly, since only one scan generator can control the microscope at a time.

Shift And Distortion



horizontal shift

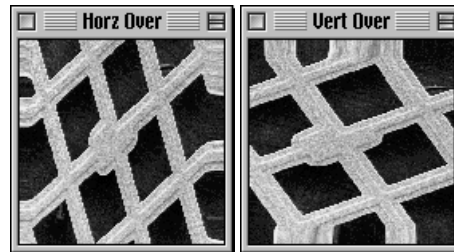
vertical shift

horizontal distortion

vertical distortion

- Horizontal and vertical **shifts** are controlled with the X-scan and Y-scan **Offset** potentiometers on the Scanning Interface Unit. Adjust both potentiometers to get the image centered properly.
- Horizontal and vertical **distortions** are controlled with the X-scan and Y-scan **Gain** potentiometers on the Scanning Interface Unit. **If the pixels happen to be approximately square, this artifact may be mistaken for a magnification setting error.** Adjust both potentiometers to remove image compressions and/or expansions.
- Virtually all installations require these adjustments to be made in the field. The process is iterative and sometimes tedious. The final result is an image whose pixels are dimensionally correct in both horizontal and vertical directions (that is, "square").

Overscanning

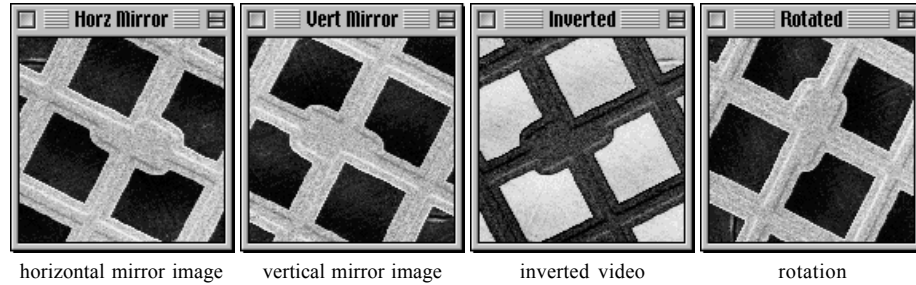


horizontal overscan

vertical overscan

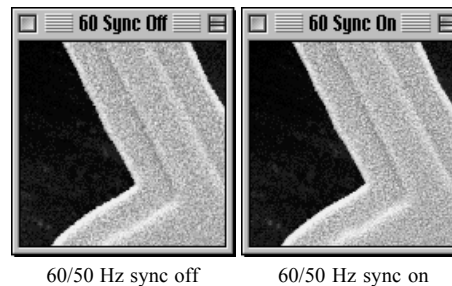
- Horizontal or vertical **overscanning** occurs when the Scanning Interface Unit's X-scan and Y-scan **Gain** potentiometers are set too high, causing the beam to be driven outside of its design range. The visual effect is to create a **repetition** of the horizontal or vertical boundaries of the image. Adjust the relevant **Gain** potentiometer **down** to eliminate overscanning. When overscanning is eliminated, adjustment of the Offset potentiometers may again be necessary.

Miscellaneous Artifacts



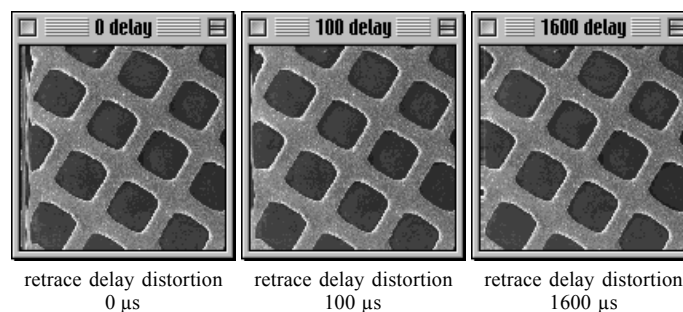
- **Mirror images** occur when the Scanning Interface box drives the beam in a direction opposite to what is required. Horizontal and vertical mirrors can be adjusted in real time from the **DAC Options** section of the [Hardware Prefs](#).
- **Inverted video** (swapped definitions of black and white) can occur for certain microscopes due to their video processing circuitry. Video inversion can be selected independently and in real time for each enabled channel from the **Channel Options** section of the [SEM Image Prefs](#).
- **Image rotation** (swapped definitions of X- and Y-scan directions) can occur for certain microscopes due to their scan generation circuitry, or for example if the X-scan drive cable is accidentally swapped with the Y-scan cable during installation. Horizontal and vertical scan definitions can be adjusted in real time from the **DAC A** and **DAC B** menus of the **DAC Options** section of the [Hardware Prefs](#).

Synchronization



- **60 or 50 Hz noise** is caused by poor isolation of the power mains from the imaging instrumentation. Generally, noise of this sort is either generated by the microscope itself or is due to improper grounding, and is more evident at higher magnifications and at edges with high contrast (see above example). If such interference cannot be eliminated by proper grounding or instrumentation adjustments, it can be minimized by synchronizing the electrom beam sweep with the phase of the electrical power sinusoid. To apply this synchronization, select the proper menu item from the **Scan Options** section of the [Hardware Prefs](#).

Retrace Delay Distortion




- A **retrace delay distortion** is caused by driving a linescan forward too soon after a horizontal retrace. As a result, the program begins acquiring linescan data at the beginning of a new line before the beam has had the chance to retrace and position itself correctly. The distortion manifests itself as a variable number of garbage pixels at the beginning of each linescan. The solution is to increase the horizontal retrace delay from the **Delay Options** section of the [Scan Generate Prefs](#). In rare instances, there may also be a vertical retrace delay artifact; the same Scan Generate Prefs can be used to adjust it. In the example above, not even 1600 μ s is enough to remove the artifact. In such a situation, it may be helpful to use the **Park Beam at Zero** control (see the **Scan Options** section of the [Hardware Prefs](#)).

Non-linear Compression Artifact

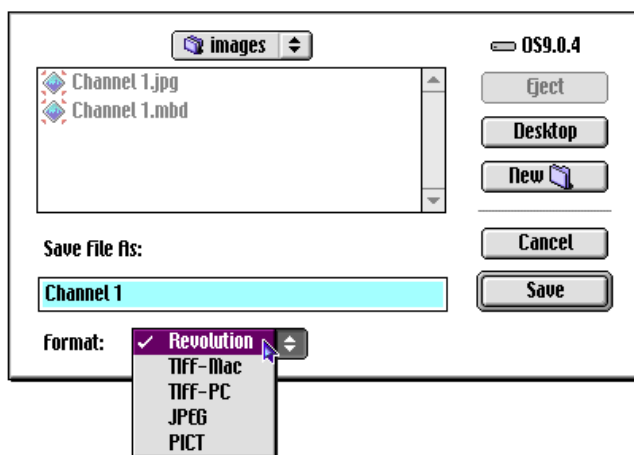
- The **non-linear compression artifact** (example images not shown) is related to the retrace delay distortion but is a more extreme example. In this case, there is not enough delay capability in the horizontal retrace delay parameter. In addition, there may be nonlinearities in the scan generator which affect the 4pi scan signal. This artifact is strongly dependent on the type and model of microscope. If this artifact is observed, the **Park Beam at Zero** checkbox of the **Scan Options** section of the [Hardware Prefs](#) should **definitely** be turned on, and the **horizontal retrace delay** (see above) set to its **maximum** value of 1600 μ s. If the artifact is still not reduced, in almost all cases it can be eliminated by increasing the amount of time that the software waits after sending a new pixel position to the electron beam and before

it starts recording data. This variable, the **pixel delay**, is set in the [Scan Generate Prefs](#). Increasing pixel delay to a large number will significantly reduce the speed of scanning and acquisition. Contact [4pi support](#) for specific information and custom troubleshooting help.

Acquiring and Saving Images

To start an acquisition, click on the acquire button → 

- The image will be captured in separate window(s), appearing in real time. The acquisition may be done while the Preview window(s) is (are) open, or without any Preview window at all.
- Images (including those that are only partially acquired) can be saved straight to disk at any time by selecting File→Save (keystroke **clover-S** on Mac, **control-S** on Windows). The same will work on a preview window.
- When the acquisition is complete, the image will stay on the computer screen, unless other image-handling options have been set in the [General Preferences](#).
- The **default** format of an image or map is selected in the [File Format Preferences](#). The data from an **acquired** image or map can be saved to disk by keystroke (**clover-S** on Mac, **control-S** on Windows), selecting Save from the File menu, or simply selecting the close box in an **acquired** window (in a **preview** window, only **File → Save** is applicable). The following dialog appears:



The selections available are:

- **Revolution**. Save the data as a proprietary file that can be reopened in Revolution. By using this format, the image presentation can be adjusted at any time after the file is saved to disk.
- **TIFF-Mac**. Save the data as a TIFF file in Macintosh format.
- **TIFF-PC**. Save the data as a TIFF file in Windows format.
- **JPEG**. Save the image as a JPEG graphic, suitable for web presentation.
- **PICT** (Macintosh) or **BMP** (Windows). Save the image in the native format of the operating system as a picture that can be pasted into a presentation program.

Micron Marker Calibration

Before the Micron Marker can be properly calibrated in Revolution, the Scanning Interface Unit [must be adjusted](#). The micron marker is controlled and calibrated from the [Micron Marker Preferences](#). The software selects a suitable marker size and adjusts itself automatically depending on the magnification you select from the **Mag** menu. Use the following step-by-step procedure to calibrate the software for the micron marker:

- Using a calibration sample (i.e., a grid with a known dimension) placed in the microscope and properly adjusted and visible in a preview window, select the microscope magnification to agree with that shown in the **Mag** menu of the [Micron Marker Preferences](#). If the available software magnification menu selections are not suitable, see the next step to learn how to create a new one.
- In the default configuration, three standard magnifications are provided in the **Mag** menu (1000, 10000, and 100000). The user can add any magnification by editing the values in the text boxes and clicking the **Add** button. The user can also **Change** or **Delete** any magnifications. Any number of magnifications can be set up and saved. all but 3 can be deleted. After entering the desired values, the user **must** click on either **Change**, **Add**, or **Delete** to make the changes stick.
- After selecting a microscope magnification to agree with that shown in the **Mag** menu of the [Micron Marker Preferences](#), you will need to edit the value in the **Global Calibration** field. The calibration constant (default = 500) is used to properly map the micron marker size into the correct number of screen pixels for display on the image. It is global in the sense that it used by every magnification record. To set the micron marker calibration, simply change this number so the micron bar in the preview window is the correct length. Although this procedure may be somewhat tedious, the micron marker calibration should not have to be changed once it has been set. The software will use this number and knowledge of the magnification to automatically draw the correct micron marker on the screen. This information will be

properly burned into the final acquired image as well. Note that unless the marker length is overridden via the **Override Auto-Sizing** checkbox, the numerical length of the micron bar is dynamically changed (auto-sized) so that the marker uses approximately the same amount of space in the image ("this is not a bug; it's a feature!"). It can be somewhat annoying during the calibration procedure, but works nicely after the calibration is complete, and keeps the user from having to select different lengths as the magnification is changed.

- For those who need to know how the calculation is performed, the equation relating the number of pixels in the marker line to the calibration factor (**Cal**) is:

$$\# \text{ pixels} = \frac{L \cdot M}{2000 \cdot Cal} \cdot Tweak$$

where **L** is the length of the micron marker in **nanometers** and **M** is the magnification currently selected. The default value of **Cal** = 500 normalizes the equation for an image width of 256 pixels. For example, a 6 mm marker at 10000 magnification with a **Cal** of 500 and **Tweak** = 1 in a 256 x 256 preview window will be 60 pixels long. Acquiring a 640 x 480 image at these settings will create a marker 150 pixels long in the final image. The precise form of the equation is not as important as the knowledge of how the different factors are used to adjust the micron marker length. The intention is not to force the user calculate the exact number of pixels that are required; the intention is to have the user adjust parameters until the marker is the right length.

- If you need to tweak the calibration factor for a particular magnification, you can do so with the **Tweak** edit field. **Tweak** is a multiplicative correction which has the effect shown in the above equation. The software will remember the tweak correction factor for each defined magnification (in contrast to the **Global Calibration** factor), allowing the greatest possible precision across all magnifications.