MSC Family
Software User's Guide

Models 791, 792, 794, and GIF MSC

Macintosh, Windows 95, Windows NT

Gatan, Inc.

5933 Coronado Lane
Pleasanton, CA 94588
Tel   (925) 463-0200
FAX   (925) 463-0204

December 1998

Revision 1

Preface

About this Guide

This *MSC Family Software User's Guide* is written for use with DigitalMicrograph 3.3 or later and provides instruction for installation of the Camera Plug-In software, some pointers for trouble shooting the installation, and basic instructions for system customization and optimal image acquisition. For additional discussion of analytical digital imaging, refer to the *DigitalMicrograph User's Guide*.

Preview of this Guide

The *MSC Family Software User's Guide* includes the following chapters:

Chapter 1, "Introduction," provides an overview of the MSC family of cameras, discusses computer requirements, and provides instruction for installation of the Camera Plug-In.

Chapter 2, "Acquisition Parameters," describes the various acquisition parameters and processes involved in setting up for image acquisition.

Chapter 3, **"Image Acquisition,"** describes image acquisition and processes involved in optimizing image acquisition, i.e., setting exposure time, calibration, etc.

Chapter 4, "Care and Maintenance," provides suggestions for the care and maintenance of the system and a list of the most often asked questions when troubleshooting.

Disclaimer

Gatan, Inc., makes no express or implied representations or warranties with respect to the contents or use of this manual, and specifically disclaims any implied warranties of merchantability or fitness for a particular purpose. Gatan, Inc., further reserves the right to revise this manual and to make changes to its contents at any time, without obligation to notify any person or entity of such revisions or changes.

Copyright and Trademarks

© Gatan, the Gatan logo is registered to Gatan, Inc.

The product names AutoFilter, BioScan, Clipring, DigiPEELS, DigiScan, DigitalMicrograph, DigitalMontage, Duo Mill, DuoPost, Gatan LowDose, GIF, Hexlok, Hexring, HotHinge, MSC, PECS, PIPS, Toggle Tilt, and Whisperlok are trademarks belonging to Gatan, Inc.

Support

Contacting Gatan Technical Support

Gatan, Inc. provides free technical support via voice, Fax, and electronic mail. To reach Gatan technical support, call or Fax the facility nearest you or contact by electronic mail:

- **Gatan Inc., USA (West Coast)**

  Tel: (925) 463 0200
  Fax: (925) 463 0204

- **Gatan Inc., USA (East Coast)**

  Tel (724) 776 5260
  Fax: (724) 776 3360

- **Gatan GmbH, Germany**

  Tel: 089 352 374
  Fax: 089 359 1642

- **Gatan, UK**

  Tel: 01536 743150
  Fax: 01536 743154

- **Nippon Gatan, Japan**

  Tel: 0424 38 7230
  Fax: 0424 38 7228

- **Singapore**

  Tel: 65 235 0995
  Fax: 65 235 8869

- **Gatan Online**

  http://www.gatan.com
  info@gatan.com
  help@gatan.com

Table of Contents

List of Figures

1 Introduction

The Gatan family of MultiScan CCD cameras (MSCs) are designed to run at different readout speeds and acquire high-quality transmission electron microscope images. The cameras eliminate the need for darkroom work and chemical waste disposal. They provide the capability for producing versatile and reproducible digital images.

1.1 Overview

All the cameras in the MSC family are controlled by the Camera Plug-In, the camera control software, which runs on Macintosh, Windows 95, or Windows NT platforms, in conjunction with DigitalMicrograph, the image-processing application developed by Gatan to display, process, analyze, print, and transfer images.

The MSC Family

Gatan manufactures many different models of MSCs.

Table 1-1   **Gatan MultiScan family of cameras.**

Model Number	Camera	CCD Size	Dynamic Range	Cooling	Mounting	Anti-Blooming	Retractable Sensor
791	MultiScan	1024 x 1024	14-bit	Peltier	35-mm port	Yes	Yes
792	BioScan	1024 x 1024	12-bit	Air	35-mm port	No	Yes
794	MultiScan	1024 x 1024	14-bit	Peltier	Bottom	Yes	Yes
794	GIF MultiScan	1024 x 1024	14-bit	Peltier	End of GIF	Yes	No
794/20	MegaScan	2048 x 2048	14-bit	Peltier	Bottom	Yes	Yes

In all models except the GIF MultiScan, the CCD sensor can be retracted to allow the electron beam to pass through the camera to other sensors mounted below. The CCD sensor on the GIF MSC does not need to be retracted as it does not interfere with any imaging devices.

The CCD sensor is automatically retracted to a sealed position when the microscope is vented to protect the cooled sensor from thermal shock and moisture. The CCD on the BioScan is not cooled and therefore does not need the protection. The CCD sensor on the GIF MSC is protected by a gate valve, which seals off the camera when the microscope is vented.

Images

Images are viewed in quasi-real time. You can select different image-acquisition parameters to "customize" the acquisition to produce high-quality images. For example, images can be binned for fast viewing, dark-subtracted to set the signal reference level, and gain-normalized to eliminate gain variations.

Shutters

The CCD camera is interfaced with the microscope shutter (below specimen) so that the beam can be blanked during CCD readout and clearing prior to each subsequent exposure to prevent smearing of the image. Some systems also have a blanker above the specimen. When closed, both specimen and CCD sensor are protected.

However, a mechanism is provided to stop beam blanking when the viewing screen is down or when the CCD sensor is retracted. This feature prevents the shutter control from interfering with specimen viewing or use of the film camera.

The MSC System

The MSC system consists of the following components:

- DigitalMicrograph 3.3 or later.

- Camera Plug-In 1.2 or later.

- MSC CCD camera.

- MSC Controller and camera cable.

   The Controller regulates the camera electronics, generates the digital data, and is connected to the camera via the camera cable.

- Gatan PCI DMA interface card and cable.

   The card provides the MSC Controller with high-speed data-transfer capabilities. The DMA cable connects the MSC Controller to the computer.

1.2 Computer Requirements

The MSC system can be operated in both Windows and the MacOS.

Hardware Requirements

- Pentium processor or PowerPC/G3 computers.

- 64 MB of RAM or more.

Software Requirements

- Windows 95/NT 4.0 or MacOS 8.0 or later

1.3 Camera Plug-In Installation

Once the MSC is installed and the interfaces to the TEM are established, the Camera Plug-In needs to be installed.

When you are installing the Camera Plug-In, make sure that:

- **DigitalMicrograph is installed on your computer.**

- **The DMA card is installed.**

  If using a PowerMacintosh, make sure the DMA card is placed in the slot closest to the power supply.

- **The DMA cable is properly connected.**

   The cable goes from the MSC Controller to the computer.

- **The MSC Controller is properly connected and turned on.**

  Check the camera cable that connects the camera to the MSC Controller.

To install the Camera Plug-In

1. **Locate DigitalMicrograph on your computer.**

   Macintosh users, be sure you find the actual application and not an alias. If it is an alias, the filename will be italicized.

   To locate the actual application, choose SHOW ORIGINAL from the FILE menu.

   For PC users, the Installer will locate the application.

2. **Open the Camera Plug-In folder from the disk provided.**

3. **Open the Installation Instruction file in the Camera Plug-In folder.**

   Follow the instructions provided in that file for either Macintosh or Windows installation.

4. **Restart your computer after installation has been completed.**

5. **Launch DigitalMicrograph.**

   The DigitalMicrograph Workplace and the MSC Command Palette will appear.

1.4 Troubleshooting the Installation

If the MSC Command Palette does not appear, verify the following:

- **Check that CAMERA appears in the main menu bar.**

   If it does not appear, the Camera Plug-In was not installed correctly. Repeat the installation procedure.

   If it does appear but its menu items are greyed out, check the DMA cable connection.

- **Check that the DMA card is properly installed.**

   If using a PowerMacintosh, make sure the DMA card is in the slot closest to the power supply.

- **Check that the MSC Controller is properly connected and turned on.**

- **Check that all the necessary files are placed correctly in their respective folders (Packages and Extensions).**

- **Restart your computer.**

2 Acquisition Parameters

This chapter discusses the various image acquisition parameters that need to be set up for actual image acquisition.

2.1 Setup

Prior to actually acquiring an image, you need to verify that the camera and the microscope are properly set up. The following steps will enable the first-time user to verify camera setup and to get up and running with DigitalMicrograph and the Camera Plug-In:

To verify set up

- **Switch on the MSC Controller.**

  Make sure the two switches on the right side of the MSC Controller are switched to "Auto" and "Computer."

  Note: The following applies only to those MSCs with water-cooled Peltier coolers. The BioScan is not water cooled.

- **Check the cooling water to the CCD sensor is flowing properly.**

  Check the Flow Meter and verify that the flow regulator is set for a range of 5–15 L/hr flow and there is indeed water flow through the regulator.

- **Turn on the Peltier cooler.**

  The Peltier cooler should not be turned on if the cooling water is not flowing. The switch for the cooler is on the front panel of the MSC Controller.

- **Let the camera temperature stabilize.**

  Although images can be taken immediately, for best results it is recommended that you wait for the camera temperature to stabilize (1+ hr depend-

ing on application) before acquiring a gain-reference image or images for storing.

While the camera temperature is stabilizing, you can proceed to enter information about the run.
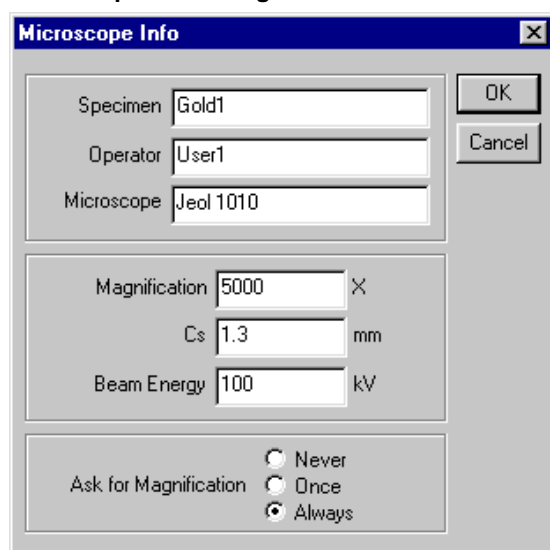
To enter information for a run

1. **Launch DigitalMicrograph.**

2. **Choose** GLOBAL MICROSCOPE INFO **under the** MICROSCOPE **menu**.

    The Microscope Info dialog will appear.

Figure 2-1   **Microscope Info dialog.**

3. **Enter the information in the dialog box.**

Specimen	Enter specimen name.
Operator	Enter operator name.
Microscope	Enter microscope name.
Magnification	Enter magnification as indicated on the microscope. If EMControl software is installed, the indicated magnification will automatically be entered by the software and there is not need to enter it here.
Cs	Enter microscope spherical aberration (Cs) value.
Beam Energy	Enter operating voltage.
Ask for Magnification	Select when you want to be prompted for microscope magnification.

This information will be stored with each acquired image.

Magnification Prompts

You may select when you want to be prompted for microscope magnification.

Note: If you have EMControl software, the indicated magnification (magnification as displayed on the microscope) will automatically be entered by the software and the prompt options are overridden.

The three options are as follows:

- **Never:** You will not be prompted for the indicated magnification.

- **Once:** You will be prompted to input the indicated magnification when you start your first acquisition**.**

- **Always:** You will be prompted for the indicated magnification each time you start to view/acquire images. **This is the recommended mode of operation**.

To change your magnification

- **Select SET INDICATED MAGNIFICATION under CAMERA in the main menu.**

  You will be prompted to enter the new magnification, which will be automatically entered in the Magnification field.

2.1.1 MSC Command Palette

You use the MSC Command Palette to set up different image acquisition parameters and to initiate/restart actual acquisition. The MSC Command Palette also contains an Acquisition Bar that displays the progress of each acquisition.
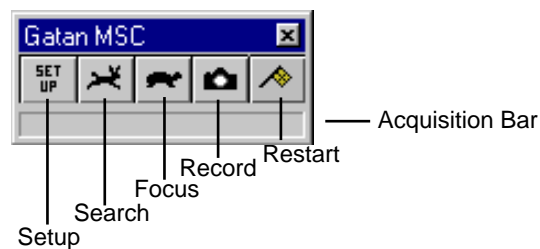
Figure 2-2   **MSC Command Palette.**

Table 2-1 summarizes the functions for the five icons in the MSC Command Palette.

MSC Command Palette icons.

Icon	Description
Setup	This icon will bring up a dialog where you can set up various acquisition parameters for each acquisition setting.
Search	This icon will initiate acquisition using user-selected parameters in Search, a fast acquisition typically used to survey a sample.
Focus	This icon will initiate acquisition using user-selected parameters in Focus, a slow acquisition of higher image quality typically used to "zero in" on a particular feature in a sample.
Record	This icon will initiate acquisition using user-selected parameters in Record, a slow acquisition of very high image quality typically used to create a file of the feature/sample for permanent storage.
Restart	This icon allows you to restart image acquisition in the frontmost image/image selection window.
Acquisition Bar	This bar will display acquisition status.

The three acquisition settings, Search, Focus, and Record, have parameters that can be configured by the user in the Setup dialog, brought up by clicking on the Setup icon. Once the parameters have been set for a specific acquisition setting, the user exits the Setup dialog and clicks on the respective acquisition icon to initiate acquisition within the predetermined parameters. If a continuous acquisition is interrupted (by pressing the Space Bar), it can be restarted by clicking on the Restart icon.

2.2 Acquisition Parameters

Image acquisition parameters can be selected via the Setup dialog accessed by clicking on the Setup icon on the MSC Command Palette (see Figure 2-2).

You can also access the Setup dialog by selecting CAMERA SETUP under CAMERA in the main menu.

User-selected acquisition parameters for each acquisition are saved by the program.
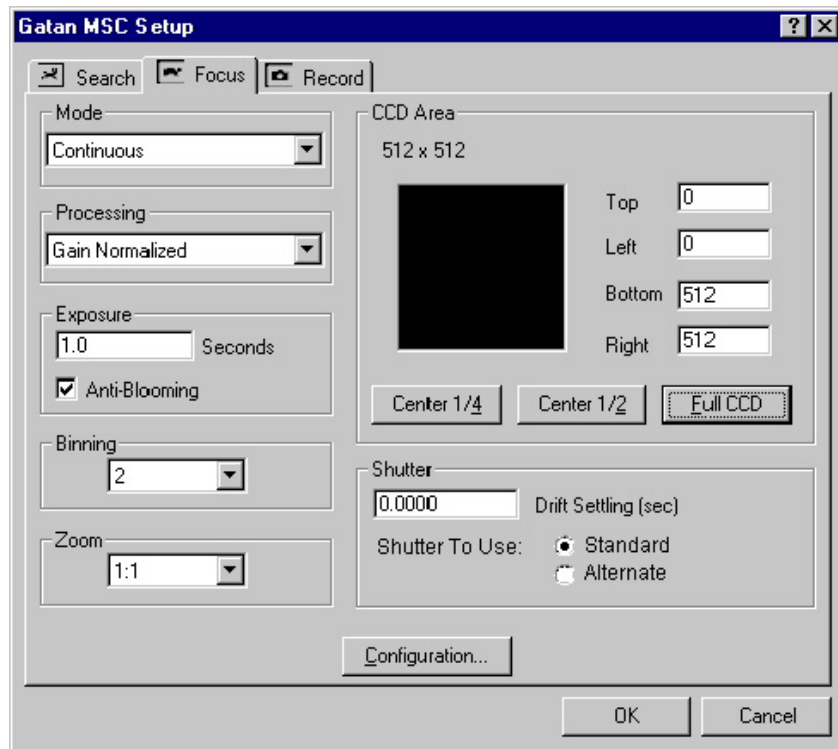
To select acquisition parameters

• **Click on the Setup icon or choose CAMERA SETUP under the CAMERA menu.**

The Gatan MSC Setup dialog appears and you can select various parameters for acquisition (see Figure 2-3).
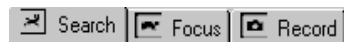
Figure 2-3   **Gatan MSC Setup dialog.**

2.2.1 Acquisition Settings

You can select from three image acquisition settings.

For each setting, you can select from a variety of acquisition parameters, i.e., the mode of acquisition, the type of processing, the exposure, the CCD area, the binning, etc. Thus, each completely independent acquisition setting can be customized by the user with specific selection of different parameters. Each of these parameters will be addressed in this chapter.

Search

Search is typically set up for fast acquisition. Images can be acquired in this setting for all three acquisition modes (see Section 2.2.2).

Focus

Focus is typically a slow acquisition setting with high image quality used to "zero in" on areas of interest and focusing a sample.

Record

Record is typically a slow acquisition setting with very high image quality used to create a file of the acquisition for permanent storage. Images in Record are acquired at a slightly slower rate than the Focus setting and typically acquired in Single Frame acquisition mode.

2.2.2 Acquisition Modes

Each acquisition setting can be customized to run under different acquisition modes.

Turbo

Note: **This mode is available only on Macintosh systems.**

Turbo is a fast acquisition mode that acquires 8-bit images for high-speed viewing, best suited for viewing sample areas and setting initial focus.

When Turbo mode is selected, the acquired image is displayed on a black screen.

If the image is too bright or too dark, hit the Enter key to have the contrast automatically adjusted.

Press the Space bar to stop image acquisition and exit from Turbo mode.

Continuous

Continuous is a mode of acquisition that continuously acquires and displays 14-bit images. This is a high-quality mode of acquisition, typically used for viewing and focusing.

Single Frame

Single Frame is the highest quality, slowest mode of acquisition, used for acquiring 14-bit images for permanent storage.

2.2.3 Processing

There are three ways in which an image can be processed.
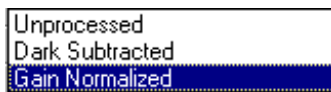
Images acquired in the Turbo mode are always unprocessed, i.e., acquired in the Unprocessed mode. Images in Continuous and Single Frame modes can be acquired with any of the three processing modes.

Unprocessed

Selecting Unprocessed will give you a raw image that has not been corrected for device noise and gain variations and is displayed "as acquired" from the camera. Images acquired in Turbo mode of acquisition are always unprocessed.

Dark Subtracted

Selecting Dark Subtracted will give you an image that has been corrected for dark current (device noise). This image is the result of subtracting a "dark image" from the unprocessed image. The dark image is acquired with the shutter closed (no beam reaching the CCD sensor) just prior to the acquisition of and with the same parameters as the unprocessed image.

Gain Normalized

Selecting Gain Normalized will give you an image that has been dark subtracted and gain normalized, resulting in an image of very high quality. With this processing, a dark image is subtracted from the unprocessed image. The difference is then corrected by the gain-reference image to provide a gain-normalized image. See Section 2.4 for a detailed discussion of gain normalization.

2.2.4 Exposure

The exposure reading on the microscope cannot be taken as the optimal exposure time for the CCD camera. You need to determine the appropriate exposure time specific to the camera. See Section 3.5 for discussion on setting the optimal exposure for the camera.

Anti-Blooming

Anti-Blooming allows you to control blooming, a phenomenon where a pixel(s) is saturated and electrons spill over into neighboring pixels on the CCD sensor. The Anti-Blooming feature is CCD dependent and allows the trapping of a certain amount of bloomed charge per second. Generally this feature functions better with longer exposures.

If blooming is not eliminated by selecting Anti-Blooming, reduce the beam intensity and increase the exposure time to spread the total dose over a longer period.

2.2.5 Binning

Binning is the process whereby the charges in adjacent horizontal and vertical pixels are summed to create larger "super" pixels. Binning by 2 will sum the charges from 4 pixels (2-pixel x 2-pixel) into 1; binning by 4 will sum 16 pixels into 1. You can select to bin by 1 through 8.

Binning decreases the net total number of pixels used in the readout, decreases the exposure time (due to more charge per pixel), reduces read-out times, and allows for faster display and processing of the image. DigitalMicrograph automatically reduces the exposure time by the square of the binning factor to maintain comparable signal levels.

2.2.6 Zoom

You can specify the zoom factor for the displayed image after it is acquired. The factor only affects the display and not the raw image data.

Zoom of 2:1 doubles the display. Zoom of 1:2 halves the display. You have the option to magnify a display by 4x or shrink it to a quarter of its original size.

2.2.7 CCD Area

You can specify the area of the CCD to be read. The smaller the area that you read out, the faster the images can be acquired.

To select the CCD area

There are two ways to select the CCD area:

- **Use the three predefined buttons for the desired area of the CCD.**

- **Input row and column values.**

  You input row values (Top and Bottom) and column values (Left and Right) from a range of 0–1023 to bound the desired area, which will be represented pictorially. This range will change if you bin because the effective number of pixels will be smaller.

2.2.8 Shutter

In this group box, you can input a Drift Settling value and select how you want to operate your shutter(s) during an acquisition.

Drift Settling

The Drift Settling field allows you to enter a value to delay the beginning of actual acquisition after the MSC Controller gives the command to open the shutter. This delay allows your system (specimen, shutter coils, etc.) to settle and prevents smearing of your image.

For routine work, use the default value of zero. If you find that you are using a >2 second delay and smearing still occurs, this could be an indication that the shutter is not functioning correctly either because it is not connected properly or because the shutter override is behaving improperly. Check with your Gatan service person.

MSC Shutters

The MSC requires a shutter to move the beam off the scintillator during CCD readout and also to protect the scintillator when the camera is idle (not acquiring) but in the beam path. When correctly configured, the shutter will automatically open during acquisition for a specified exposure period and close when acquisition is complete.

When the user is viewing images at any post-shutter location (e.g., the viewing screen, or, in the case of the GIF, on the TV camera), an automatic shutter override is used to keep the shutter open. In this mode, a signal from the microscope indicates that the camera is not in the beam path and the shutter is left open so as not to obstruct the beam for any post-shutter devices. On some systems, a manual override may be needed when exposing film. See subsequent section on "Shutter Override" for more detail.

The shutters that can be used include the pre- and post-specimen microscope shutters and the GIF shutter (for those MSCs that are attached to GIFs).

The advantage of a post-specimen shutter is that the sample is continuously illuminated thus reducing sample drift. For this reason, most non-GIF MSCs are connected to the post-specimen microscope shutter.

A pre-specimen shutter, on the other hand, contributes to reduced beam damage of the specimen and is, therefore, useful in low-dose applications since the beam can be blanked when the user is not using the camera or the viewing screen.

GIF shutters are used with MSCs mounted on the back of a GIF. The shutter is located inside the GIF, after the BF/DF detectors. This configuration allows for simultaneous STEM dark-field imaging and spectrum acquisition. However, the GIF shutter is slower than some microscope shutters, so for the very short acquisition times used in spectroscopy, it may be preferable to use a microscope shutter. For this reason, many GIF MSCs are connected to both the GIF shutter and one of the microscope shutters.

The MSC has the capability to support two shutters—a "Standard" shutter and an "Alternate" shutter (see Figure 2-3). The shutter connections are on the back of the MSC Controller and are labeled accordingly. Both shutter connections may be connected individually to any one of the pre-/post-specimen shutter on the microscope or to the GIF shutter. The shutter designation (Standard vs. Alternate) is made at installation based on the capabilities of the microscope and the applications for which it is used and is strictly arbitrary. At installation, the Gatan Service Engineer will try to configure the system hardware and software such that the shutter operation is as transparent as possible to the user.

To choose a shutter

In the Gatan MSC Setup dialog for each of the three acquisition settings (Search, Focus, and Record), you may select the shutter to use.

- **Select Standard or Alternate from Shutter to Use.**

If only one shutter is connected, the shutter of choice in the Gatan MSC Setup dialog should be Standard shutter.

On systems with two shutters connected, only one shutter will blank (close) at a time; the other will be unblanked (open). You may select which shutter to use in each of the acquisition settings. For instance, if you know the characteristics of the different shutters that are available, you might select a faster, pre-specimen shutter for Turbo viewing in the Search mode.

To select shutter position when not acquiring

You have the option of having the shutter "Closed" or "Open" when the camera is in the beam path but not actively acquiring images (idle). The recommended setting is "Closed" to reduce the risk of damage to the scintillator.

- **Click on the Configuration button and bring up the MSC Configuration dialog (see Figure 2-4).**

- **Select Closed or Open under Shutter Position (When Not Acquiring).**

In selecting the setting, you should consider the location of the shutter. For example, in the case of the pre-specimen shutter, this setting will control whether the specimen is being continuously irradiated when the camera is idle.

Note: The Shutter LED on the MSC Controller indicates whether the shutter is open (LED lit) or closed.

Figure 2-4   **MSC Configuration dialog.**
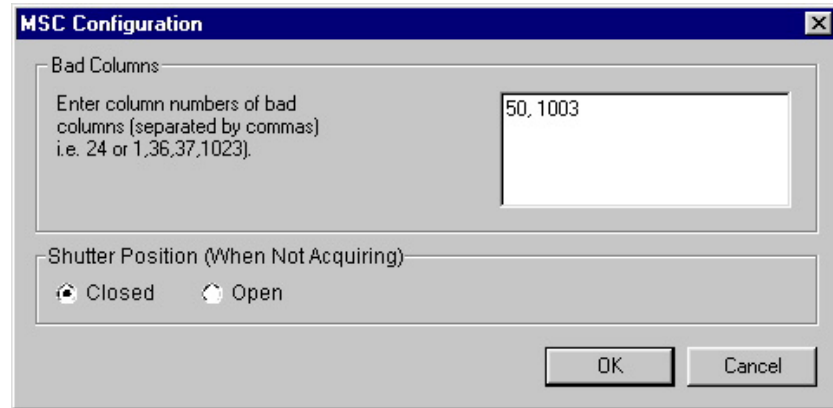
On most systems, the Shutter Position (When Not Acquiring) option does not apply when the camera is not in the beam path. When the camera is retracted or the screen is lowered (in the case of a bottom-mount camera), the camera releases control of the shutter, which will then remain open. This way, the shutter does not affect normal image viewing on the screen or acquisition on film.

Manual Shutter Override

However, some microscopes do not provide the necessary external control to allow the "switch over" from camera to microscope control of the shutter to occur. On those systems, the shutter may remain closed and the Shutter LED on the MSC Controller may remain off even when the viewing screen is lowered.

Case 1: For systems without the necessary external controls.

A manual override feature is provided for those systems that do not have the necessary external control and only applies to those systems on which the shutter does not automatically open when the viewing screen is lowered.

To manually override the shutter

- **Flip the Shutter switch on the MSC Controller to the Open position.**

    Remember to switch it back to Auto when you are ready to acquire images with the camera.

Case 2: To expose film with a bottom-mount camera.

You also need to use the manual shutter override feature if you want to expose film with a bottom-mount camera inserted, but idle.

Consider the following scenario: The screen is raised, the camera is inserted but idle, and you have selected to keep the shutter closed when the camera is idle. A film exposure will fail for the shutter will not open.

To expose film in this scenario, you can either retract the camera so it is no longer in the beam path or use the shutter override feature described above.

To use script acquisition

The shutter selected in the Record panel of the Gatan MSC Setup dialog is used in the script acquisition functions.

2.2.9 Configuration

Additional parameters can be customized by clicking on the Configuration button in the Gatan MSC Setup dialog.

Bad Columns

Sometimes a CCD may have defects that result in entire columns appearing very bright in the acquired image. The software can compensate for these bad-column defects.

Once defective columns are identified, their numbers can be entered into the Bad Columns field in the Configuration dialog. The values for these columns will be replaced by the mean of the pixels in the adjacent columns in all acquired images. Consecutive bad columns will be replaced by interpolated pixels from both sides of the consecutive bad columns.

To correct for bad columns

1. **Acquire an image that clearly shows the column defect(s).**

2. **Use the Magnifying tool and zoom up on the defective column.**

3. **In the Target palette, click on the Ruler (by the Image radio button) and switch to the "uncalibrated" mode.**

4. **Place the cursor over the defective column.**

    The coordinates of the cursor position will appear in the Image Status window. The "x" coordinate is the column number.

5. **Click on the Setup icon in the MSC Command Palette and then select the Configuration button.**

    This will bring up the MSC Configuration dialog (see Figure 2-4).

6. **Enter the column number(s) in the Bad Columns field, in ascending order separated by commas.**

Exit both the Configuration and the Gatan MSC Setup dialogs.

Shutter Position

The selection of shutter position when the camera is idle has been discussed in the section of "To select shutter position when not acquiring," under MSC Shutters.

2.3 Recommended Operating Parameters

Your acquisitions can be customized by various combinations of the acquisition parameters. The following are some recommended settings as a starting point.

Table 2-2   **Recommended operating parameters.**

Parameters	Search	Focus	Record
Mode	Continuous	Continuous	Single frame
Processing	Unprocessed	Gain normalized	Gain normalized
Exposure	0.05 sec	0.5 sec	Optimized
Anti-blooming (if available)	Selected	Selected	Selected
Binning	3	1	1
Zoom	1:1	1:1	1:2
CCD Area	Full CCD	Center 1/4 or 1/2	Full CCD
Drift settling	0 sec	0 sec	0 sec
Shutter normally closed	Selected	Selected	Selected
Alternate shutter	Selected	Selected	Selected

2.4 Gain Normalization

There is no perfect detector. There will always be gain variations from pixel to pixel, across the CCD, in the fiber optics, and in the coupling between the fiber optics and the CCD. Although the variations amount to only a few percent, they contribute to the "gain variation" and can become bothersome for they will show up as a fixed reproducible pattern superimposed on every newly acquired image. This gain variation, however, can be completely removed by a process called gain normalization.

The process starts with an acquisition of a dark image—an image obtained with no illumination to the camera. This dark image will only contain contributions from dark current (device noise). An unprocessed image is acquired and the dark image is subtracted from it, resulting in an image that has contributions from the gain variations. This "dark-subtracted" image is then divided by a gain-reference image, which is obtained by acquiring a dark-subtracted image of an evenly illuminated CCD. The quotient is the final dark-subtracted, gain-normalized image, I_F, given by the following equation:

$$I_F = (I_{raw} - I_{dark}) / I_{gain}$$

where:

I_{raw} = the unprocessed image,

I_{dark} = the dark image,

I_{gain} = the dark-subtracted, gain-reference image.

In practice, the dark subtracted, gain-reference image is calculated as a $1/I_{gain}$ value so the gain normalization calculation is in actuality a multiplication process. When viewing a gain-reference image, the reciprocal value of the gain-reference image will display the dark areas in gain variation as bright areas.

Preparing a Gain-Reference Image

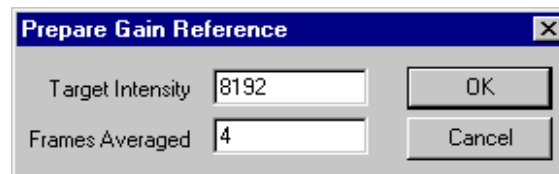A gain-reference image is needed to perform gain normalization.

To obtain a gain-reference image

• **Choose PREPARE GAIN REFERENCE under the CAMERA menu.**

   This will bring up the Prepare Gain Reference dialog.

Figure 2-5   **Prepare Gain Reference dialog.**

Enter the target intensity of each dark-subtracted, gain-reference image and the number of frames to be averaged. Averaging helps to minimize the random noise in the gain-reference image.

Suggested starting point for 14-bit cameras is a target intensity of 8200 counts (half the dynamic range) and 4 frames to be averaged.

Suggested starting point for 12-bit cameras is a target intensity of 2500 counts (approximately half the dynamic range) and 4 frames to be averaged.

The gain-reference image is stored on disk and does not need to be acquired again until the gain characteristics have changed enough for traces of a fixed pattern to appear in the gain-normalized images.

If you are not sure whether you need a new gain-reference image, acquire a gain-normalized image of an evenly illuminated CCD. If a fixed pattern is visible in the image, a new reference is needed.

To view a gain-reference image

Each freshly acquired gain-reference image is processed to facilitate quick gain corrections and then automatically saved to disk. It is not displayed. It can be viewed, however.

• **Acquire the gain-reference image and obtain a gain-normalized image.**

• **Go to the WINDOW menu**

You will see the gain-reference image listed at the bottom.

To stop gain reference preparation

The entire gain-reference preparation procedure can be aborted at any time during the process. Press the ESC key anytime during the operation.

3 Image Acquisition

This chapter provides information on basic image acquisition and additional discussion on optimizing your acquisition with topics such as selecting the optimal exposure time, image calibration, magnification table, etc. For more detailed information on image manipulation, analysis, and processing, please refer to the *DigitalMicrograph User's Guide*.

3.1 Image Acquisition

Image acquisition can be controlled through the MSC Command Palette or through menu selection under CAMERA in the main Menu.

To acquire an image

1. **Be sure the MSC camera is inserted (if retractable).**

2. **Click on the Setup icon in the MSC Command palette.**

   This will bring up the Gatan MSC Setup dialog (see Figure 2-3).

   Set up the desired acquisition parameters in the Gatan MSC Setup dialog. See the suggested operating parameters from Table 2-2.

3. **Click OK to close the Gatan MSC Setup dialog.**

4. **Click on the appropriate acquisition icon on the MSC Command Palette to initiate image acquisition.**

   Use the Enter key to adjust image display contrast.

5. **Hit Space Bar to terminate continuous image acquisition.**

To resume acquisition

- **Click on the Restart icon in the MSC Command Palette or choose RESTART VIEW under the CAMERA menu.**

Acquisition will be resumed with the same acquisition parameters customized (for the frontmost image) prior to termination of acquisition.

To view a subarea

You can select to view an area within an acquired image.

- **Use the Rectangle ROI tool to frame a desired subarea on the image.**

- **Choose VIEW SELECTED AREA from the CAMERA menu.**

    The subarea you have selected will be displayed.

3.2 Commands and Manipulations

As images are acquired, certain keystroke commands can be used to facilitate various aspects of the acquisition and image manipulation. Table 3-1 contains a list of keystroke commands.

Table 3-1　　　　　**Keystroke commands.**

Goal	Description
Double exposure time	Up arrow key.
Halve exposure time	Down arrow key.
Recalculate image display contrast	Enter key (in Turbo mode).
Switches to Turbo FFT	F
Switches back to Turbo View	T

Table 3-2 contains a list of simple operatives for image manipulation. For more detail, refer to the *DigitalMicrograph User's Guide*.

Table 3-2　　　　　**Operatives.**

Goal	Description
Resize window	Click with Pointer tool and drag at corner.
Move image (target: page)	Click on image with Pointer tool and drag.

Table 3-2   **Operatives.**

Move image (target: image)	Click on image, hold down Option key, and drag with Pointer tool for Mac systems. Click/Alt in Windows.
Move page	Click with Move Page tool (Hand) and drag page.
Stop acquisition immediately	Space bar. Stops acquisition at the end of current frame.
Restrict selection to be a square	Hold down Control key while making a selection with the Rectangle tool.
Restrict selection to dimensions that are a power of 2	Hold down Option key while making a selection for Mac systems. Use Alt key in Windows.
Restrict a line to 45° or 90°	Hold down the Control key while making the selection.

3.3 Save Numbered Series

You can save a series of acquired images and have DigitalMicrograph automatically number them for you.

To save a numbered series

1. Choose GLOBAL INFO under the FILE menu.

Select Auto-save under General in the Global Info dialog.

Figure 3-1   **Global Info—General-Auto-save.**

Series name	Input name for the series.
Series index	Input starting number for the series.
Save Preview	Select to save a preview.
Update Image Database	Select to automatically save to disk.
Select Folder	Select destination folder for the series.

2. **Click OK.**

3. **Acquire the first image of the series.**

4. **Choose SAVE NUMBERED under the FILE menu.**

    The image will be saved as Seriesfilename.1. Acquire another image and used the same command to save as Seriesfilename.2.

5. **To terminate one series and start another, return to the Global Info Auto-save dialog.**

    Input the new series filename, index, etc., and proceed with the acquisition of the new series.

3.4 Acquiring a Time Sequence

You can acquire a timed sequence of images and save them to disk. Set up your acquisition parameters and optimize your image.

1. **Choose ACQUIRE SERIES under the CAMERA menu.**

    You will bring up the Acquire Time Sequence dialog.

Figure 3-2   **Acquire Time Sequence dialog.**
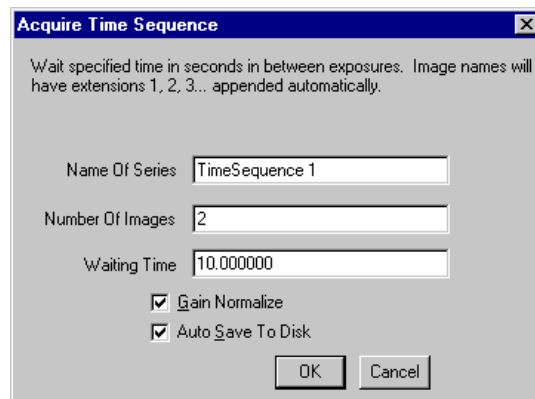
Name of Series	Input name of sequence file.
Number of Images	Input number of images in the sequence.
Waiting Time	Input the delay time between successive exposures (as measured from the beginning of an exposure to the beginning of the following exposure).
	The delay time must be longer than the sum of the exposure time and any other time associated with acquiring an image
Gain Normalize	Select to acquire gain-normalized images.
Auto Save to Disk	Select to automatically save to disk.

The speed and the number of images you can save is determined by the available space on your storage system—be it RAM or disk. Writing to RAM is much faster than writing to disk, but the number of images will be limited by the total amount of RAM allocated to DigitalMicrograph. If higher-speed acquisition is desired, do not select Auto Save to Disk and images will be kept in RAM. If speed is not an issue, select Auto Save to Disk and save to disk to gain larger storage capacity.

2. **Click OK**

3. **Start acquisition of your series.**

3.5 Exposure

The exposure reading on the microscope cannot be taken as the optimal exposure time for the CCD camera. You need to determine the appropriate exposure time (displayed in the Acquisition Status palette) specific to the camera.

To do so, you need to know the average intensity of your image. Place the cursor on the image. The intensity of the pixel under the cursor is displayed in the Image Status palette (Value). Move the cursor over the image to determine the approximate average intensity of your image.

To obtain the appropriate exposure for the camera

- **Acquire a gain-normalized image.**

  Be sure you have uniform illumination across the CCD sensor.

- **Adjust beam intensity and exposure time to obtain the suggested intensities given below:**

    - 14-bit camera—5000-10,000 counts (in the bright regions).

    - 12-bit camera— 2000-3000 counts (in the bright regions).

Saturation of the CCD camera

Under the electron beam, the pixels of the CCD sensor can become saturated when their accumulated charge reaches the maximum charge-storage capacity. For a 14-bit camera, this occurs when the intensity is over 16,384 counts. For a 12-bit camera, saturation occurs when the intensity is over 4096 counts.

If an image contains saturated pixels, image detail in those pixels are lost. You will, however, observe in the saturated region a "fixed pattern" characteristic to the CCD, but which is not relevant to your sample.

If saturation occurs, decrease either the exposure time or the beam intensity. The Up/Down Arrow keys provide an easy way to double/halve the exposure time in Continuous mode.

It should be pointed out that the CCD camera has excellent linearity, i.e., the recorded intensity and exposure time are extremely linear within the dynamic range. Due to this linearity, a "new" exposure time can be easily estimated from the "old" intensity/exposure time.

3.6 Magnification Correction

The magnification at the viewing screen and that at the CCD camera are different due to the location of the latter below (or above) the viewing screen. Without magnification correction, the software will treat the magnification at the screen (indicated on the microscope) as the actual magnification at the camera. Thus, it is necessary to perform a magnification calibration to correct for this magnification discrepancy.

3.6.1 Magnification Calibration

The magnification calibration process is quite straightforward.

To calibrate an image

1. **Choose GLOBAL MICROSCOPE INFO under the MICROSCOPE menu (see Figure 2-1).**

   Select "Always" in the Ask for Magnification group box.

   For DigitalMicrograph prior to version 3.3, choose GLOBAL INFO under the FILE menu. Select "Ask for magnification before each acquisition."

2. **Acquire an image of a sample with a feature of known size.**

   When you start the acquisition, the Indicated Magnification prompt will appear. Enter the magnification as displayed on the microscope console.

Magnification Correction

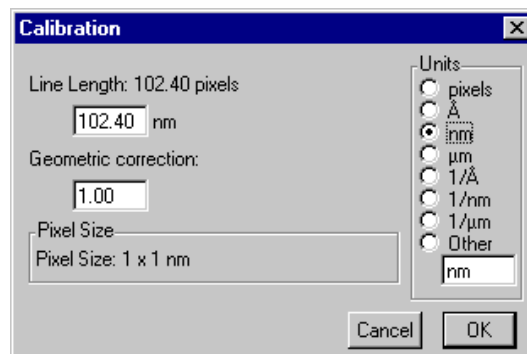**Figure 3-3**   **Indicated magnification prompt.**
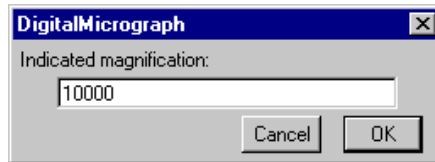
3. **Use the Line ROI tool and mark off the length of the feature in your image.**

4. **Choose CALIBRATE under the ANALYZE menu.**

   This will bring up the Calibration dialog.

Figure 3-4   **Calibration dialog.**

5. **Input the length of the feature in the Line Length field.**

   Make sure the correct unit is selected.

   Note: The Geometric Correction field defaults to 1. This parameter corrects for distortion in the pixel dimensions. Square pixels have a Correction factor of "1."

6. **Click OK.**

7. **Choose SET MAGNIFICATION FACTOR under the CAMERA menu.**

   The software will automatically log your "indicated" magnification, calculate a "correction factor," and then calculate the corresponding "actual" magnification at the camera. These values will be entered in a magnification table.

MSC Family Software User's Guide   **3-7**

8. **You can view/edit the magnification table by choosing** EDIT MAGNIFICATION TABLE **under the** CALIBRATE **menu.**

This will bring up the Edit Magnification Table dialog.

Select Use Magnification Table to view the magnification table.
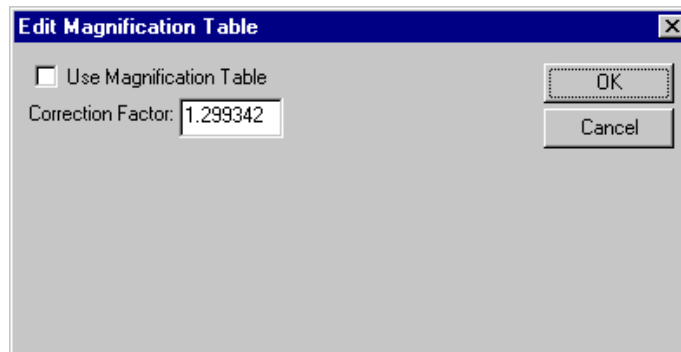
Figure 3-5   **Edit Magnification Table dialog.**
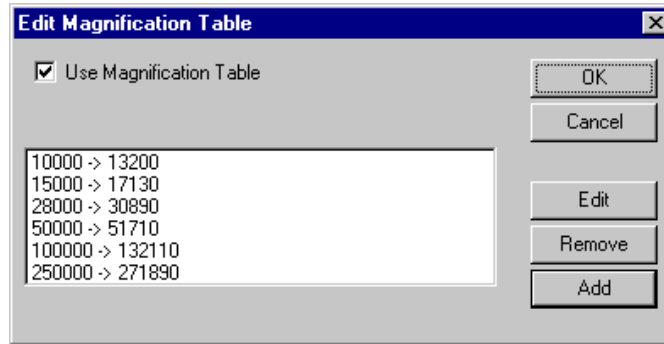
If the field is unselected, the Magnification Correction Factor the program used to calculate the actual magnification at the camera will be displayed.

Figure 3-6   **Magnification Correction Factor.**

To generate a table with several commonly-used magnifications, repeat the calibration procedure (Steps 2-7 above) at these magnifications.

It is advisable that you check the magnification table often to ascertain that all entries are correct. If an incorrect entry is found, either edit or delete it.

To edit values in the magnification tables

1. **Click on the Add button in the Edit Magnification Table dialog.**

This will bring up the Indicated Magnification dialog.

Indicated and Actual Magnification dialogs.
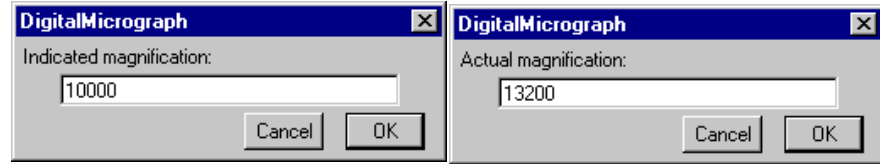
2. **Input the indicated (microscope) magnification.**

3. **Click OK.**

    This will bring up the Actual Magnification dialog. See Figure 3-7.

    Input value.

4. **Click OK.**

    This will add this set of values into the magnification table.

3.7 Data Bar

To enter a data bar

DigitalMicrograph can automatically add a data bar (scale marker) to images that you acquire.

1. **Choose GLOBAL INFO from the FILE menu.**

    The Global Info dialog will appear (see Figure 3-8).

    The Data Bar panel will appear in the right side of the dialog box.

2. **Select Data Bar on the left side of the dialog box.**

3. **Select Add Data Bar After Acquisition in the right panel to automatically place a scale marker on your acquired images.**

4. **Click OK.**

Figure 3-8   **Global Info**—**Data Bar.**
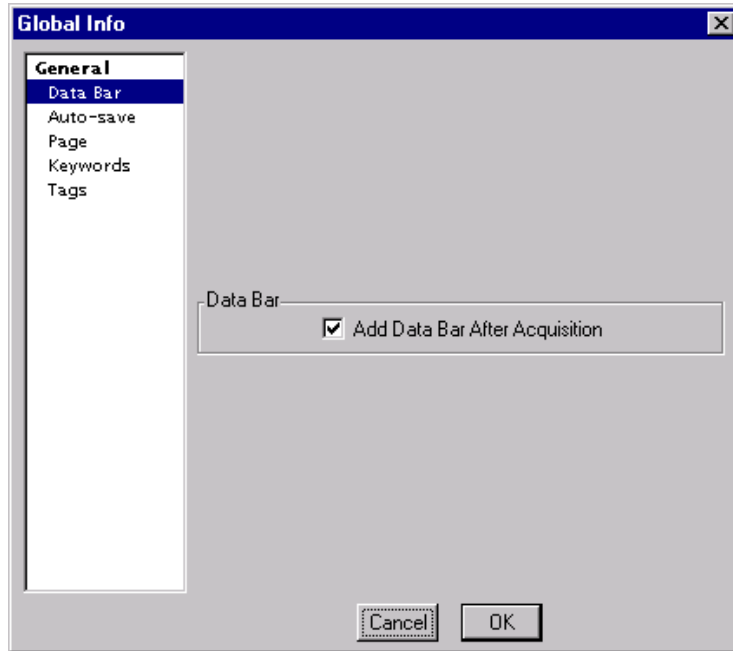
4 Care and Maintenance

This chapter contains some general tips on the care and maintenance of your MSC. It also contains a list of questions that are often asked by users. We have provided them as an aid in troubleshooting your system.

4.1 General Precautions

The following are some general precautions users should adhere to in the care and maintenance of their cameras.

Note: The following items apply to those cameras with Peltier coolers.

- **The Peltier cooler should not be switched on if the cooling water is not flowing.**

  The Peltier cooler consumes about 12 W of power. If this heat is not removed by the cooling water, there is a possibility that damage to the Peltier cooler and/or the CCD may result. **Check the flow of the cooling water periodically.** If the flow rate of the water has deviated significantly, make sure that the water lines are not obstructed and adjust the flow meter to bring the flow back to the original level. A quick way of checking to see if the camera is properly cooled is to place your hand on both the connector side and the microscope side of the camera. The latter should feel warmer than the former.

- **The Peltier cooler should not be left on when the TEM cooling water is turned off or fails.**

  The Peltier cooler is cooled by water from the TEM Chiller. If the Chiller is turned off or fails for a long period, there could be severe damage to the camera. Be aware that for some microscopes the cooling system turns off in the Stand By mode. Be sure the cooler of the camera is also turned off in that situation. Gatan will not be responsible for damage to the camera caused by lack of TEM cooling water.

- **The cooling water connections for the Peltier cooler should be checked on a regular basis.**

  The water fittings in the water lines are checked by the Gatan Service Engineer after installation. Users should subsequently check the lines on a regular basis over time. Gatan will not be responsible for damages caused by leaks in the water lines.

- **Do not leave the CCD electronics switched on if the microscope viewing chamber is to remain at air pressure for more than 24 hr.**

  When isolated by the gate valve, the CCD vacuum is good for about a day after which slight frosting on the CCD may occur if it is kept cooled.

  If you have a small amount of ice, it will just sublimate once the high vacuum is restored and the CCD gate valve is opened. With large amounts of condensation, you need to restore the high vacuum and switch off the CCD electronics for about 30 min so that the CCD can warm up to help dissipate the condensation under high vacuum.

Note: The following apply to all cameras.

- **Avoid exposing the scintillator to the electron beam as much as possible.**

  As a general rule, avoid saturating the CCD and you will prolong its lifetime. Each time any CCD pixels are intensely saturated, the efficiency across the image field of the scintillator may vary or the scintillator may be permanently impaired. If you are not using the CCD camera, retract the camera to protect the scintillator from beam damage. Also, a retracted camera will not collect debris or abrasive particles that have fallen down from the TEM's film-transport mechanism.

- **Check and monitor the scintillator routinely for contamination.**

  Perform the following procedure to first obtain a reference image from your "clean" scintillator and then subsequently to obtain images to monitor contamination of the scintillator:

  - With a low accelerating voltage between 40–60 KV, acquire an unprocessed image to obtain a reference image of your clean scintillator. Record the beam intensity.

  - After a week of operation, repeat above step and check the image for signs of contamination (oil droplets) or reduction in sensitivity on the image.

  - If signs of contamination are present, warm up the camera overnight to vaporize diffusion pump oil that may have been trapped on the cold surface of the scintillator and run the check again.

  - If no signs of contamination are present, increase the time interval for the next check.

  - Store all these images for subsequent comparisons.

If the camera is used too long without warming, the electron beam can harden the trapped oils making the scintillator difficult to clean. If oil accumulates even with regular warming of the scintillator, it is recommended that the vacuum system be checked.

It is OK to run the warmer overnight. **Do not run the warmer when the camera is at air pressure or if the cooling water is not flowing.**

If the surface of the scintillator becomes dirty or scratched, ship the camera to Gatan for service. Do not attempt to clean the scintillator yourself. Damage may occur.

4.2 Most Often Asked Questions

The following are some of the questions most frequently asked by camera users. If you are having a problem with your camera, check and see if the tips listed below are helpful.

- **The CAMERA menu items do not activate (are greyed out), what should I do?**

Check if the MSC Controller is turned on.

Check if the DMA card is correctly inserted in the first slot near the power supply.

Check both ends of the DMA cable (MSC Controller to the DMA board) to see if each is correctly plugged in and tightened down with the set screws.

- **There is no image on the CCD, what should I do?**

Check if the cable from the MSC Controller to the camera is properly connected.

Check the CAMERA menu to see if the camera has been inserted.

Check if the Shutter switch on the MSC Controller is set to Auto.

Check if your beam has drifted.

Check if the viewing screen is lifted (for bottom-mounted cameras).

Check if you have a film plate stuck above the camera in the TEM (for bottom-mounted cameras).

Perhaps you really do have an image, but you cannot see it. Move the pointer around to get an idea of the counts.

For 14-bit cameras, if the value is over 15,000 counts, you have saturated the CCD. The value is 4000 for 12-bit cameras. Reduce the beam intensity or shorten your exposure time.

For 14-bit cameras, if the value is 600-1100 counts, you do not have enough beam on the specimen. For 12-bit cameras, check and see that you are above 300 counts.

- **Why do I have to blank the beam when the CCD is reading out?**

  You do not want to expose the CCD to a signal when it is reading out. It's like continuing to expose the film during its retraction. Blanking the beam will prevent any electrons from reaching the CCD.

- **How often do I have to acquire a gain reference?**

  Whenever image quality seems to be deteriorating. The most reliable way to see if a new gain reference is advisable is to take a gain normalized image of uniform illumination with no specimen in the beam and look for any spurious pattern. If one is seen, update the gain reference.

- **What is the shortest or longest exposure time I can use?**

  The shortest time is actually determined by the shutter speed of the TEM shutter, but an exposure of 0.025 sec is close. Exposures over 1 minute are unusual.

- **Is the image quality the same when I use Continuous mode and stop it as when I use Single Frame mode?**

  The image quality is slightly better in Single Frame mode as compared to Continuous mode, except for Turbo in Search. Turbo is set up for the fastest viewing mode and its signal-to-noise ratio is less than that for the other viewing modes. The only cases where Single Frame mode is noticeably better than Continuous mode is when the signal level is very low, say less than 50 counts/pixel or so.

- **Why does Turbo view have so many horizontal noise lines in it?**

  Turbo is set up for the fastest viewing mode and its signal-to-noise ratio is less than that for the other viewing modes. To improve image quality, try increasing the signal to the image, selecting a higher binning factor, and/or increasing the exposure time.

- **My images are noisy, why can I not increase the gain of the camera?**

  Increasing the gain will not solve the problem because the noise is almost always from the shot noise in the image not the camera. Try increasing the exposure or current density of the beam.

  Do not use the Turbo mode to acquire images. This mode is noisy and results only in an 8-bit image. Use Record with Single Frame mode.

- **My images are slowly deteriorating, what should I do?**

  Determine if the CCD is warming up. Check that the water lines are not clogged. Check the water temperature of the chiller—should be approximately 20°C. Be sure to acquire a new gain-reference image.

- **The image is brighter (or darker) on the top than on the bottom, what should I do?**

  Check that the CCD has been cooled for at least 2 hr.

  Check that the switch on the MSC Controller is set to Auto.

  It could be a bad gain-reference image. Check all the gain-reference images under WINDOWS. Locate the bad one, delete it, and resume acquisition.

  It could be a bad gain reference image. Retake another one.

  Check that the shutter is functioning correctly. If it is, the shutter indicator light and MSC Controller would go on and off as you take an image.

- **When viewing, the image sometimes gets suddenly bright/dark, what is wrong?**

  Sudden changes in the contrast are due perhaps to stray x-ray photons that saturate the cross-wire survey pixels. Use the pointer and peruse the image to find these pixels. Use the Zapper tool to delete these blemishes.

- **Can I damage the scintillator/CCD of the camera when acquiring diffraction patterns?**

  There is no defined cutoff where the scintillator will be damaged. Rather, damage can result from prolonged exposure of the scintillator to a very high beam intensity. If you have no saturation with a short exposure (roughly 13,000 counts at 0.1 sec for 14-bit cameras, 4000 counts for 12-bit cameras), then there is no danger. You can then increase the exposure time and still not harm the scintillator as it is the beam intensity on the scintillator that causes the problem and not the accumulated signal in the CCD.

  If you think you have damaged the scintillator, take an unprocessed image of uniform illumination. If the spot turns out dark, you have damaged the scintillator! If it is bright, spread out the beam and set the illumination so you have about 10,000 counts/sec (2500 counts/sec) and switch the shutter switch on the MSC Controller to Open. Expose the scintillator for 3 min, switch back to Auto, and take another picture. The bright area should be gone and the scintillator should operate normally.

- **How long is the life-time of the CCD?**

  None of Gatan's CCDs has failed due to old age so it is hard to answer. So far, there are no known mechanisms for aging.

- **How often and for how long should the camera be warmed?**

  If you have a clean vacuum in the camera chamber area, between once or twice a month is enough. But if you have backstreaming from your diffusion pump, you will need to do it more frequently. The camera should only be warmed for several hours or overnight, but not longer.

  Refer to the procedure for monitoring contamination of the scintillator provided in Section 6.1.

- **Can I run the camera cooled during the week and turn it off for the weekend?**

  It is not recommended. If you have any reason to believe you have a vacuum leak, water, or any other problem with the camera or your TEM, do not do this. If you do not have a Penning gauge to monitor the vacuum of your camera chamber, do not do this as you will only know you have a leak when you see ice on the CCD camera.

- **The camera takes a long time to insert/retract, what should I do?**

  Check your compressed-air pressure. It should be between 30-60 N/cm^2. Also check if you have a leak in the pneumatic valve in the MSC Controller or in one of the tubes supplying pressure to the MSC.

  If the camera inserts very fast but retracts very slowly, you can increase the pressure in the Red line by turning the regulator screw on top of the pressure regulator several times in the clockwise direction.

- **How come I cannot expose photographic film with the MSC on and inserted?**

  With the camera inserted, the beam will automatically be blanked. Retract the MSC camera and acquire an image on film. Be sure to select Shutter Normally Closed to protect your scintillator from damage from diffraction patterns when screen is lifted.

- **I see a ghost image that won't go away, what should I do?**

  It could just be a bad dark-reference image; try retaking another one.

  Try recording an unprocessed image with uniform illumination. If you still see the ghost image, you may have temporarily over exposed the scintillator. But don't worry, this image will go away in time.

  If it doesn't, you can set the shutter switch on the MSC Controller to Open and adjust the illumination so you have about 10,000 counts/sec. After three minutes, close the shutter and record an unprocessed image. The ghost image

should be gone. If the ghost image is still visible, you might have to warm up the CCD and recool it. Or, you may have burned out your scintillator.

Index