User Manual

About Sniper

Sniper is a software program used by Hauptman-Woodward Institute / HarkerBIO for automated color imaging of high-throughput crystallization experiments. There are two major aspects of the software: coordinating stage ("table") movement to align experiment wells with the camera, and utilizing the camera to capture images of the wells. The images are saved to a network repository and subsequently packaged for distribution with corresponding chemical information.

Requirements

- 64-bit PC running Windows 7
- at least 1 GB of available RAM
- Microsoft .Net Framework 4.0 or later
- Microsoft Visual Studio Express 2012 (for debugging)
- 2 Ethernet ports (1 for network, 1 for controller)
- IEEE 1394 (FireWire) port for connecting to the camera
- 9-pin serial (RS-232) port to configure Ethernet settings (USB adapter can be used)
- monitor/screen resolution of at least 1366 x 768
- Parker/Compumotor's *Motion Planner* software
- QImaging's *QCapture Suite (QCapture x64)*

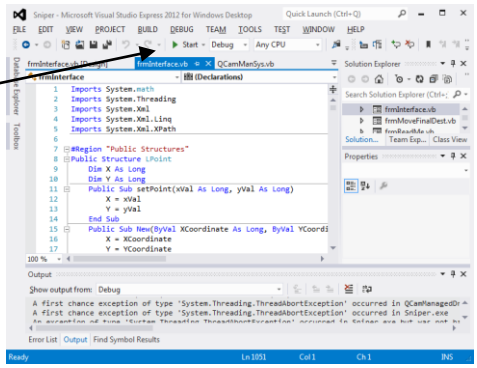
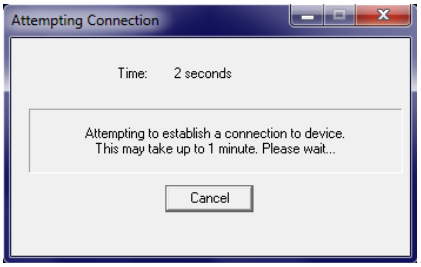
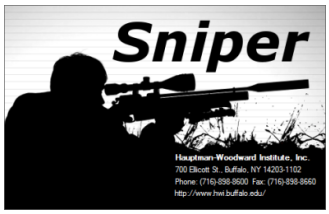
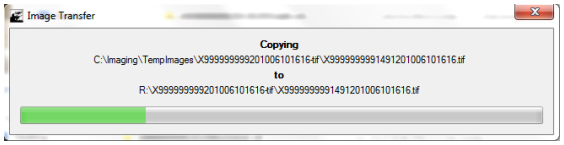
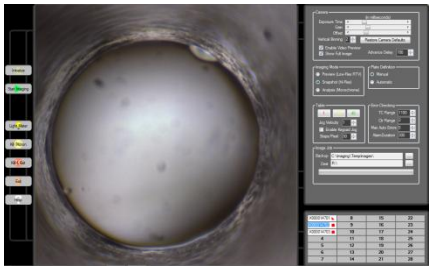
About this Manual

This manual contains two sections. Section I describes the options, commands, and procedures required for users to do day-to-day plate imaging. Section II contains more technical information about hardware and troubleshooting, and is intended more as a refresher for support staff. Some program features may not be described in this manual; and some features described here may not be available in the program. All information contained in this manual is subject to change without notice.

Section I:
Basic Operation

Running Sniper

Since *Sniper* is non-commercial, non-distributed software, we run it as a project within the Visual Studio IDE rather than as a standalone executable program. This affords the developer more convenience in modifying the Visual Basic code, easier debugging, and a better chance of retaining progress if an uncaught exception occurs during execution.

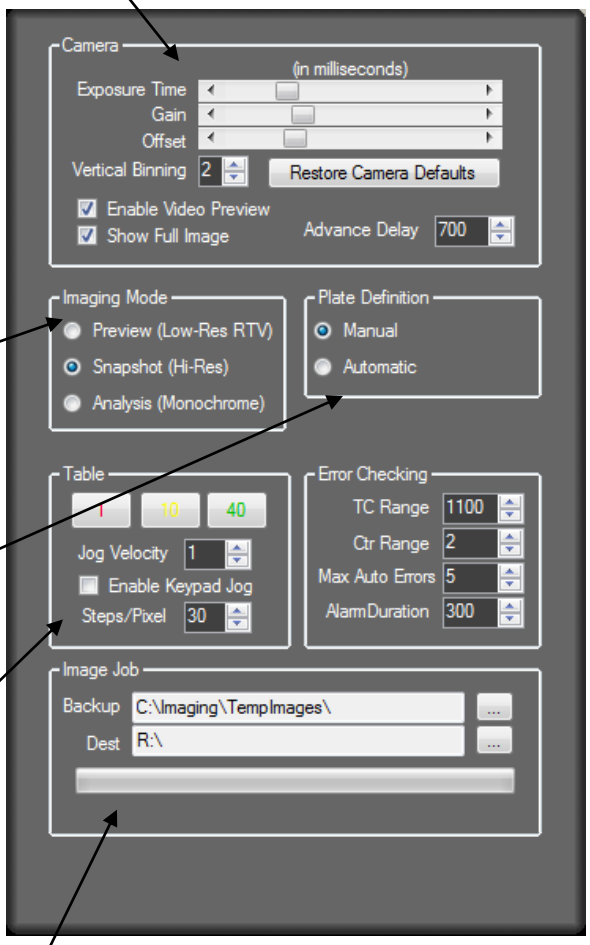
1.	Launch *Sniper* by opening the Sniper Solution in Visual Studio 2012 (double-click Sniper.sln) and then click the "Start" button (Start). ***DO NOT OPEN MULTIPLE INSTANCES OF THE SOLUTION:*** since they share the same settings, this may cause erratic behavior when the settings are modified.	
2.	When Sniper is launched, a connection window may appear while the software attempts to connect to the control. It should connect within a few seconds; if it doesn't, see the section entitled "Establishing an Ethernet Connection".	
3.	Splash screen appears. It will go away within a few seconds. (You can also click to close it.)	
4.	If any images are stored in the backup directory (due to recent network outage), Sniper will attempt to move them to the destination directory.	
5.	*Sniper*'s main window appears.	

Using Sniper

Options and Settings

Camera

Exposure Time: A higher number increases the amount of light, but also shows more blurring if the camera or subject is moving.
Gain & Offset: These map the analog input range to the digital output range. Changing the gain changes the 'width' of the analog input range in the digital scale and changing the offset shifts the analog input range up and down the digital scale.
Vertical Binning: Should always be set to ‘2’ for imaging.
Restore Camera Defaults: Resets Exposure Time, Gain, and Offset to standard settings; use if the default settings are not loaded from the camera on startup.
Show Full Image: When checked, scales down the image if necessary to ensure that the entire captured image is shown.
Advance Delay: # of milliseconds to pause between automatic well-to-well advancement and snapping the well image. A lower # speeds up the imaging process, but may result in blurrier images.



Imaging Mode

Preview: A low-resolution mode that allows a higher frame rate, ideal for real-time positioning.
Snapshot: The high-resolution mode used for imaging.
Analysis: A mode that displays the grayscale image used for some center-finding algorithms.

Plate Definition

Manual: Prompts the user to manually center 3 corners to define the orientation of each imaged plate.
Automatic: Enables the currently set automatic well-centering procedure.

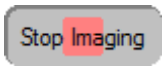
Table

Jog Velocity: Speed of keyboard jogging.
1/10/40: Preset velocity buttons. 1 is good for centering a well; 10 is good for well-to-well navigation; 40 is good for plate-to-plate navigation.
Steps/Pixel: Defines an approximate relationship between the image’s pixel size and the motors’ step size.

Image Job

Backup: Path where images are placed temporarily in case of a network outage.
HWI: Destination path for “HWI” reads
HarkerBIO: Destination path for “HarkerBIO” reads

Command Buttons

	**Initialize**: This command moves the stage to the X-Y coordinate currently defined as the origin (0,0). This is done to check that the system has an acceptable origin point defined, since it is the foundation for all stage positioning. (For more information, see section entitled “Stage Initialization”.)
	**Start Imaging**: This command starts the imaging process. The Start button is only shown when the table is not imaging; and changes into the Stop button when the imaging process begins. (Before imaging actually starts, the system proceeds through a plate definition routine.)
	**Stop Imaging**: This command stops the imaging process, including any sub-processes such as plate definition. When Stop is pressed, imaging cannot be resumed; it must be restarted from the beginning.
	**Pause Imaging**: This command pauses the imaging process in such a way that it can be resumed by pressing the button again.
	**Light Meter**: This command displays the Light Meter dialog, which is used to check the light level and set white balance.
	**Kill Motion**: This command immediately kills current motion on all axes. However, if a process is currently running, Sniper may still send additional motion commands to the controller, resulting in continued movements. For emergencies, use “Kill & Exit”
	**Kill & Exit**: This command is mostly for use in emergencies. It immediately kills motion on all axes AND shuts down Sniper to prevent it from sending additional motion commands to the controller.
	**Exit**: Closes Sniper.
	**Help**: Displays the program’s ReadMe file.

Preparing to Image Plates

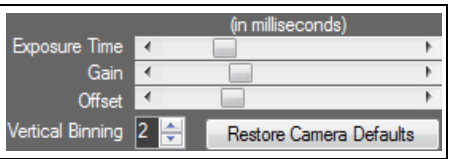
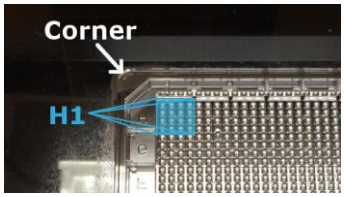
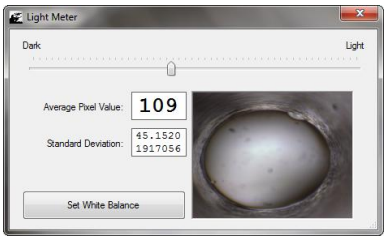
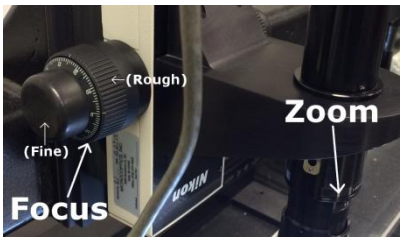
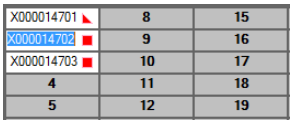
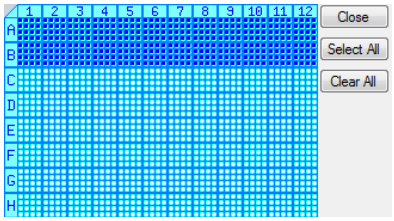
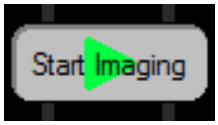
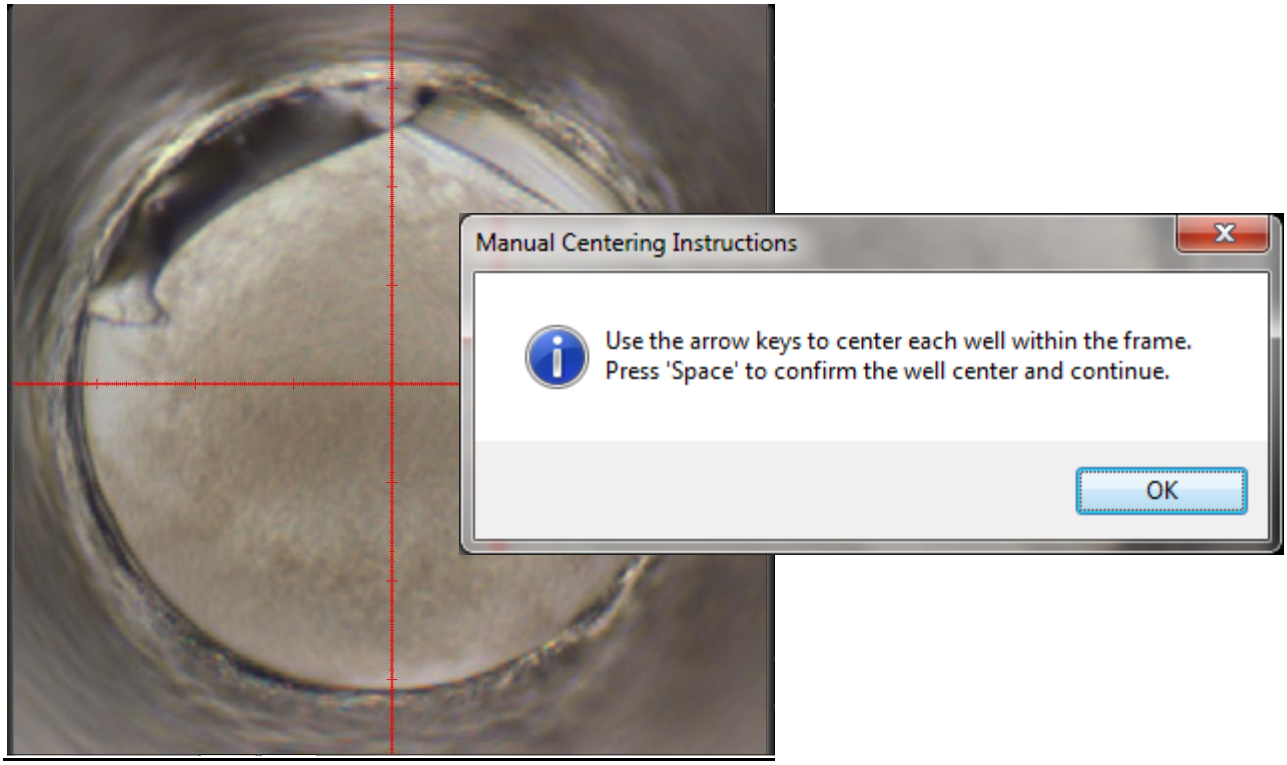
1.	Verify that camera settings are correct (exposure time, gain, offset). If needed, click the “Restore Camera Defaults” button.	
2.	Add plates to the table. (Upside-down, with section H1 as the far-left corner, fitting snuggly against Plexiglas guide or other plates.)	
3.	Click the “Initialize” button to return the stage to its origin. Verify that the stage is properly initialized. (For more information, see section entitled “Stage Initialization”.)	
4.	The camera should be directly over the first plate. If the image appears blue, it needs to be white balanced. (This needs to be done whenever *Sniper* is started.) Click the “Light Meter” button and click “Set White Balance”. Exit the Light Meter Dialog.	
5.	Verify that the camera’s zoom and focus are acceptable.	
6.	Enter plate numbers.	
7.	*Optional: Click the red square to modify the selection of wells to be imaged. A full red square indicates that the entire plate is selected to be imaged (default). A partial red square (triangle) indicates that only part of the plate will be imaged.*	
8.	Click the “Start Imaging” button. If manual plate definition is selected, you will be guided through a plate definition procedure for each plate. (See section entitled “Plate Definition”.)	

Plate Definition

Plate definition is a procedure which gives *Sniper* the precise location of 3 corner well centers for each plate, establishing the position and orientation of the plate. Plate definition occurs immediately before imaging, and may be manual or automatic.

If automatic plate definition is selected, *Sniper* will move to and define each plate in sequence using a center-finding procedure.

In the case of manual definition, the table will move to each well (3 per plate) and wait for the user to center the well. Use the arrow keys to jog the table, centering each well under the camera; then press [spacebar] to continue.

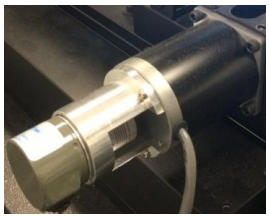


Section II:

Troubleshooting & Advanced Operations

Stage Connections and Preparations

Each imaging stage (aka “table”) is connected to several hardware components:

Component	Function	Photo	Technical
2 stepper motors	move the stage along X and Y axes		ZETA57-83-MO NEMA 23, 2 STACK
2 drives	control stepper motors		ZETA4 DRIVE
controller	interprets commands for coordinated axis motion		6K 2 AXIS CONTROLLER
I/O module	connects limit switches		VM25 I/O MODULE W/CABLE
I/O expansion module	allows connecting a joystick (no longer used)		EVM32-II BASE BOARD
power supply	provides power		POWER SUPPLY 60 WATT, 24 VDC

Connecting Hardware

Ensure the Controller is connected to other components as follows:

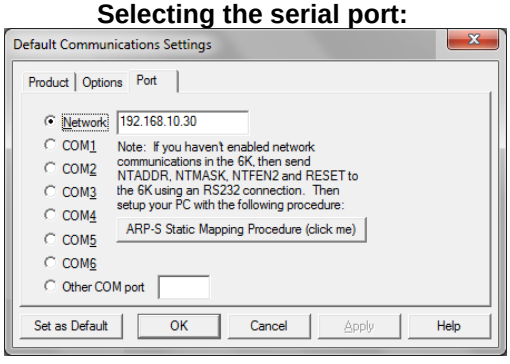
“Encoders” → Stepper Motors.
“Drives” → Drives
“Limits/Home (1-12)” → I/O Module
“+24 VDC PWR” / “DC RETURN” → Power Supply
“Expansion I/O” → I/O Expansion Module
“Ethernet” → 2nd Ethernet Port (PC)
“RS-232” → 9-pin serial port or USB port via converter (PC) (the serial connection is only used temporarily to set up the Ethernet connection)

Establishing an Ethernet Connection

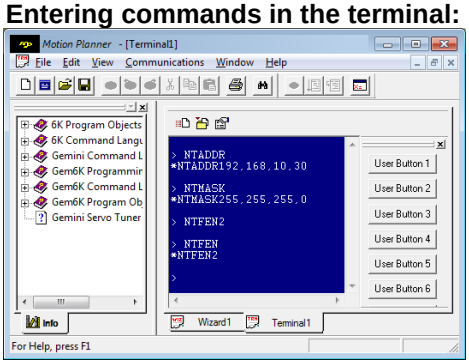
For *Sniper* to control the stage, the PC must have an Ethernet connection to the 6k2 controller. This is achieved using Parker’s *MotionPlanner* software to configure the Ethernet options via an RS-232 (DE-9) connection.

1. Connect the 6k2’s RS-232 port to the PC via DE-9 cable (or a USB converter).
2. Run *MotionPlanner* and open a new terminal.
3. Select the appropriate serial port for the connection (COM1, COM2, etc.)
4. In the terminal window, enter
5. Close *MotionPlanner* and disconnect the DE-9 cable.

Selecting the serial port:



Entering commands in the terminal:



Camera Connections and Preparations

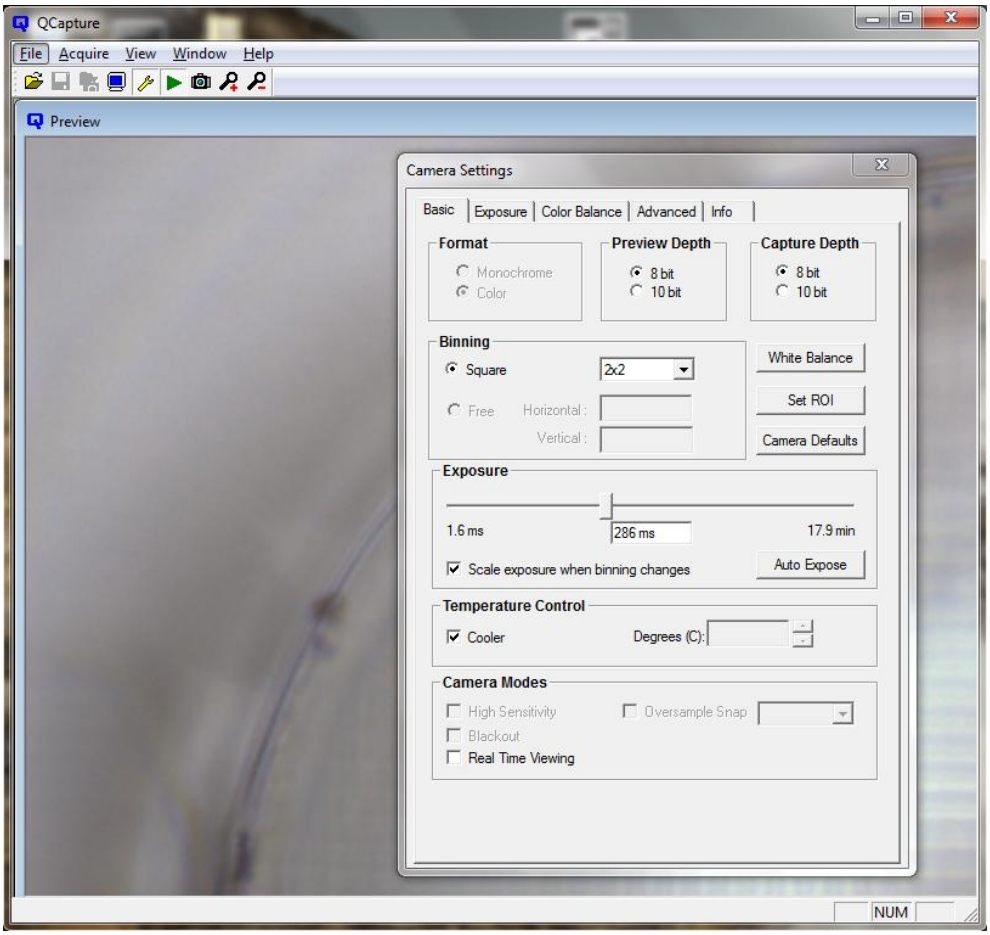
Connecting the Camera

Connect the camera to the PC via a IEEE 1394 (aka “Firewire”) cable. The PC should have an updated QCam driver installed from QImaging (http://www.qimaging.com/).

Following a shutdown, power failure, or reinstallation of the camera, the camera’s internal settings may cause the PC to crash when *Sniper* is started.

To circumvent the issue (before starting *Sniper*):

1. Open the *QCapture x64* software.
2. If the camera was detected, the “Preview” button will be enabled/green. Click it. (If the button is disabled/gray, the camera was not detected. Close *QCapture*, disconnect/reconnect the camera, and try again.)
3. A window will appear with camera settings. If you get this far, you have succeeded. Close *QCapture* and start *Sniper*. It should no longer crash.



Stage Initialization

Click the “Initialize” button to return the stage to its origin (point 0,0).

> *If for any reason the “Initialize” action causes the stage to move in directions that will not bring the top-left corner of the stage under the camera,* ***use a Kill Motion command immediately*** *and reset the origin point as described below.*

The camera should be directly over the top-left corner of the first plate. Press [Ctrl+I] to view the red initialize guide and verify that the inside curve of the plate is properly aligned with the guide as shown in the image.

If the guide is not congruent with the plate’s curve, either the camera orientation is off or the stage origin is incorrect. If the camera has been moved, replaced, or forcefully unplugged recently, the camera orientation may be off, making the arc of the plate curve differ from the arc of the initialize guide. If this is the case, see the section entitled “Matching Camera Orientation to Stage Motion”.)

On the other hand, if the two arcs match but are not congruent after initializing, the stage’s origin is off. This is fairly common, and may occur if the stage was moved in an irregular manner, such as trying to jog the axes when it is physically impossible to do so. It may also change gradually over time.

<u>To set the stage origin point:</u>

1. Press a plate against the Plexiglas guide in the top-left corner of the stage. The plate should be upside-down, with section “H1” (well #48) in the top-left corner.
2. Press [Ctrl-I] to display the initialize guide.
3. Enable keyboard jog and use the arrow keys to align the initialize guide with the inside curve of the plate which is up and right of the top-left well of the plate (#48), as shown in the image above.
4. Press [Ctrl+D] to show the Debug panel, and click the “Init Here” button.
5. To test the origin, click the “Get Pos” button; the position display should read 0 for both axes.

Matching Camera Orientation to Stage Motion

Aligning the camera so that its x and y sides closely match the x and y directions of the stage axes is critical for all automatic well-finding procedures, as they direct the axes to move based on pixels in the image. It is also important to the consistency of images over time and/or across the different tables. The challenge here is to transform the stage motion into a visible standard to which the camera orientation can be matched.

The following procedure has been used with much success:

1.	Print out a sheet of paper with a 1-pixel width black line from one end to the other.	
2.	Place a piece of strong, thick piece of tape (Gorilla duct tape is great) of 2-3 layers about 3-4” from the left imageable side of the table.	
3.	Use a thumb-tack to precisely pin one end of the line to the tape, rendering the paper unmovable but rotatable on that point.	
4.	Use Ctrl+C to enable the crosshairs on Sniper; move the table so that the crosshair is centered near the pin-point on the line.	
5.	Straighten the paper/line until the line looks reasonably straight/congruent/parallel with the horizontal crosshair beam.	
6.	Move the table to the right until the end of the line is visible.	
7.	Hold the pin firmly in place, making sure that end of the line does not move; rotate the free end of the paper until the line on screen has the same relationship to the crosshair beam as the pinned end of the line did.	
8.	Hold down the paper with a heavy, flat object, ensuring that it does not move.	
9.	Travel the axis back to the start of the line, testing for straightness. The distance relationship between the line and crosshair beam should remain from end to end.	
10.	***At this point, the line matches the axis motion.*** Turn the camera until the line is straight/congruent/parallel with the crosshair beam.	
11.	Tighten screws holding the camera in place, ensuring that it does not move. The camera is now aligned with the axis motion.	