PicoLE Video System User's Manual

v 1.0

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Video System Overview

The NAVITAR zoom lens system provides an optical magnification range of 2.1x-13.5x to the camera. Placing a small or large Molecular Imaging scanner in the optical path increases the magnification range by 1.8x, to achieve a total magnification to the camera of 3.8x-24.3x. The degree of magnification at the monitor depends on the ratio of the monitor size to the CCD chip size. The camera uses a 1/3" CCD (6mm diagonal). Using a 12" monitor (305mm diagonal) with the 1/3" CCD chip, the total magnification of the system would then be $(13.5) \times (1.8) \times (305/6) \approx 1230$

A coaxial illuminator is used to project light downwards to the sample surface.

Hardware Setup:

The system comes partially assembled. Some assembly is required by the user.

List of Partially Assembled Components (as they are shipped)

The camera, lens and some of the other components arrive from the factory having already been assembled. The major components to be assembled by the user are listed below.

- Camera and lens assembly
- Stop-ring assembly
- Vertical translator
- X-Y translator
- Vertical video post
- Video platform (base)
- Light source
- Optical fiber cable w/adapter
- RCA video cable
- RCA to BNC adapter

List of All Individual Components

This list is included to inform the user of the components that may be assembled/disassembled by the user.

Light Source Assembly

- Dolan Jenner MI-150 light source
- Black anodized fiber optic plug
- Flexible light fiber
- Light fiber/light source adapter

Stand Assembly

- Video platform
- Vertical video post
- Stop-ring assembly
- Vertical video translator
- X-Y translator

Camera and Lens Assembly

See **Figure 1** below for more information.

- CCD Video camera
- NAVITAR zoom lens
- NAVITAR 2x adapter tube
- NAVITAR 1.5x bottom lens
- NAVITAR C-mount coupler
- Camera power supply (with 120V or 230V adapter)
- RCA video cable
- RCA to BNC adapter

Tools

- 5/32" hex wrench
- 5/64" hex wrench

The video system can be used without the bottom 1.5x lens to achieve a greater field of view at lower magnification. See **Figure 6** on page 6 for details on special positioning requirements.

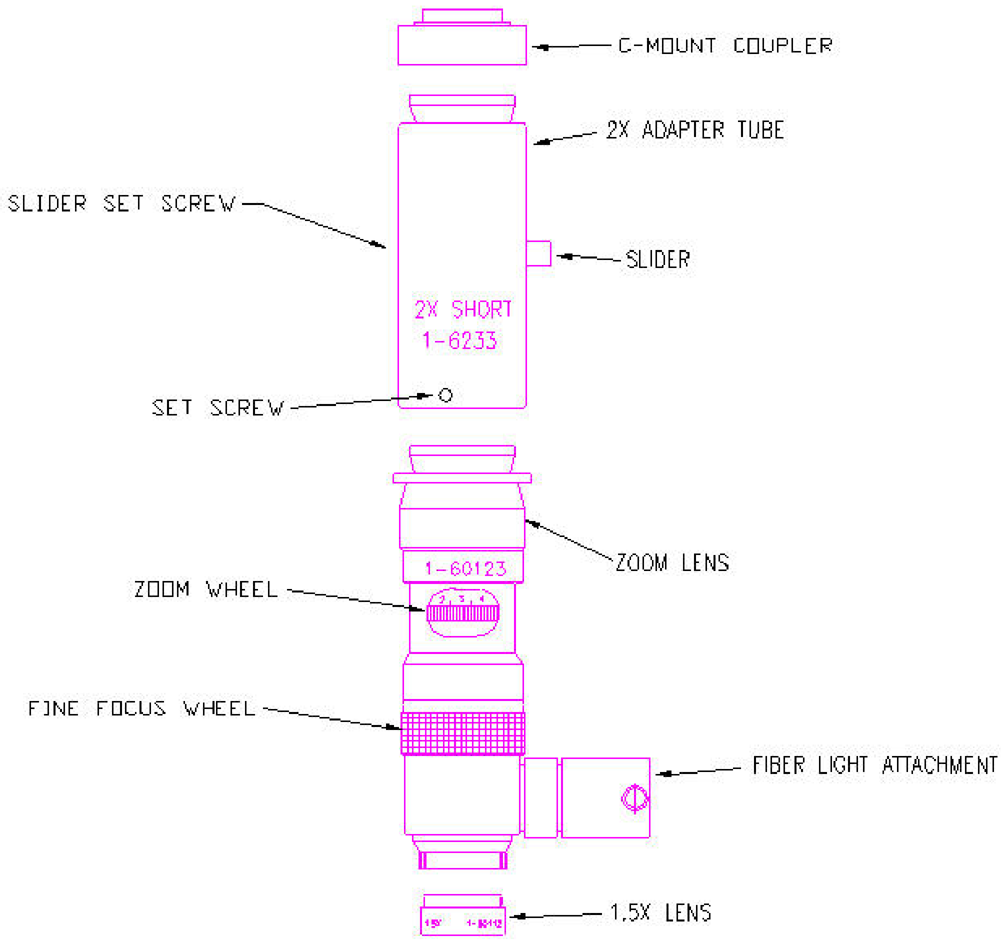

Figure 1 (Camera and lens assembly)

Assembly Instructions

1. Screw the video post into the video platform. The stop ring assembly is adjusted with the 5/32" hex wrench. The stop ring should be located in the position shown in **Figure 2** below to assure that the system will focus properly. The position can be moved for special cases after the user is familiar with the focusing procedure.

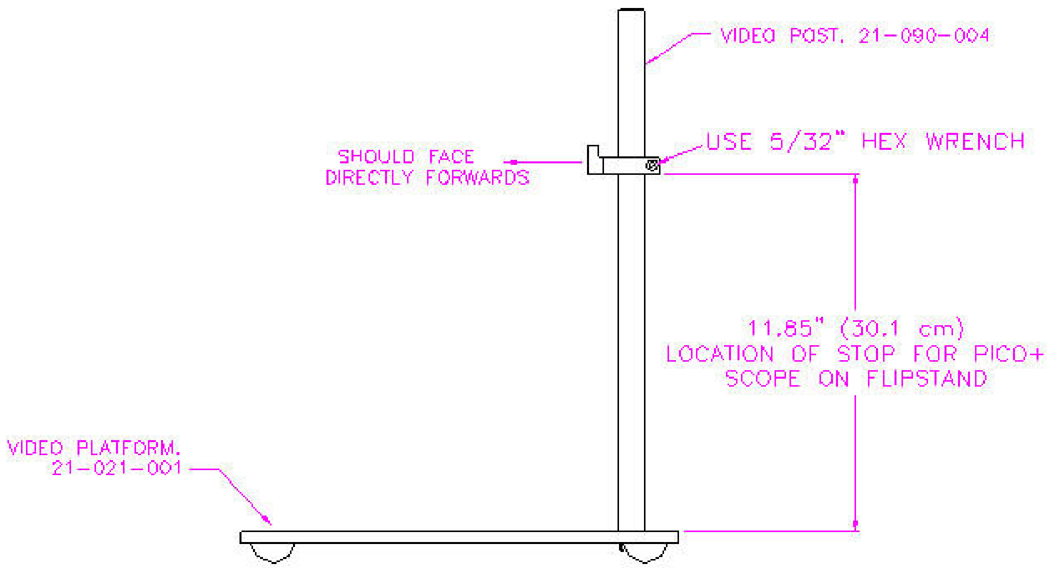

Figure 2 (Base assembly with stop-ring)

2. Place the **X-Y Translator** into the **Vertical Translator**, and lock it in with the clamp knob (located at the rear of the Vertical Translator assembly). The knobs of the X-Y assembly should face as shown in **Figure 3** below.

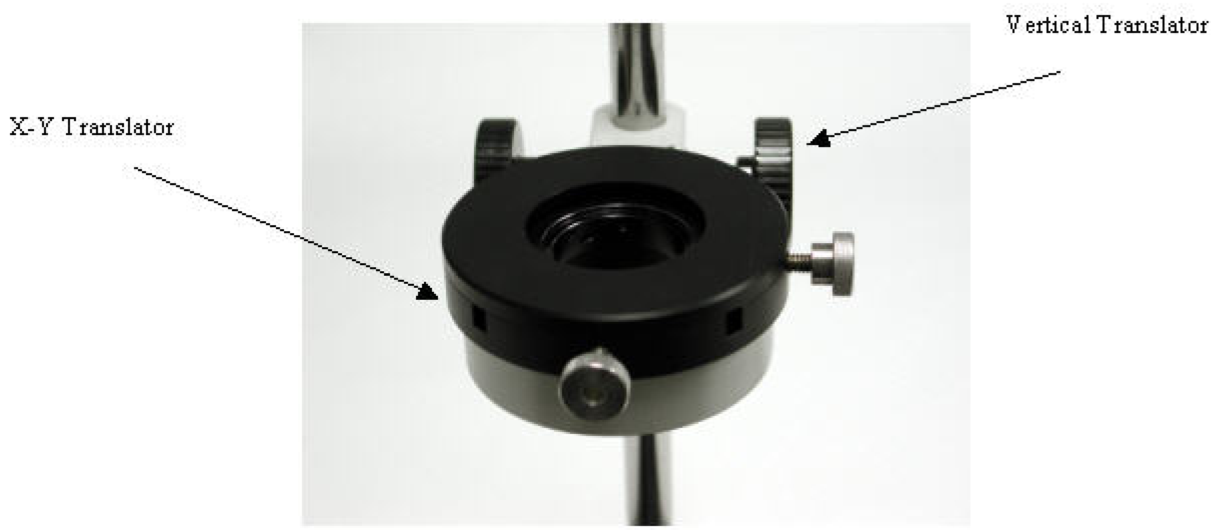

Figure 3 (X-Y translator correctly placed into vertical translator)

3. In order to place the NAVITAR camera assembly though the **X-Y Translator**, the Fiber Light attachment of the NAVITAR zoom must be unscrewed (counter-clockwise). See **Figure 4** (left) below. When removed, slide the NAVITAR/camera assembly in as far as it will go. Replace the **Fiber Light** attachment immediately to prevent contamination from getting into the system. Align the assembly as shown, and tighten the NAVITAR/camera assembly in place with the 2 setscrews shown in **Figure 4** (right) below. Use the included 5/32" hex wrench.

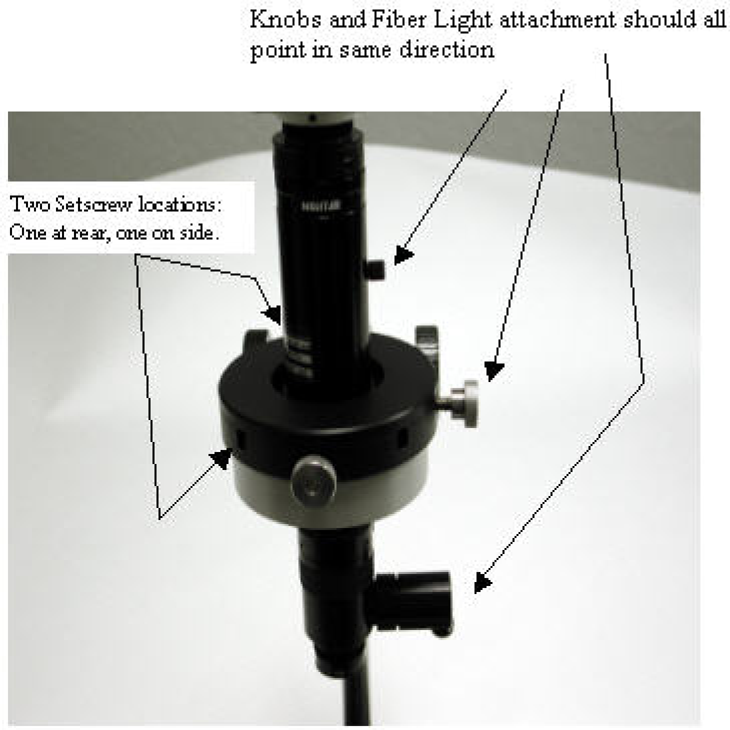

Figure 4 (Left: camera and lens assembly with fiber optic connector detached to permit placement into the X-Y translator. Right: camera and lens assembly in place with fiber optic connector reattached.)

4. Attach the small black fiber optic plug to one end of the flexible fiber light cable with a 4-40x3/16 setscrew. See **Figure 5** below.

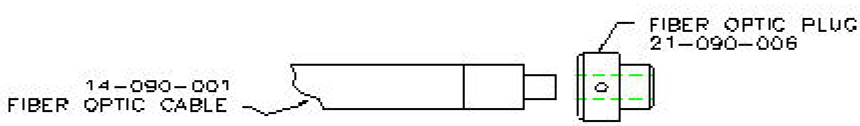

Figure 5 (The plug is used to connect the fiber optic cable to the light source)

Video System Alignment

Follow this procedure.

1. Plug the anodized fiber optic plug into the MI-150 light box, and attach the other end of the flexible light cable to the NAVITAR zoom. Plug the MI-150 into a power source.

2. Connect the 12-volt switching power supply cable to the camera and provide the appropriate voltage source.

3. Attach one end of the RCA cable to the monitor/video card of the computer. Plug the other end into the BNC port on the top of the camera using the RCA to BNC adapter.

4. Make sure the zoom control is set at lowest magnification (zoom collar reads 0.7).

5. A PicoLE microscope, flip stand and a multi-purpose scanner, are needed to test the video performance. A 10µm x 10µm grating held on a sample plate is a good target to view. Set the microscope in position on the video platform with the scanner in place. The sample stage should be as close as possible to the cantilever tip, without risking contact between the tip and the grating.

6. Make sure the stop ring on the pole is set at the correct position. (See **Figure 2**)

7. Start the video capture program if one is being used.

8. Switch on the MI-150 light source, and set to approximately 30% power.

9. The end of the video camera should be positioned relative to the scanner image lens as shown in **Figure 6** below.

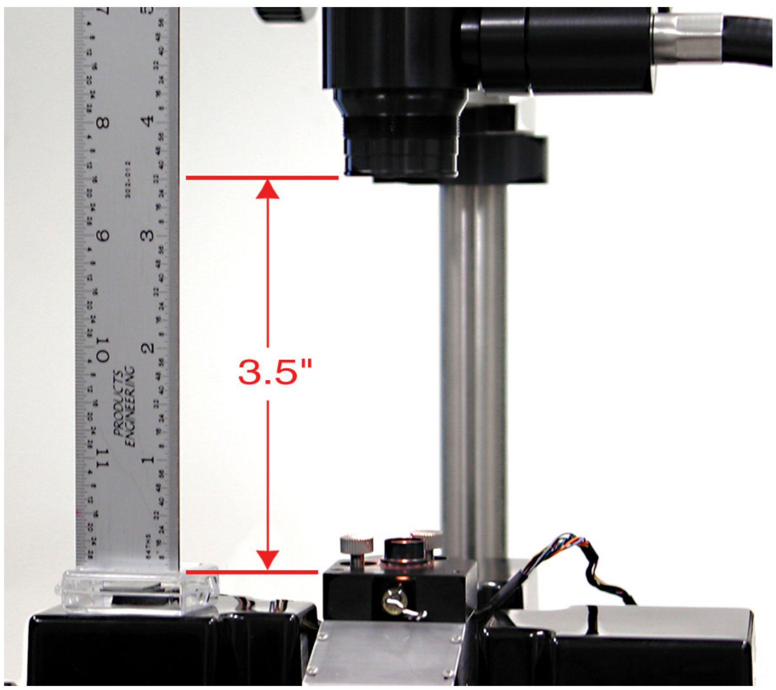

Figure 6 (Vertical spacing of the camera assembly above the microscope)

10. Begin searching for the image of the tip or grating. The grating must be positioned correctly, as only certain areas can be seen. Move the X-Y micrometers on the <u>microscope</u> to move the sample.

11. Use the X-Y translator to move the camera over the area of interest.

System Use:

Once the Video System has been properly assembled and aligned, using the system is relatively straightforward.

Software

The Video System software, once installed, can be opened from the Start\Programs menu under AVerTV. Open the application called AVerTV in this submenu. A viewing window will open and, with the default settings, should begin showing the CCD video camera image.

Using the Video System to Align the Laser

As an alternative to aligning the laser as described in the PicoLE System module, the Video System can be used to center the laser spot on the apex of the cantilever. The procedure is described below.

1. Mount the sample in the microscope as level (horizontal) as possible.

2. Place and focus the lens and camera assembly according to specifications.

3. It is recommended that the zoom be set all the way out in order to maximize the depth of field.

4. Use the **Vertical Translation** knob (see **Figure 3**) to adjust the vertical position of the camera and note the position at which the cantilever is in focus and then, closer to the microscope, the position at which the reflection of the cantilever is in focus. Then adjust the camera height to the midpoint of these two positions.

5. Using the microscope controls, manually approach the sample to the tip until the reflected image of the cantilever tip comes into focus. When the reflected image comes into focus, stop the approach, locate the midpoint again (as described in the preceding step) and resume the approach.
   Note: The closer the tip is to the sample, the less the vertical adjustment between the real image focus position and reflected image focus position. Also, the higher the magnification setting, the narrower the depth of field (the smaller the vertical range that will be in focus at any one position).

6. Adjust the laser position using the two adjustment knobs on the top of the scanner (see the **Aligning the Laser** section of the **System** module). If the reflected laser spot is visible, the laser is not aligned on the cantilever. When the laser is hitting the back of the tip of the cantilever, the "star-shaped" diffraction pattern should be in the reflected image.

Using the Video System to Approach the Sample Quickly

The microscope approaches the sample to the cantilever by extending the Z piezoelectronics to "feel" for the sample. If no deflection of the cantilever is detected by the photodiode detector, the Z piezo retracts, the stepper motor(s) raise the sample stage slightly and the cycle starts over. Even though this process happens quickly, the distances covered by each cycle are very small so that an **Auto Approach** from a macroscopic distance can take several minutes. It is possible to use the Video System to manually approach the sample quickly to within a microscopic distance of the cantilever tip, where **Auto Approach** will safely continue approaching the cantilever to within imaging distance. The procedure is described below.

1. Mount the sample in the microscope as level (horizontal) as possible.

2. Place and focus the lens and camera assembly according to specifications.

3. It is recommended that the zoom be set all the way out in order to maximize the depth of field.

4. Use the **Vertical Translation** knob (see **Figure 3**) to adjust the vertical position of the camera and note the position at which the cantilever is in focus and then, closer to the microscope, the position at which the reflection of the cantilever is in focus. Then adjust the camera height to the midpoint of these two positions.

5. Using the microscope controls, manually approach the sample to the tip until the reflected image of the cantilever tip comes into focus. When the reflected image comes into focus, stop the approach, locate the midpoint again (as described in the preceding step) and resume the approach.
   Note: The closer the tip is to the sample, the less the vertical adjustment between the real image focus position and reflected image focus position. Also, the higher the magnification setting, the narrower the depth of field (the smaller the vertical range that will be in focus at any one position).

6. When the tip-sample distance is small enough, it will be possible to see both the cantilever tip and the reflection of the cantilever tip off the sample surface simultaneously. The closer the sample is to level, the closer the two images will be.

7. Continue to manually approach the sample while watching the two images of the tip (real and reflected) in real time in the video display. The closer the sample is to level, the more slowly the two images will approach each other.

8. When the two images of the tip are on top of each other, the tip is touching (or very close to touching) the sample surface.

9. With the tip this close to the sample, let the microscope software **Auto Approach** to avoid crashing the tip into the sample.

Sample Images

Shown in the figures below are three progressively greater magnifications of an AFM "diving board type" cantilever over a grating with 10 μm between lines.

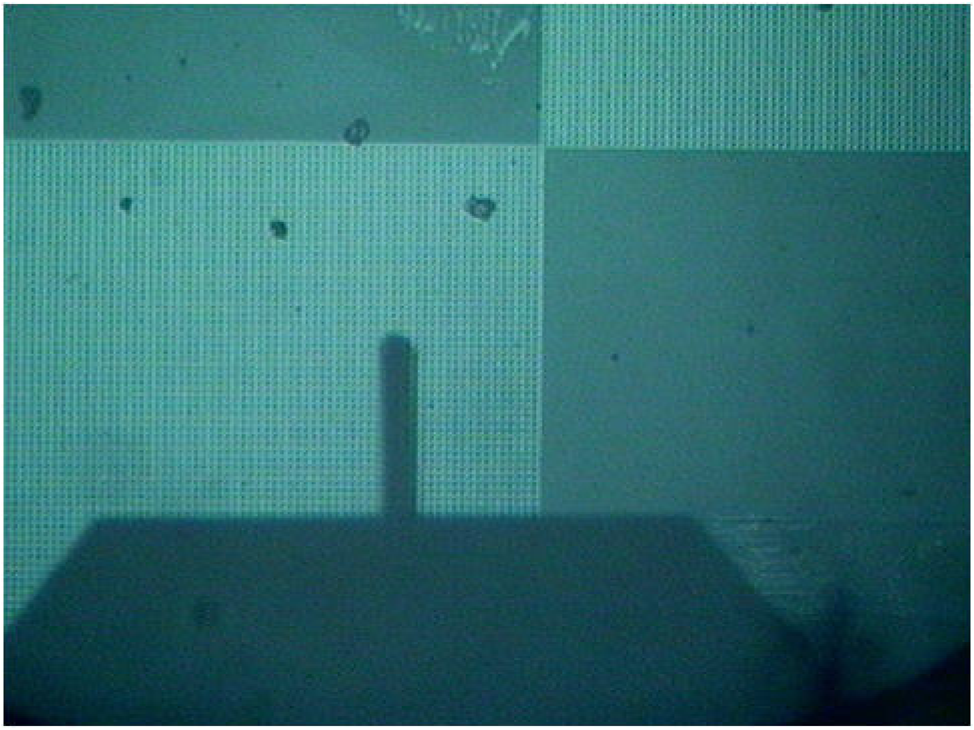

Figure 7 (Lowest magnification, over a 10μm grating)

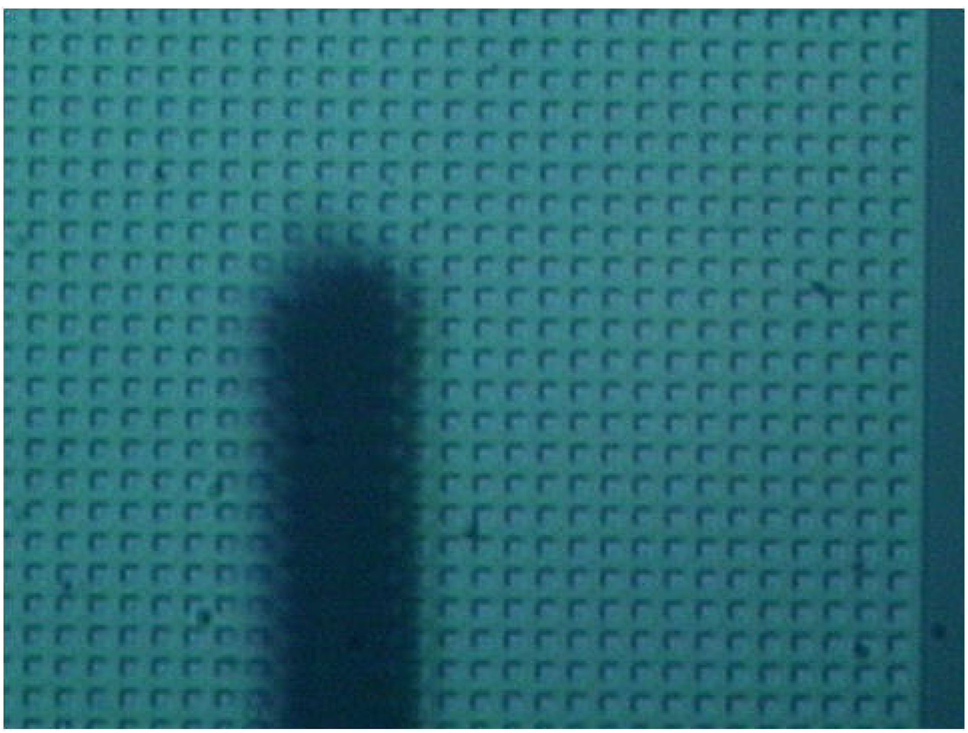

Figure 8 (Magnification set at #2 position, over a 10μm grating)

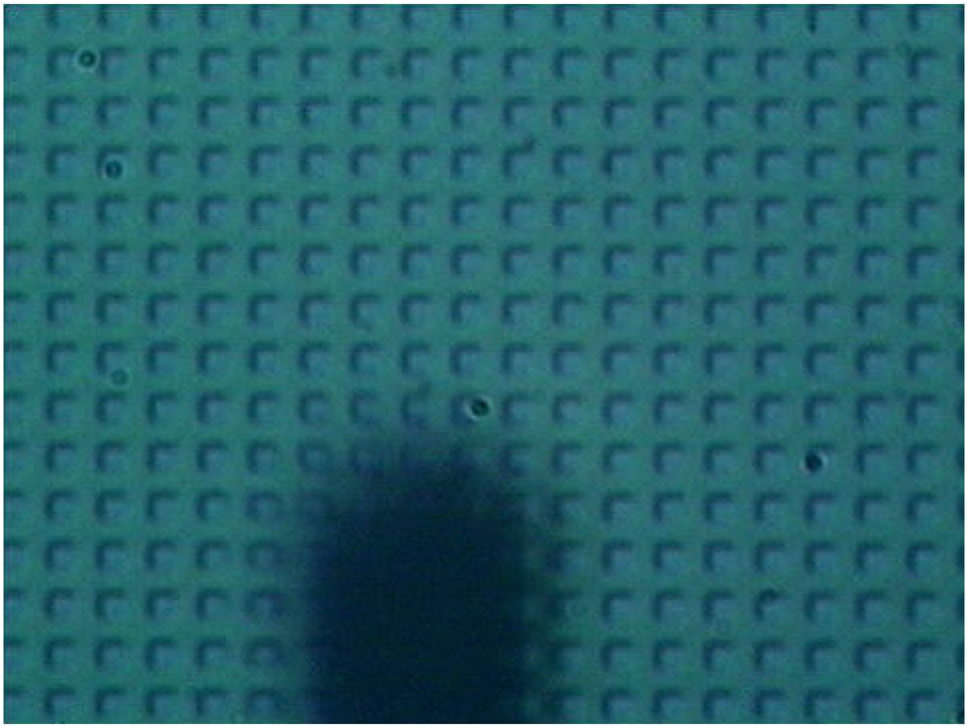

Figure 9 (Highest magnification, over a 10µm grating)

Troubleshooting:

There are some procedures that the user can perform that may improve image quality.

Adjusting Focus Slider

If the image goes out of focus when changing from low to high zoom, and the fine focus wheel doesn't have enough range to compensate, use the following procedure.

1. Loosen the side set screw on the body of the zoom lens (see **Figure 1**).
2. Set the lens to maximum zoom, then adjust the vertical translator (rack and pinion holder) knob to bring the object into focus.
3. Now set the lens to minimum zoom. Loosen the knurled knob on the zoom slider and adjust the vertical slide position until a clear image can be seen. Carefully retighten the knurled knob.
4. Lock the slide setscrew on the body of the zoom lens.

The image should remain clear for all zoom positions. Slight changes in focal quality are corrected with the bottom focus wheel.

Appendix: Verification of Resolution

Purpose: The purpose of this experiment was to verify the absolute resolution of the video access with the PicoPlus.

Definition: The *resolution* of a microscope objective is defined as the smallest distance between two points on a specimen that can still be distinguished as two separate entities.

Experiment: An image of a calibration standard was obtained with an AFM Scanner and the video system. The spacing of features can be measured to within 10% by the AFM Scanner used, and those distances can be used to judge resolution on the video image.

Data: Figure A.1 shows the AFM Topography image of the calibration standard used for the experiment. Note from the image that the lines are only about 1 µm wide and the pits are about 2 µm wide.

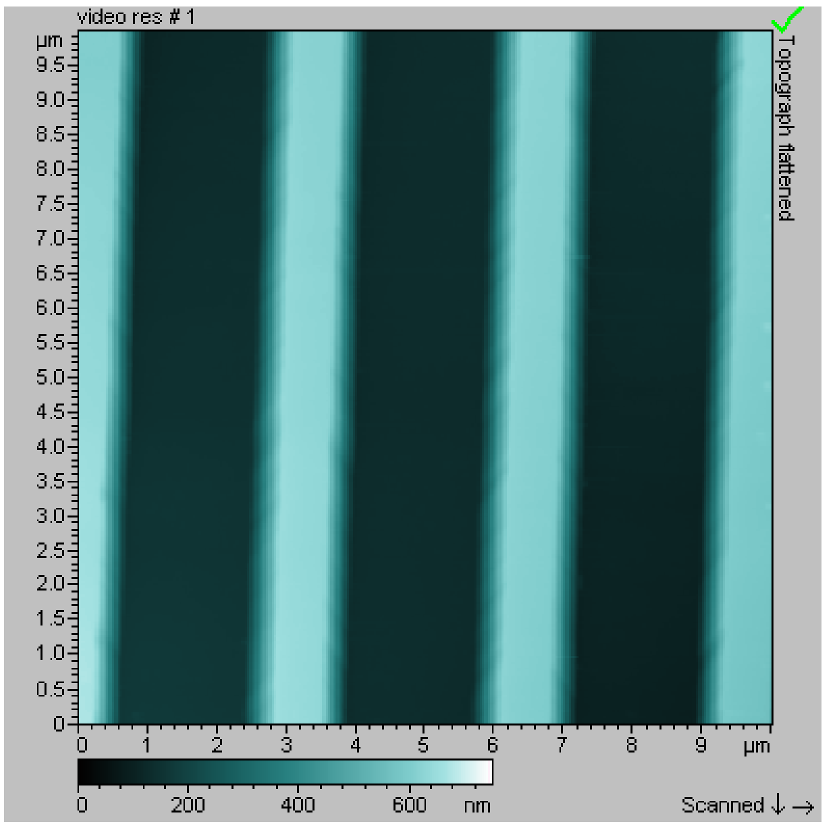

Figure A.1 (AFM topography image of a calibration standard)

Figure A.2 shows the video image from the same sample. Note the image of the standard clearly shows repeating structures, the largest of which are ~2 µm wide.

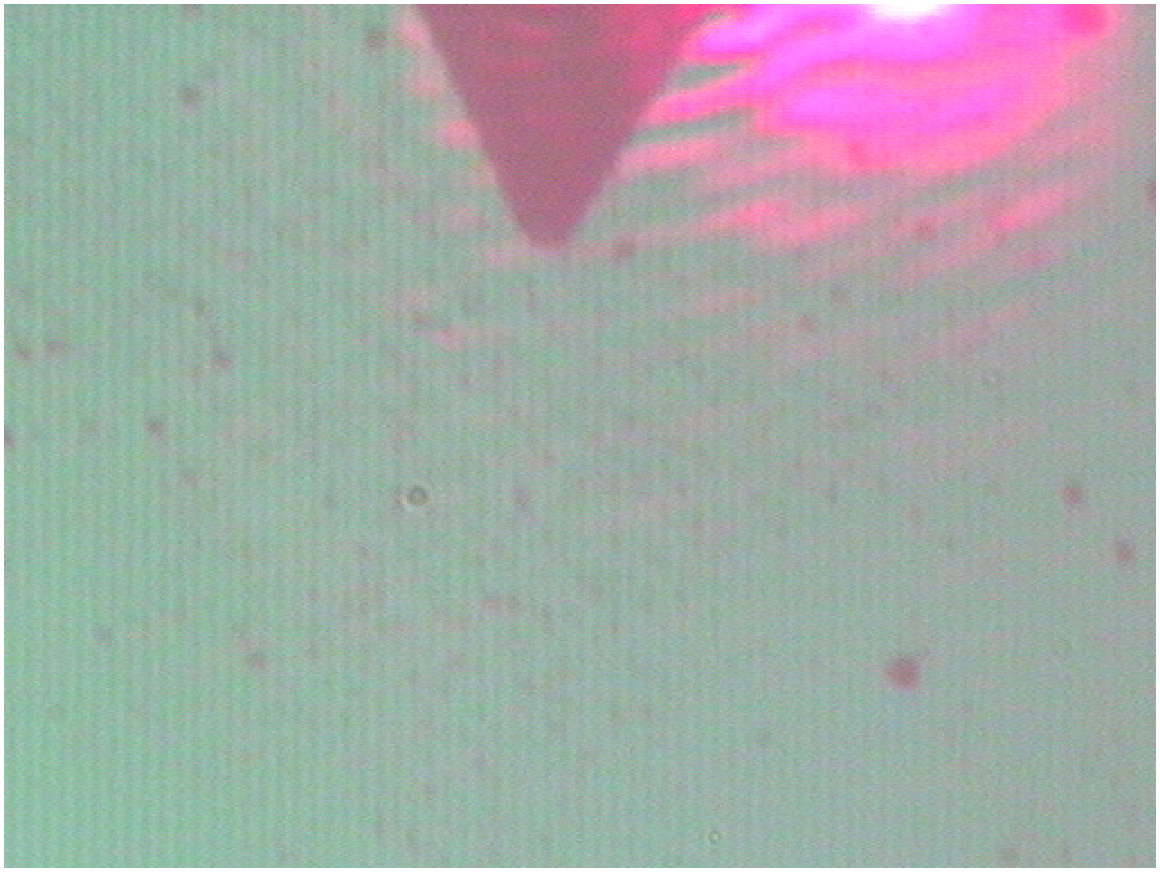

Figure A.2 (Video image of the AFM tip and the calibration standard)

Conclusion: This clearly shows that the resolution of the video system is less than 2 μm as stated in the specification sheets.