To make these tests you must have an operational controller and host computer with GUI software installed. See file “Electrical Check” for details.

Initial optical alignment

The initial alignment is done without installing objective lenses. However the prism boxes and objective mounts containing the quarter wave plates are installed. The Plain Beam Block and the Retro Beam Block are not installed yet.

1. Center the Television Field Lens on its mounting holes. Retract the filter slider for maximum sensitivity so that there are no filters in front of the TV camera.
2. Place a small mirror underneath the left Prism Box (CCD-side) to direct light from a distant object (>20 ft away) up through the Prism Box bottom aperture. The light should then travel through the Prism Box and up through the achromatic TV Field Lens into the television camera. Adjust the Television Focus Tube up/down until you get a sharp image of the distant object on the monitor screen. Fix the Television Focus Tube in place with a set screw.
3. Install fiber wigglers and plug them into the lasers. Turn on the laser source opposite the CCD (Laser B) to a low power, say~10% of maximum.
4. Place an IR Indicator Card near the right Prism Box and detect light coming out of the front aperture and bottom aperture. We want to adjust the fiber wiggler with three screws to roughly collimate the beam and also make it enter the opposite prism box. CAUTION! Do not allow laser light to strike your eyes.
5. Observe the focal spot move in the CCD camera when you adjust the collimation of laser B. Adjust 3 screws on right wiggler for smallest focal spot in TV monitor. Remove corner adjustment rod from top of wiggler to retain best focus.
6. Place Aperture Iris Buttons (see TOOLS) in the front apertures of both Prism Boxes. Adjust 2 remaining rods on right wiggler so light passes into left aperture button and is visible on TV. Move rods to obtain round symmetric spot. Such optimum spot is not usually at center of TV.
7. Slide the TV Field Lens on its magnetic mount so the spot laser focus appears at the center of the TV monitor. Mark the position of the TV lens with a permanent marker or else glue it in place.
8. Turn off Laser B and install the Plain Beam Block on RH Prism Box. Remove both Aperture Iris Buttons.
9. Turn on the laser source near the CCD (Laser A) to a low power, say~10% of maximum.
10. Place an IR Indicator Card underneath the left Prism Box and detect light coming out of the bottom and front apertures. We want to adjust the fiber wiggler with three top rods on Wiggler A to roughly collimate the beam and also make it enter the opposite prism box. CAUTION! Do not allow laser light to strike your eyes.
11. Install the Retro Beam Block with Retro Reflector under the LH Prism Box. Observe the reflected spots move in the CCD camera when you rotate the Retro Reflector in its

mount. Adjust the collimation of laser A with 3 screws on left wiggler for smallest spots and smallest pattern in center of the TV monitor. Finally, remove corner adjustment rod to retain the best focus.

Adjusting Polarizations

Plug in the four detectors into the four preamps according their color codes.
Green – Trap A force detector – bottom ADC board, left preamp
Blue – Trap A light level detector – bottom ADC board, right preamp
Yellow – Trap B force detector– upper ADC board, left preamp
Red – Trap B light level detector– upper ADC board, right preamp
Then perform the following tests:

1. Turn on lasers one at a time and observe the sum signals on the host program. Twist and bend the input fibers to the wigglers to obtain maximum sum readings. The highest sum position should correspond to minimum light lost out of the bottom aperture, as seen by the IR Indicator Card. Tape the fibers to some surface in order to retain good transmission.
2. Run the A laser only and place a round 1”-diameter mirror flat against the small end of the Objective Centering Mount and facing left. Light now travels from the A laser, through the left quarter-wave plate, bounces off the mirror, comes back through the left quarter-wave plate, and falls on the left detector (yellow).
3. Insert the quarter wave plate wrench into the quarter waver plate mount holes and turn until the left detector (yellow sum) is maximized. The far side detector (green) should be near zero. Remove the mirror and observe that the far side (green) goes up, and the near side (yellow) goes down. Now light coming from Laser A through the left-side quarter wave plate is circularly polarized. We use this beam to adjust the right side quarter wave plate.
4. Insert the wave plate wrench in the right side quarter wave plate mount and turn to maximize the green reading. The wave plates are now adjusted for maximum detector efficiency for light traveling in either direction.

Centering the objective lenses

Screw one objective lens into the left hand *Objective Centering Mount.* Remove the right-hand *Objective Mount* from the DS-40X stage and screw the other objective into that detached mount. Replace the mount onto the DS-40X stage and tighten with the set screw. Objectives should be ~1mm apart at faces.

Turn on laser B to direct light toward TV camera so the spot can be seen on the monitor. It is recommended to have blue LED turned off and the filter slider retracted because the spot is hard to find. Remove the IR filter from the focus tube lens cap if necessary. Slightly loosen the four screws that hold the centering mount onto the objective bracket. Turn 3 screws in the centering bracket to adjust the X and Y offsets of the left objective. Look for maximum reading in the

yellow detector sum and a dim light appearing in the CCD camera. Focus the right objective in/out (along Z-axis) by turning the Worm Gear. Adjust 3 screws of the centering bracket to bring the focused light to the center of the CCD camera. Tighten 4 screws on Centering Mount to fix objective in final position. Put the IR filter and lens cap back on the camera focus tube.

Adjusting the light lever

To be done for both left and right sides.
Both lasers turned on, with laser spot is in the center of the TV screen.
Remove the light lever mirror bracket by unscrewing the set screws.
Place the IR Indicator card in front of the Light-Lever Pick-Off Tube. Look for infrared light coming out of pick-off tube. Observe the collimation of the beam by moving the card back and forth. CAUTION: do not look directly down the pickoff tube with lasers turned on. Place Focusing Wrench inside Pickoff Tube to engage Lens Slider (see TOOLS). Move lens slider to focus the spot to about 9” from the end of the pick-off tube and 5” above the table.
To raise/lower the spot, move the whole pickoff tube in/out. If the spot focuses too close to the tube, push the lens slider deeper into the tube with the focusing wrench. If it focuses too far (diverges) from the tube, then pull the lens slider farther out in the pick-off tube.
Reinsert the light lever Mirror Bracket and tighten its set screws.
To confirm that the twist of the pellicle is aligned, use the host program and zoom in on the graphical representation of the light lever position. Then twist the wiggler adjustment rods in either horizontal of vertical axes to see how orthogonal and how symmetrical to the X-Y axes the movement is. If they are rotated, loosen the screw in the light lever standoff spacer and twist the spacer until it is as close to non-rotated as possible. The best signal/noise ratios are obtained when the SUM readings on the position detectors are between 20,000 and 30,000. See the document “How to Optimize SUM readings”.

Adjust Wiggler Tilt

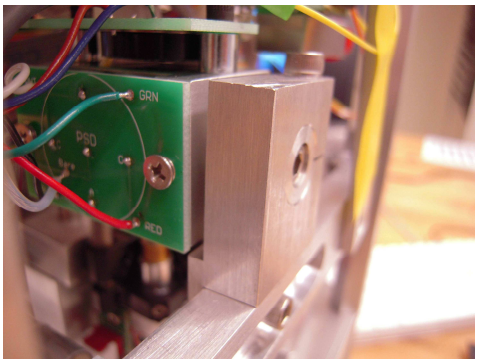
There are many degrees of freedom associated with a fiber wiggler, but the ones which matter physically are those defining the light cone where it comes out of the fiber end. The vertex of the cone must start on the optic axis (X=0 and Y=0) and be one focal length above the lens (Z=f). Then the tilt of the light cone axis has then 2 angles associated with it (Theta=0 and Phi=0) relative to the XYZ axes..

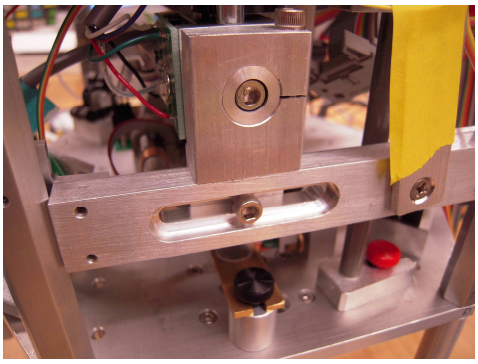
You can change the tilt of the fiber (and hence the tilt of the cone) by changing the piezo screw adjusters. This adjustment will also change the fiber tip position so you will need to bring the tip back onto the optic axis by turning the X, Y, Z rods while watching for the focus spot to be in the middle of the TV. The reason you can change the tilt independent from the position is because the fiber tilts on a 9-mm radius but the wiggler Piezo Tube rotates on a longer radius (~60 mm) which changes its tilt more slowly with changes in position.

The tilt angles should be adjusted so that the light cone hits the center of the collimating lens when the wiggler is in the center of its electrical range (command "CenterTrap") and the tip is on the optic axis.

A crude way to check the fiber tilt is to look at the SUM reading on the PSD detector when the spot is in the middle of the TV screen. Then move the spot with the control rods and observe how the SUM falls off when the spot goes past the edge of the TV screen. That power should fall off the same amount when you move the same distance either right or left or up or down from center. In other words, the center spot of the TV should correspond to the center of the SUM intensity readings when you move the control rods. If it does not, then the fiber is tilted wrong so that the light cone is closer to one edge of the collimating lens than the other, even when the tip of the fiber is on the optic axis.

A quantitative test for adjusting the fiber tilt is shown below. It is done with the objective lenses removed but the objective mounts installed with the waveplates adjusted for maximum transmission. Put the Force Detector brackets in the middle of their range. That is, put the cross-bar slider screw in the middle of the slot and the PSD Holder Handle even with the back of the Cross Bar Slider (see photos).



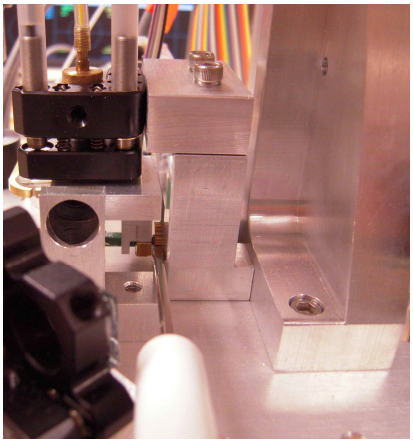


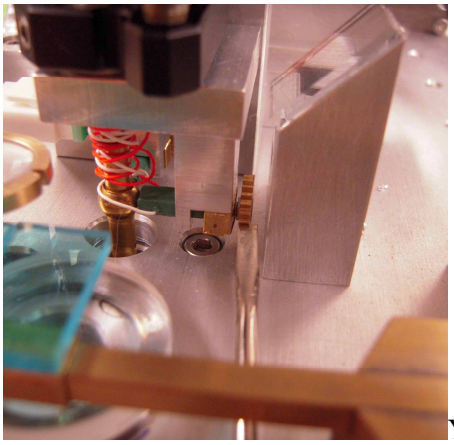
Install both Beam Blocks, with the retro-reflector under Laser A. Turn on the instrument controller and host program and click "CenterTrap" in the GUI control. Then turn on Laser A and adjust the control rods so the retro-reflector star appears at the center of the TV screen. Then turn off the laser, remove both Beam Blocks and install Aperture Iris Buttons in both Prism Boxes. Turn on Laser A and observe the PSD window in the Host Computer while increasing laser A current until you get a SUM reading of several thousand. Push the Main Control Board reset button once to cancel any PSD zero offset. Then

move the top relay lens (under detector A) until the PSD spot is near the middle of the circle in the PSD window. Thus you should be able to minimize X and Y numbers to less than 1% of the SUM.

Figure.3 Adjusting Relay Lens by hand.

Then remove both Aperture Iris Buttons and watch for changes in the position of the PSD spot. The brightness SUM will go up when the buttons are removed. However if the wiggler tilt is correct then the numbers for X and Y will remain less than 10% of the new SUM. If the deflection becomes larger than 10% of the SUM in the X direction, you should change the X-axis wiggler tilt by turning the toothed wheel on the end of the X-axis piezo screw with the edge of a flat-blade screw driver (see photos). The same procedure works for the Y axis by using the other screw.


X-axis

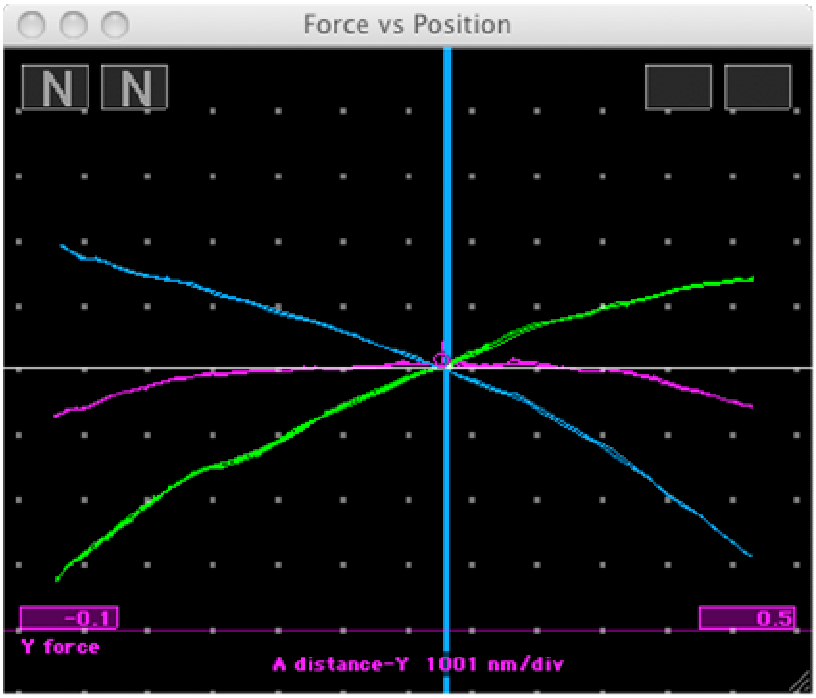

Y-axis

Observe the TV screen when turning the screw and you will see the position of the trap move away from the TV center when you have succeeded in turning the screw. Avoid pushing sideways on the toothed wheel (up/down is OK) because sideways force might push the mirror mount off the control-rod screws. After the trap is seen to move, restore it to the center of the TV screen with the control rods. Then observe changes in the deflection signals on the PSD screen. If the deflection problem gets worse, then you are turning the piezo screw in the wrong direction. Stop adjusting when both deflections get near zero with the trap seen in the center of the TV. Then repeat the same procedure for the trap B wiggler. Run only one laser at a time for these tests.

Adjust Force-Detector Heights

Here we try to make the force detector reading independent of the trap position by setting the detector height at a conjugate image plane to the Fiber Wiggler pivot point. Although the fiber tip generally moves, and hence the trap moves, we do not wish the beam hitting the force PSD to move unless there is an object in the trap exerting a force on the light beam. The light path can be thought of as passing through the wiggler pivot point at a constant position, even when the fiber tip moves. Therefore re-imaging that pivot point to the center of the PSD prevents trap movement from causing any movement of the light on the PSD surface. Here we move the force PSD up and down on the Detector Cross Bar and tighten the 2 screws at the proper height. To test for the proper height, first install a chamber with water and focus the laser beams as though making a trap. Then turn off one laser and use the Force-Position window to plot the apparent force from the remaining laser versus trap position.

Below is a Force-Position plot made by running Laser A alone and plotting the Y-force versus A-distance-Y while moving the traps in the Y direction with the mouse.



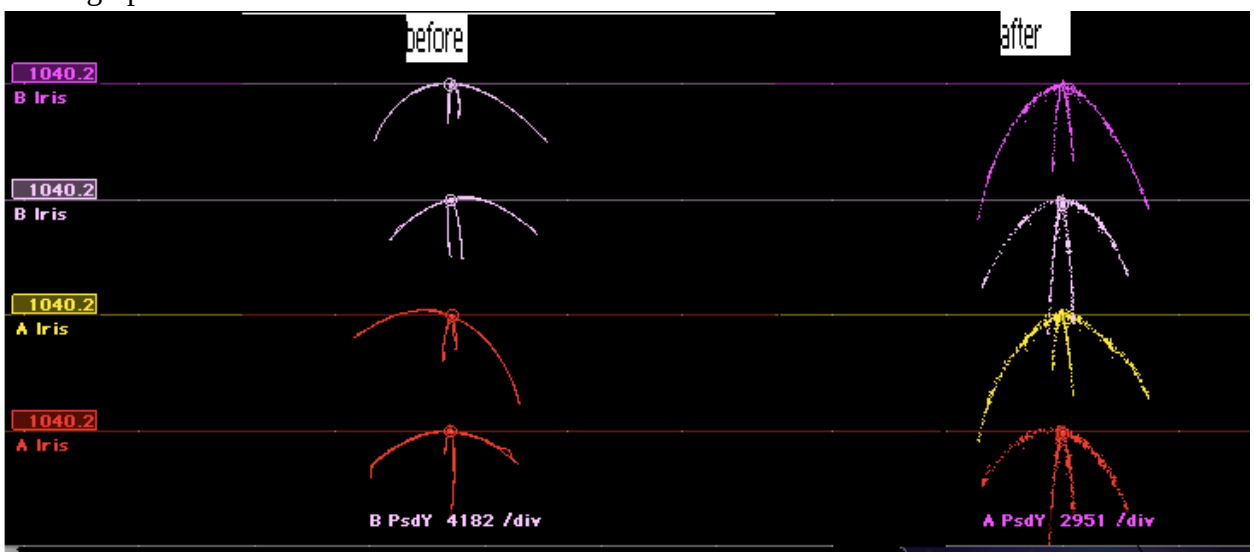
Magenta curve is the null position (correct detector height)
Green curve was taken with the detector ~3mm above null position
Blue curve was taken with detector ~3mm below null position

Note that the vertical scale is fine (0.1 pN/div) and the horizontal scale is coarse (1000 nm/div). Here the worst position gives 0.5 pN baseline shift in 10 micron range. Thus an experiment running over a 0.1 um range, e.g. unfolding a protein molecule, would give an error of only 0.005 pN even before correcting the detector height. The error for such a short range near the middle becomes zero after setting the correct detector height.

Several strategies can be used if the height adjustment is not sufficient to nullify tilt in the force baseline. You can flip which side is up (convex or planar) in the top relay lens. Flat side up lets you lower the detector slightly. You can shorten the Relay Lens Standoff Spacer from 0.6” to 3/8”. You can trade the upper relay lens for one with a shorter focal length; say 63mm instead of 75mm.

Center the Bullseye Filters

The bullseye filters are used to derive the axial force on the trapped bead. They must both be centered on the optic axis to work properly. Otherwise the instrument will indicate a false axial (Z) force whenever a transverse (X,Y) force is applied. The bullseye positions can be changed by loosening 2 mounting screws and moving the entire iris-detector circuit board with attached photo-diode and bullseye filter. To test whether the bullseye is centered, perform the following test: Install a fluid chamber, turn on the lasers and center the traps on the TV screen. Press the Main-Control board reset button to remove any zero offset in the PSD signals. Re-position both upper-relay lenses until the beams are centered on the PSDs as indicated in the PSD window. If the X and Y signals are less than 1% of the SUM, then that is close enough. Now start to create a new graph window as shown below.



First delete all the plots in the Oscilloscope window except the bottom plot.
Change the x axis of that plot to be “A_PsdX”. Anti-zoom the x sensitivity to be ~ 3000/div.
Change the y axis of that plot to be “A_Iris”. Anti-zoom the y sensitivity to be ~ 1000/div.
Create a new copy of that plot (press N key).
Change x-axis of that plot to be “A_PsdY”
Create a new copy of that plot (press N key).
Change x-axis of that plot to be “B_PsdX”
Change y-axis of that plot to be “B-Iris”
Create a new copy of that plot (press N key).
Change x-axis of that plot to be “B_PsdY”

Next trap a bead in the optical trap and Autoalign the beams. Confirm that both traps are engaged with the bead by seeing equal thermal noise patterns in the PSD window. Turn Autoalign off and select TrapA only. Move TrapA alone in the X direction only by selecting “X” button under the “Move Trap” button in the “traps” window. Observe opposing symmetric deflection signals in the PSD window when you move the trap. Move the mouse both right and left while observing that a wide arc is formed in those plots where the x axis is labeled “A_PsdX” and “B_PsdX” whereas a narrow arc is formed where the x axis is “A_PsdY” and “B_PsdY”. Now turn Autoalign ON briefly and then turn Autoalign OFF to center the beams along the x axis. Select the “Y” button under the “Move Trap” button in the “traps” window. Now move the mouse up and down and watch that similar arc patterns form on the remaining two plots.
If a wide arc is not centered on its narrow arc (2nd plot from bottom of inset “before”) then the bullseye is not centered on that PSD in that direction. Try to move the iris-detector circuit boards until all 4 plots are centered, as in the right-hand panel (inset “after”). In some cases it may be necessary to enlarge the mounting hole on the circuit board.