Mini Tweezers Basic User Guide

Chamber Construction and Plumbing:

See "How to construct a microchamber."

Construction is similar to the protocol for the old Bustamante lab tweezers, with two changes. First, use number 2 glass slides which accomplishes two things. It reduces any variance due to deformations in the glass slide (greater thickness minimizes this effect). Also, it leaves less space between the lens and the chamber, which allows for the new emersion liquid to better stay in place.

Second, do not use a bearing tube to thread the pipette tip into place. Instead seal the pipette tip in between the parafilm. This reduces drift from the movement of the pipette. Attach the PE 10 tubing with the UV setting glue.

For plumbing lines, use the substitute PE 50 tubing. (Small Parts Inc, 800-220-4292, PTFE Tube light wall 24G 100' Part # - STT-24-C). If using a shunt use the dispenser size tubing, otherwise vibrations from the fluid can increase the noise of the bead.

Note: There is a new gasket design ("pinched gasket").

Preference File Information:

Preference files store user changes to machine variables (i.e., pulling protocols, feedback gains, stored locations, data recording, etc…). These values are loaded when the program starts or when you import preference values. They may be saved by exporting preference values.

As an example, settings shown below are for the Bustamante lab "blue" mini tweezer using a 3.1 um bead in the trap.

@ ForceRateDiv 0	*Force feedback speed, 0 = fastest (4000 Hz), 255 = slowest*
@ ForceGain 55	*Adjust gain to prevent oscillation*
@ PosRateDivA 0	*Position A feedback speed, 0 = fastest (4000 Hz), 255 = slowest*
@ PosRateDivB 0	*Position B feedback speed, 0 = fastest (4000 Hz), 255 = slowest*
@ PositionGainA 64	*Adjust gain to prevent oscillation*
@ PositionGainB 32	*Adjust gain to prevent oscillation*
@ GotoSpeedX 15	*Initial motor speed in X*
@ GotoSpeedY 15	*Initial motor speed in Y*
@ GotoSpeedZ 7	*Initial motor speed in Z*
@ MaxDataFileSize 100000000	*File size in bytes. Will record files A – G then start to rewrite over A*
@ HopDistance 2	*Trap displacement during hopping test. 1 unit = 1.6 Å*

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The following lines control what data are recorded in your output: 1 = recorded; 0 = not recorded

@ *CycleCount/n____ 1	*Sequential cycle count from main controller PIC (4000 Hz)*
@ *A_PsdX_____ 0	*Trap A, Force PSD X output*

@ *A_PsdY_____ 0	*Trap A, Force PSD Y output*
@ *A_PsdSum_____1	*Trap A, Force PSD SUM output (total light intensity)*
@ *A_Iris_____ 1	*Trap A, Iris detector output (bullseye filtered intensity)*
@ *A_LeverX_____ 0	*Trap A, Lever PSD X output*
@ *A_LeverY_____ 0	*Trap A, Lever PSD Y output*
@ *A_LeverSum_____ 0	*Trap A, Lever PSD SUM output (total light intensity)*
@ *B_PsdY_____ 0	*Trap B, Force PSD Y output*
@ *B_PsdSum_____1	*Trap B, Force PSD SUM output (total light intensity)*
@ *B_Iris_____ 1	*Trap B, Iris detector output (bullseye filtered intensity)*
@ *B_LeverX_____ 0	*Trap B, Lever PSD X output*
@ *B_LeverY_____ 0	*Trap B, Lever PSD Y output*
@ *B_LeverSum_____ 0	*Trap B, Lever PSD SUM output (total light intensity)*
@ *A_Temperature___ 0	*Temperature sensor A [deg C]*
@ *B_Temperature___ 0	*Temperature sensor B [deg C]*
@ *X_force_____ 1	*Calculated force in X direction [pN]*
@ *Y_force_____ 1	*Calculated force in Y direction [pN]*
@ *Z_force_____ 1	*Calculated force in Z direction [pN]*
@ *Tension_____ 1	*sqrt(F_x^2+ F_y^2 +F_z^2)*
@ *A_dist-X_____ 0	*Calculated A trap displacement in X [nm]*
@ *A_dist-Y_____ 1	*Calculated A trap displacement in Y [nm]*
@ *B_dist-X_____ 0	*Calculated B trap displacement in X [nm]*
@ *B_dist-Y_____ 1	*Calculated B trap displacement in Y [nm]*
@ *Motor_X_____ 0	*Calculated chamber displacement in X [µm]*
@ *Motor_Y_____ 0	*Calculated chamber displacement in Y [µm]*
@ *Motor_Z_____ 0	*Calculated chamber displacement in Z [µm]*
@ *MotorVel_X_____ 0	*Calculated chamber velocity in X [µm/s]*
@ *MotorVel_Y_____ 0	*Calculated chamber velocity in Y [µm/s]*
@ *MotorVel_Z_____ 0	*Calculated chamber velocity in Z [µm/s]*
@ *A_trapPIC_Err___ 0	*Error report from A trap PIC*
@ *B_trapPIC_Err___ 0	*Error report from B trap PIC*
@ *comPIC_Err_____ 0	*Error report from communications PIC*
@ *A_CycleCount____ 0	*Cycle count from A trap PIC (16 bit, 4000 Hz)*
@ *B_CycleCount____ 0	*Cycle count from B trap PIC (16 bit, 4000 Hz)*
@ *Motor_CycleCount 0	*Cycle count from motor PIC*
@ *time(sec)_____ 1	*Calculated time from communications PIC cycle count [s]*
@ *Status_____ 1	*Indicates state of pulling protocols (i.e., waiting, hopping, pulling, stretching…)*
@ *Null_____ 0	*Skipped channel*

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@ samples/line_HI 400 *Data rate divider: low speed = 4000Hz / 400 = 10 Hz*
@ samples/line_LO 4 *Data rate divider: high speed = 4000Hz / 4 = 1000 Hz (don't go lower than 4)*

****

Coordinates of motor "go to" positions. Only valid for 1-7. Usually represent dispenser and pipette tube positions.

goto0 0 0 0
goto1 8388608 8388608 8388608

```
# goto2 8327896 8402399 8388608
# goto3 8328928 8374564 8389522
# goto4 8373693 8378995 8399187
# goto5 0 0 0
# goto6 0 0 0
# goto7 0 0 0
# goto8 0 0 0
# goto9 0 0 0

**********************************************************************
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# yourXYZint 123 456 789    Optional user variable for C programming- advanced
$ MaxForceP1          20.000000          Maximum force in P1 pulling protocol [pN]
$ MinForceP1 2.000000          Minimum force in P1 pulling protocol [pN]
$ PullRateP1   100.000000          Pulling rate in P1 pulling protocol [nm/s]
$ refoldTime1 0.000000          Time stall at minimum force [s]
$ MaxForceP2          20.000000          Maximum force in P2 pulling protocol [pN]
$ MinForceP2 2.000000          Minimum force in P2 pulling protocol [pN]
$ PullRateP2   100.000000          Pulling rate in P2 pulling protocol [nm/s]
$ refoldTime2 0.000000          Time stall at minimum force [s]
$ constForce1  5.000000          Constant force 1 [pN]
$ constForce2  18.000000          Constant force 2 [pN]
$ yourFloat          1.234000          Optional user variable for C programming- advanced
* yourXYZfloat          1.000 2.000 3.000          Optional user variable for C programming- advanced
End
```

Initial chamber alignment:

Make sure there is water or buffer in the chamber. Apply immersion fluid to both sides of the chamber. Use the new immersion liquid (Cargilie 3421) that does not evaporate. This should last for several days. (Note: Although the refractive index is the same, Cargilie's surface tension is less than water. See the note on chamber construction. Re-apply a little at the beginning of each day to avoid any problems later in the day.)

Open the software. You may need to restart the program several times until the lines on the screen are wiggling. If they are straight with no variation, the program is not communicating correctly with the instrument.

Find the pipette tip. Reset the motor position by resetting the motorPIC (under Tools ->Reset -> motorPIC). This does the same function as the reset button on the control board. This reset should be done when the trap is positioned slightly above the pipette tip. Set the position by pressing alt-1. Find the positions of the dispenser tubes and mark the position with alt-2 and alt-3. Now, pressing the number 1, 2, or 3 will automatically take you to the memorized position. If during the movement, the mouse is moved, it will abort and stop moving. You can save these preferences under File -> Export Preferences. You can import the previous days' settings under File -> Import Preferences. Opening the program automatically uploads the preference file.

Turning on the lasers:

Make sure the six metal Side Panels around the hexagon are in place. Then make sure the black plastic Beam Blocks under the Prism Boxes are also in place. Note in log book that all Beam Blocks are in place.

Turn on the laser power supply with toggle switch on the right of the box. Rotate both "laser current" knobs to reduce the current to zero in both lasers. Press both red "enable" buttons, which should illuminate both green "enabled" lights. Make sure both range switches select "power monitor." Then, gradually turn the "laser current" knobs to bring the "power monitor" reading up to HALF of the typical running values written on the tape next to the meters.

Pull out the Filter Slider knob to visualize the lasers on the TV monitor. There should be several focal spots. The single spot is from the yellow laser and should be moved to the middle of the star shaped pattern formed by the green laser. You can rotate the laser spots by turning the Retro Prism Holder, underneath the left beam block. Adjust the lasers in the x and y axis using the orange and green knobs sticking out of the top of the box.

Troubleshooting alignment: Verify that the "yellow" laser shows as a focused spot on the TV. If you cannot see the yellow laser spot, turn off the LED (Motors -> LED). This will dim the background on the screen and allow you to better locate the beam and focus it. To focus the single spot, use an Allen wrench to adjust the screw above the right objective in the z-axis (in and out from the chamber). There is a long lag time between movement of the wrench and movement of the objective, so go slow. Clockwise rotation brings the lenses closer together. Check that there is no air between the chamber and the lens. An Airey disk, which is a concentric ring pattern around the focus, indicates air somewhere in the optical path, which must be remedied by adding immersion fluid between the chamber and the lens.

Bring both lasers up to the full running values written on the tape next to the meters. These values are specific to each laser due to variations in the internal laser detector sensitivities. Higher powers allow higher pulling forces but shorten the lifespan of the laser.

In the log book, record the following readings for each laser: Power Monitor, Laser Current, PSD sum, and Iris/Sum (empty trap). Obtain the Laser Current value by turning the range switch on the laser power supply. Please turn the range switch back to Power Monitor when you are done.

Precise alignment of the lasers – X, Y and Iris/Sum value (daily):

This part of the alignment process precisely aligns the foci of each laser beam, first in the x and y axis, and then in the z axis. The alignment of the z axis is indicated by the value of the iris/sum value. Ideally, if the foci of each laser are coincident, the iris/sum value will be the same without and with a bead in the trap.

First, relax the piezos by selecting "Center Trap". Move the spots into the center of the TV screen using the Adjustment Rods on the top of the optical head. If the PSD X and Y values are greater than 10% of the PSD sum, the detectors may be out of alignment, making it necessary to adjust the relay lenses within the optical head- call someone who knows how. Alternately, the piezo tension may need to be adjusted inside the fiber wigglers- call someone who really knows what they're doing! Next, zero the PSDs by selecting "Zero PSDs". Only zero the PSDs with no bead in the trap. This will set the X and Y values to zero in the software.

Grab a bead of the size that will be in the trap during an experiment. With a bead in the trap, the trap deflections should become equal and opposite, as seen in the green and yellow spots in the PSD windows. Prior to hitting "Autoalign" minimize the X and Y deflections manually using the Adjustment Rods on the top of the optical head.
Select "Autoalign" and the lasers will move to minimize the X and Y values even closer to zero.

With the trap aligned in the X and Y directions, next align the focus in the Z axis. With the bead in the trap, note the Iris/Sum values for the green and yellow lasers and average the two values together. Lose the bead by knocking it out of the trap with the pipette tip. Note the Iris/Sum values and their average. The average values with the trap full and the trap empty should be close to each other, no greater than 0.015 apart. Closer is better. Adjust the focus by moving the right objective using a 5/64 inch Allen screwdriver placed into the Worm Gear assembly (don't drop the objective mount by turning the wrong screw). Move this slowly as there is a lag in the response of the system. Again try to minimize the difference in the average Iris/Sum values. These values should be recorded in the log book as well.

Alternately, it is also effective to focus the lasers in the Z axis by minimizing the size of the yellow laser trap displayed on the TV screen. However, the TV camera must be set to a consistent position for this method to work.

Alignment of the Light Levers (daily):

With the trap aligned properly in the X and Y axis, adjust the light levers by inserting a wrench through the holes in the beam blocks to adjust the light levers in the X and Y positions. The X and Y values should be no greater than 10% of the total strength hitting the detector. Try to get them as close to zero (centered on the detector) as possible.

NOTE: The mini-tweezers instrument only measures force and distance. Therefore, it is important to regularly check the force and distance calibrations.

Stokes Test (weekly):

1. Zero the PSDs (with no bead in the trap- see above)
2. Trap a bead from a known size distribution
3. Select "Autoalign"
4. Select "Autoalign" again (to turn it off)
5. Select Stokes' test (Tool->Stokes' test)
6. Select "Zero force"
7. Select "Move motors"
8. Move the mouse back and forth to move the motors in X and Y. Move the motor in Z with the scroll wheel (or by selecting "Move motor Z"). Be careful not to hit the pipette or chamber walls.
9. Freeze the Stokes' Test screen ("F")
10. Rotate the X and Y patterns to horizontal with "Q" and "E"
11. "E" will display the calculated bead diameter in the lower left corner of the screen.
12. Repeat for several beads (unfreeze the screen- "F", clear the data- "shift-~", rezero the PSDs, get a new bead)

If the averaged calculated bead size matches the manufacturer's reported bead size, the instrument is properly calibrated. This test assumes the fluid has the viscosity of pure water. Room temperature changes are compensated via the thermometer readings within the optical head. Beware of buffers containing glycerol and PEG and beads that are not round. For more information, see "How to calibrate."

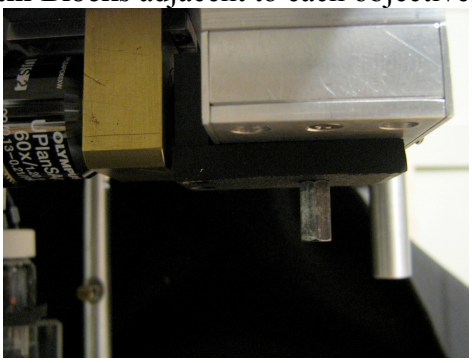
Checking Distance Calibration (monthly):
The light levers measure the position of the traps. They report their positions in the data file in nanometers under the columns "Lever Distance X" and "Lever Distance Y." These values are calculated using the lever sensitivity calibration factors. These factors should be checked by comparing the Lever readings with motor shaft encoder readings for a trapped bead on a pipette using the "Motor/Lever" graph. See the document "How to calibrate the minitweezers."

Basic Mini Tweezers User Guide: Part II

Glossary of terms:

Light Levers report the relative positions of the optical traps. The light lever window reports the brightness SUM and X and Y deviations for each laser in ADC (analog digital convertor) units. The red values are associated with the yellow laser trap while the blue values are associated with the green laser trap.

Beam blocks prevent laser light from escaping into the room. The most relevant ones are the six rectangular **Side Panels** of the instrument head; these should all be in place when the lasers are on. In addition, there are also **Beam Blocks** adjacent to each objective, as shown below:



The Beam Block is the L-shaped black plastic piece beneath the silver Prism Box

Controller boards are in the stack of green circuit boards attached to the mini tweezers Controller Power Supply. They facilitate rapid feedback monitoring between the host computer and the optical head. They control constant force, constant position, and motor go-to feedback functions.

Iris/Sum values report the ratio of Iris detector output to the PSD SUM output. They are used to determine if the two laser beams are focused at the same point along the Z axis by comparing the values with the traps centered, and no bead in the trap, and with the traps under auto-align, with a bead in the trap. See "Basic Mini Tweezers User Guide 1."

The correction collars are adjustments on the objective lenses for specific thickness cover glasses. For example, #1 cover glasses require a correction collar setting of 0.15, while #1.5 = 0.17 and #2 = 0.21. Using the correct settings will give the sharpest image and strongest traps.

The Worm Gear is a screw that you turn to slide one of the objectives relative to the other in the Z direction. It is on top of the rightmost objective (*not* the thing with the lock icon next to it) and is controlled with a 5/64" hexagonal wrench. It has *significant* mechanical lag, so you have to make several turns before it starts actually moving the objective.

The layout of the mini-tweezers apparatus:

- The upper region of the mini-tweezers apparatus should have all six rectangular plates affixed to the sides of the instrument; these are the "side panels" and they prevent stray light from escaping the tweezers. Only to be removed if the lasers are off.
- There are two pairs of holes on the side plates of the tweezers; each hole leads to a small hexagonal-tip screw. These screws are used to center the light levers.
- On top of the tweezers are a camera, a hitch for the bungee cord, and two pairs of screws (green, orange). Each of these pairs controls the X and Y positions for one of the traps.
- Attached to the mini-tweezers and the computer is a stack of circuit boards.
- These control the input of data from the instrument to the computer. If you want to reset the hardware, there is a small gray button on top of this stack. The on/off switch is a silver toggle.
- The big gray box labeled **Dual Diode-Laser Driver** controls the laser diodes
    - The on/off switch for the laser diode controller is a rocker switch on the right side of the box.
    - For each laser, there is a selector switch controlling the readout on the LCD screen: power, current, etc... Always make sure that you are looking at the right information!
    - The lasers are activated by hitting the red **"Enable" buttons.**
    - The knobs down at the bottom of the gray box control the laser current.
- There is a large syringe on the desk, which attaches inside the mini-tweezers to the fluid reservoir full of buffer. It has a four-way valve that allows you to vent the syringe, vent the buffer reservoir, apply pressure to the buffer reservoir, or cut off the buffer reservoir completely.
- There is a plunger on the desk that controls the filters in front of the camera (the **Filter Slider**); it has three settings: at full depression, the plunger cuts off the laser light almost completely; at 50% depression, some but not all of the laser light gets through; and when pulled out completely a lot of the laser light gets to the camera.

Inserting the chamber between the objectives:

-Slide it up, being careful to not scrape either of the objectives. Tighten both screws to hold it in place.

Turning on the lasers:

Turn on the laser power supply with the toggle switch. Turn the **Laser Current** knobs down to zero.. Press the enable buttons. Select **Monitor Power** on the range switches. Increase the **Laser Current** knob until the **Monitor Power** (the #s under 'Dual Diode Laser Driver') come up to 50% of maximum for both lasers. The 100% max powers are usually written on tape next to the meters.

Relax the wiggler piezos with the computer **center trap** button, then center the traps by looking at them on the video screen (the focused dot and the diamond of four points created by the retro reflector) and turning the X-Y control rods on top of the instrument head.

One of the lasers will have a lower output intensity (Force PSD SUM) than the other; select that one and increase its **Laser Current** knob while watching the **Power Monitor** up to 80% of maximum power. This level is sufficient for most experiments while maintaining good laser life.

Make the other laser's output intensity (the top numbers on the computer monitor in the **PSD window)** match that of the first laser. The second laser's internal **Monitor Power** should then end up below 80% of its written maximum.
In the notebook, write that the beam blocks are still in place. Record the monitor powers, the laser currents (turn the knobs next to the five red LEDs to display currents—make sure to switch back to 'Laser Power' when you're done!), and the laser output intensities at the top of the **PSD window** in the logbook
Do the Stokes' Law Test (below), recording the iris/sum ratios and the measured and actual diameters of the bead in the notebook.

The purpose of the **Log Book** is to record the instrument user and condition at various times. Specifically we want to know if the lasers are failing with time, or if the output polarization is changing as indicated by changes in the light-lever to the force-detector sums. Also it is useful to remind users to check their safety guards and beam blocks which must be in place to prevent eye injuries. The log book can also be used to record calibration checks such a Stokes' drag test or a Motor/Lever distance calibration. Finally, the log book is a good place to record bugs and complaints and suggested improvements. It is best to record bugs all together on the back pages of the book.

Mini-Tweezers Log Page

Date:

Name:

Check here if all beam blocks are in place before turning on lasers ☐

	Green Laser	Yellow Laser
Power monitor		
Laser current		
PSD sum		
Iris/Sum (empty trap)		
Iris/Sum (bead in trap)		
Light lever sum	(blue)	(red)

Notes:

Getting ready to record data

- Hit **center trap**, and align the lasers manually with the knobs on top of the miniTweezers (do this rarely—after manual adjustment, things drift for about 20-30 minutes!).
- To view the lasers' positions relative to one another, adjust the plunger controlling the light filters to its center position, so some (but not all) of the laser light is sent to the camera.
- **Zero the PSDs** before trapping the bead (important for measuring force)
- Trap a bead, hit **Autoalign** (control-A), then **turn off Autoalign**
- If desired, manually adjust light levers (through the holes in the sides of the miniTweezers head) Again, do this rarely! The optics take a while to settle after adjustment.
- **Zero the light levers** (for measuring extension) with the button in the program. This is only really necessary for constant-position protocols (e.g. P2) but it is a good habit
- Turn on **autoalign**, then hit the **zero force** button
- Record data
- Publish

The protocol for taking data and catching new beads when everything has been adjusted and the lasers are stable is more straightforward:

1) Catch a small bead, and mount it on the pipette tip with suction.
2) With the trap empty, **center** the trap. **Zero PSDs.**
3) Catch a big bead, bring it close to the pipette.
4) Make sure that the pipette has zero suction on it (it will mess with the force when
1. the beads are close together)
5) **Autoalign** with the big bead in the trap, hit **zero force** button
6) Bring the trapped bead to near the pipette bead, then fish by moving the trap in Y.
7) When you catch a fiber, make sure that the X and Z forces are zeroed when the Y force is about 5-10pN.

Open a new data file and start collecting data.

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Stokes' Law Test:

- **Zero the PSDs** with no bead around (only do it by hitting the button if they're not too far off, i.e. below 1000)
    - If the PSDs are more than 1000 off, hit the **main_reset** button on the circuit board stack to reset the hardware, or adjust the PSDs with the screws on the top of the mini-tweezers.
- Catch a bead
- Move at least half of a screen away from any glass surfaces or bubbles
- The PSDs should be deflected in symmetrical and opposite directions
- **Turn on auto-align (control-A)** and turn it back off again
- Go to **Tools** à **Stokes Test**
- Record the iris/sum ratio with the bead in the logbook as 'full'
- Lose the bead, then record the iris/sum ratio again in the logbook as 'empty'

    - If the ratio goes up when the bead is trapped, this means that more light is being caught by the iris—i.e. that you may need to adjust the objective focus slightly. This is probably only necessary once, when installing a new chamber.
    - Adjusting Objective Focus:
      § #1 cover slips require about a ~0.17 mm setting on the correction collar
      § Hit **center trap** on the program before adjusting the objective focus
      § The Worm Gear is a small horizontal cylinder just above the objective lens on the right
      § The iris/sum ratios should ideally be within ~0.010 of one another; differences significantly greater than this would suggest adjusting the objective focus
- Re-capture the bead, move the bead at least 10 diameters away from the pipette
- **Do the Stokes law test with autoalign off**
- Move trap around; you will see some parallel diagonal lines. Can scroll the system in the Z direction to form the third line
- Hit **f** to freeze the screen
- Use **Q/E** to make the lines horizontal, then hit E to display the corresponding bead diameter in the lower left of the screen
- Record the "diameter" and the actual value given by the company on the diameter of the beads in the logbook

Hopping Test (optional: used to detect trap drift or check trap stiffness):
- Catch a bead in the trap, autoalign, then get rid of the bead
- Move trap over a bead held on the pipette
    - Move the trap such that the PSDs are centered
- Hit autoalign
- Center the PSDs by moving the trap again
- Turn off autoalign
- View the window for hopping in the program (Graph à Hopping)
- Execute the 'hopping' procedure (under the "move trap" button) to make the traps wiggle back and forth in opposite directions (not required)
- Non-zero slope indicates that the pipette is drifting with time
- It's possible to obtain the trap stiffness by trapping a bead and hitting the 'stiffness' protocol at several different forces

System Shutdown
- Dial down the laser diodes to zero power
- Hit the red "enable" buttons
- Hit the main rocker switch on the control box to turn off the diodes, and turn off the circuit board stack as well.

Troubleshooting:
- **Known software bugs:**
    - Sometimes the program crashes in the middle of data collection. It's not a super-common event, but it has happened a few times for me in six months.

    - Sometimes the "Tools" menu on top of the screen no longer works, making it impossible to change the parameters of a pulling/clamping/etc. protocol without actually running it and hoping that the parameters window will pop up for you.
    - Rebooting the program is the only solution I have found to this.
- **The average Iris/sum ratios of the two lasers change by more than 0.010 between "auto-aligned with trapped bead" and "no bead with autoalign off" conditions**
    - One option is to lose the bead, adjust the Worm Gear slightly with the filter slider set at the intermediate position until the center laser looks like it's in focus, and try again; a short cut is also to keep the bead in the trap, with auto-align on, and adjust the Worm Gear until the values are close to what they were without the bead.
- **One of the lasers isn't visible on the camera, even at moderate power (50%).**
    - Sometimes the Worm Gear on the right objective needs to be adjusted, to bring one of the lasers into focus. Removing and replacing the chamber has also been known to fix this issue in the past. Probably there is air between one lens and the chamber. Add immersion fluid and move chamber to get it to flow in.
- **Things in the chamber used to be focused and visible, but now they aren't**
    - There could be a bubble in the chamber, or between the chamber and the two objectives. Try to flow some buffer through the chamber, or to put more fluid between the chamber and the objectives.
- **When I move the chamber a long distance in the Z direction, suddenly the chamber gets super fuzzy**
    - This happens when using the Cargille fluid instead of water on the objectives: since this fluid creates a more dramatic meniscus than you get with water, so you can't move things in Z as far as you could before.
- **The pipette and trap beads are out of alignment with each other in the Z plane**
    - Use pageup/pagedown to step the chamber up or down in the Z plane. Pagedown lowers the net Z force, and pageup raises it.
- **A light lever's deflection (X or Y) value is very high, even after I have centered both traps**
    - Make sure that you hit "A&B" before hitting "center traps"
    - If this fails, you must adjust the appropriate centering screws on the side of the miniTweezers box (the ones recessed in the body of the tweezers and accessible with a 5/64" hexagonal wrench). The X coordinate is controlled by the upper of the two holes; the Y coordinate is the lower of the two.
- **A light lever's power is unusually low, or zero**
    - Rarely a piece of tubing within the instrument (behind the beam blocking plates on the mini-tweezers) has gotten in the way of the light lever beam. This has been corrected in Laser Tweezers East with some tape, but it may still slip.
    - Otherwise, manipulate the light lever with the screws in the sides of the mini-tweezers; the light lever may be totally missing its sensor. You may find that scanning in one or both axes will eventually get you into a range where the light lever is hitting the sensor again.
- **In force-ramp mode (P1) the trap doesn't ever get down to the minimum force, so it keeps on moving the beads towards each other until they collide.**

    - You probably have some issues with the force calibration, due to residual flow in the chamber (or suction from the pipette), a mis-alignment of the beads (most often in the Z axis!), or the fact that the force on the trapped bead wasn't zeroed properly before catching a fiber.
- **The trapped bead feels an increase in force as it approaches the pipette**
    - Carefully focus the pipette bead in and out on the Z axis and look for a decrease in the force error. Always use the big bead in the trap and the small bead on the pipette.
    - There may be some residual suction in the pipette that is biasing your force values when the bead gets too close. Make sure that the plunger in the syringe connected to the pipette has been removed completely.
- **Something *very* strange is happening with the data readouts (things are frozen, or a number suddenly got enormous, or no data are being displayed on the screen at all)**
    - Try resetting the hardware, using the button on top of the circuit board stack attached to the miniTweezers.
    - There are two reset buttons on the stack of horizontal circuit boards:
        - The **usb_reset** button resets the USB (not frequently used)
        - The **main_reset** button resets all the piezos and other tweezers hardware. Use this if the numbers go on the fritz.
- **The program starts up but isn't collecting data from the instrument**
    - Try closing and re-starting the program several times, or resetting the tweezers hardware with the button on the circuit board stack.