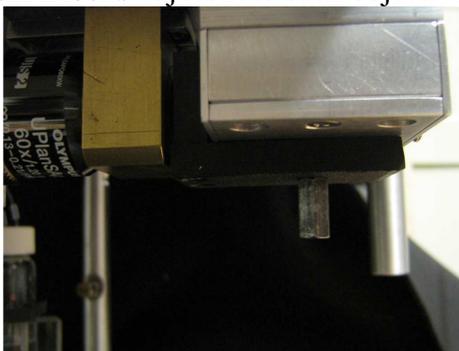


## Basic Mini Tweezers User Guide: Part II

### *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 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.

-

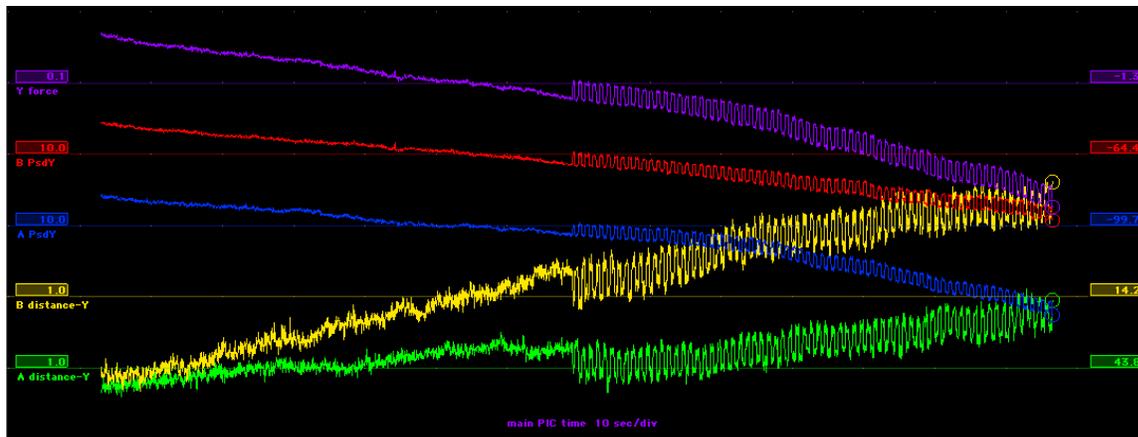
## Stokes' Law Test:

- **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**  **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  $\sim 0.17$  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  $\sim 0.010$  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, electrical noise and dirt noise):**

We make a test of instrument noise and drift by immobilizing a bead onto the end of a glass pipette and positioning it in the dual-focus laser trap. The focus is moved up and down once every second by changing the 16-bit DAC that drives the piezo wigglers. The hopping distance can be changed by editing the PREF file. For instance, 3 units equals  $\sim 1/2$  nm ( $3 \times 0.15 \text{ nm} = 0.45 \text{ nm}$ ). The figure below shows the force and position detector outputs averaged to 10 Hz bandwidth. The trace runs for 3 minutes and clearly shows drift, that is, tilt in the trace due to some long-term effect whose frequency is unknown because we did not measure long enough. The drift of  $\sim 1$  nm/minute in this figure (no feedback) is primarily due to expansion of the wiggler piezos with changes in room temperature, but pipette motion can also be seen. Finally, there is a source of error inside the light-lever measurement of position due to a drift in polarization at the output of the fibers which affects the power split at the pellicles. Note the half-nm step at 1 Hz is clearly visible. Indeed our smallest piezo step (1.5 Angstrom) would be barely visible in such a graph (data not shown).



Drift and noise from hopping test. Red and Blue = raw ADC readings from force PSDs @ 10 units/div. Purple = sum of red and blue converted to force @ 0.1 pN/div. Yellow and green = light lever PSD converted to distance @ 1 nm/div. The top trace (purple) shows a 0.03 pN force change for 0.45 nm displacement indicating trap stiffness of 0.066 pN/nm.

To make the hopping test:

Catch a bead and autoAlign the traps.

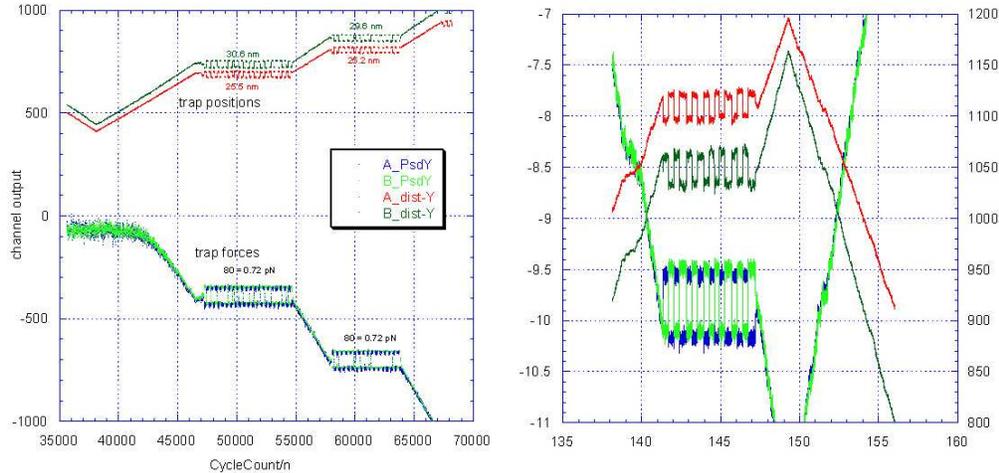
- Turn off autoAlign and suck the bead onto a pipette
- Move pipette with motors until pipette bead is centered on traps (force is less than 5 pN)
- View the window for hopping in the menu bar (Graph->Hopping)
- Execute the 'hopping' procedure (under the "move trap" button) to make the traps wiggle up and down
- Look for square-wave signal to indicate low noise conditions
- Non-zero slope indicates that the pipette is drifting with time

Some sources of noise that obscure the square wave could be electrical interference affecting the A/D convertors or else dirt in the buffer which has settled onto the trap and is wiggling by Brownian motion. To check for the later source, try moving the pipette out of the trap and flushing with clean buffer to remove dirt. Baseline drift is always present and may be due to air currents or temperature changes. Try wrapping the bottom fluidics section with saran wrap. Try the "soundproof" box. Wait ½ hour after touching any metal parts with your hands. One nanometer per minute is considered "good" whereas one nanometer per second is considered "bad".

### Stiffness Test

Normally when stretching a molecule, the slope of a force-position curve is a measure of stiffness for the trap spring in series with the molecular spring. To get the optical trap stiffness alone requires a special test. The dual counter-propagating trap geometry enables us to measure stiffness *in-situ* at various forces with the molecule attached. The simplest way to measure trap stiffness would be to move the trap focus a known distance relative to the bead and see how much the force changes (as shown in figure above), where the force is measured by changes in light momentum flux. Unfortunately for tethered beads, changing the focus position will change the force and thus cause the tether molecule to change its length and thus move the bead by some unknown amount, dependent on the molecular elasticity. Note, however, the molecule responds to the *total* light force on the bead which, for a dual trap, is the sum of forces from trapA and trapB. What happens if we move one trap toward the

pipette (lowering its force) and the other trap away from the pipette (raising its force)? Now the sum of forces remains constant and the average bead position remains fixed. We know how far each trap has moved (by light levers) and how much the force changes for each trap, so we could calculate an independent stiffness for each trap. The combined trap then has the stiffness that is the sum of the two values.



*Combined-trap stiffness test. Force and position changes for 1 kbp dsDNA attached to a 2-um polystyrene bead in the trap and another on a pipette. Force is negative for tension in the molecule. (A) Traps are moving but tether is slack between cycle counts (time) 35000 and 40000. First stiffness test done at -400 ADC units equals 7.2 pN. For a force change of 0.72 pN, trapA moved average 25.5 nm (stiffness = 0.028 pN/nm), and trapB moved 30.6 nm (stiffness = 0.023pN/nm). The test was repeated at -700 ADC counts (both traps) which is 12.6 pN. There, for the same force step (0.72 pN), trapA moved 25.2 nm and trapB moved 29.6 nm for a combined stiffness of 0.0526 pN/nm.*

The figure above shows data from a mixture of two pulling protocols. Initially the combined focus was moved at a constant velocity to stretch a DNA tether attached between the trap bead and a pipette. Note the molecular tension rose (indicated in -Y direction) when the contour length of 1000 basepairs was reached. At some point during the pull (say 7 pN), an order was given to measure trap stiffness. Then both traps were set to constant-force feedback mode and the two feedback setpoints were made slightly different (say 3.2 pN and 3.8 pN). However the forces still summed to 7 pN so the molecular stretch did not change. Then the setpoints were switched at a regular interval (3.2/3.8....3.8/3.2....3.2/3 etc) always summing to the same value. After ~10 cycles, the feedback was released and the constant-velocity pull protocol was resumed. Data in this figure indicate a combined stiffness of 0.0513 pN/nm at 7.2 pN force and 0.0526 pN/nm at 12.6 pN. This trap became stiffer when the tension was raised in this range. Such a trend is the result of the non-linear refraction with offset in a sphere, a form of “spherical aberration”. This inflection will reverse when the bead pulls farther out of the focus and starts to escape the trap.

It’s easy to obtain the optical trap stiffness while stretching a molecule and recording a data file. Suppose you are running a force-position protocol such as *PI*. When the molecule reaches some force where you want to know the trap stiffness, press the *Stiffness* radio button and you will see that the force stop increasing and ConstForce feedback indicators come on. Observe the plot closely and you will see that the distance coordinate (*A\_DistY*) hops back and forth every second. After such hopping has continued for say 8-10 cycles, press the pulling protocol button again (say *PI*) and the pulling curve will continue. Repeat this test at several forces along the pulling

curve. It is important for the accuracy of this test that both lightlever beams be active (working) and fairly well centered on their PSD targets.

For a special program that was created to find regions in a data file containing stiffness tests, see the Software section under “Stiffness Test Analyzer”. Note that specific columns must be included in the data file for this program to work, namely: *CycleCount/n*, *Y\_force*, *A\_dist-Y*, *B\_dist-Y*, and *Status*. To make a demonstration graph such as the figure above also requires recording *A\_PsdY* and *B\_PsdY* but these columns are not needed for the program to compute stiffness because the amount of the opposing force jumps are constant and are written into the GUI source file *ltControl.c*. Note that older versions of the program specified a force jump of +/-40 ADC counts per trap (as seen in *A\_PsdY* and *B\_PsdY*) and this number corresponded to 0.72 pN in the figure above. Newer versions of the GUI specify two, possibly different, integer offsets for the two traps:

```
const int forceJumpA = round (1/ data->instrument.trapAForceSensitivity.y);  
const int forceJumpB = round (1/ data->instrument.trapBForceSensitivity.y);
```

where these numbers always represent +/-1.00 picoNewton of force offset in each trap.

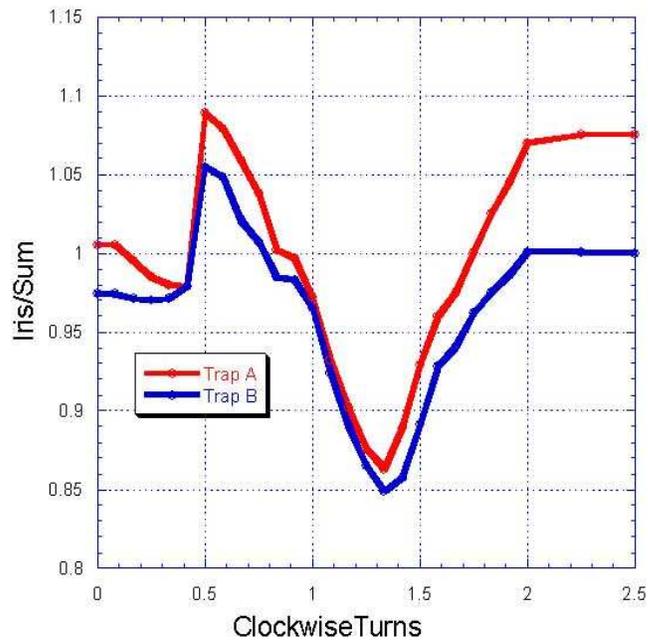
### System Shutdown

- 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.
- **Turning the focus-adjust screw doesn’t cause the Iris/Sum values to have the same average with a trapped bead as with an empty trap.**
  - 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. Be sure the Autoalign feedback is running while you turn the focus screw. Remember that the two Iris/Sum values will never be the same with the trap empty versus full, but the average of the two Iris/Sum values needs to be made the same, within say +/- 0.01.

Note that Iris/Sum values are very sensitive to focus position, but only when both foci are within the same trapped bead. The figure below shows the trap response using a 2-micron bead. The correct position is somewhere near the middle of the transition, which there happened to be at +1 CW turn of the screw.



- **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.