

Mini Tweezers Basic User Guide 1

Chamber Construction and Plumbing:

See document “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 non-evaporating immersion 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 PE-10 tubing, otherwise vibrations from the fluid can increase the noise of the bead.

Note: There is a new gasket design (“pinched gasket”) which allows us to view both ends of a dispenser tube and check for blockage.

Preference File Information:

Preference (PREF) 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. This file is located in each user’s home directory. You must check this file before recording your first experiment to see if the channels you want (such as Y-force) will be recorded. **If your PREF file is missing or wrong, you will think you recorded your experiment but find later that the files are useless.**

As an example, settings shown below are for the Bustamante lab “Blue Mini” tweezers using a 3.1 um bead in the trap. Feedback gain setting may change for other size beads.

| | |
|--------------------|--|
| @ ForceRateDiv 0 | <i>Force feedback speed, 0 = fastest (4000 Hz), 255 = slowest</i> |
| @ ForceGain 55 | <i>Adjust gain to prevent oscillation</i> |
| @ PosRateDivA 0 | <i>Position A feedback speed, 0 = fastest (4000 Hz), 255 = slowest</i> |
| @ PosRateDivB 0 | <i>Position B feedback speed, 0 = fastest (4000 Hz), 255 = slowest</i> |
| @ PositionGainA 64 | <i>Adjust gain to prevent oscillation</i> |
| @ PositionGainB 32 | <i>Adjust gain to prevent oscillation</i> |
| @ GotoSpeedX 15 | <i>Initial motor speed in X</i> |
| @ GotoSpeedY 15 | <i>Initial motor speed in Y</i> |
| @ GotoSpeedZ 7 | <i>Initial motor speed in Z</i> |

@ 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 = about 0.18 nm assuming ideal wiggler action where 2¹⁶ DAC counts would move the trap focus by 12 microns*

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

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

@ 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. 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
```

```
# 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]
$ unfoldTime1    0.000000       Time stall at max force [s] It8E and higher
$ MaxForceP2     15.000000     Maximum force in P2 pulling protocol [pN]
$ MinForceP2     1.000000     Minimum force in P2 pulling protocol [pN]
$ PullRateP2     300.000000    Pulling rate in P2 pulling protocol [nm/s]
$ refoldTime2    8.000000       Time stall at minimum force [s]
$ unfoldTime2    0.000000       Time stall at max force [s] It8E and higher
$ MaxForceP3     25.000000     Maximum force in P1 pulling protocol [pN]
$ MinForceP3     0.000000     Minimum force in P1 pulling protocol [pN]
$ PullRateP3     150.000000    Pulling rate in P1 pulling protocol [nm/s]
$ refoldTime3    0.000000       Time stall at minimum force [s]
$ unfoldTime3    3.000000       Time stall at max force [s] It8E and higher
$ 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 placement:

Before you place the chamber between objective lenses, you should assemble all fluidics connections and then run buffer through the chamber and check it for leaks or

trapped bubbles. This test can be done outside the instrument by placing the fluidics cassette under a magnifier lens or microscope. Then make certain there is enough room between the objectives to slide the chamber in without scratching the objectives or cracking the chamber. The normal inter-lens working gap is 880 μm (for coincident foci) whereas the thickness for a normal chamber comprising 2 Nescofilm gasket layers and 2 coverslips (size #2) is 660 μm . Thus we have a close fit if all is normal. Do not try to insert the chamber at a twisted or tilted angle to the optic axis or else it will not fit. Do not force the chamber past the lenses or else it will scratch them.

Apply immersion fluid to both sides of the chamber. NEVER ALLOW BUFFER OR SALT WATER TO TOUCH THE LENSES. You can use distilled water or else a non-evaporating immersion liquid with the same refractive index as water, such as Cargille Labs. 43421. Then one application should last for several days. (Note: Although the refractive index is the same, Cargille's surface tension is less than water. Use thicker #2 cover glasses to reduce gap width and retain fluid. Re-apply a little fluid 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. This is a problem in USB device assignment.

Find the pipette tip. Reset the motor position by pressing Motor->ZeroMotor. 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:

There are dangerous laser beams inside the instrument head. Do not take off side panels without wearing protective eyewear. In normal operation, 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 (trapB) and can be moved with the right-hand set of control rods. The star pattern of dots is from the green laser (trapA). You can move this star with the left-hand set of control rods and rotate the pattern by turning the Retro Prism Holder which is underneath the left beam block. Adjust both traps to the center of the TV screen by using the control rods sticking out of the top of the instrument head.

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. 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 Airy 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.

Focus test: With Laser-B alone turned on and the sliding filter pulled completely out, you should see a diffraction pattern (square array of spots) around the bright central focus. If the center focus is fuzzy and no spots appear, try turning the objective positioning screw a little. If you never see any spots, check again that immersion fluid (or water) is covering both objective lenses and that the objective correction collars are set correctly for the chamber coverslip thickness.



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 Z focus (daily):

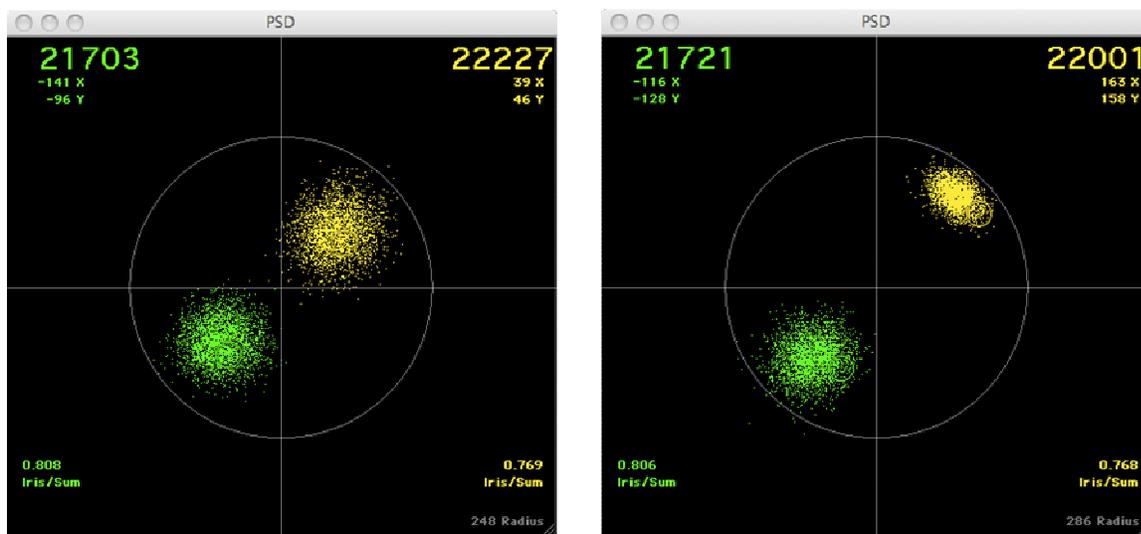
This part of the alignment process precisely aligns the foci of the 2 laser beams, first in the x and y axis, and then in the z axis. The alignment of the z axis is indicated by the value of the iris/sum value. Ideally, if the foci of both lasers are coincident, the iris/sum value will be the same without 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. Place the TrapB spot in the center of the TrapA retro-reflector pattern. Push the main board reset switch to remove any PSD offset. If the PSD X and Y values are greater than 10% of the PSD Sum, the detectors are out of alignment, making it necessary to adjust the relay lenses within the optical head. Get someone to help who knows how this is done. *Adjusting the relay lenses involves removing the side panels with the lasers running, so be sure to wear protective eye gear. The relay lenses, which are mounted on the lower deck directly below the Force Detector assemblies, may be moved by loosening their single mounting screw and pushing the lens holders by hand. Observe the PSD window to put the signals near the middle where $x=0$ and $y=0$. Replace all side panels and beam blocks when you are finished.*

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. (see below). 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 by turning the objective focus screw. Then turn the Autoalign OFF and PSD deflection scatter plots will start to drift slowly apart. Zoom into the PSD plot (press ‘z’) to make the Brownian noise visible on the PSD plot (see figures below).



The Brownian noise patterns should be symmetric and equal, as seen in the left panel. If one pattern is small or asymmetric (as in the right panel) then probably the foci are not together on the optic axis and the bead is closer to one focus than the other. Move the objective focus screw until both patterns are circular and equal. If that cannot be achieved, consider the possibility that one laser is weaker than the other, or one beam is focused better than the other. Check that both objective lenses have their correction collars set to the proper coverslip thicknesses.

Another way to check the Z-axis trap focus is as follows: 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. See document User Guide 2 for troubleshooting this alignment.

Alignment of the Light Levers (daily):

With the trap aligned properly in the X and Y axis, adjust the light levers by inserting a 5/64" hex wrench through the holes in the side panels 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 XYZ"
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 bead against the pipette or chamber walls.
9. Freeze the Stokes' Test screen by pressing "F" key.
10. Rotate the X and Y patterns to horizontal with "Q" and "E" keys.
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."