Piezo tracking introduction

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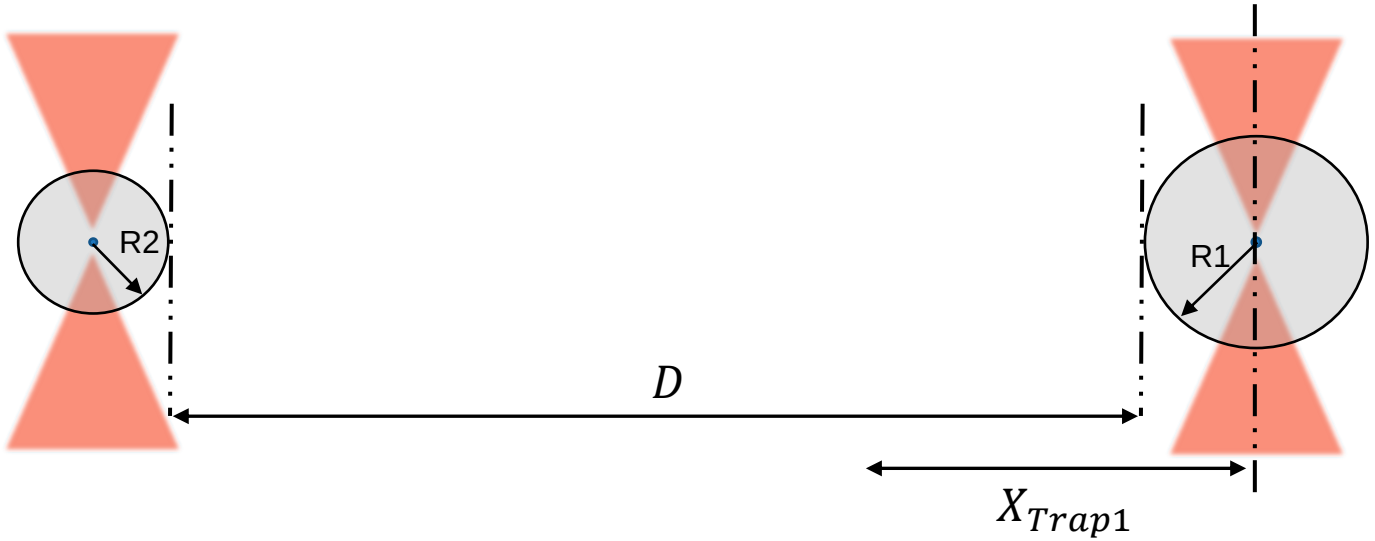
What is piezotracking (PT)?

- Short construct (~<5 kbp) = poor brightfield camera tracking (template merging)
- Need another way to get the distances → piezotracking
- Piezotracking = calculates distance between the two beads using the displacement of trap 1 but has several limitations

What is piezotracking (PT)?

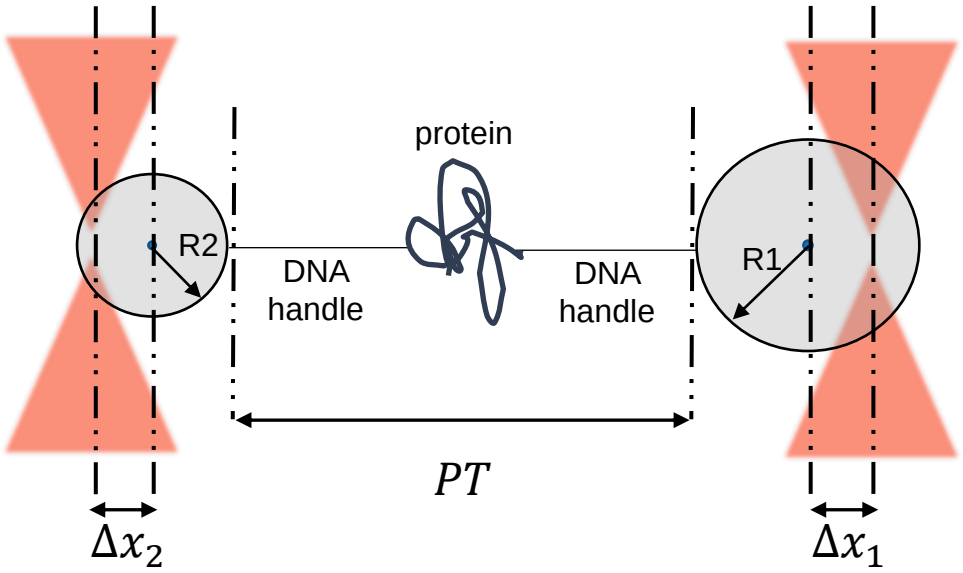
zero forces



Known distance D from brightfield template tracking at larger distances and at zero forces

(R1 and R2 already subtracted)

with forces



$$PT_{theory} = D - ?$$

$$PT_{theory} = D - X_{Trap1}$$

$$PT_{theory} = D - X_{Trap1} - |\Delta x_1| - |\Delta x_2|$$

$$F = k\Delta x$$

$$PT_{theory} = D - X_{Trap1} - \frac{|F_1|}{k_1} - \frac{|F_2|}{k_2}$$

Limitations of piezotracking

1. Trap 1 piezomirror sensor position calibration
2. Baseline force
3. Distance offset (D_{off})

$$PT_{theory} = D - X_{Trap1} - \frac{|F_1|}{k_1} - \frac{|F_2|}{k_2}$$

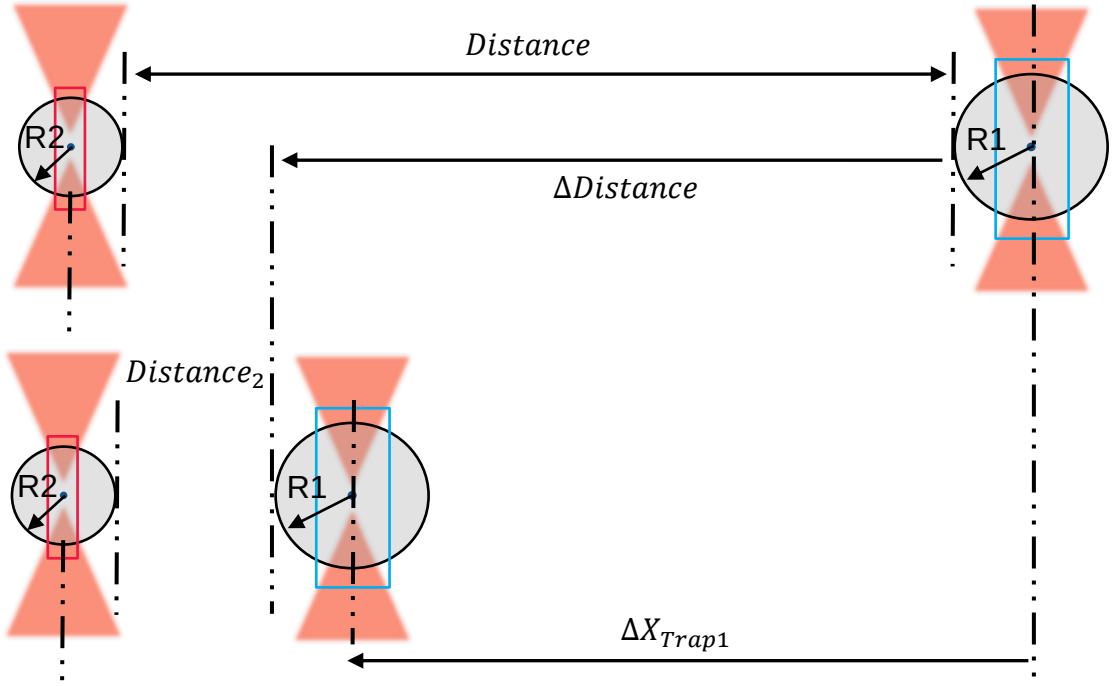
$$PT_{practical} = D_{off} + D - m \cdot X_{Trap1} - \frac{|F_1 - F_{1_{baseline}}|}{k_1} - \frac{|F_2 - F_{2_{baseline}}|}{k_2}$$

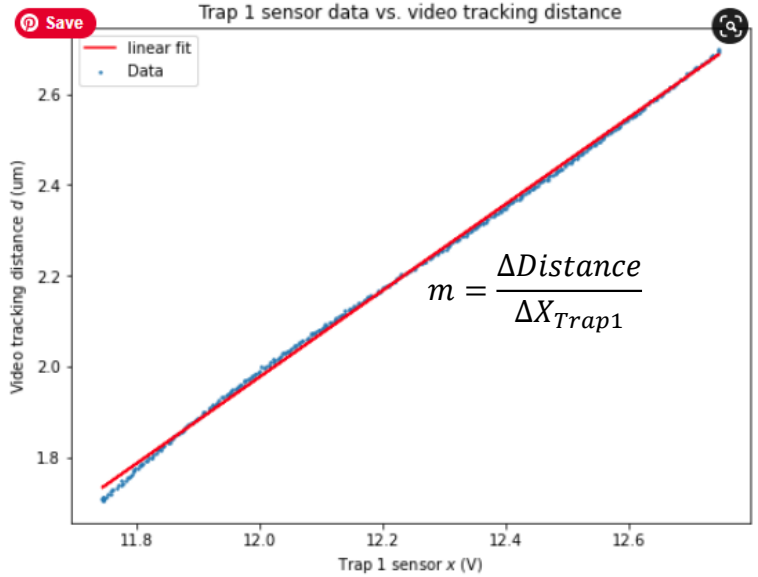
Limitations of piezotracking

1. Trap 1 piezomirror sensor position calibration

$$PT_{practical} = D_{off} + D - m \cdot X_{Trap1} - \frac{|F_1 - F_{1_{baseline}}|}{k_1} - \frac{|F_2 - F_{2_{baseline}}|}{k_2}$$

- Bluelake converts Trap 1 piezomirror sensor output from volts to um using an average sensor calibration value
- Because the piezomirror motion is not linear across its full range of motion, this leads to sources of errors
- Solution: perform the sensor calibration ourselves using the template-tracking distance and Trap 1 position in the range where we obtain our data, then, apply this calibration during post-processing





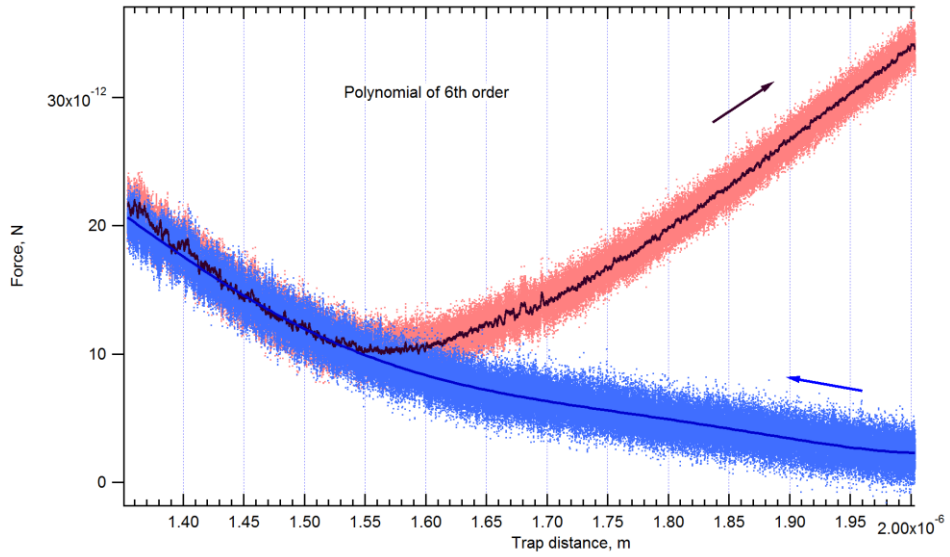
This fit only works for distances where camera tracking is still possible, and also only if there is no forces on the bead. In post processing, it may be necessary to select that range manually

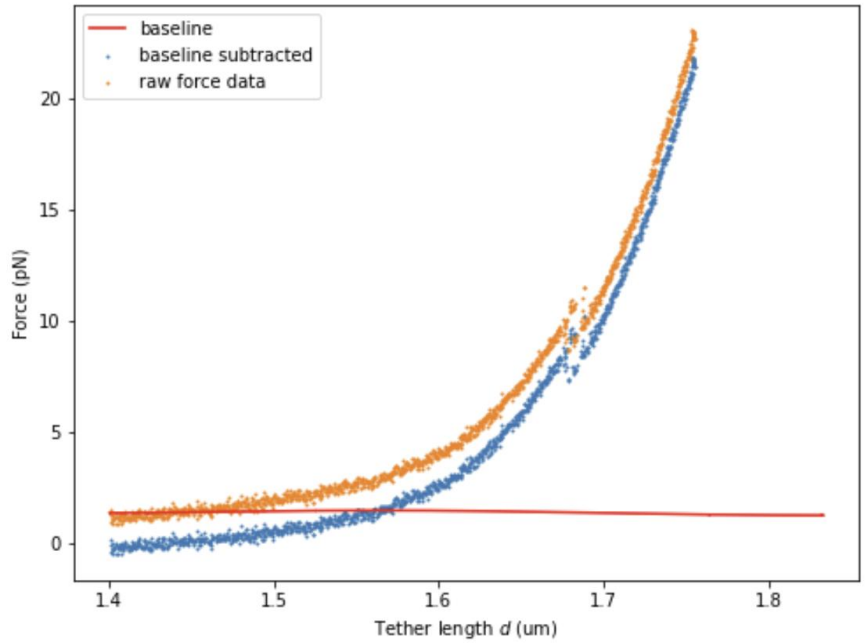
Limitations of piezotracking

2. Baseline force

$$PT_{practical} = D_{off} + D - m \cdot X_{Trap1} - \frac{|F_1 - F_{1_{baseline}}|}{k_1} - \frac{|F_2 - F_{2_{baseline}}|}{k_2}$$

- At any given force measured by the detectors, a small part of that force is an artifact force that is detected when two beads are held very close to each other
- This is mainly due to the two beads feeling each other's trap, but also other phenomenon (likely not interference)
- Bluelake 1.6 does not have baseline correction implemented
- Solution: measure a baseline curve ourselves and subtract this artifact force during post-processing





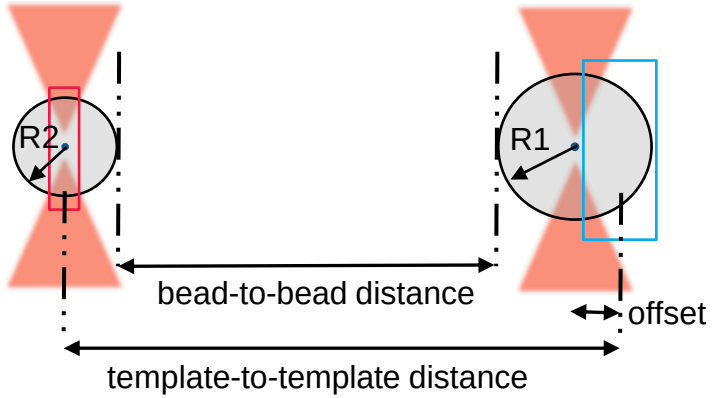
Limitations of piezotracking

3. Distance offset

$$PT_{practical} = D_{off} + D - m \cdot X_{Trap1} - \frac{|F_1 - F_{1_{baseline}}|}{k_1} - \frac{|F_2 - F_{2_{baseline}}|}{k_2}$$

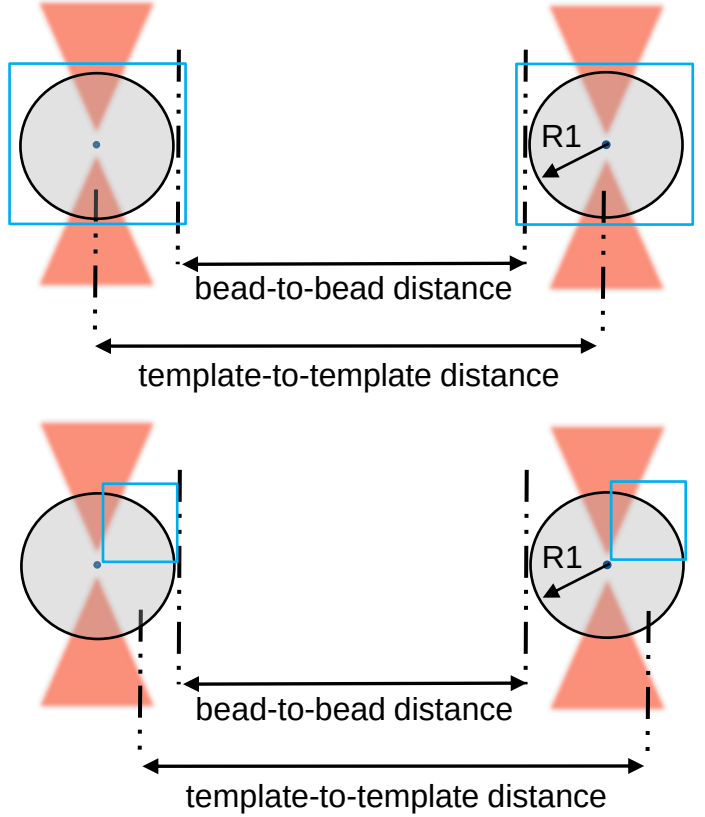
- To track the distance between two beads of different sizes, we must use two different templates on Bluelake
- A resulting distance offset inevitably occurs and its magnitude depends on how accurate the templates are positioned by the user
- Solution: draw the different templates as centered as possible and use the WLC fitting in post-processing to correct for the offset

Template tracking using **different** bead sizes



bead-to-bead distance = template-to-template distance – R1 – R2 – offset

Template tracking using **same** bead sizes



bead-to-bead distance = template-to-template distance – R1 – R1

What does the script do?

- Step 1: Calibration of sensor (converts Trap 1 movement to trap-to-trap distance)
- Step 2: Baseline correction
- Step 3: Subtraction of off-center bead displacement
- Distance offset remains but can be corrected while fitting the WLC

$$PT_{practical} = D_{off} + \underbrace{D - m \cdot X_{Trap1}}_{\text{Step 1}} - \underbrace{\frac{\left|F_1 - F_{1_{baseline}}\right|}{k_1} - \frac{\left|F_2 - F_{2_{baseline}}\right|}{k_2}}_{\text{Step 2 (baseline)}\ \text{Step 3 (off-center bead displacement)}}$$

remains, but can corrected further by fitting the WLC

General workflow steps

On the data viewer screen, under the F,d panel, change the eWLC model to the expected length of your full construct (say 5 kbp). Change the force channel to the one of your choice, and use the distance channel 'Piezo Distance'. Remember this eWLC curve might not correspond to what you will measure due to the limitations of the piezotracking on Bluelake. Once you have a better idea of how long your overall construct appears on your system, you can adjust the eWLC contour length to use it as a visual guide.

After catching two beads in different channels (1 and 3 for example) and going to the buffer channel:

1. Stop the flow, vent
2. Position your beads horizontally about ~5 um apart, with Trap 1 on the right of Trap 2. You can store this Trap 1 position ‘’calib’’ using the waypoint system
3. Calibrate your beads. If the beads do not have the same diameter, you will have to calibrate twice and only apply to the relevant trap
4. Zero the forces
5. Activate PT (find the "PT" icon and click on it). This will lock the Y movement of Trap 1 (to increase accuracy) and will use the current brightfield template tracking distance as an initial distance.
6. Move Trap 1 to the left and start 'fishing' at different distances, with decreasing distances and increasing wait time, much like the regular lambda DNA workflow (except no flow). However, this time, keep in mind the piezo distance measured may be off significantly. In addition, you will measure some artifact forces when the two beads get close to each other.
7. Once you have a tether, you can relax the tether and start a forward Fd curve. After you rupture the tether, it is important to also take a 'background' Fd curve. You can do so by taking another Fd curve backwards, for example, after the tether ruptured. The background curve will be useful for both the Trap 1 distance sensor calibration and the baseline correction
8. Once you have your data, export both the data curves and the corresponding background curves. Make sure to export all force channels, the distance 1 and piezo distance channels, the Trap 1 (X & Y) position channels. You technically do not need the 'piezo distance' channel but it can be useful for visualisation in post-processing. Remember that the data to export should have the 'download' icon lit up.

Important workflow points

- Always get one background curve per bead pair that can be used for both step 1 and step 2 of the script
- The background curve should cover as much as possible the full range of the data
- Always input the right diameter of the beads in the templates
- Try your best to center the templates in the middle of the respective bead to reduce the distance offset
- Make sure the force calibration is good
- Make sure you only have a single bead per trap (you can release the trap while the flow is off to see if multiple objects diffusing together)
- It requires some experience and several tethering attempts before having a good understanding of how many tethers are being tethered and at what distances. This can be checked using the overstretching plateau forces, or counting the amount of tether breaking events (large staggered jumps)