Measuring the Force of the Myosin Protein in Onion Cells with Optical Trapping

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8.13 Oral Presentation

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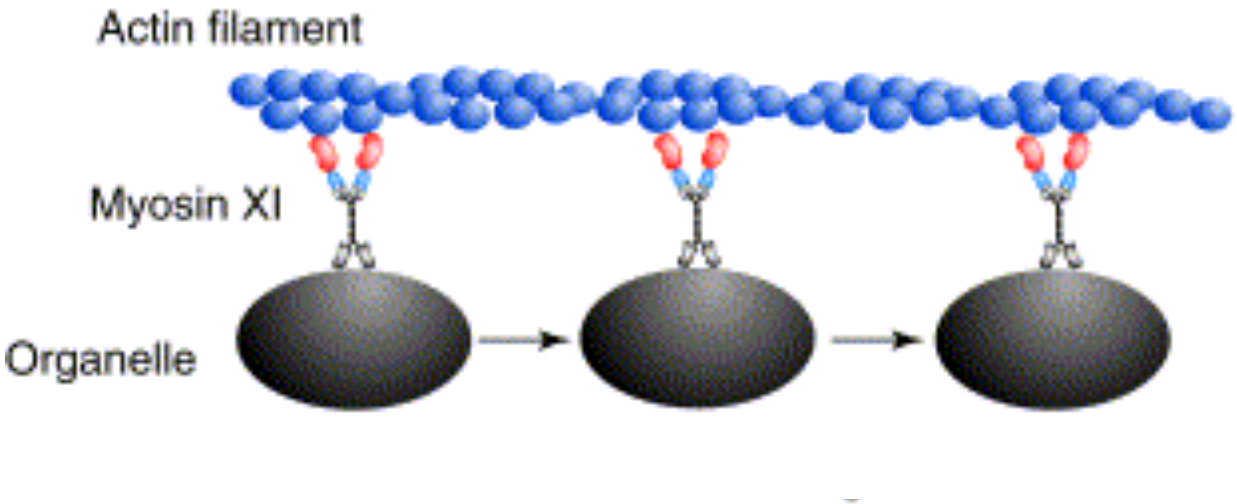
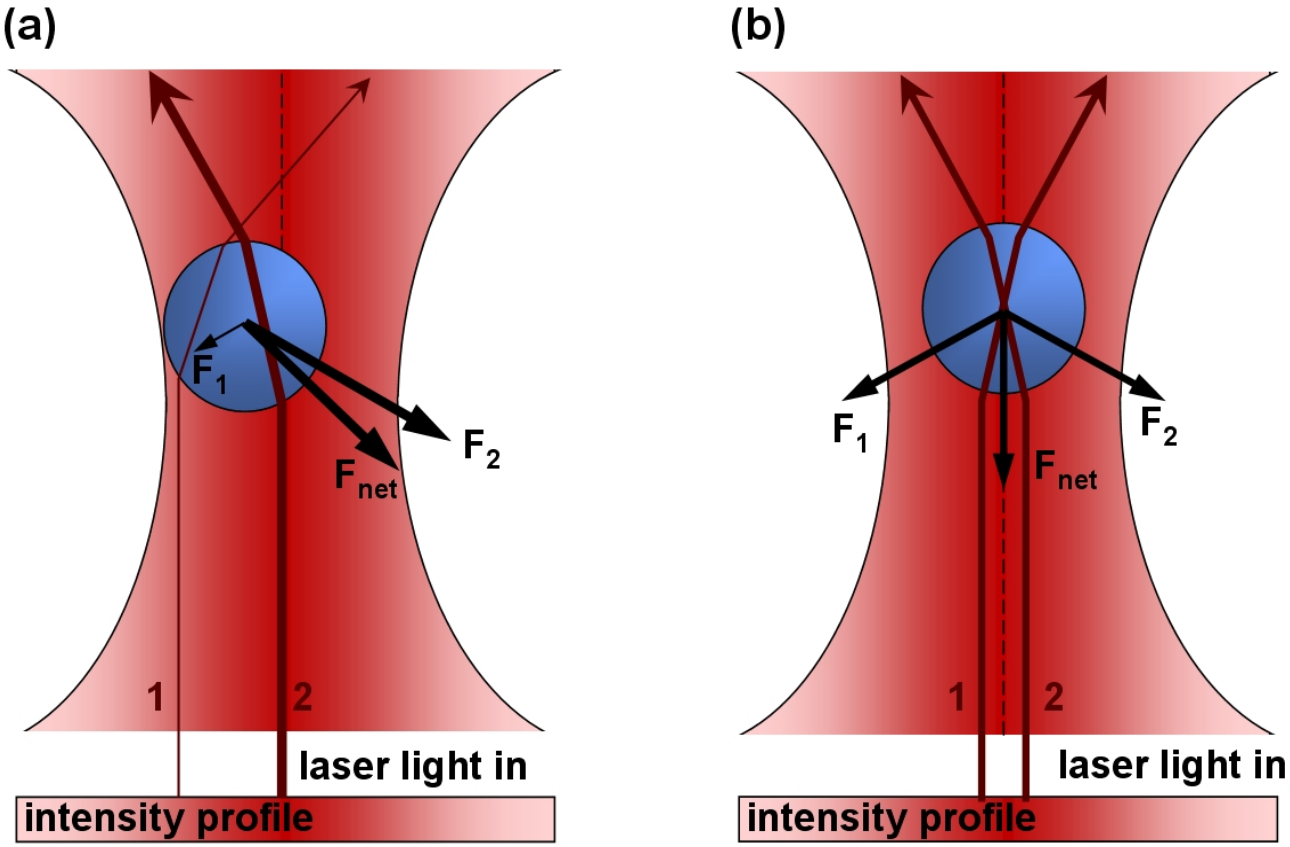
We set out to measure the force exerted by myosin on the vesicles it transports



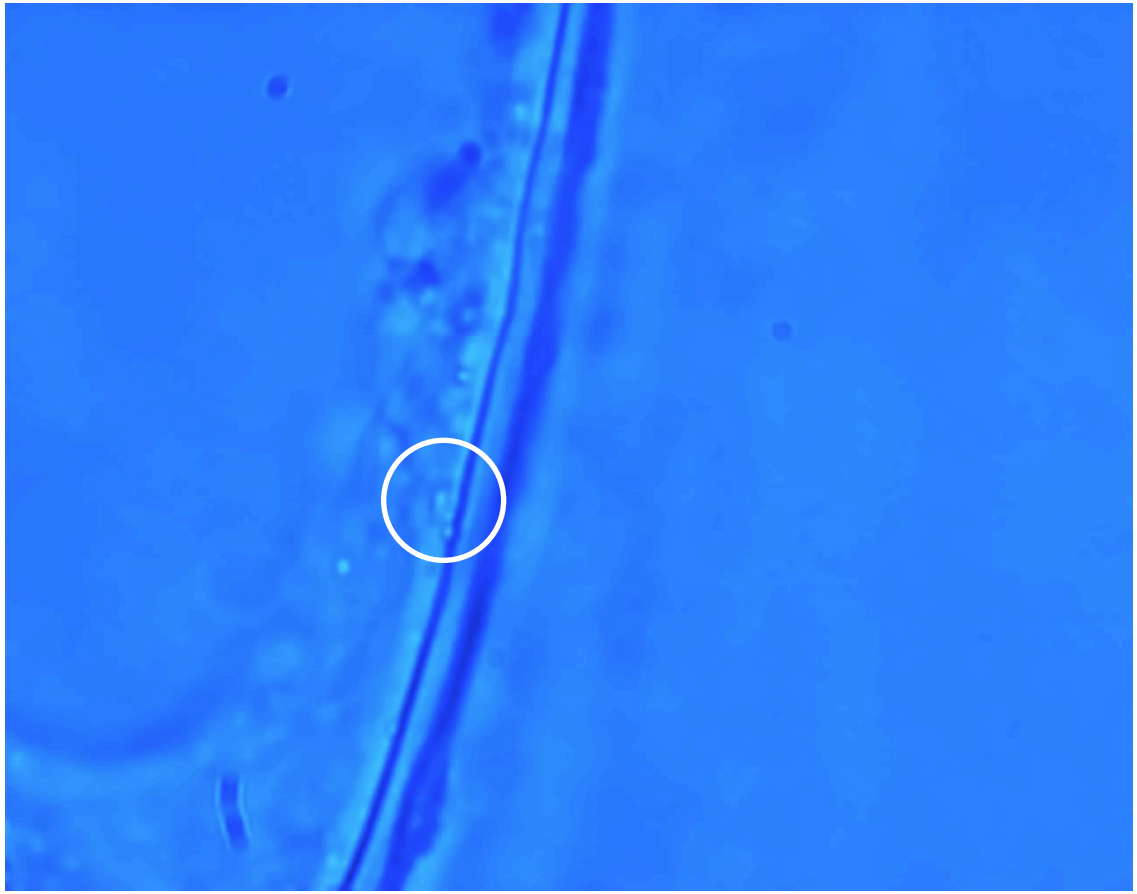
Source: “Optical Trapping”. UC Berkeley Physics 111.

Forces exerted from photons in a laser can trap a small object



Source: Junior Lab Staff, "Optical Trapping". MIT Department of Physics

Laser power was increased until moving vesicles were stopped

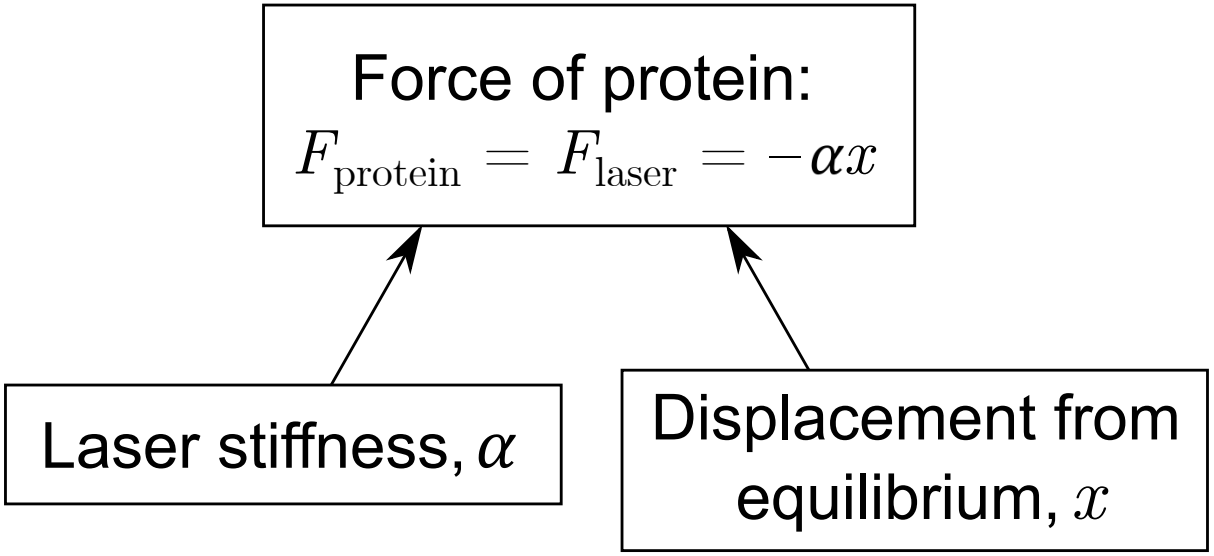


We will approximate the force of the laser as a harmonic oscillator

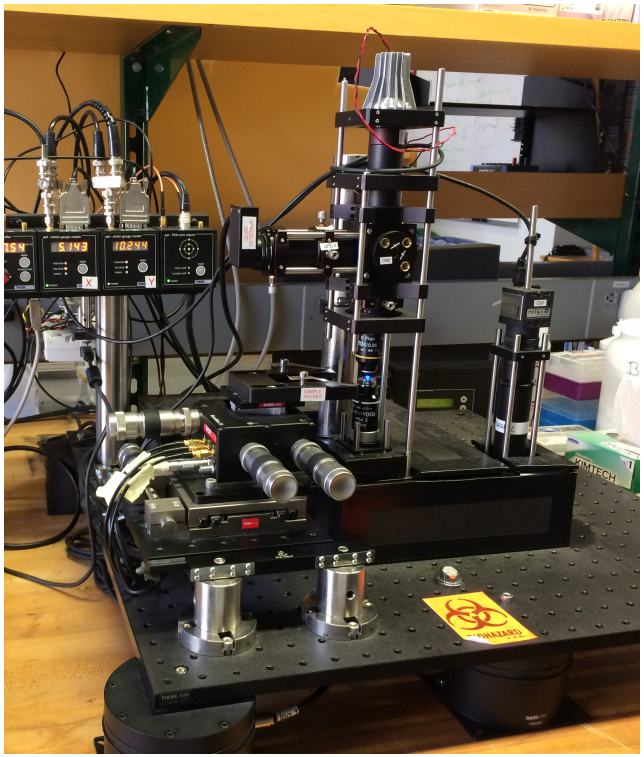
Force of protein:

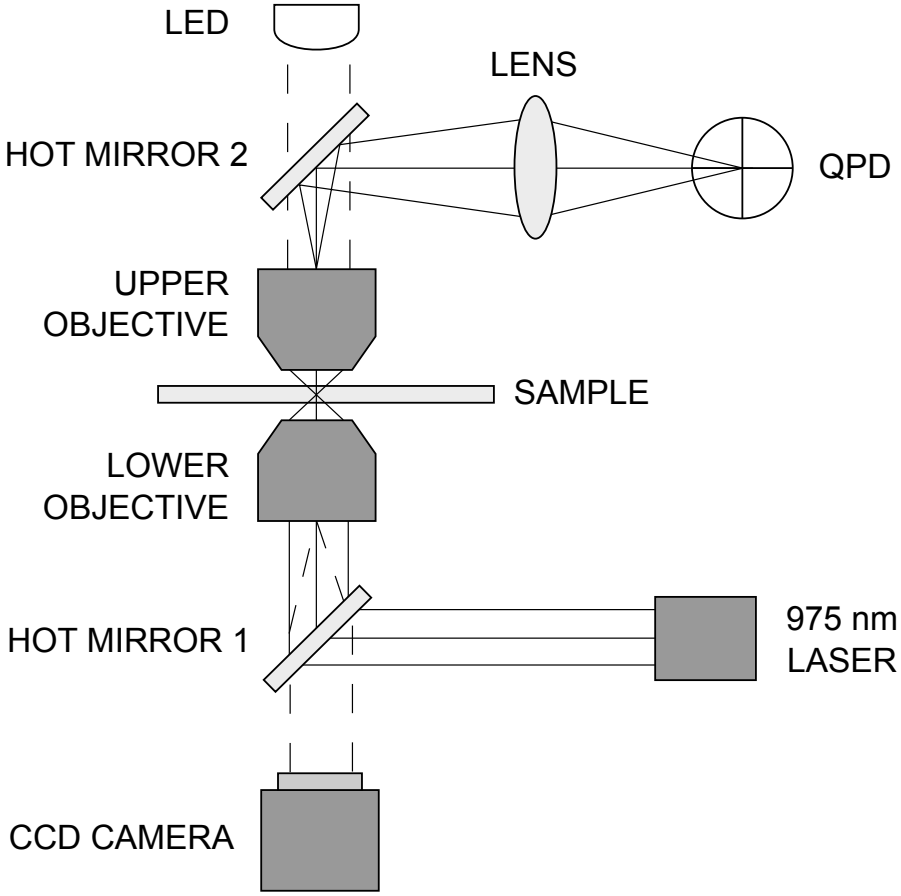
$$F_{\text{protein}} = F_{\text{laser}} = -\alpha x$$

We will approximate the force of the laser as a harmonic oscillator

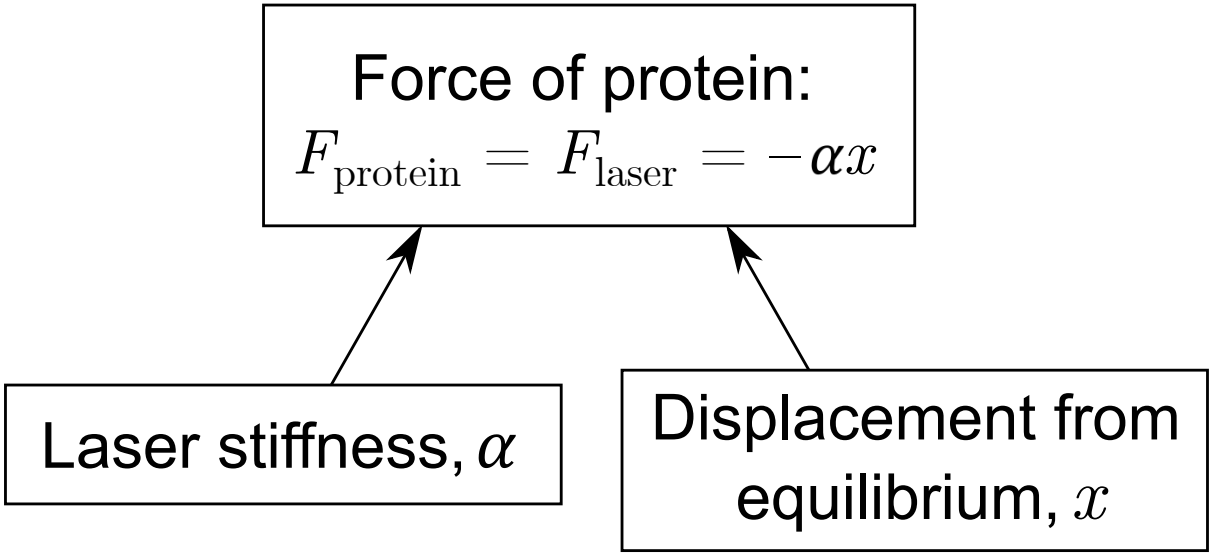


Laser intensity is measured by the QPD to give data on the position of trapped objects





Steps of analysis to arrive at myosin protein force



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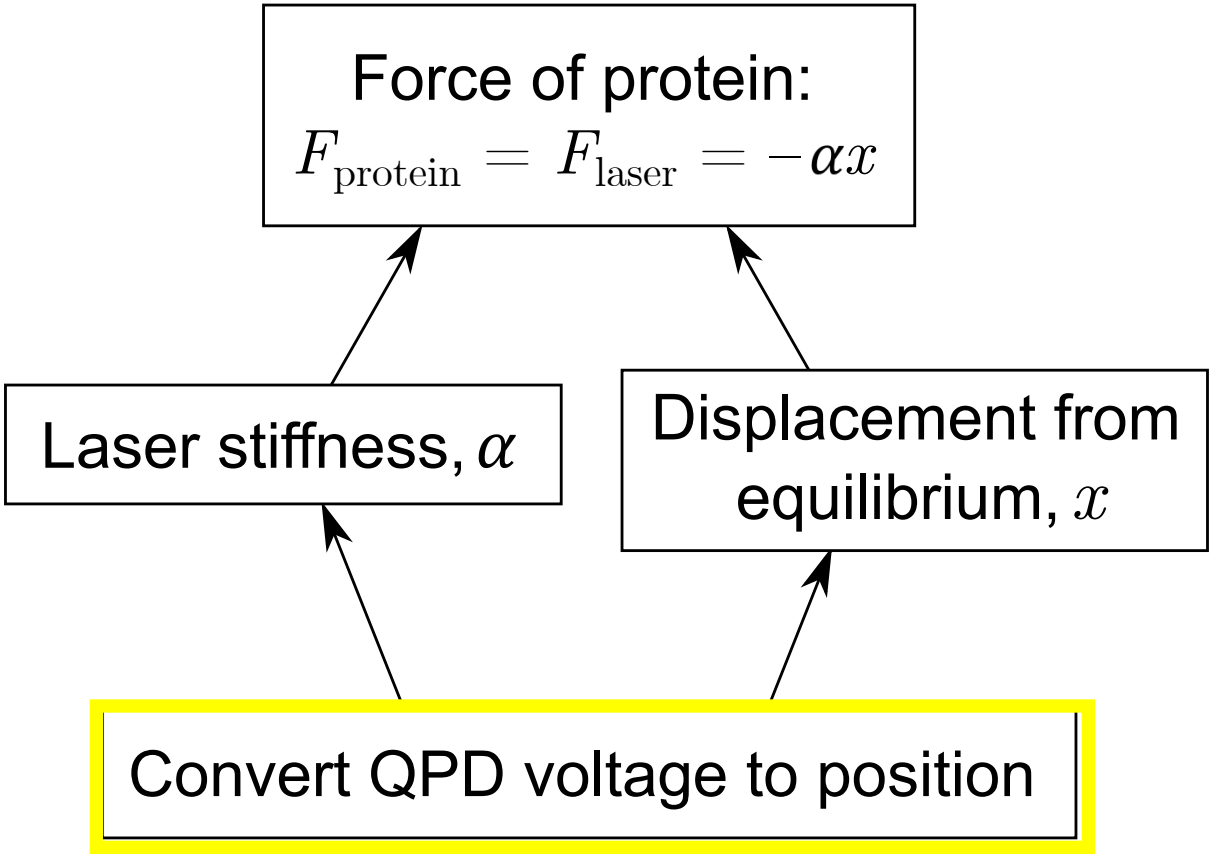
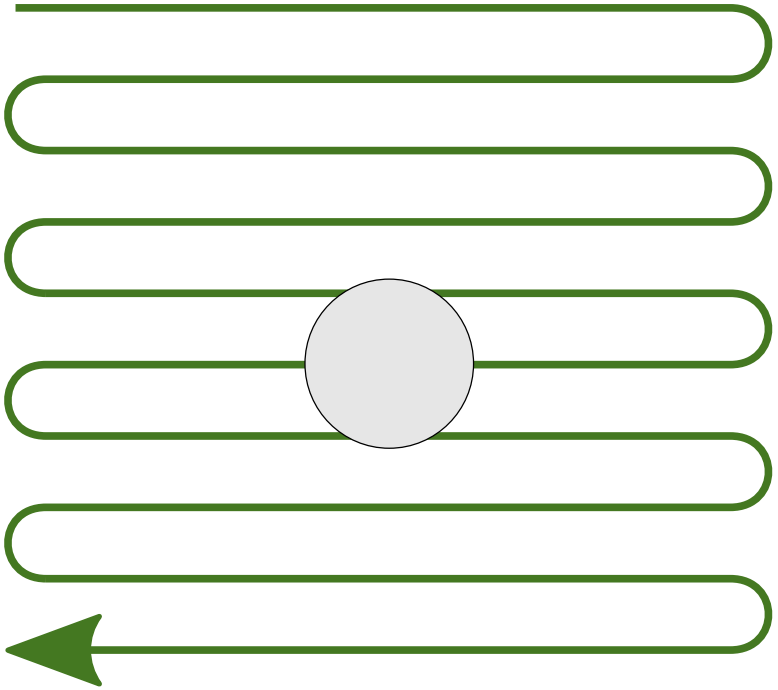
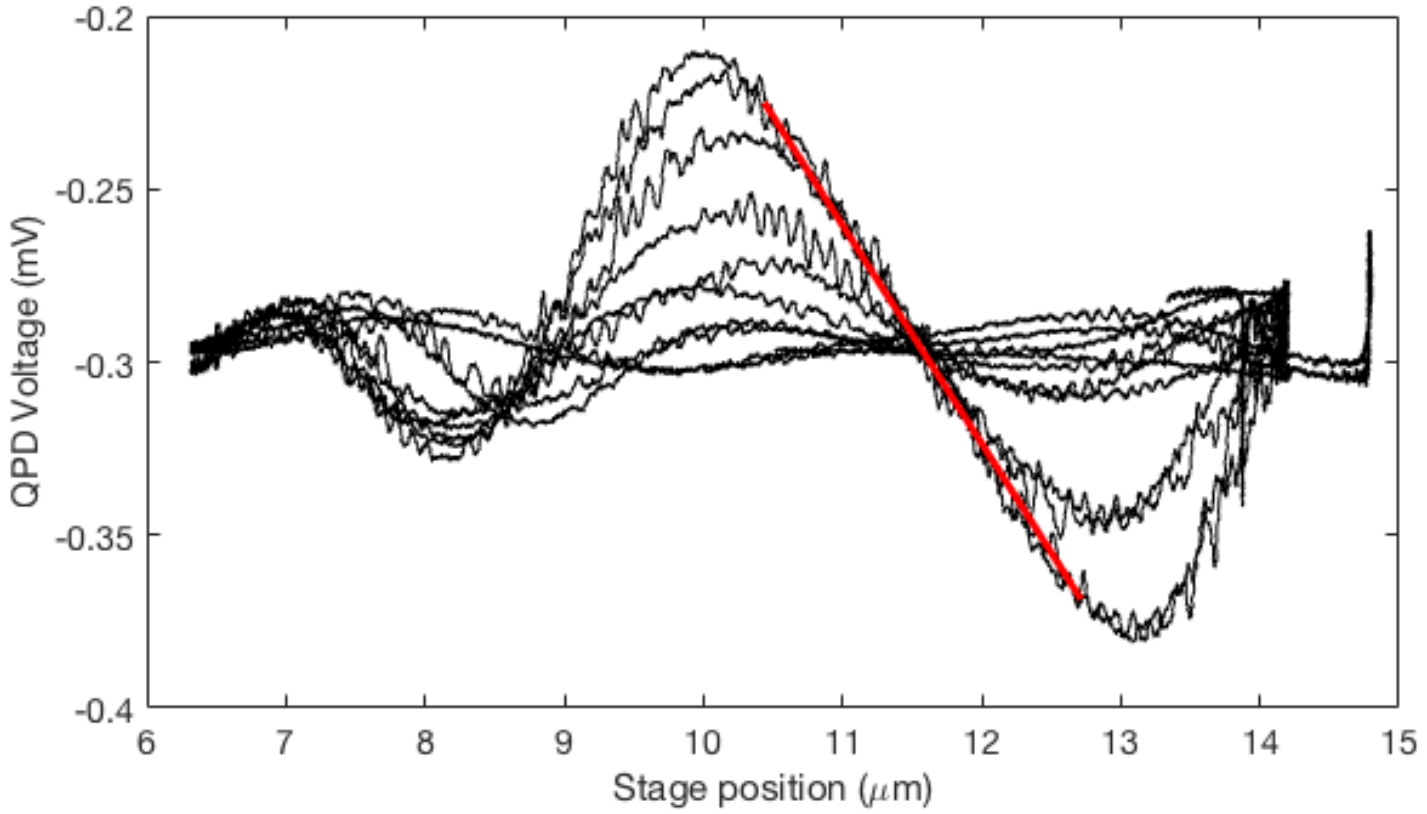


Calibrate QPD voltage to displacement by scanning over a fixed silica bead



Slope of steepest curve gives conversion from QPD voltage to strain gauge voltage

250 mA Laser Current along the X-Axis



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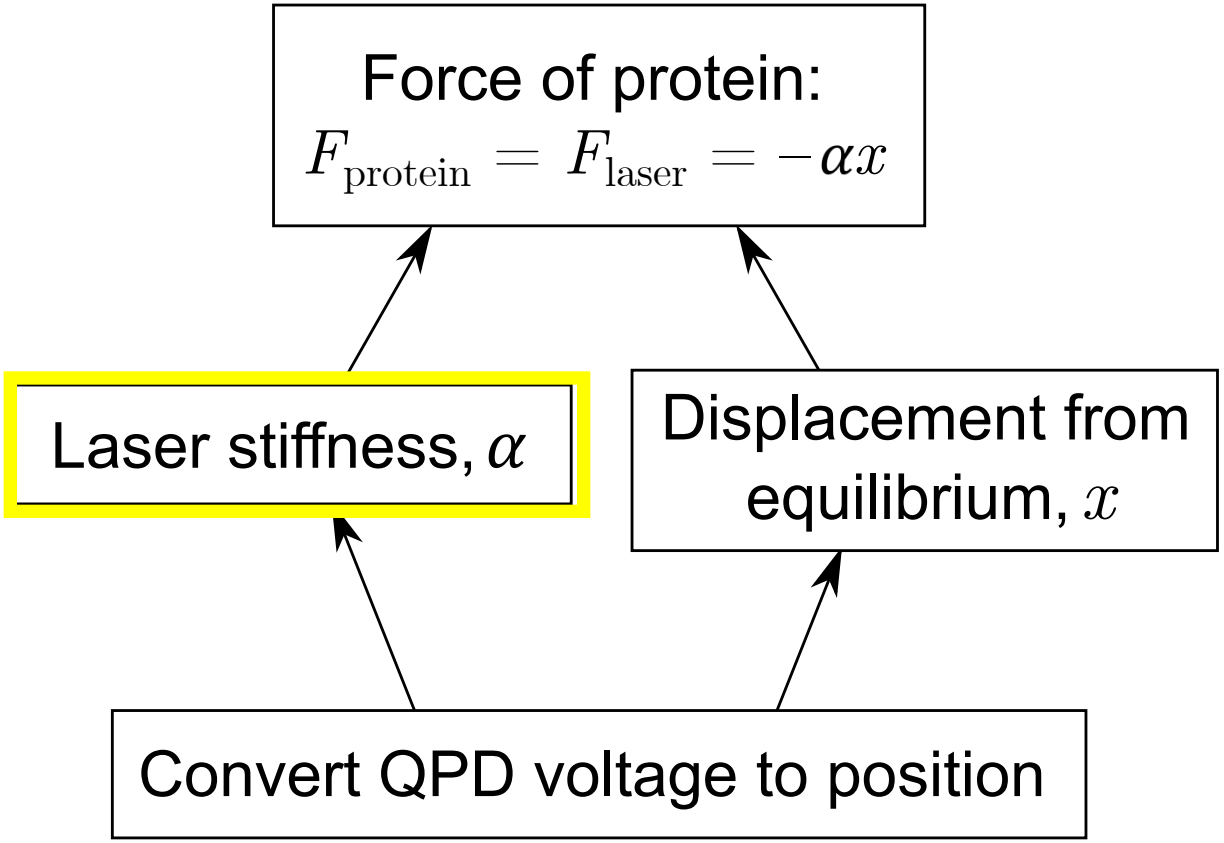


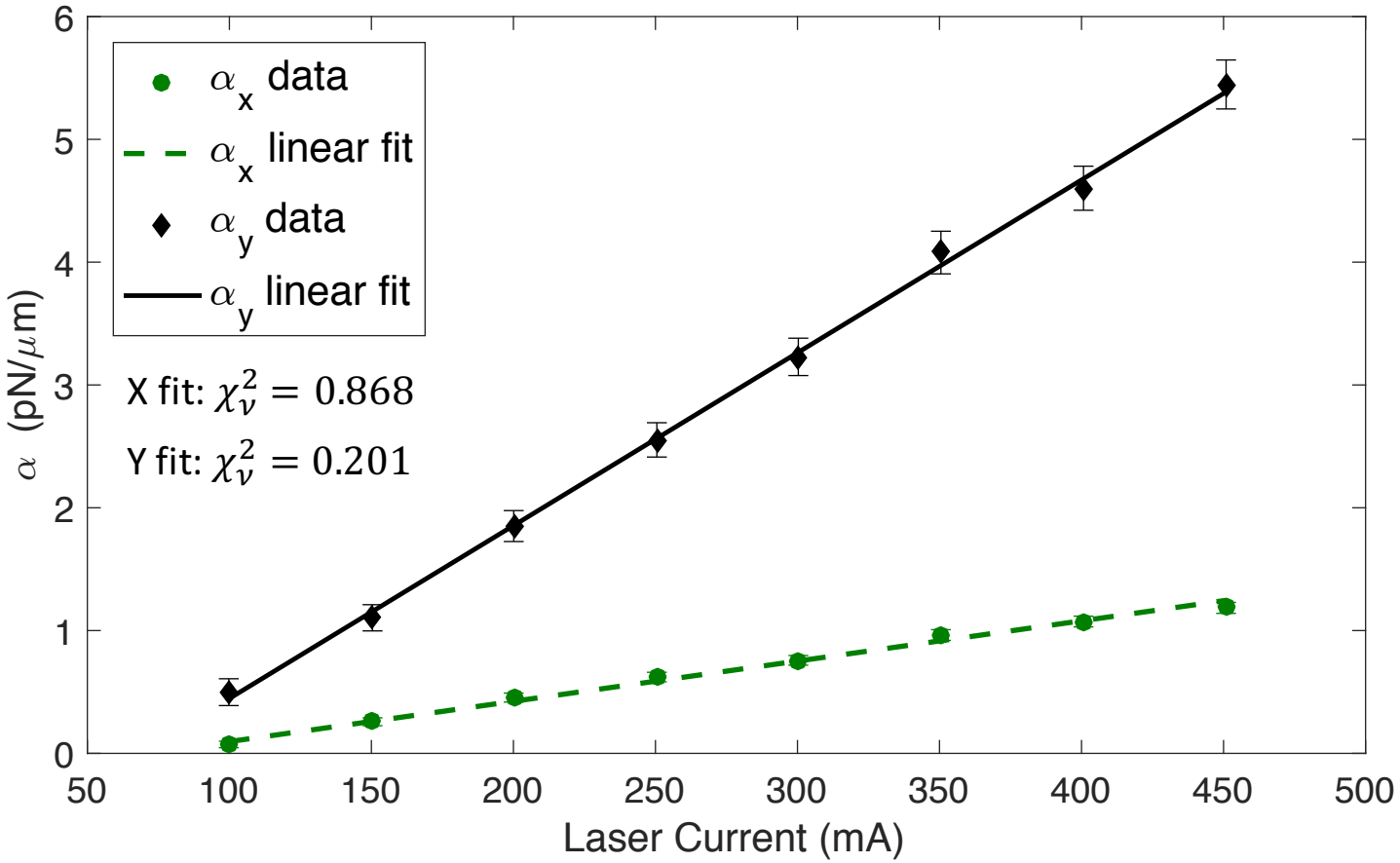
Use physics of Brownian motion to calculate trap stiffness

Equipartition theorem: a system at equilibrium has an energy of $\frac{1}{2}k_BT$ for each degree of freedom.

$$\frac{1}{2}k_BT = \frac{1}{2}\alpha_x\langle x^2\rangle$$

$$\frac{1}{2}k_BT = \frac{1}{2}\alpha_y\langle y^2\rangle$$

Equipartition theorem method arrives at trap stiffness increasing linearly with laser current

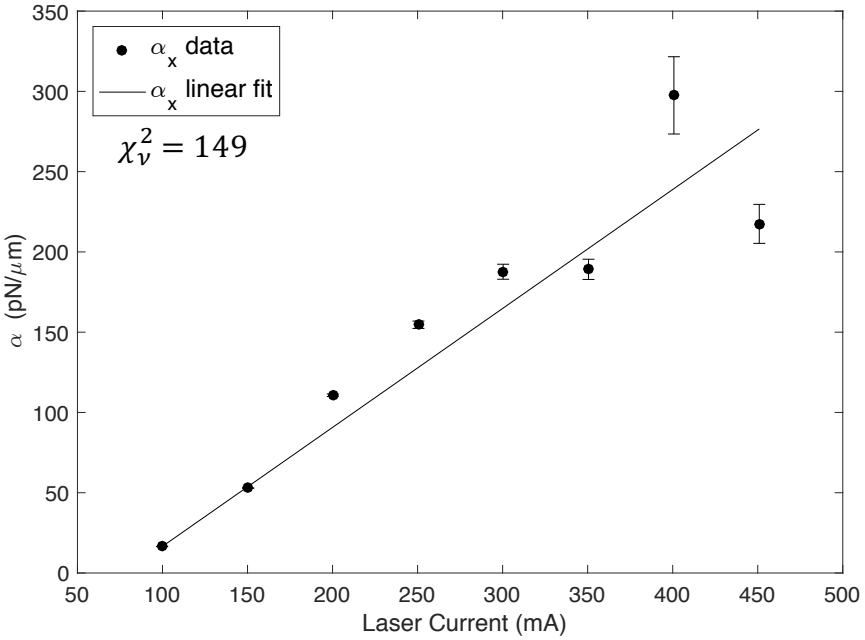


Alternate method: the PSD function tells us about Brownian motion in more detail

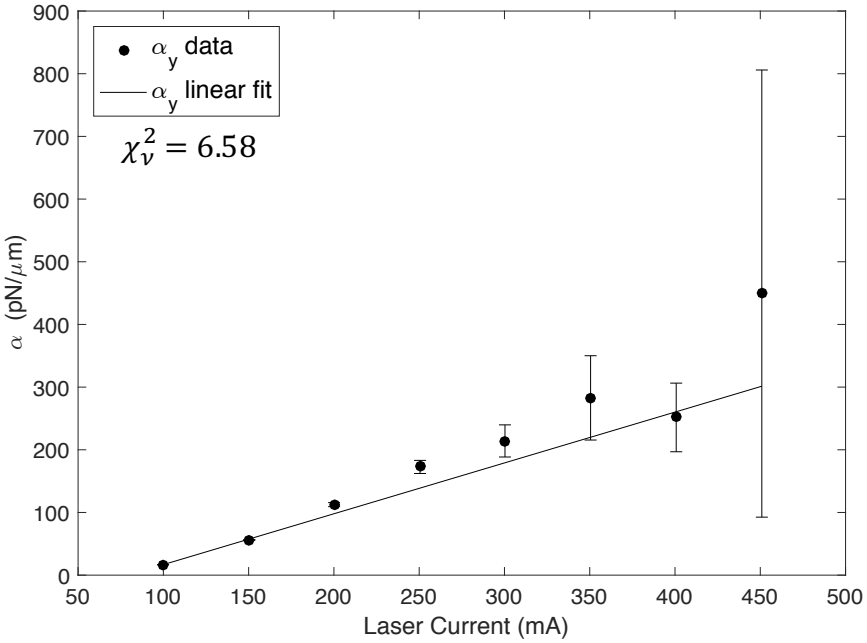
$$S_{xx}(f) = \frac{k_B T}{\pi^2 \beta \left(f^2 + \left(\frac{\alpha}{2\pi\beta} \right)^2 \right)}$$

PSD function stiffness values were not linear and much bigger

Trap Stiffness along X-Axis



Trap Stiffness along Y-Axis



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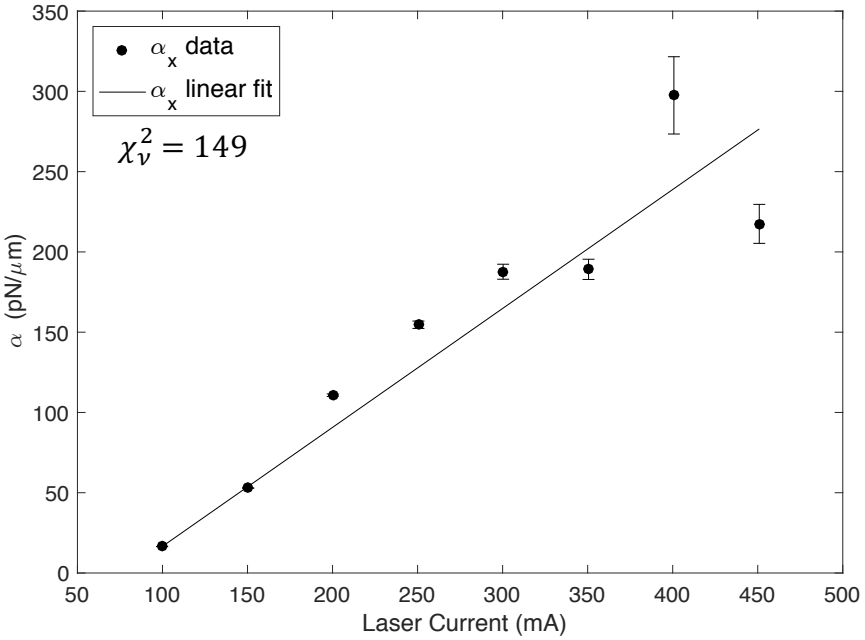

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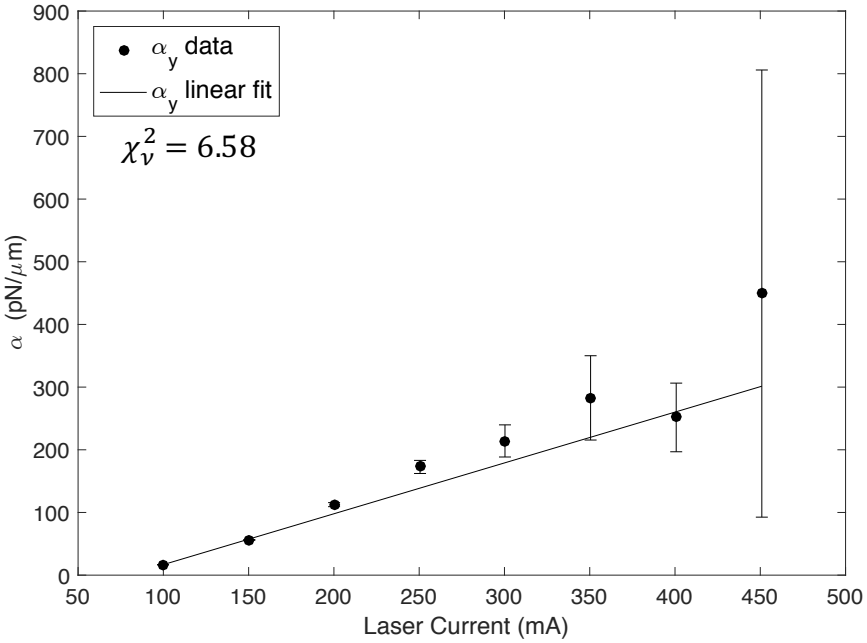
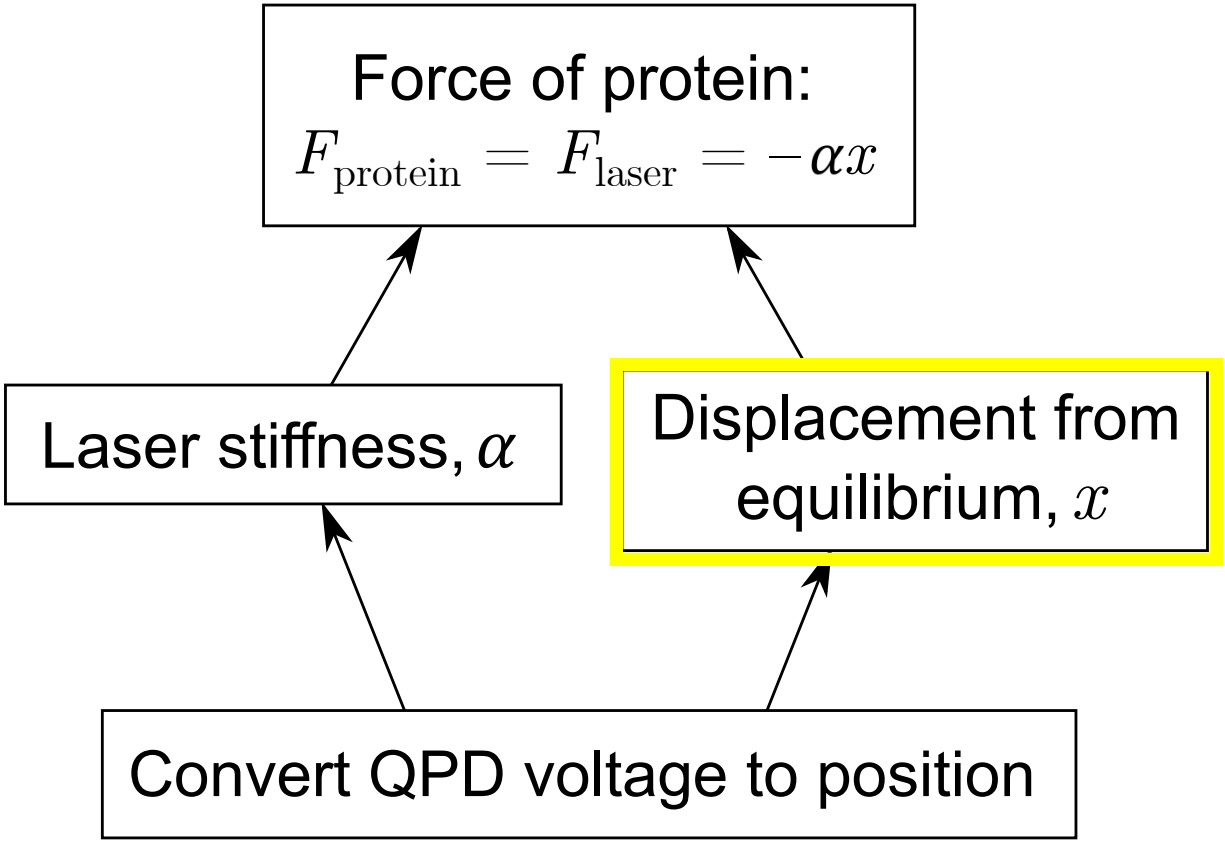


Experimental value: $k_B = (4.9 \pm 1.2) \times 10^{-6}$ pN $\cdot$ μm/K

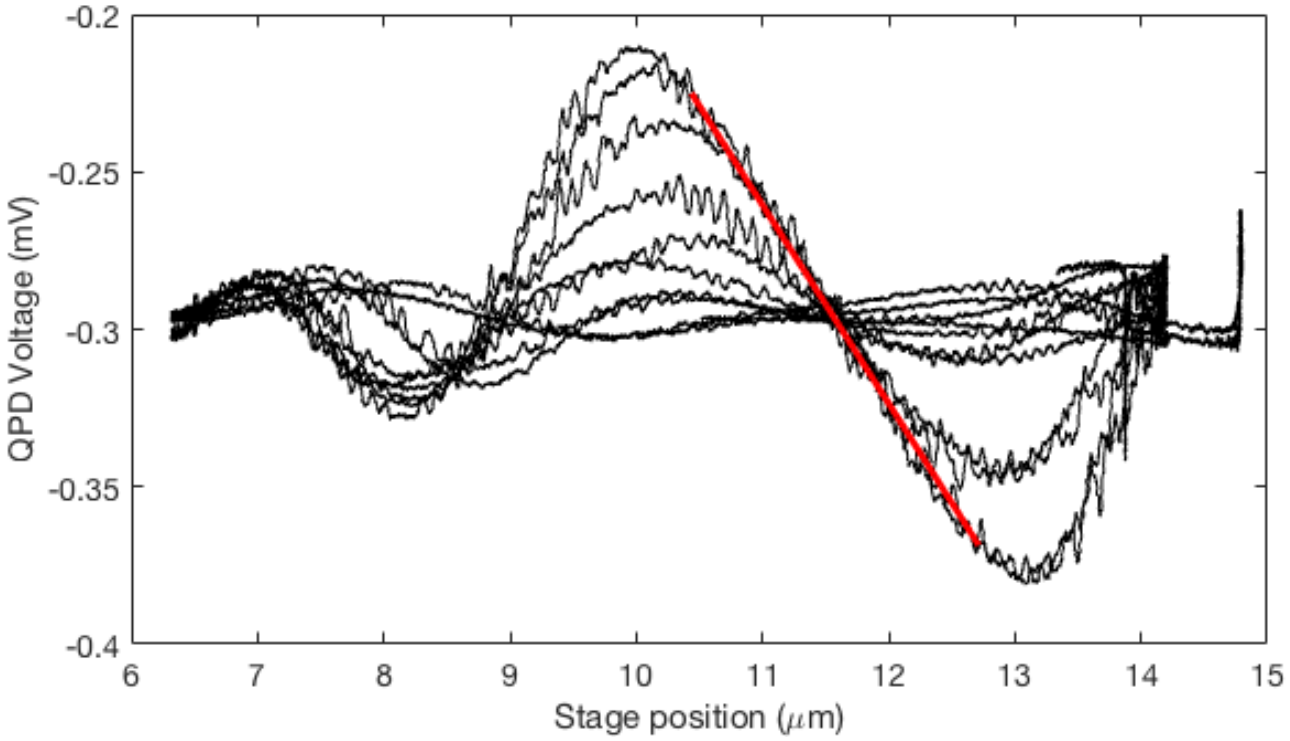
Accepted value: $k_B = (1.38) \times 10^{-5}$ pN $\cdot$ μm/K

Steps of analysis to arrive at myosin protein force



Maximum force applied by the laser is trap stiffness multiplied by the width of the trap

$$F = -\alpha x$$



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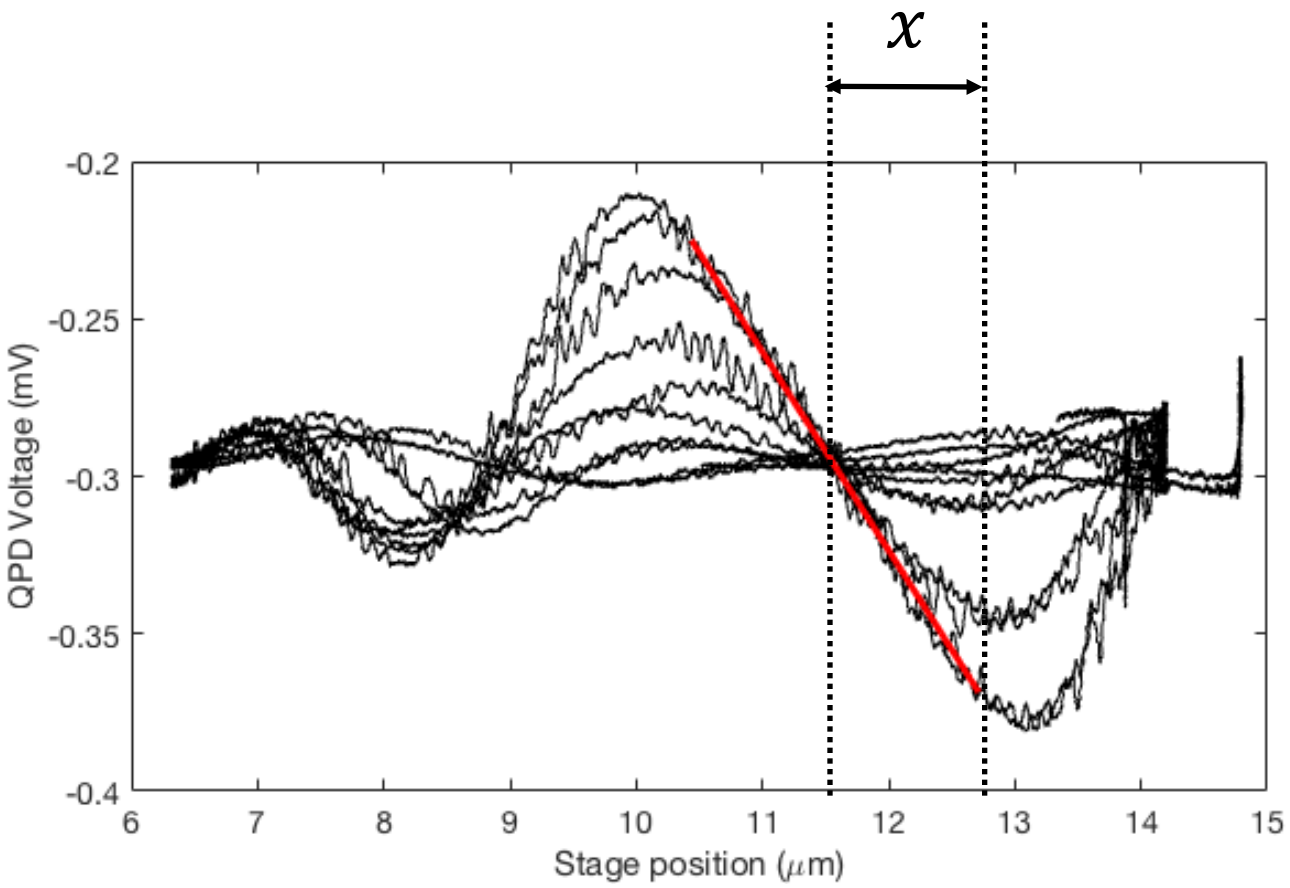

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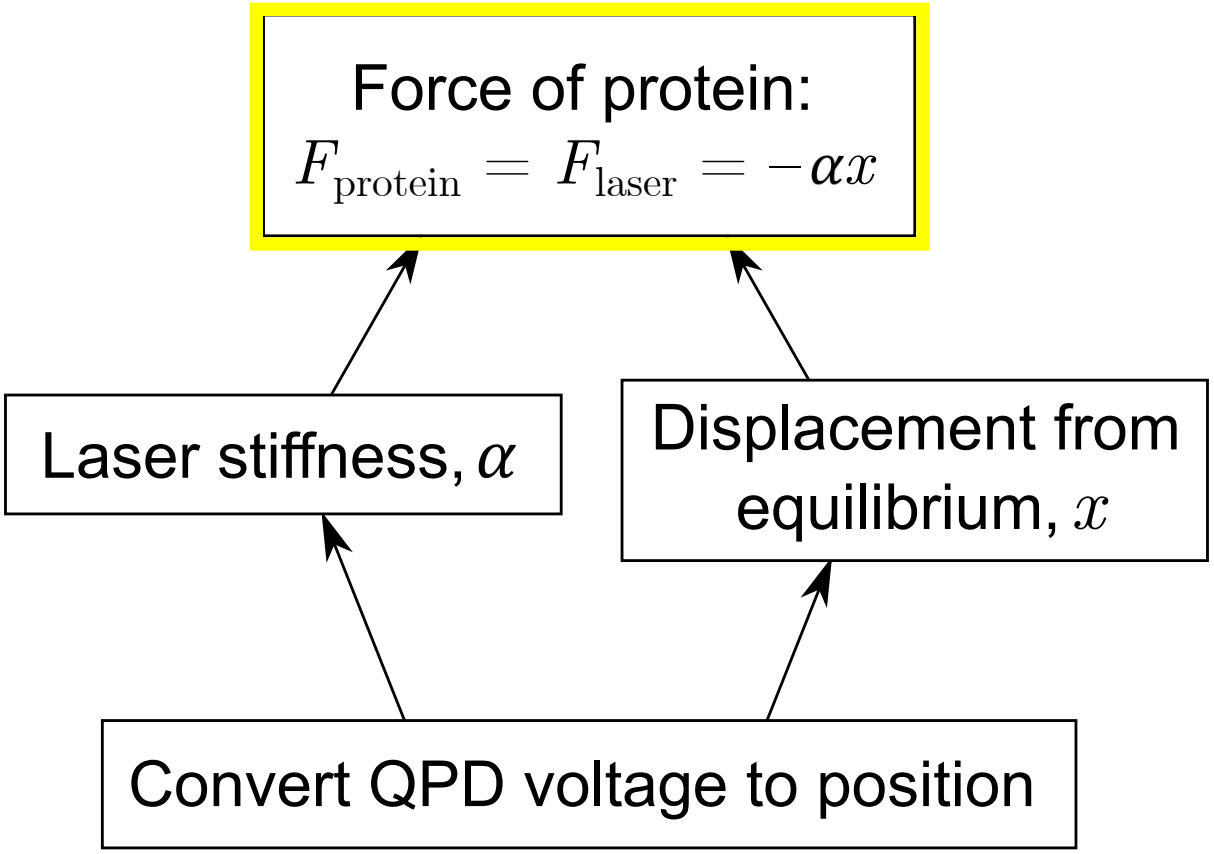
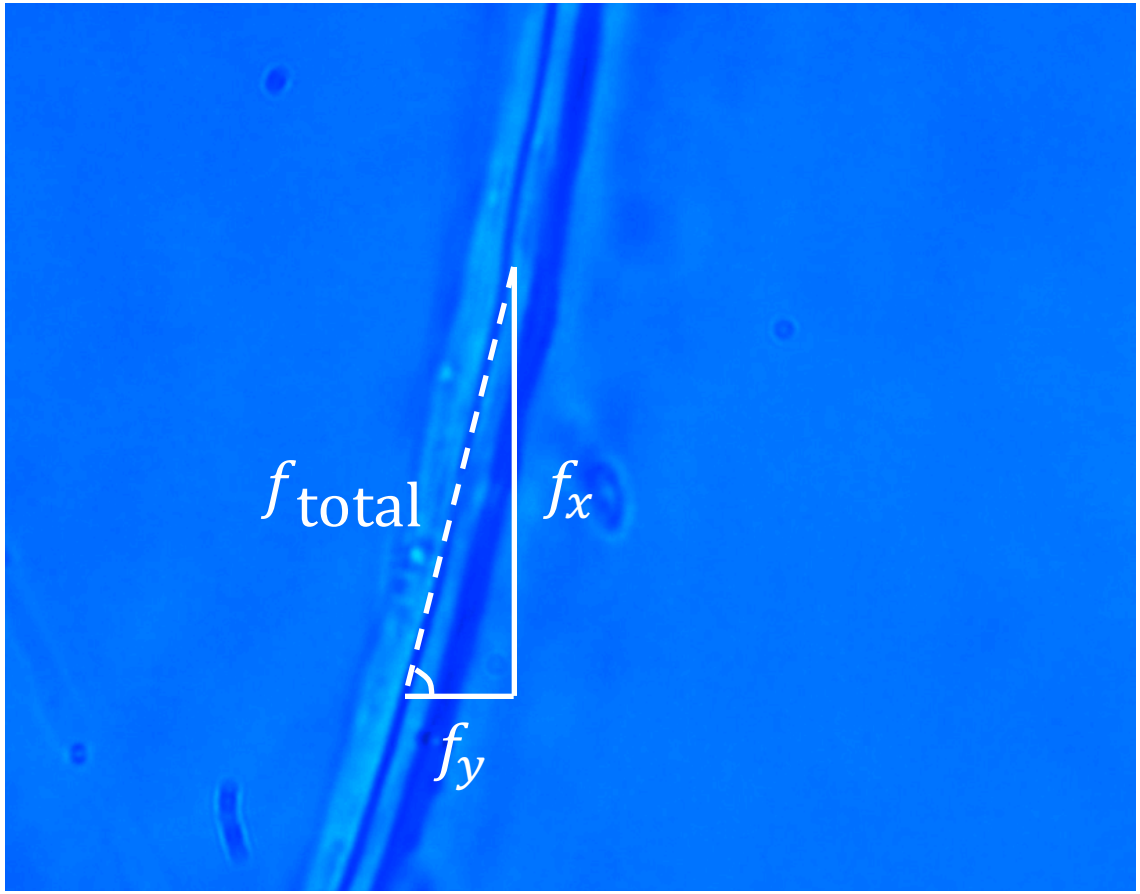


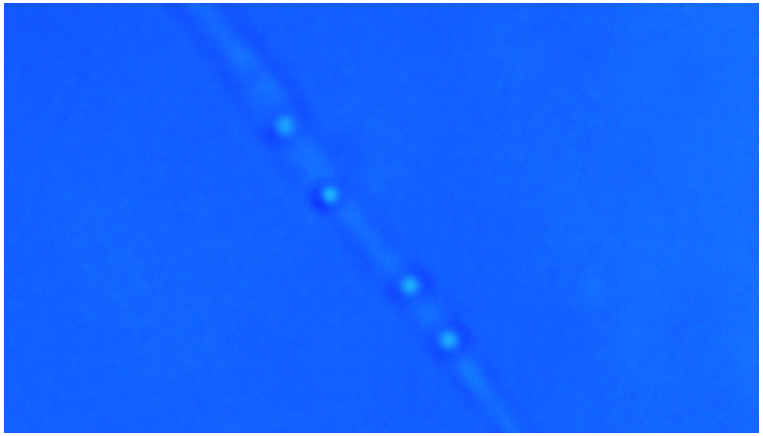
We treated the x and y axes separately and calculated the sum of force vectors



Systematic error for the myosin force: mostly from differences between environments

Source of Error	Percent of F_{protein}
Uncertainty in trap width	**5.6 %**
Difference in α between water and cytoplasm	3.4 %
Uncertainty in angle of vesicle motion	1.2 %
Uncertainty in laser cut-off current	1.1 %
Uncertainty in temperature measurement	0.4 %

Force of myosin protein matches previously measured values



$$\text{Force} = 5.52 \pm 0.57_{\text{stat}} \pm 0.63_{\text{sys}} \text{ pN}$$

Accepted values of myosin force are between 1 and 5 pN

(Source: Lodish H, *Molecular Cell Biology. 4th edition*)

Acknowledgements

- Carissa Skye
- *Junior Lab staff:*
  - Prof. Joseph Formaggio
  - Spencer Tomarken
  - Dr. Sean Robinson
  - Kay Lowden
  - Amelia Smith