Correcting the stiffness calibration for optical traps

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What is an optical trap?

- Infrared laser creates a potential energy well around a bead
- Applies a force on the bead proportional to displacement
- Allows us to use piconewton scale forces on membranes to probe their physical properties

$$F = -kx$$

- Displacement (x) is calculated by a tracking algorithm on images taken via camera
- Experimental calibration is needed to determine trap stiffness (k) for accurate force (F) measurements

The equipartition calibration method

- Observe the trapped bead over time without additional external force and calculate the variance in position
- Brownian motion describes the expected position distribution of the bead over time

$$k = \frac{k_B T}{V_{bead}}$$

- The stiffness depends on k_B the Boltzmann constant, T the temperature, and V_{bead} the variance of bead position calculated by the tracking algorithm
- Other methods include the drag force approach – observing the motion of the trapped bead in moving fluid
  - Produces more accurate results but not a viable method in certain trapping setups, whereas the equipartition method can always be used

The problem

- Camera readout noise leads to imprecision in the bead tracking algorithm, reducing the accuracy of variance calculations and thus the overall equipartition calibration method.

A model for calibration correction

- Say the errors in bead tracking from camera noise have variance V_{noise} and assume these errors are **independent** of the bead location
- Then, the total variance of the bead position V_{total} as calculated by tracking follows the relationship

$$V_{total} = V_{noise} + V_{bead}$$

and the stiffness calculated from the calibration method follows

$$k = \frac{k_B T}{V_{bead}} = \frac{k_B T}{V_{total} - V_{noise}}$$

- No direct way to calculate V_{noise} from images
- We can **simulate** bead motion to estimate V_{noise} and obtain a more accurate calibrated stiffness

Creating a realistic simulation

- Take experimental videos of optically trapped beads and recreate the images while specifying the exact location
- Simulate the position of the bead over time to generate a movie

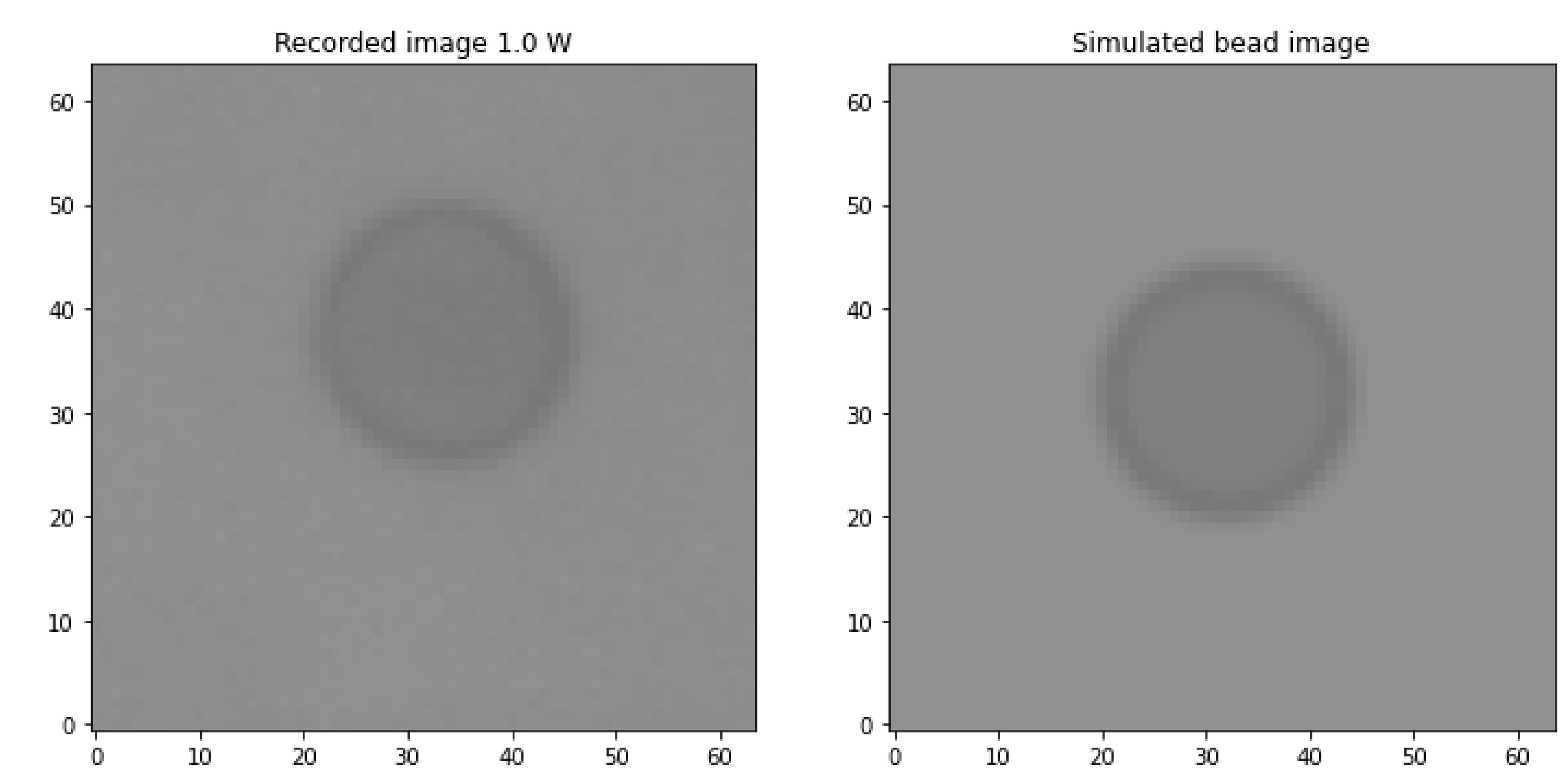

Figure 1. Comparison between an experimentally captured image of a bead trapped by a 1.0 watt laser (left) with a simulated image (right) used for testing. The simulated bead was designed to appear similar. Simulated beads have known locations, providing a "ground-truth" to compare tracking results against.

Fitting the model

- Apply the tracking algorithm to a simulated movie and calculate V_{noise} to correct the estimate for trap stiffness
- Test this correction on other simulated videos with various trap stiffnesses – new estimate looks much better

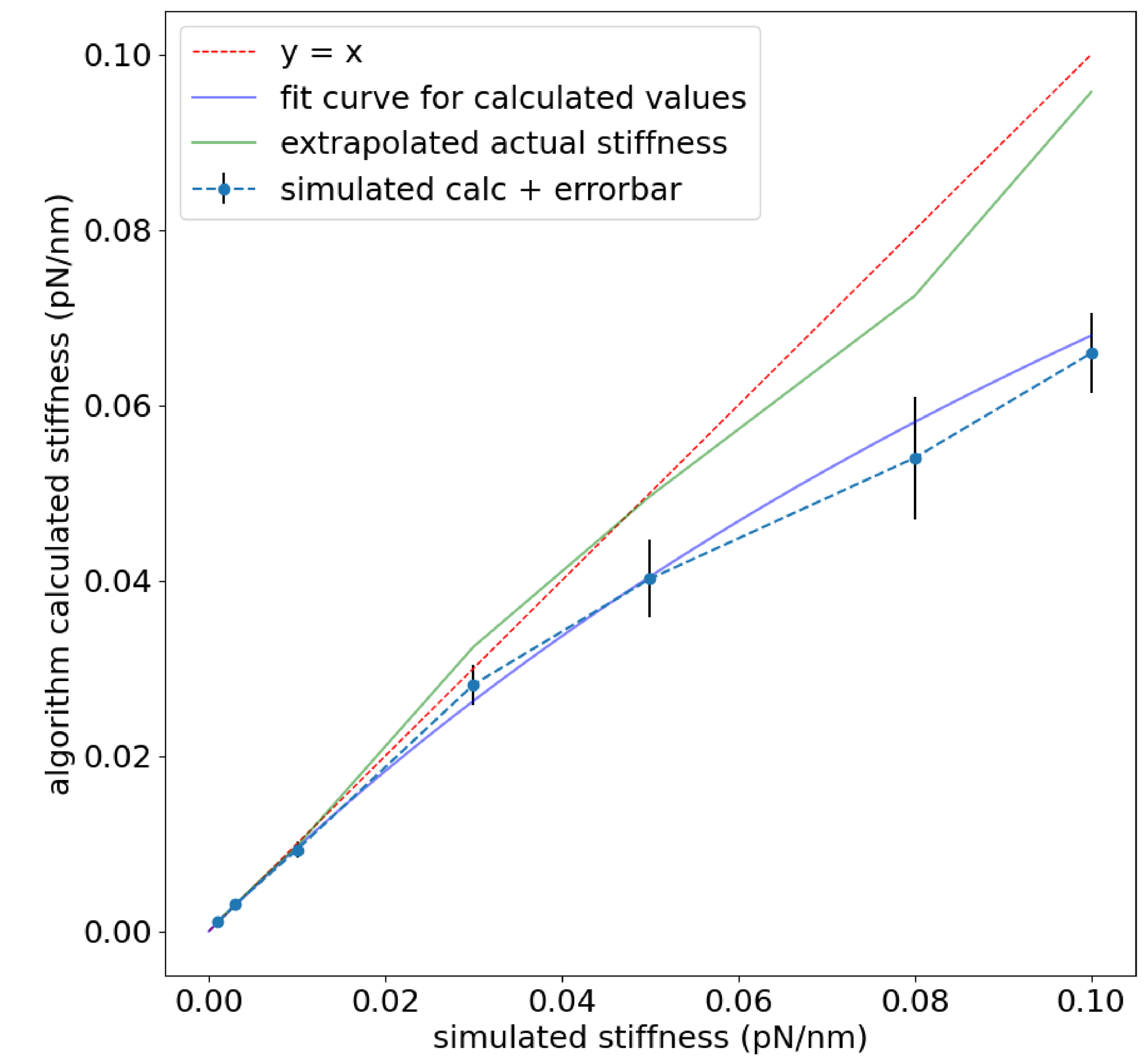

Figure 2. The results of the correction method applied onto simulated data. The actual stiffness of the trap in the simulation is given on the x-axis, and the calculated stiffness from bead tracking is given on the y-axis. A stiffer trap leads to less accurate tracking, but the proposed correction (with V_{noise} = 2.7e-4) helps produce more an accurate calibration as shown on the right hand side of this plot.

Future work

- Experimentally confirm the simulated results by comparing results with the drag-force method
- Use the optical trap - run experiments to calculate how the insertion and extraction of lipids or proteins physically affects lipid bilayers

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