Learning from Cells to Create Transportation Infrastructure at the Micron Scale

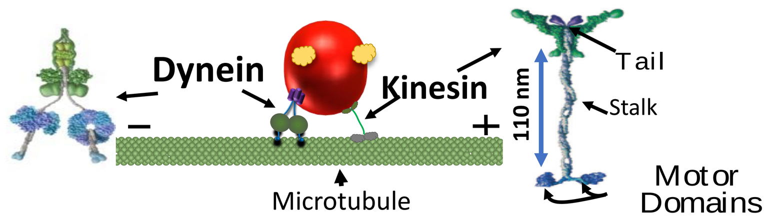
Murti Salapaka[1], Tryphon Georgiou[2] and Thomas Hays[3]

[1]Nano Dynamics Systems Lab, Department of Electrical Engineering, University of Minnesota, Twin Cities, USA
[2]Department of Mechanical and Aerospace Engineering, University of California, Irvine, USA
[3]Genetics, Cell Biology and Development, College of Biological Sciences, University of Minnesota, Twin Cities, USA

UNIVERSITY OF MINNESOTA
Driven to Discover℠

INTRODUCTION

INTRACELLULAR TRANSPORT

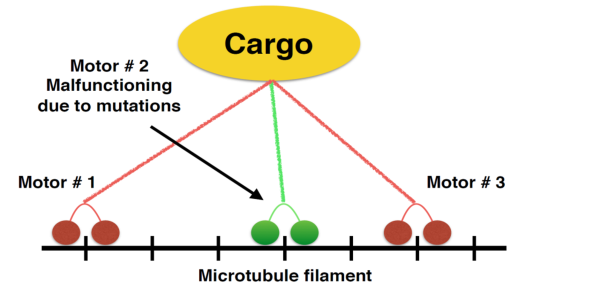


Transport of cargo within cells – Key Components

- **Cargo** : Organelles, Vesicles
- **Carriers** : Molecular Motor Proteins Kinesin, Dynein, Myosin
- **Fuel** : Adinosine TriPhosphate (ATP)
- **Tracks** : Microtubule (Directed polymer lattices)

Deciphering intracellular transport mechanism may aid in creating transportation infrastructure at micron scale

TRANSPORT BY ENSEMBLES OF MOLECULAR MOTORS

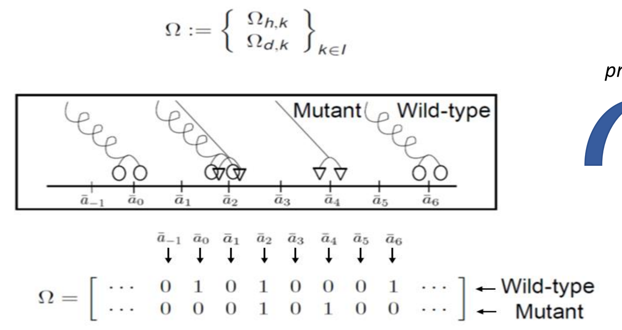


- In vivo, several motors work in teams to transport common cargo
  - Improves run-length
  - Provides ensemble robustness
  - Can carry larger loads
- Multiple motors of different types also form teams
  - E.g. Kinesin and Dynein together enable bidirectional transport
- Motors can be affected by ***disease causing mutations***

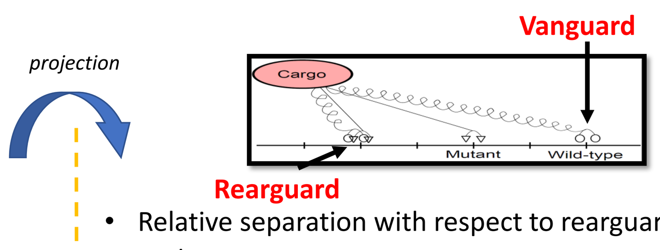
Relative number and type of motors affects cargo transportation characteristics

PREDICTIVE COMPUTATION ENGINE

GROUP BEHAVIOR CAN BE PREDICTED USING INDIVIDUAL MOTOR BEHAVIOR



- Infinite Dimensional Model : Master Equation
  - $\frac{\delta}{\delta t} P_\Omega(\Omega, t) = -P_\Omega(\Omega, t) \sum_{\Omega' \in A} \nu_\Omega(\Omega', \Omega) + \sum_{\Omega' \in A} \nu_\Omega(\Omega, \Omega') P_\Omega(\Omega', t)$
- Transition rates can be determined from single motor model for step, detach, attach
  - $P_\Omega(\Omega', t + \Delta t | \Omega, t) = \nu_\Omega(\Omega', \Omega) \Delta t$



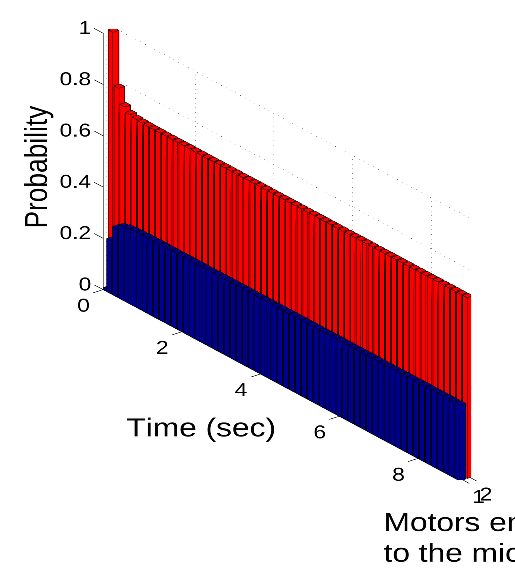
- Relative separation with respect to rearguard motor
- Projection Operator Υ maps Ω to $\Upsilon(\Omega)$
- Finite Dimensional Model : Master Equation
- $\frac{\delta}{\delta t} P_\vartheta(\vartheta, t) = -P_\vartheta(\vartheta, t) \sum_{\vartheta' \in H} \nu_\vartheta(\vartheta', \vartheta) + \sum_{\vartheta' \in H} \nu_\vartheta(\vartheta, \vartheta') P_\vartheta(\vartheta', t)$
- Solving,

$$\frac{d}{dt} P(t) = \Gamma P(t),$$
$$P(t) = e^{\Gamma(t - t_0)} P(t_0)$$

where $\Gamma \in \mathcal{R}^{N \times N}$ is completely defined by transition rates $\nu_\vartheta(\vartheta', \vartheta)$

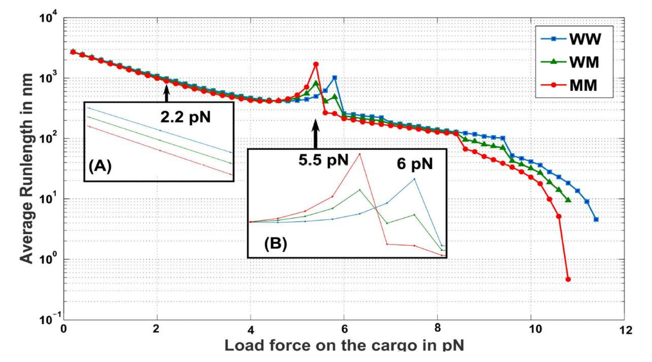
INSIGHTS GAINED FROM SIMULATION ENGINE

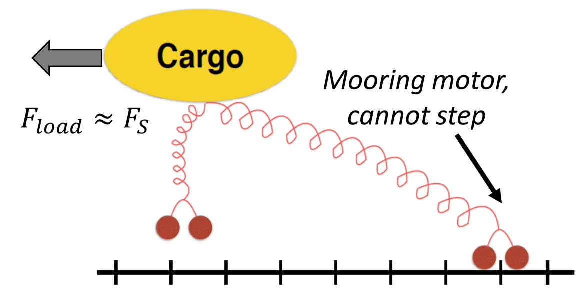
MOTOR CONFIGURATION REACHES STEADY STATE



Ensemble of two motors, under constant load force

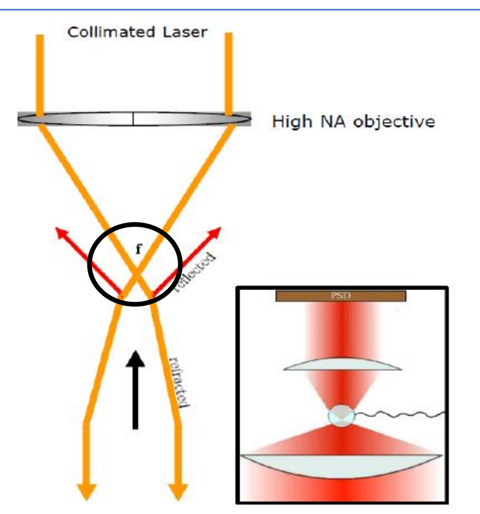
AVERAGE RUN-LENGTH AND MOORING





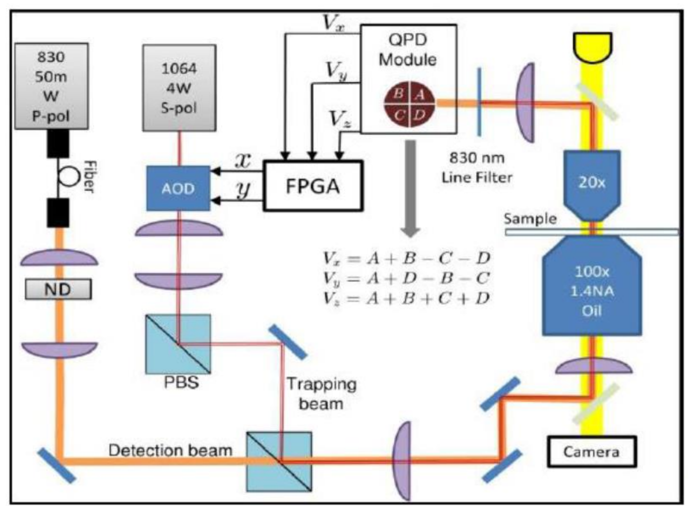
EXPERIMENTAL TESTBED

PRINCIPLES OF OPTICAL TRAPPING



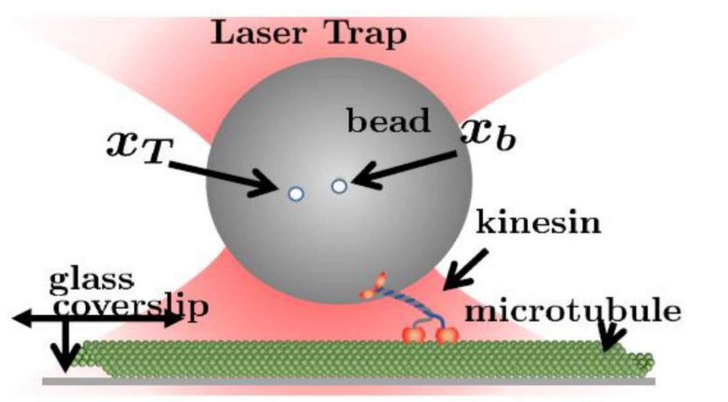
- Collimated laser passed through high NA objective
- Creates stable trap near focus
- Trapping of sub-micron particle possible
- Another laser passed through bead and collected on a photo-sensitive detector (PSD)
- The laser spot on the PSD gives a measurement of bead position

OPTICAL TWEEZERS : EXPERIMENTAL SETUP



- Laser steered using ‘Acousto-Optical Deflector’
- Trapped bead can be moved very fast & precisely
- High speed FPGA acquires and processes data from photodiode
- FPGA generates actuation commands for AOD, according to the control law

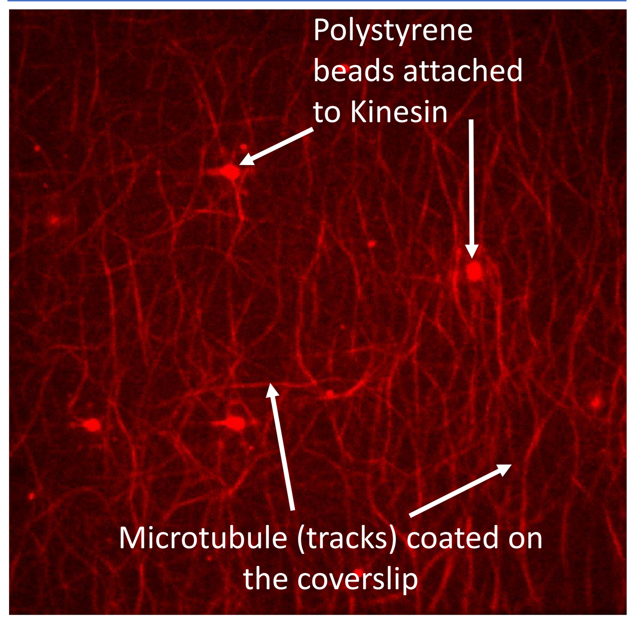
TRACKING BEADS ATTACHED TO LIVE PROTEINS



Schematic of an optically probed Kinesin

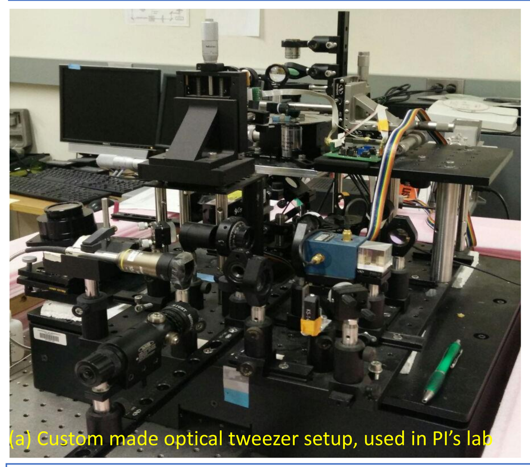
- Live proteins attached to polystyrene beads (Cargo)
- Surface coated with microtubule filaments (Tracks)
- Using laser, bead is trapped and brought near the surface, enabling protein to ‘walk’
- Motion of bead detected on the PSD, through which motion of kinesin protein is inferred
- Motion of kinesin detected by labeling kinesin with fluorescent Quantum Dots and using Total Internal Reflection Fluorescence (TIRF) Microscopy

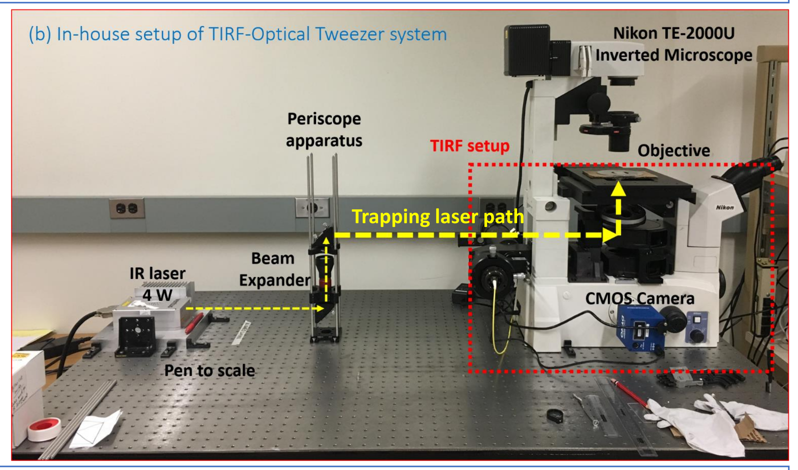
BIO-CHEMISTRY



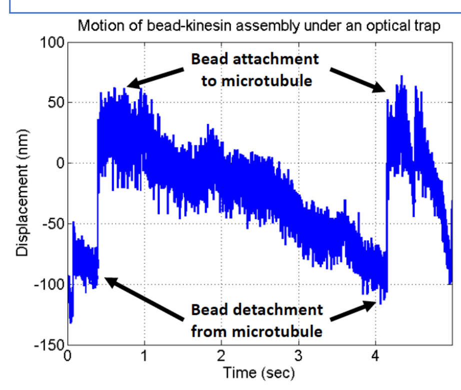
In-vitro Transportation infrastructure using kinesin motors, polystyrene beads and microtubule. Biochemistry realized in PI’s lab.

INSTRUMENTATION



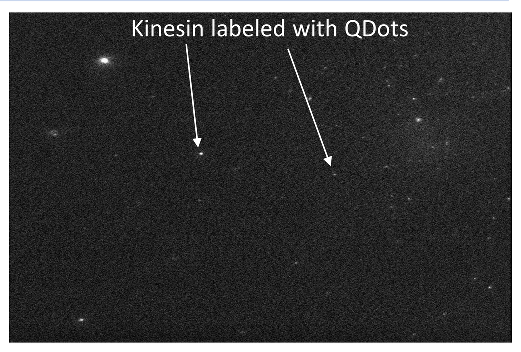


DATA



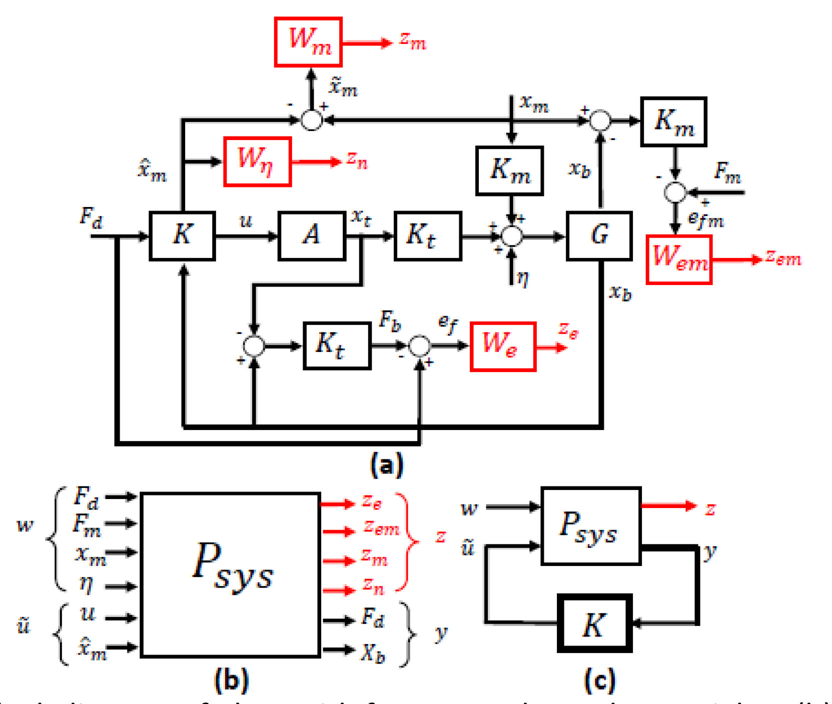
(Left) Typical motion of cargo (i.e. beads) being carried by motor protein Kinesin, captured using optical tweezer setup

(Right) TIRF image of quantum dot labeled kinesin

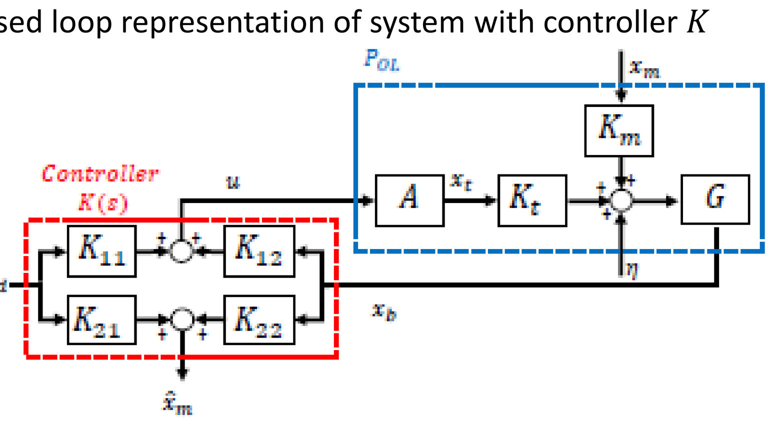


SUB-PICONEWTON LEVEL FORCE REGULATION

NOVEL CONTROLLER DESIGN USING MODERN CONTROL FRAMEWORK

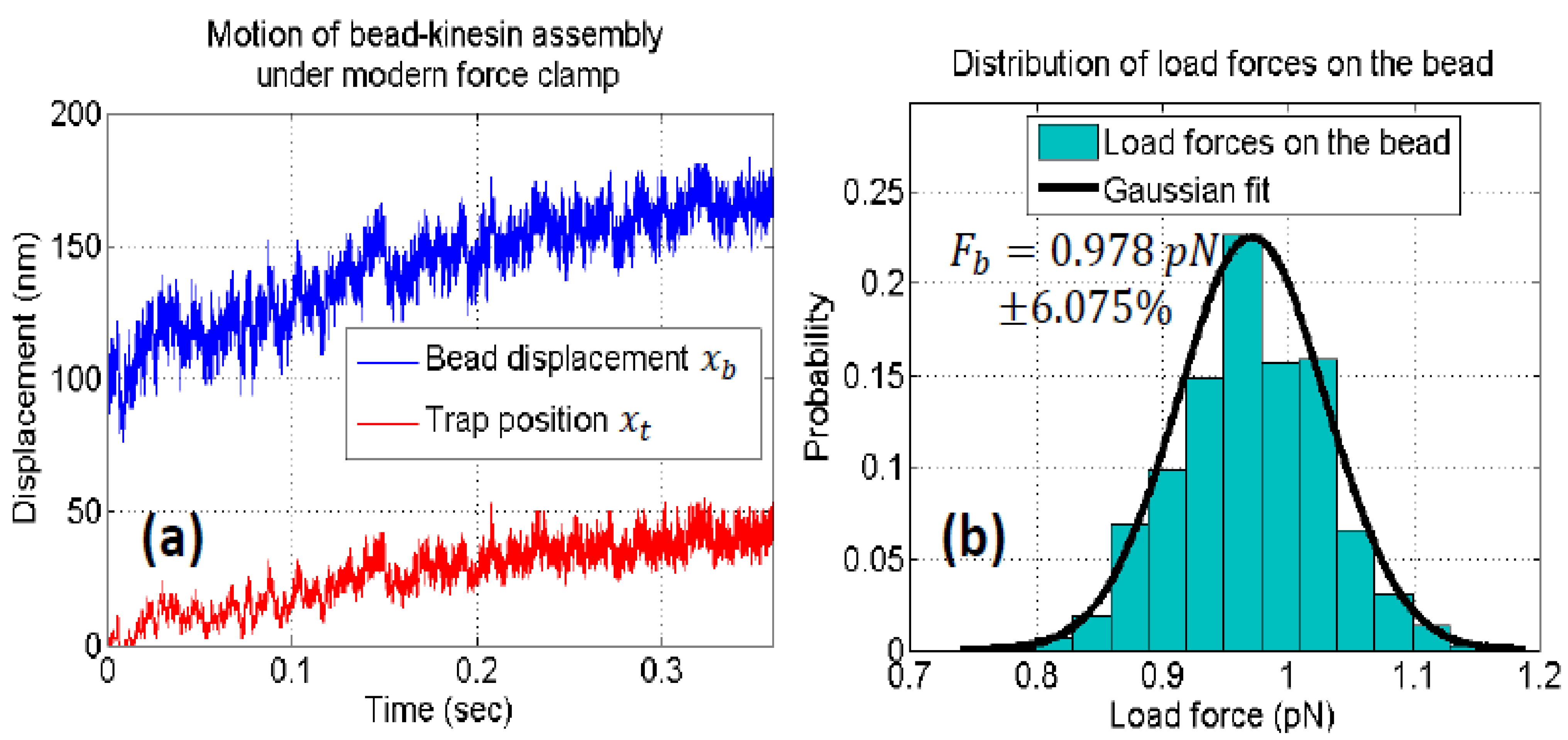


(a) Block diagram of plant with frequency dependent weights. (b) Arrangement of the system in terms of inputs $[w; \tilde{u}]$ and outputs $[z; y]$. (c) Closed loop representation of system with controller K



Block diagram of closed loop system with open loop plant P_{OL} and controller $K = \begin{bmatrix} K_{11} & K_{12} \\ K_{21} & K_{22} \end{bmatrix}$

TESTING ON LIVE MOTOR PROTEIN



(a) Modern force clamp in action (b) Distribution of load force maintained on the bead

ORDER OF MAGNITUDE IMPROVEMENT IN FORCE REGULATION COMPARED TO PREVIOUS STATE-OF-THE-ART

KEY OUTCOMES AND WAY FORWARD

ACHIEVEMENTS

1. Development of experimental platform to simultaneously probe and visualize molecular motors
2. Order of magnitude improvement in sub-pN level force regulation
   - Previous: 17% error
   - Our method: ~ 6% error
3. 10x improvement in tracking bandwidth
4. Predictive power of simulation engine demonstrated for wild type, mutant type and for ensembles of bidirectional motors

FUTURE DIRECTIONS

1. Experimental demonstration of simulated outcomes
2. Optimization of bio-chemistry to simultaneously attach beads and quantum dots to molecular motors
3. Development of biochemistry to control exact number of motors on cargo using DNA scaffolds

This will enable precise control over velocities and transported distance

RECENT PUBLICATIONS

1. Shreyas Bhaban, Donatello Materassi, Mingang Li, Thomas Hays, Murti Salapaka. Study of Emergent Transport Properties of Molecular Motor Ensemble. PLoS Computational Biology, 2016
2. Saurav Talukdar, Shreyas Bhaban, Murti Salapaka. Steady State Dynamics of Molecular Motor Ensemble Reveals Load Dependent Cooperativity. 55th IEEE Conference on Decision and Control, 2016
3. Shreyas Bhaban, Saurav Talukdar, Mingang Li, Thomas Hays, Peter Seiler, and Murti Salapaka. Single Molecule Studies Enabled by Model Based Controller Design. IEEE/ASME Transactions on Mechatronics, 2018
4. Rachit Shrivastava, Shreyas Bhabhan, Sivaraman Rajaganapathy, Thomas Hays, Murti Salapaka. Transport Properties of Molecular Motor Ensemble with Bi-Directional Motors: A Computational Approach. American Society of Cell Biology Annual Meeting, Philadelphia, PA, December 2017

For further queries and discussions:

Visit Lab website: http://salapakalab.ece.umn.edu/
Or contact:
Rachit Shrivastava, Department of Electrical and Computer Engineering, University of Minnesota, Twin-cities, shriv058@umn.edu