Learning from Cells to Create Transportation Infrastructure at the Micron Scale

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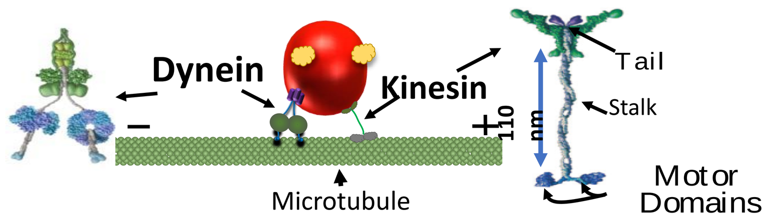
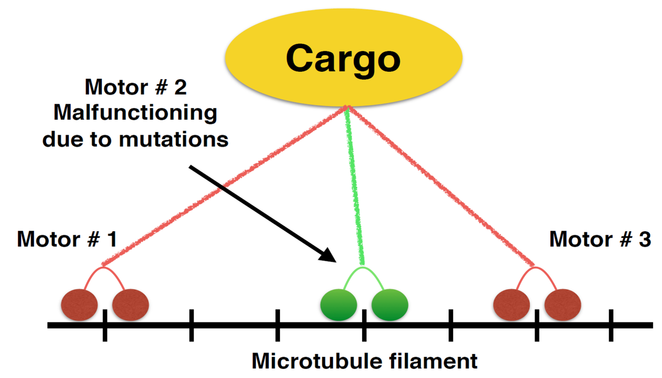
INTRODUCTION

INTRACELLULAR TRANSPORT



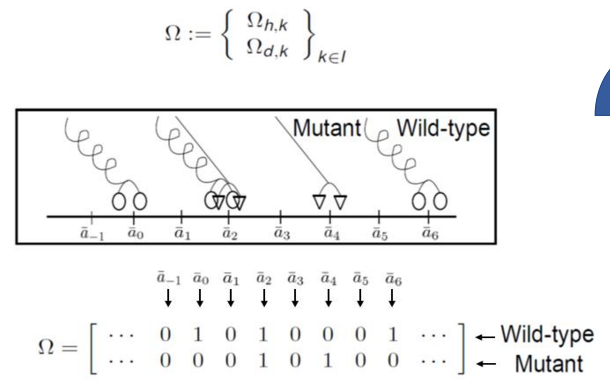
- Transport of cargo within cells
  - Cargo : Organelles, Vesicles
  - Carriers : Molecular Motor Proteins Kinesin, Dynein, Myosin
- Molecular Motors convert chemical energy to mechanical work
  - E.g. Kinesin hydrolyzes 1 ATP molecule per step
  - $M + ATP \underset{k_{OFF}}{\overset{k_{ON}}{\rightleftharpoons}} M\,ATP \overset{k_{CAT}}{\rightarrow} M + ADP + p_i + \Delta E$
- Tracks : Microtubules
  - Directed polymer lattices
- Disruptions of molecular motor functionality is linked to several diseases
  - Neurodegenerative diseases : Huntington's, Alzheimer's, Parkinson's, Amyotrophic Lateral Sclerosis (ALS)
  - Muscular disorders, High blood pressure etc

TRANSPORT BY ENSEMBLES OF MOLECULAR MOTORS

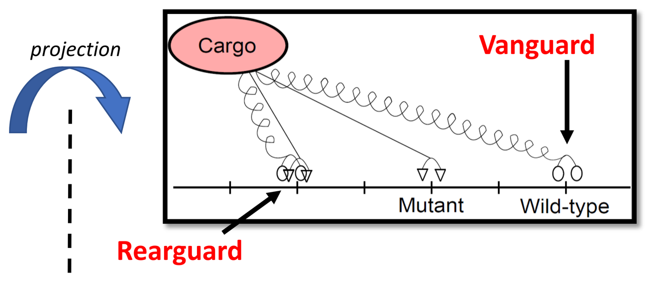


- In vivo, several motors work in teams to transport common cargo
  - Improves run-length
  - Provides ensemble robustness
  - Can carry larger loads
- Multiple motors of different types also form teams
  - E.g. Kinesin and Dynein together enable bidirectional transport
- It is likely that some of the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

Absolute and Relative Configurations



- 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
  - $P_\Omega(\Omega', t+\Delta t | \Omega, t) = \nu_\Omega(\Omega', \Omega)\, \Delta t$



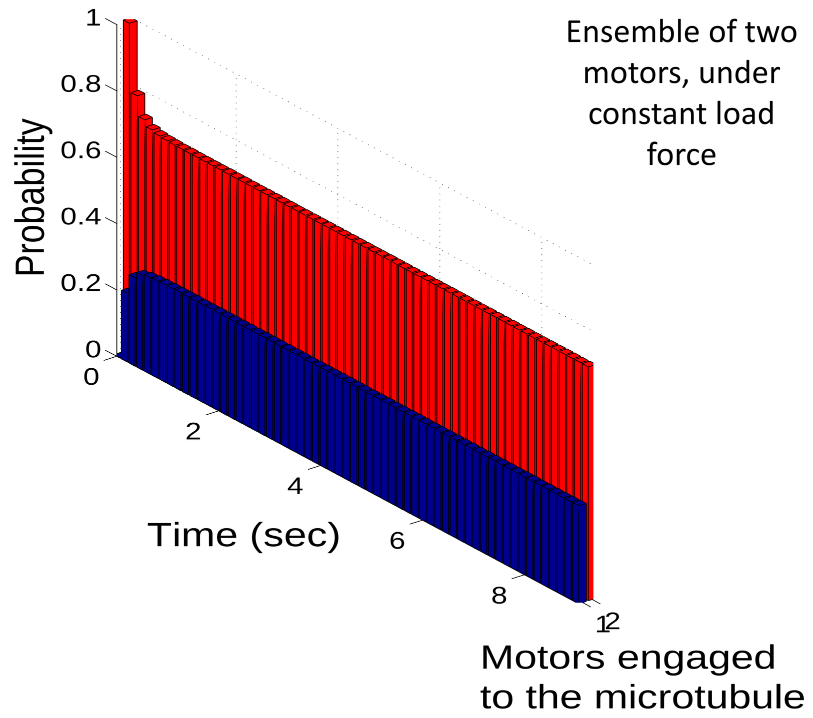
- Relative separation with respect to rearguard motor
- Projection Operator Υ maps Ω to $\Upsilon(\Omega)$
- Finite Dimensional Model : Master Equation
- $\frac{\delta}{\delta t} P_\vartheta(\vartheta,t) = -P_\vartheta(\vartheta,t)\sum_{\vartheta' \in H} \nu_\vartheta(\vartheta',\vartheta) + \sum_{\vartheta'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

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

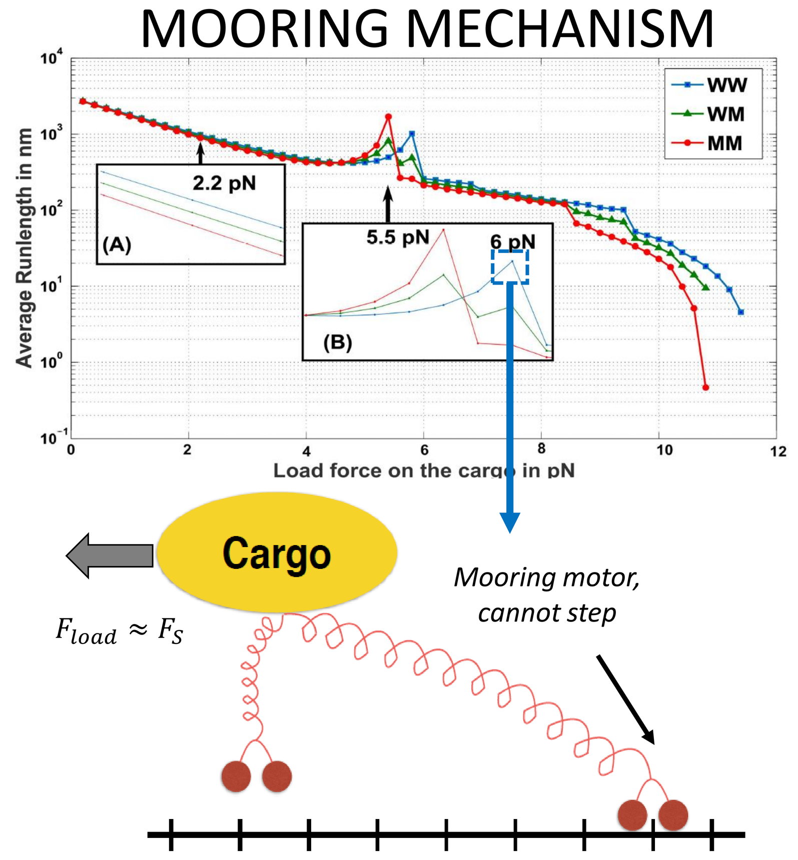
where $\Gamma \in \mathcal{R}^{N\times N}$ is completely defined by transition rates $\nu_\vartheta(\vartheta',\vartheta)$

ANALYTICAL RESULTS

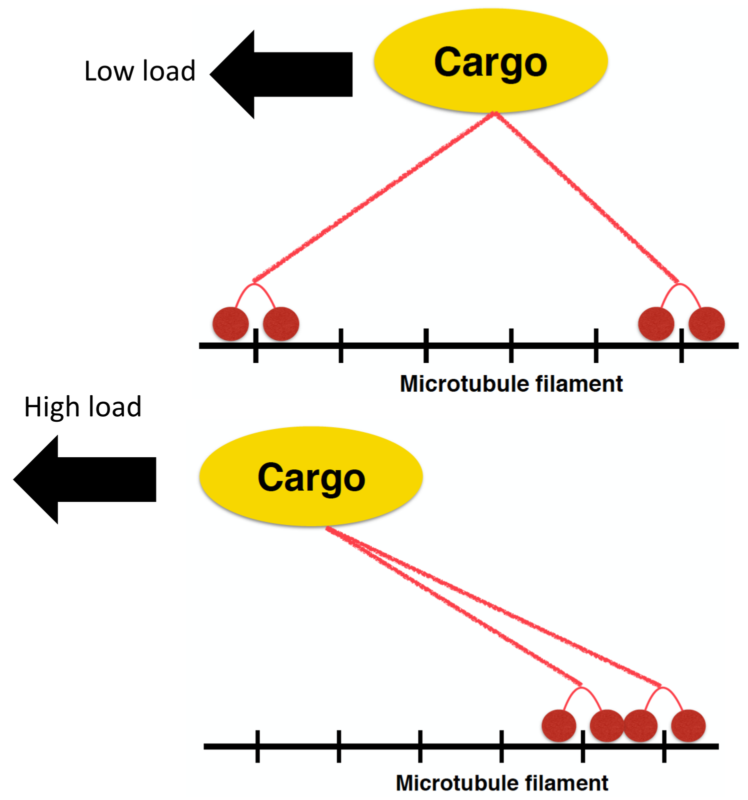
MOTOR CONFIGURATION REACHES STEADY STATE



AVERAGE RUN-LENGTH AND MOORING MECHANISM

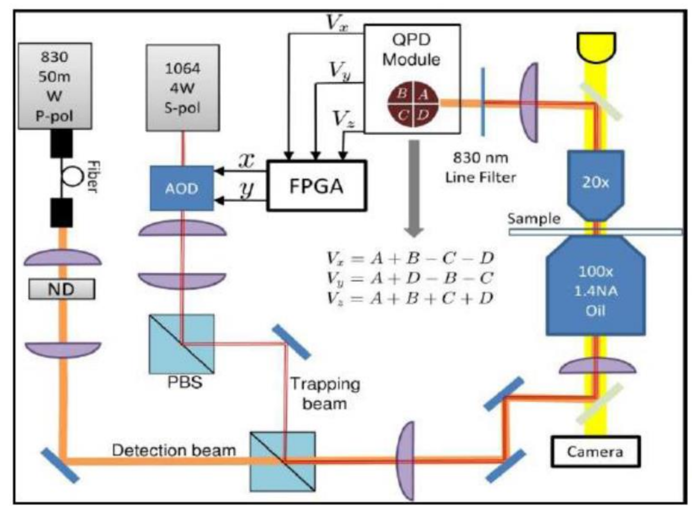


MULTIPLE AGENTS CLUSTER UNDER HIGH LOADS



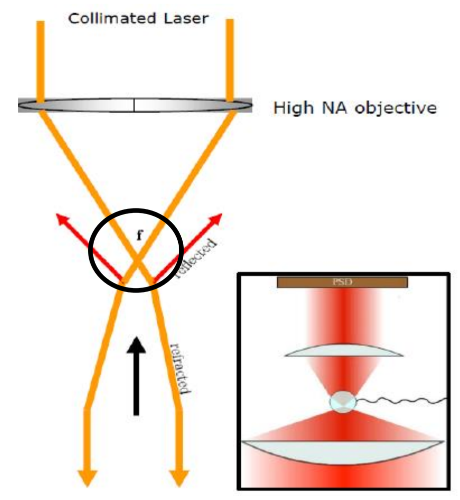
APPROACH : EXPERIMENTAL METHOD

OPTICAL TWEEZERS : EXPERIMENTAL SETUP



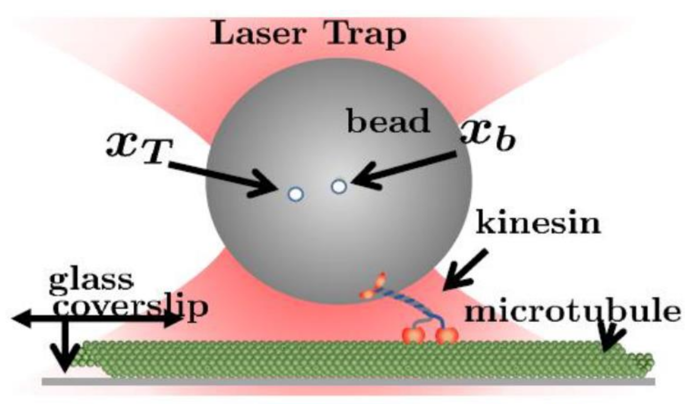
- Trapping laser is steered using an 'Acousto-Optical Deflector (AOD)' to manipulate trapped particle in the sample plane
- Conjugate lens pair ensures deflection of beam by AOD translates to the sample plane
- Thus, trapped bead can be moved very fast and precisely in the X-Y plane
- High speed FPGA acquires and processes data from photodiode and generates actuation commands for AOD, according to the control law

PRINCIPLES OF OPTICAL TRAPPING



- Collimated laser passed through high NA objective (NA >1.3) creates stable trap near focus
- Trapping of sub-micron particle with suitable refractive index is possible
- A second laser is passed through the bead and collected on a photo-sensitive detector (PSD)
- The laser spot on the PSD gives a measurement of bead position

TRACKING BEADS ATTACHED TO LIVE PROTEINS



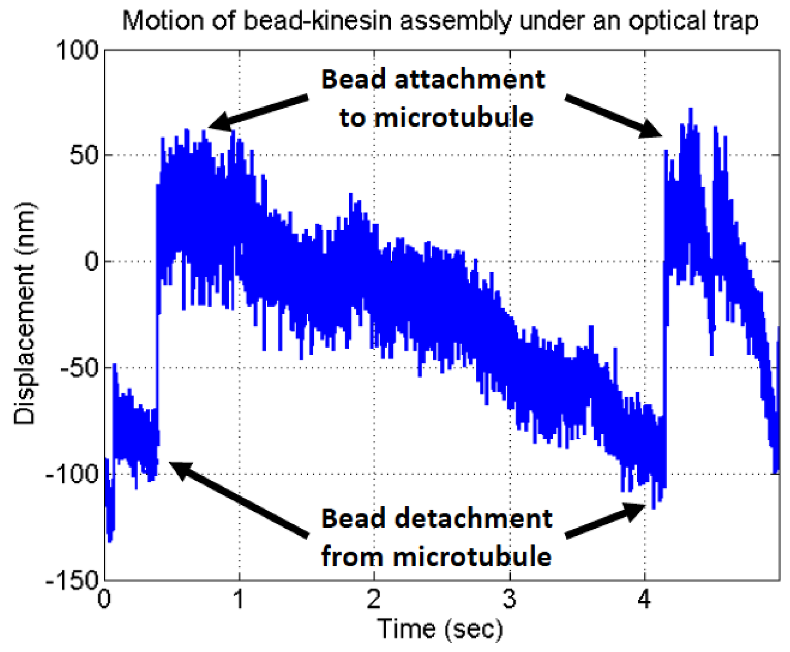
Schematic of an optically probed Kinesin

- Live proteins, like kinesin, attached to polystyrene beads through appropriate biochemistry
- Surface is coated with microtubule filaments to provide platform for kinesin to 'walk'
- Using laser, bead is trapped and brought near the surface, enabling it to 'walk'
- Motion of bead detected on the PSD, through which motion of kinesin protein is inferred

EXPERIMENTAL ANALYSIS

ANALYSIS USING OPTICAL TWEEZERS

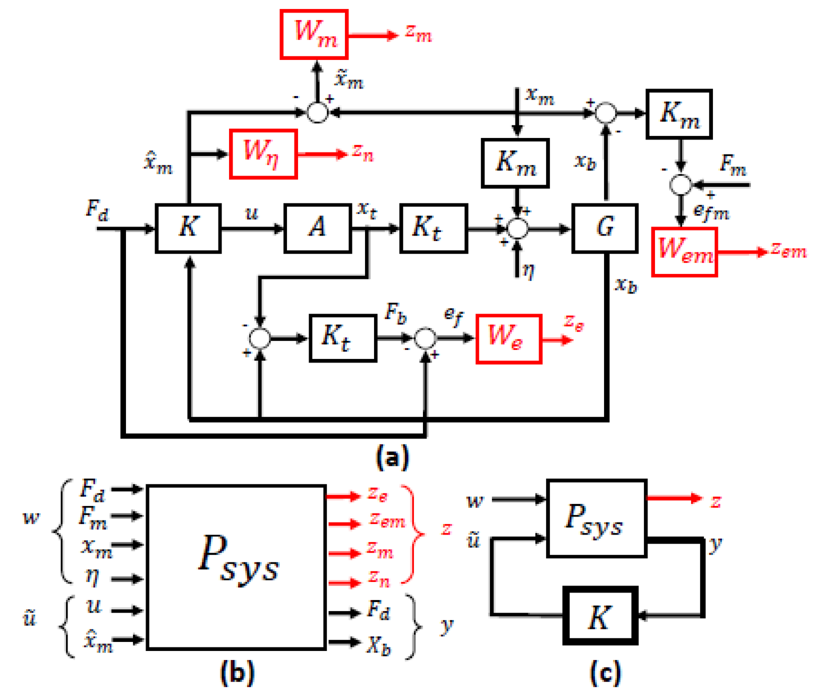
Custom made optical tweezer setup, used in PI's Nanodynamics lab at the University of Minnesota, Twin Cities



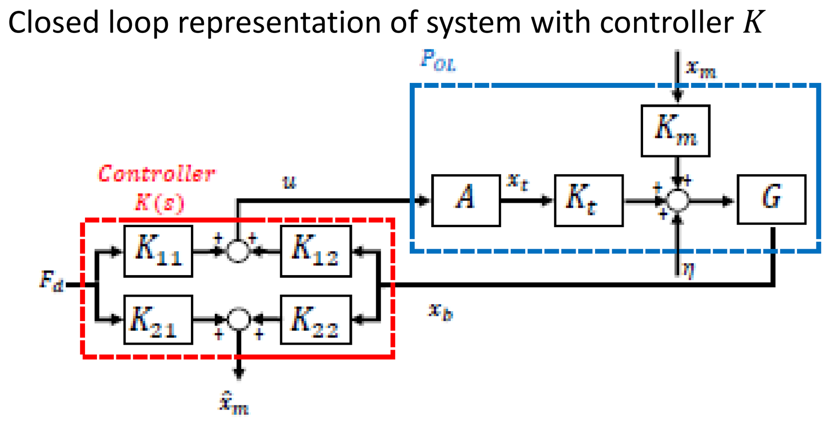
Typical motion of cargo (i.e. beads) being carried by motor protein Kinesin, captured using optical tweezer setup

CONSTANT FORCE STUDIES USING MODERN CONTROLS FRAMEWORK

CONTROLLER DESIGN

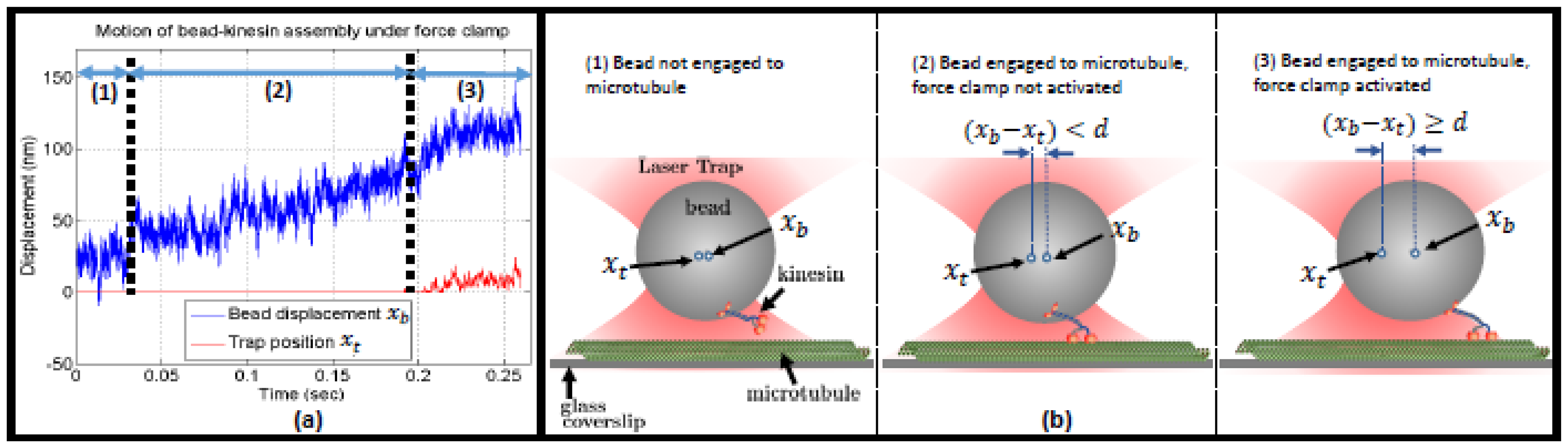


(a) Block diagram of plant with frequency dependent weights. (b) Arrangement of the system in terms of inputs $[w; \tilde{u}]$ and outputs $[z; y]$. (c) Closed loop representation of system with controller K

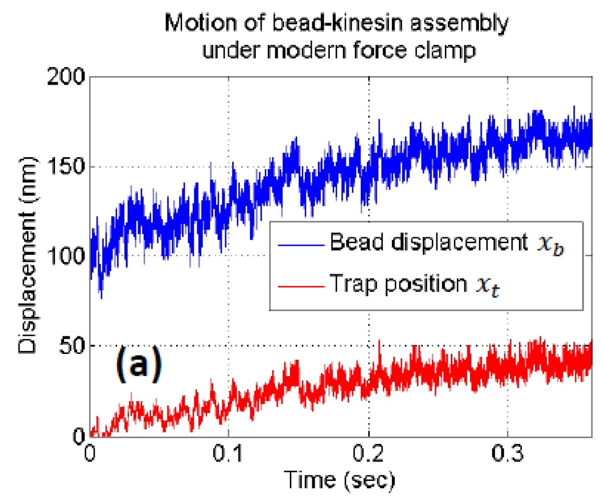


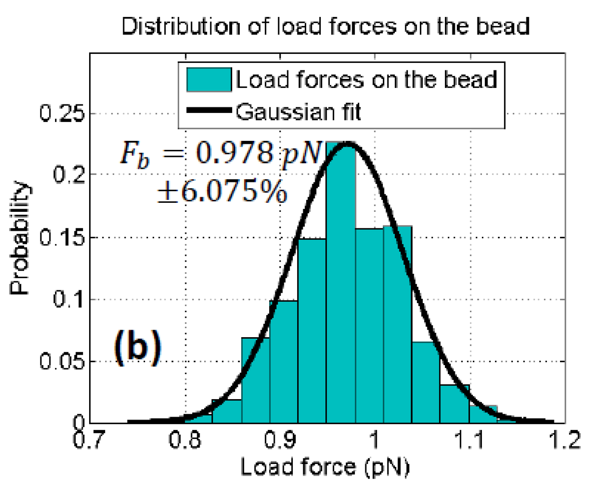
Block diagram of closed loop system with open loop plant P_{OL} and controller K = $\begin{bmatrix} K_{11} & K_{12} \\ K_{21} & K_{22} \end{bmatrix}$

TESTING ON LIVE MOTOR PROTEIN



(a) Experimental data on bead motion due to kinesin motor protein, analyzed under constant force. (b) Representation of 'pick-and-place' experiment, corresponding to sections 1,2 and 3 in (a)





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

ACHIEVEMENTS

1. Order of magnitude improvement in force regulation. Previous state-of-art demonstrated 17% error while our method shows ~ 6%
2. Regulation performance demonstrated in very low force range (< 1 femto Newton)
3. 10x improvement in tracking bandwidth
4. Ability to track and study faster moving motors (e.g. dynein) achieved
5. Disturbance estimation paradigm applicable to wide array of systems including scanning probe microscopy, microfluidic flow estimation, high density data storage etc.