Motor Proteins
Investigation and Modelling

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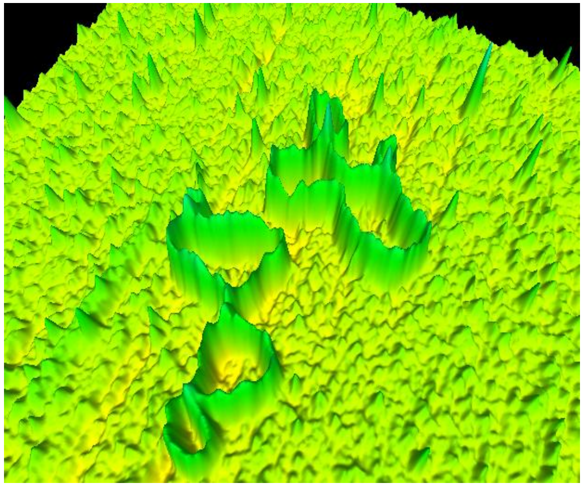

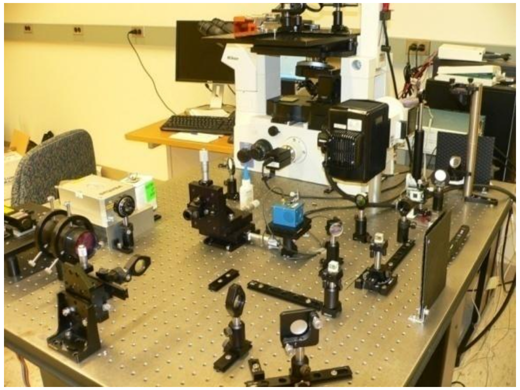

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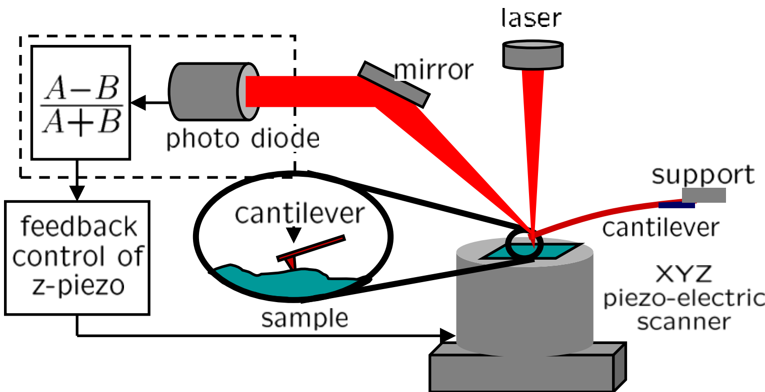
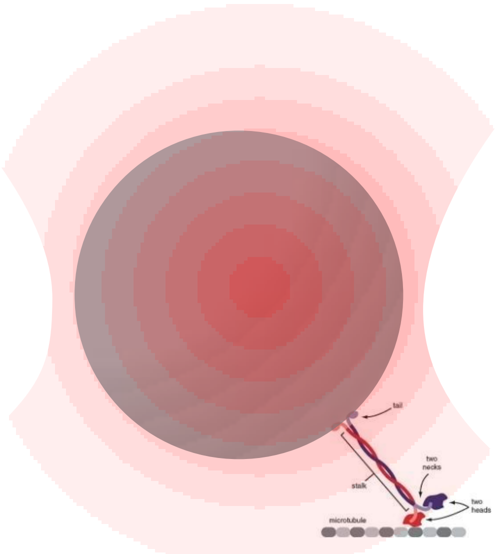


Outline

- Motor Proteins
  - What are they?
  - Role in human physiology
  - Single molecule studies
    - Why single molecule and comparison with ensemble studies
    - Challenges
  - Modes of Investigation
    - Optical Tweezers for Single Molecule Experiments
  - A new Step detection method
    - Application to Kinesin walk detection
  - Cooperative behavior amongst molecular motors
    - Understanding cargo transport in cells with multiple motors

Motor Proteins



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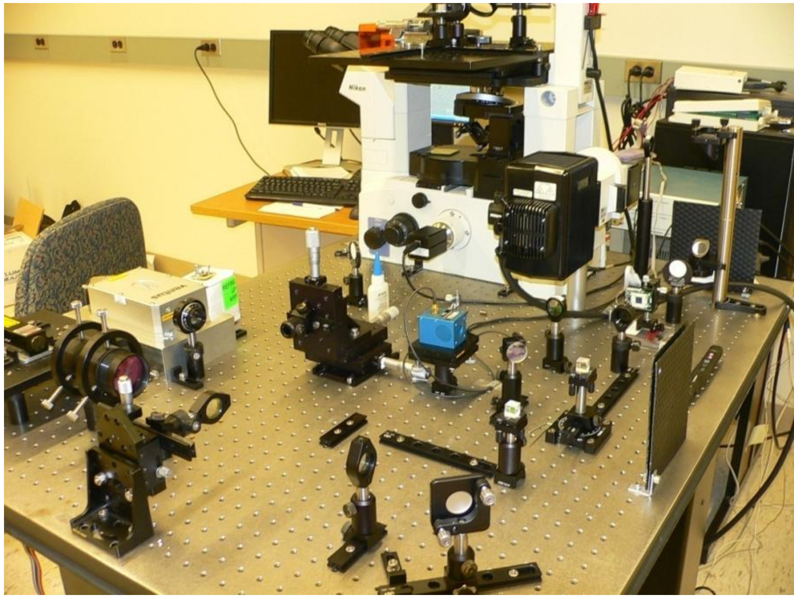
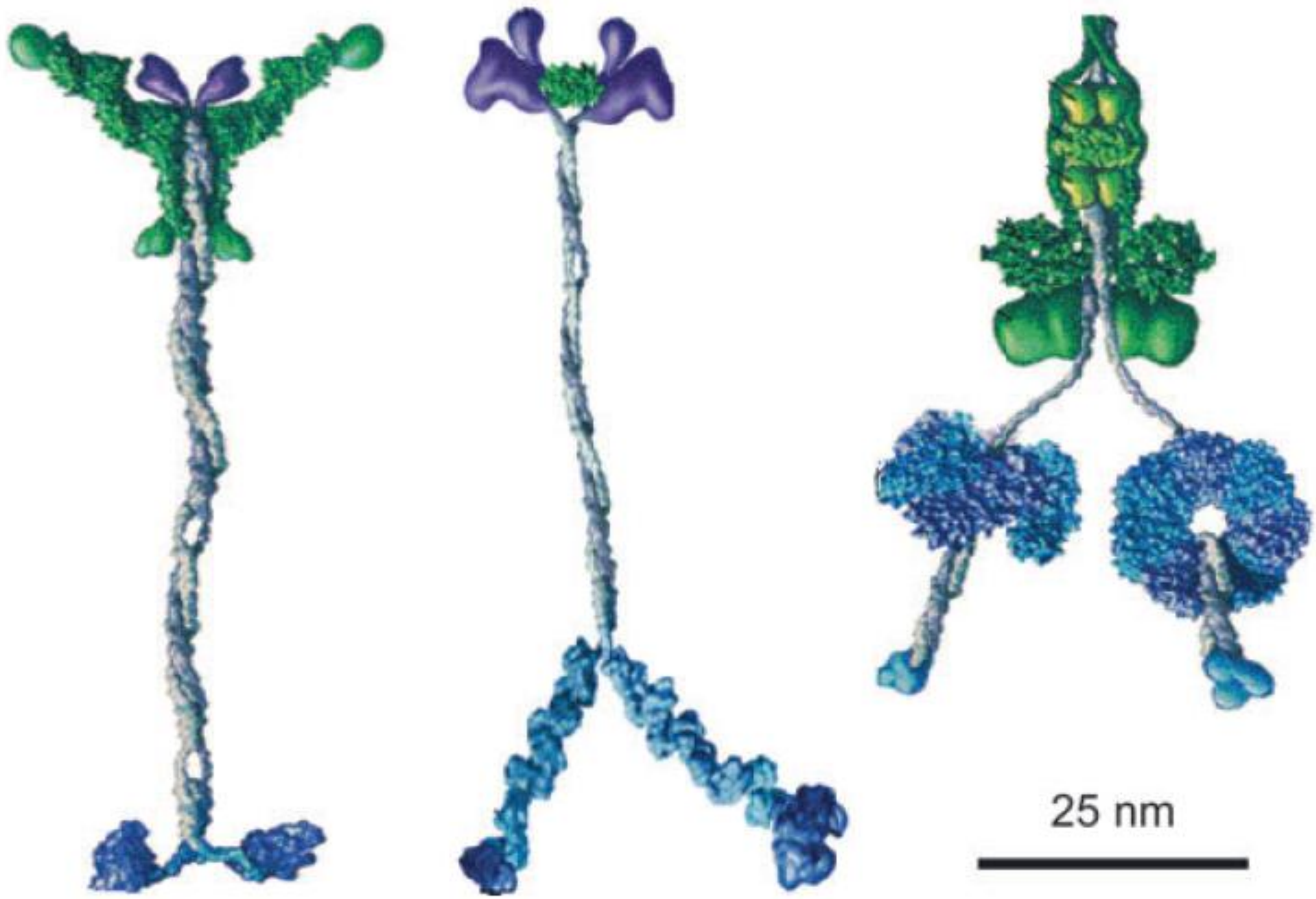


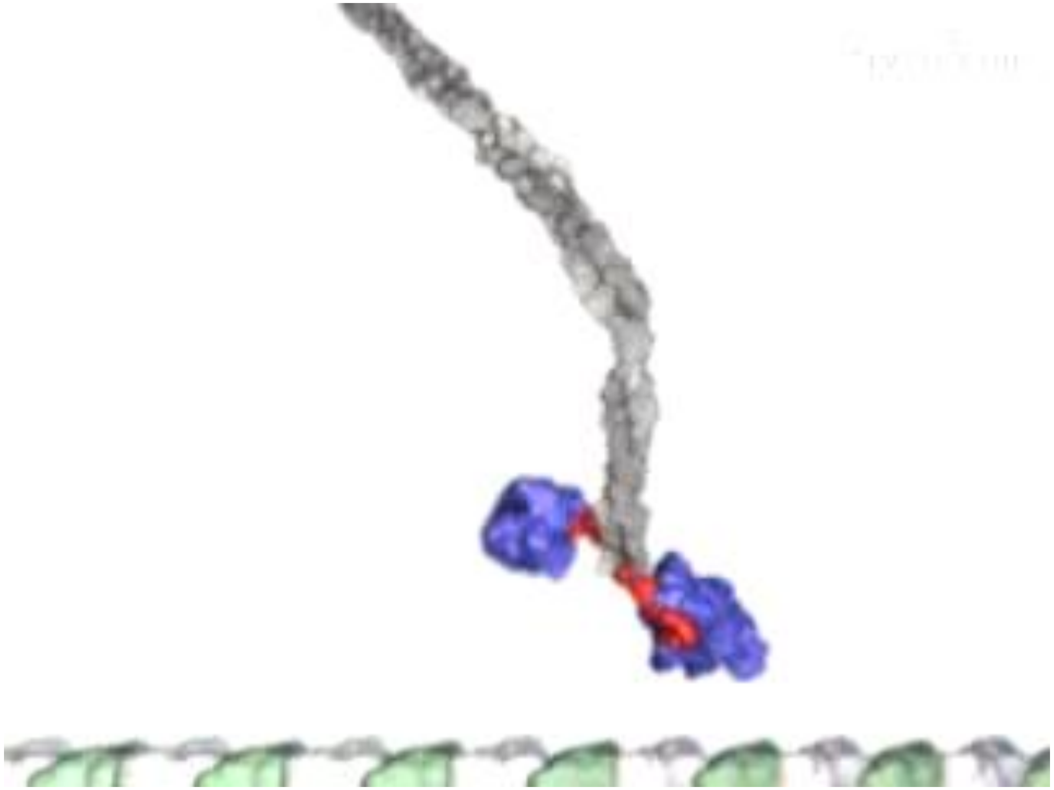
What are they



Kinesin Myosin Dynein

Pictures from: Kolomeisky, A. B., Fisher, M. "Molecular Motors: A Theorist's Perspective", Anuual Review of Phys. Chem, 58, 675-695, 2007

Transport in cells



Kinesin Motor

- Processive motor
  - Two heads
  - walks over microtubule
  - Length of Kinesin molecule is about 100 nm
  - Cargo can be about a μm in dimension

Animation

This video is from: Essential Cell Biology, 3rd Edition Alberts, Bray, Hopkin, Johnson, Lewis, Raff, Roberts, Walter ISBN: 978-0-8153-4129-1

What are they

Gliding Assay: Microtubules transported by Kinesin on substrate (work at NDSL)

- Motor Proteins
  - ⋆ Enzymatic molecules that convert chemical to mechanical work and motion
  - ⋆ Processive motor proteins
    - – Kinesin, Dynein and Myosin
    - – Step unidirectionally along linear tracks carrying a cargo

Physiological Relevance (Examples)

- Kinesin
  - ⋆ Left-right body determination (KIF3) (body axis)
  - ⋆ Suppression of tumorogenesis
  - ⋆ KIF17 and KIF5 involved in higher brain functionality (long term memory)
  - ⋆ Tau factor regulation implicated in Alzheimers

Hirokawa, N. and Noda, Y. and Niwa, S., "Kinesin superfamily motor proteins and intracellular transport", Nature, Reviews, Vol. 10, pp. 682-696, 2009

Challenges

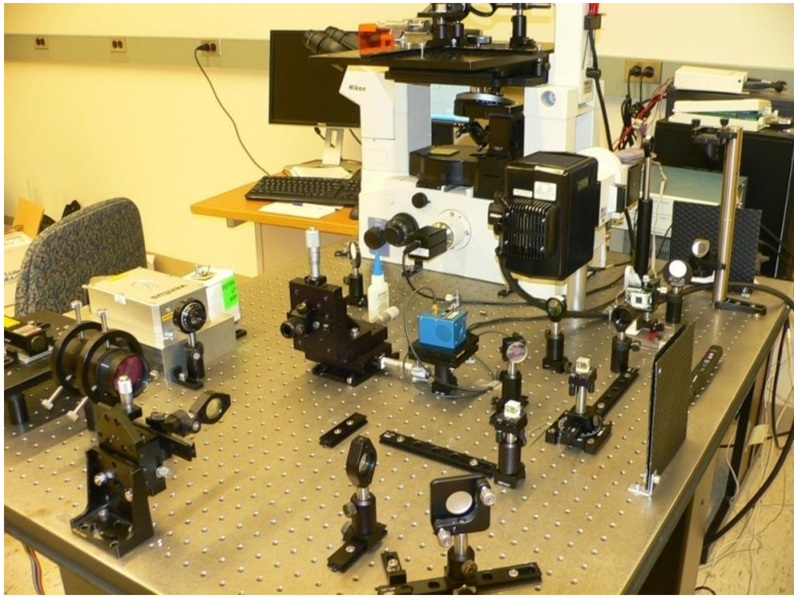
- Are there substeps
- Backward steps; are they real
  - ⋆ Rare event detection
- Is the biocehmical pathway altered when an assitive force is provided
- Browninan ratchet or power stroke
  - ⋆ Modeling to identify parameters to control
- How do multiple Kinesin coordinate motion intransport of the same cargo
  - ⋆ Typically less than five kinesin involved.
- How do multiple motor protein types (Dynein and Kinesin) achieved tasks (like mitosis)
  - ⋆ Typically six to seven dynein Vs 1 kinesin.

Single Molecule Vs Ensemble Studies

- Biological systems form is inextricably linked to function
  - ⋆ Corollary of Darwins evolution theory
  - ⋆ Huge effort in determining static structure of molecules
- Study of dynamics of molecules
  - ⋆ Ensemble methods
    - Need to synchronize all elements of ensemble to the same initial state
    - Initial state memory lasts only for a short time with thermal forces lead to decoherence
    - Studies can be done for millisecond time range after synchronization
    - Processes have time scales in much larger time scales; protein folding in the minutes time scale
    - Rare events cannot be studied
    - Multimodal behavior cannot be unravelled
- Single Molecule methods
  - ⋆ Advantages
    - No synchronization issues
    - Rare occurences can be studied by tailoring the effect
    - Effects of forces and torques can be studied
    - Larger time scales are accesible
    - Internal noise effects can be better understood
  - ⋆ Challenges
    - Signal is twenty orders smaller
    - Dynamics can be quite complex

Modes of investigating Single molecule physics

Optical Tweezer
Atomic Force Microscope
FRET



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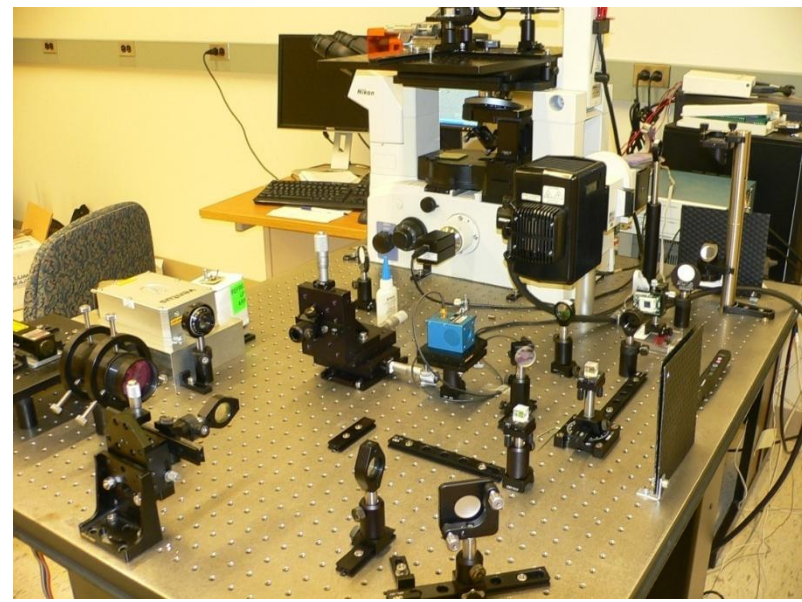
Optical Tweezer

Harnessing the momentum of Light



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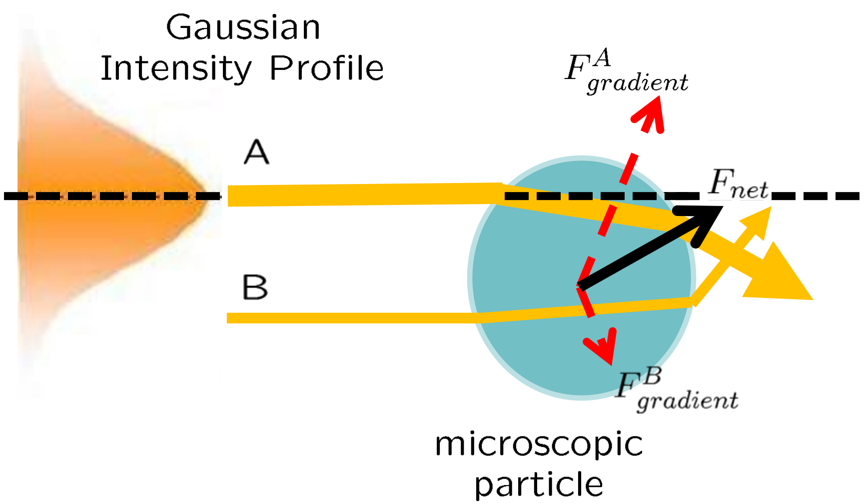
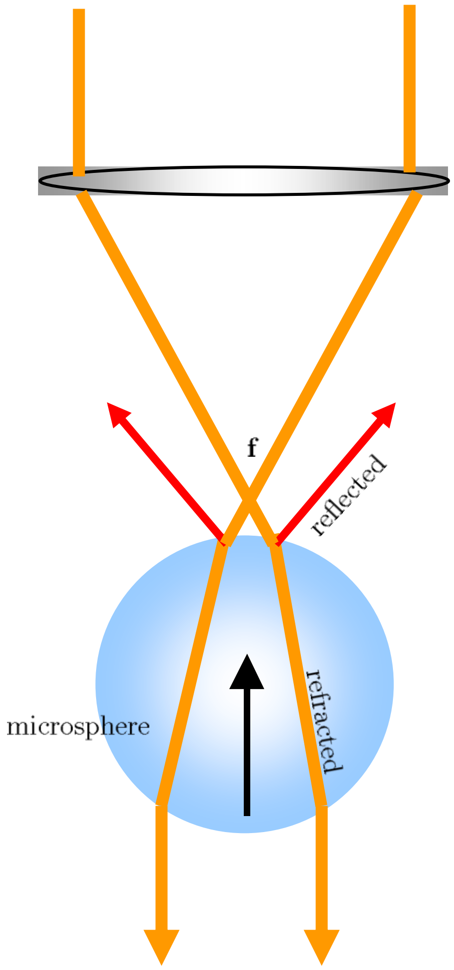
Physics



- Optical trap
  - ⋆ Radiation pressure of light
- Scattering force
  - ⋆ In the direction of beam propagation
    - – A destabilizing effect
- Gradient Force
  - ⋆ Stabilizing influence
- Numerical Aperture Large
  - ⋆ Leads to a stable trap with a Single Laser beam

A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and Steven Chu. Observation of a single-beam gradient force optical trap for dielectric particles. Opt. Lett., 11(5):288, 1986

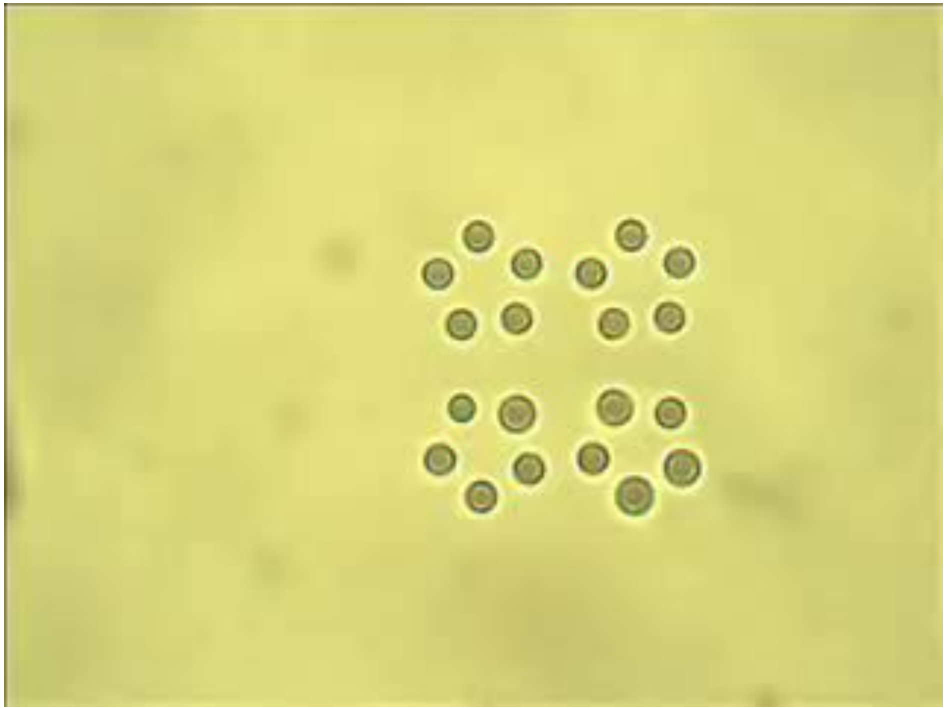
Optical Trapping



- Optical trap
  - ⋆ Maximum force: 100pN
  - ⋆ Hookean spring constant
    - 0.01pN/nm to 0.5pN/nm
- Manipulation range
  - ⋆ 50 μm
- Bead size
  - ⋆ on the order of micron

A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and Steven Chu. Observation of a single-beam gradient force optical trap for dielectric particles. Opt. Lett., 11(5):288, 1986

Single Beam Time Multiplexed traps

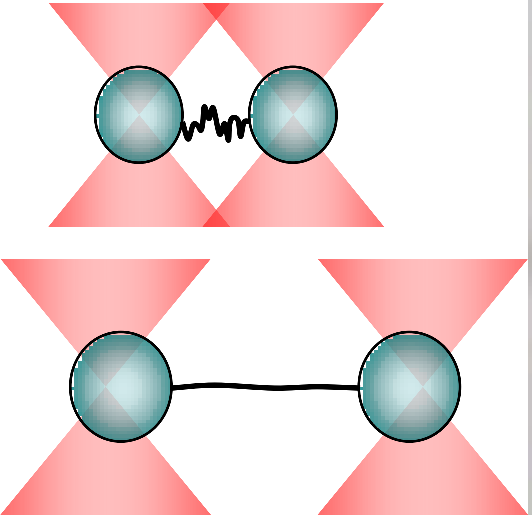


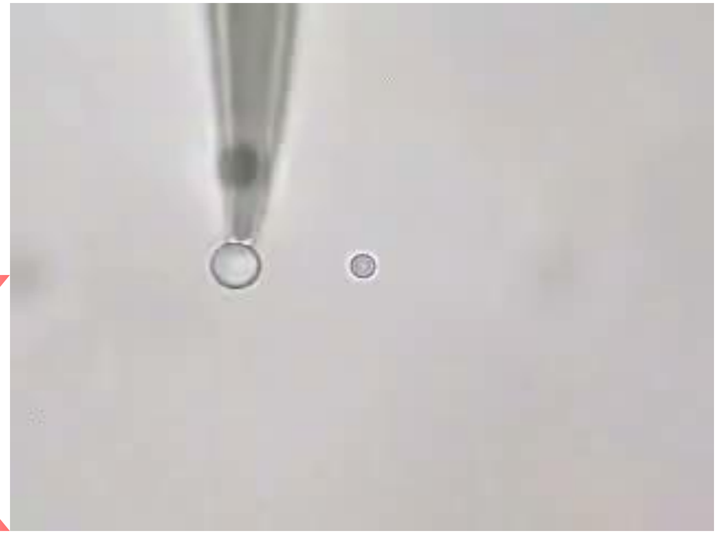
Achieved at NDSL

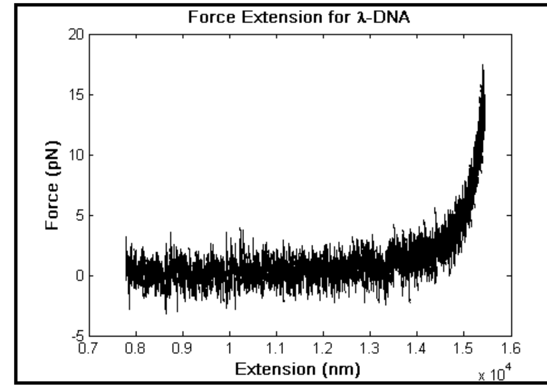
- Dynamic Patterns
  - ⋆ Generate MicroFluidic flows
    - – Realized experimentally at NDSL
  - ⋆ Projects
    - – Using CCD output as measurement maintain a robust pattern

Achieved at NDSL

Single Molecule Force Spectroscopy







Achieved at NDSL

- Force Spectroscopy
  - ⋆ Identify domains
    - – Protein Folding
  - ⋆ Projects
    - – Identification of events

Coordinated Motion of Molecular Motors: Modeling and Analysis

Dr. Donatello Materassi

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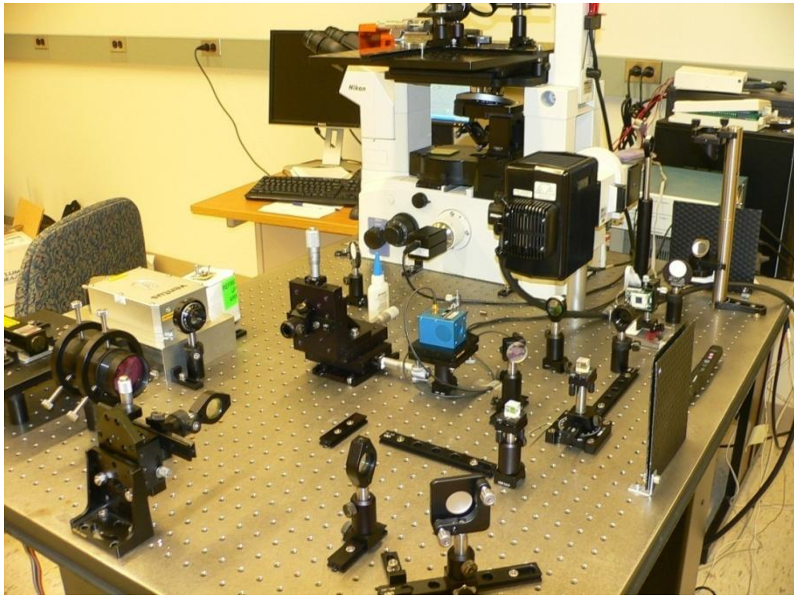
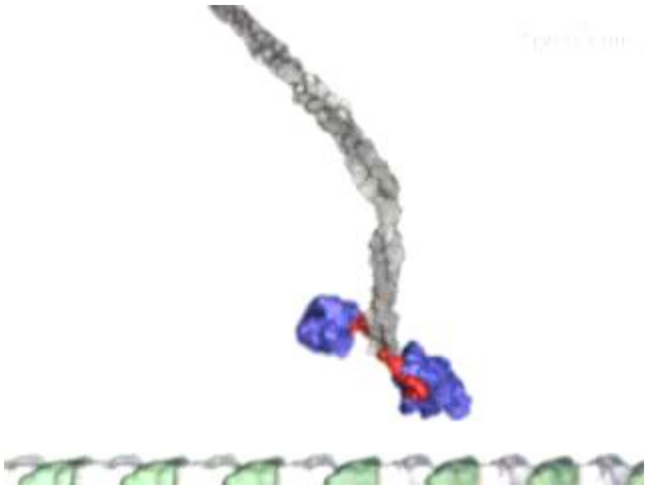
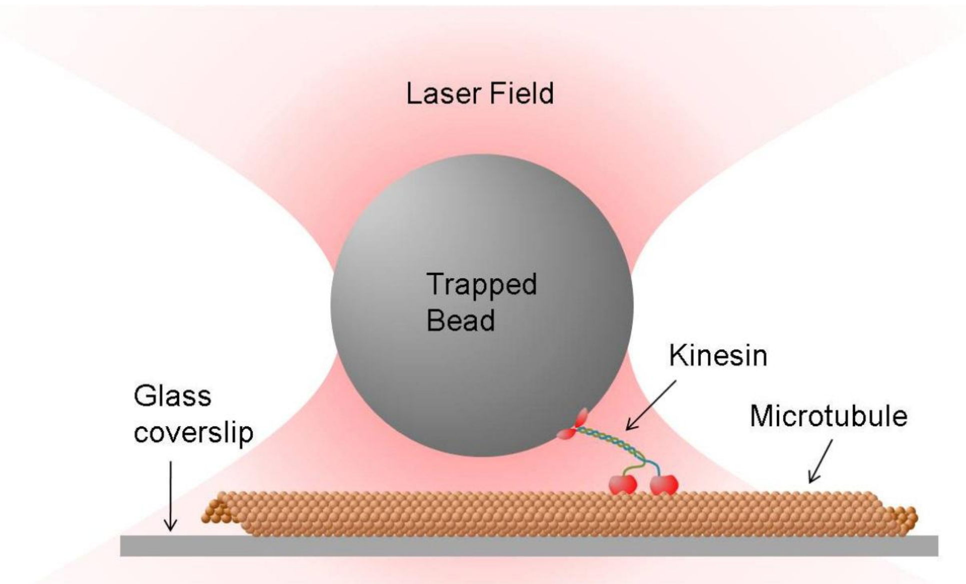


The Individual Motor model

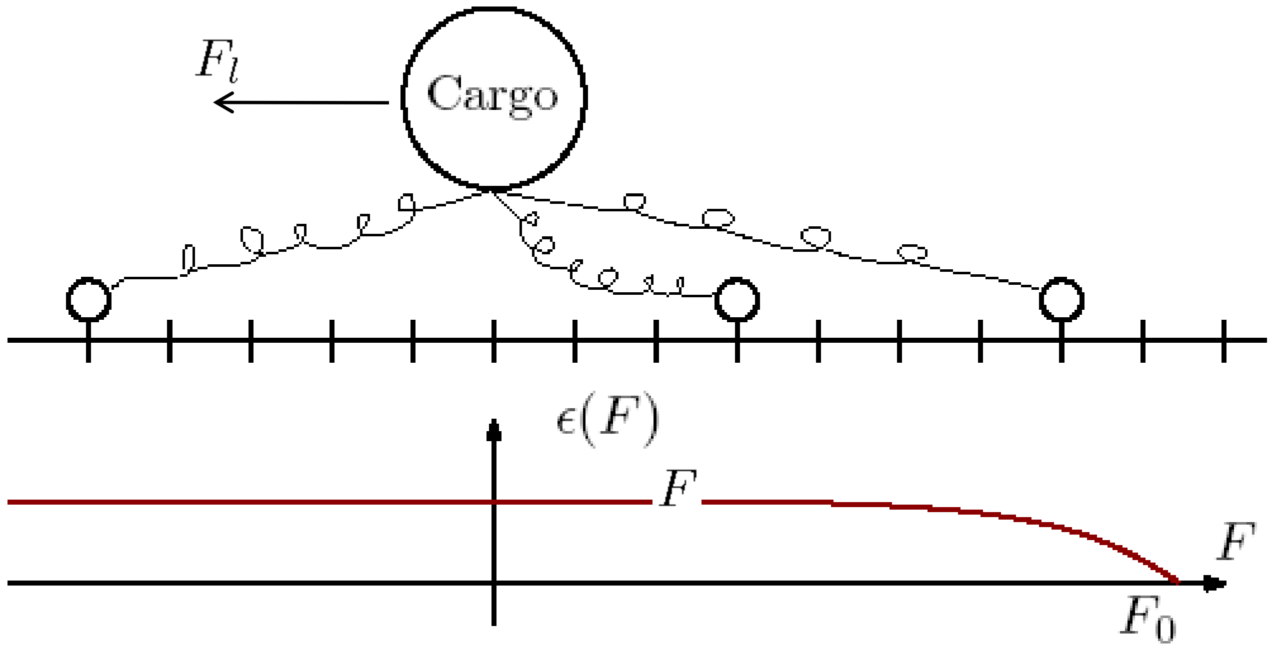




- Stepping Probability
  - ⋆ The Michaelis-Menten dynamics leads to
    $$\texttt{Hydrolysis rate} = \frac{k_{cat}[ATP]}{[ATP] + k_m}$$
    where $k_m = (k_{cat} + k_{off})/k_{on}$ is the Michaelis-Menten constant
  - ⋆ A step is successful with rate ϵ (efficiency of a step)
  - ⋆ The step size is d. We have
    $$P_{step} = \frac{k_{cat}[ATP]}{[ATP] + k_m}\epsilon$$

$$V = P_{step} d = \frac{k_{cat}[ATP]}{[ATP] + k_m} d\epsilon$$

The Individual Motor model



- Influence of force on the motor
  - ⋆ The efficiency $\epsilon = \epsilon(F)$

$$\epsilon(F) = \begin{cases} 1 & \text{if } F \leq 0 \\ 1 - \left(\frac{F}{F_0}\right)^2 & \text{if } 0 < F < F0 \\ 0 & \text{otherwise} \end{cases}$$

  - ⋆ F_0 is the “stall force”

The Individual Motor model

- Modeling detachment
  - ⋆ k_{off} increases with increasing forces [Gross et al, 2005]

$$k_{off} = k_{0off} e^{Fd_l/K_bT}$$

  - ⋆ We have the probability of a successful step under a force F

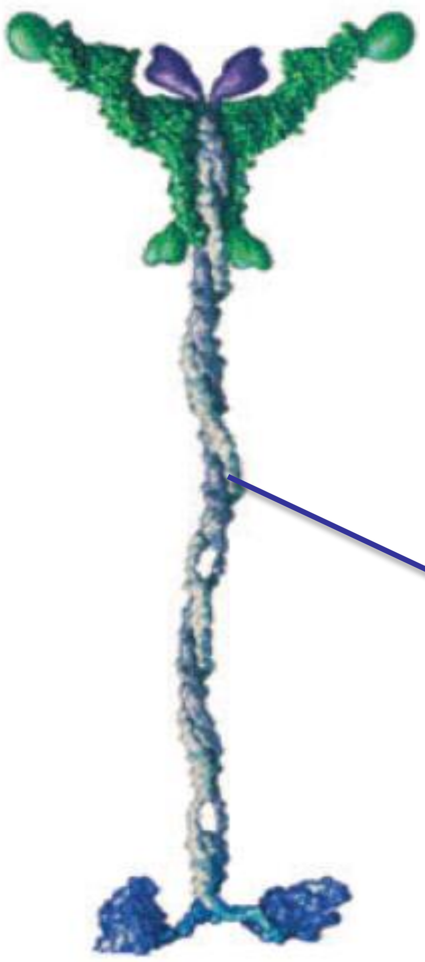
$$P_{step} = \frac{k_{cat}[ATP]}{[ATP] + \frac{k_{on}+k_{off}(F)}{k_{cat}}}\epsilon(F)$$

$$= \frac{k_{cat}[ATP]}{[ATP] + \frac{k_{on}+k_{0off}e^{Fd_l/K_bT}}{k_{cat}}}\left[1 - \left(\frac{F}{F_0}\right)^2\right]$$

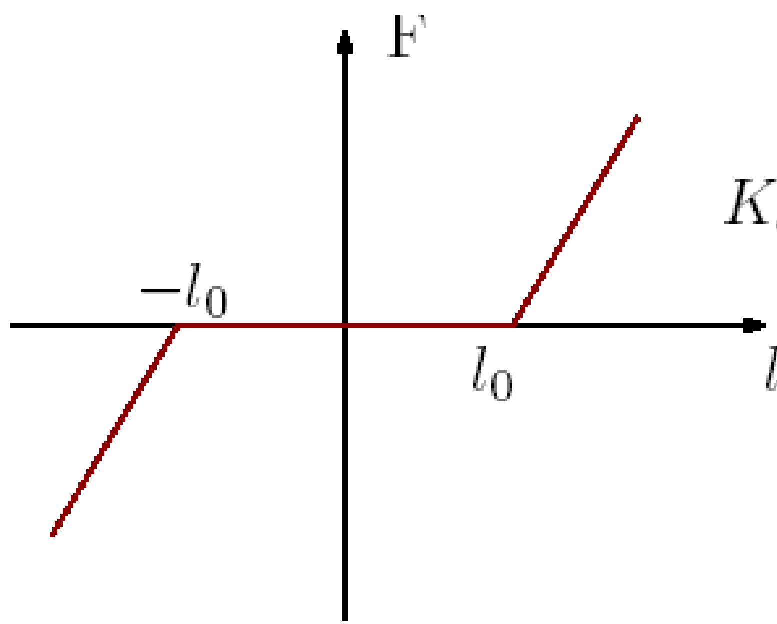
The Individual Motor model

- Modeling detachment
  - ⋆ From [Schnitzer et al. 2000], the processivity L is
    $$L = \frac{d[ATP]Ae^{-F\delta_l K_b T}}{[ATP] + B(1+A)e^{-F\delta_l K_b T}}$$
  - ⋆ Then
    $$\frac{P_{step}(F)}{P_{detach}(F)} = \frac{L}{d} = \frac{[ATP]Ae^{-F\delta_l K_b T}}{[ATP] + B(1+A)e^{-F\delta_l K_b T}}$$
- The probability of reattachment is $P_a = 5s^{-1}$.
- A motor can only reattach at a location whose distance from the cargo is less than l_0.

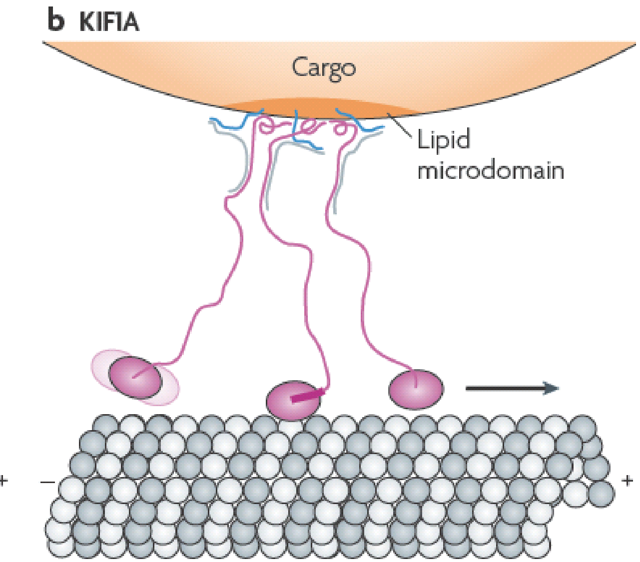
The Individual Motor model

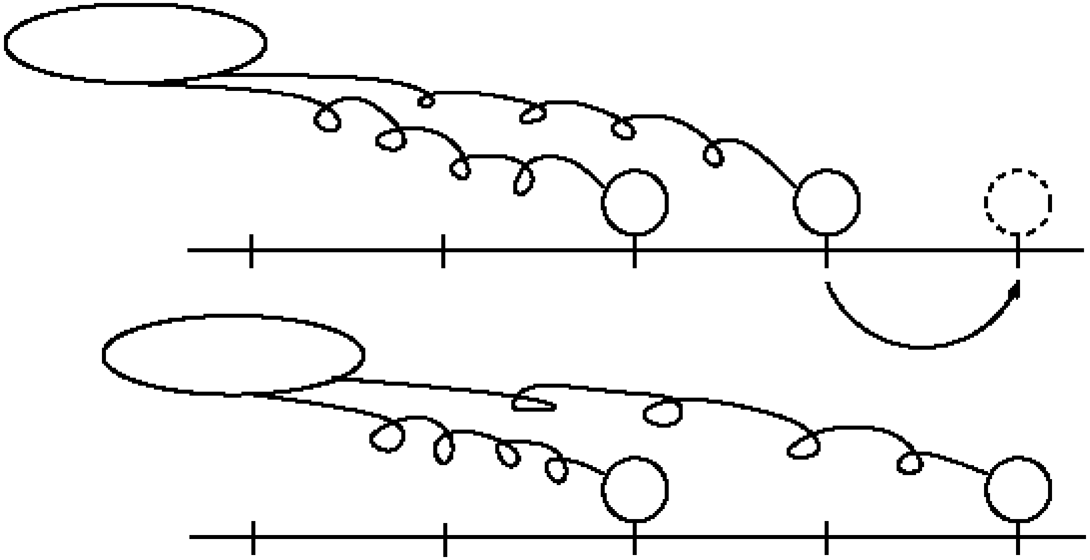


- Model of the stalk
  - ⋆ is a linear spring with constant K_{el}
  - ⋆ has a rest length equal to l_0
  - ⋆ it buckles down with no resistance when compressed



Cargo Model





★ Cargo position determined entirely by force balance of forces exerted by motors and Force applied

– If a motor steps, the cargo reaches the new position instantaneously
– If there are m_a "active" motors we observe a step in the cargo with length d/m_a
– A shift of the configuration is equivalent

A Equivalent Model

- Elements
  - ⋆ bi-infinite *"Microtubule"* with equally spaced locations $\{a_k\}_{k \in \mathcal{Z}}$
    $$a_k = x^{(0)} + kd_s$$
  - ⋆ m *engaged motors* on the Microtubule
  - ⋆ the i-th motor position $x_i \in \{a_k\}_{k \in \mathcal{Z}}$
  - ⋆ z_k: number of motors in the location a_k
  - ⋆ a *configuration* is the bi-infinite sequence
    $$Z = (\ldots, z_{-1}, z_0, \ldots, z_k, \ldots)$$

A Equivalent Model

- Transitions
  - ⋆ $\lambda(Z_2, Z_1)$ is the rate of transition $Z_1 \to Z_2$
  - ⋆ A step from a_k to a_{k+1}

$$Z = \begin{pmatrix} \vdots \\ z_k \\ z_{k+1} \\ \vdots \end{pmatrix} \xrightarrow{\lambda(Z+R_k^{(s)},Z)=P_{step}} \begin{pmatrix} \vdots \\ z_k \\ z_{k+1} \\ \vdots \end{pmatrix} + \begin{pmatrix} \vdots \\ -1 \\ +1 \\ \vdots \end{pmatrix} = Z + R_k^{(s)}$$

  - ⋆ A detachment/attachment at location a_k

$$Z = \begin{pmatrix} \vdots \\ z_k \\ z_{k+1} \\ \vdots \end{pmatrix} \xrightarrow{\lambda(Z\pm R_k^{(a)},Z)=P_{det}/P_{att}} \begin{pmatrix} \vdots \\ z_k \\ z_{k+1} \\ \vdots \end{pmatrix} + \begin{pmatrix} \vdots \\ \pm 1 \\ 0 \\ \vdots \end{pmatrix} = Z \pm R_k^{(a)}$$

  - ⋆ A translation in space preserves the rates

A Equivalent Model

- An equivalent model
  - ⋆ Transitions $Z \to Z + R$ define a Gillespie Model
  - ⋆ $P(Z,t)$ (probability of configuration Z at time t) satisfies the Master Equation
$$\frac{\partial}{\partial t}P(Z,t) = -P(Z,t)\sum_{Z' \in \mathcal{Z}} \lambda(Z',Z) + \sum_{Z' \in \mathcal{Z}} \lambda(Z,Z')P(Z',t),$$
  - ⋆ Enumerate the configurations $Z_1, ..., Z_j, ...$ and define
$$\mathcal{P}(t) := (P(Z_1,t), ..., P(Z_j,t), ...)^T$$
  - ⋆ The Master Equation becomes
$$\frac{\partial}{\partial t}\mathcal{P}(t) = \mathcal{A}\mathcal{P}(t)$$

Spread is finite

- Spread
  - ⋆ For a configuration $Z = (..., z_k, ...)$, define the *spread*
$$s(Z) := \max_{z_k \neq 0}\{a_k\} - \min_{z_k \neq 0}\{a_k\}$$
Also define
$$s^{(max)} := \max\left\{\frac{\overline{m}F_s - F_{load}}{K} + d_s, \frac{F_{load}}{K}\right\} + 2l_0.$$
Under the KVXG rules for any $S \geq s^{(max)}$, if $s(Z(t_0)) \leq S$
$$Pr\{s(Z(t)) \leq S\} = 1 \qquad \text{for } t > t_0.$$

Ensemble Representation

- Tracking number of motors at each location relative to spread

  ⋆ Define the *regular dimension* of the ensemble
  $$n := \left\lceil \frac{s^{(max)}}{d_s} . \right\rceil$$
  Given $Z = \{z_k\}_{k \in \mathcal{Z}}$, define its *ensemble representation* as the n-vector
  $$Q = (z_{k_1}, ... z_{k_1+n-1})^T$$
  where
  $$k_1 := \inf\{k \in \mathcal{Z} | z_k \neq 0\}$$

  ⋆ We write $Q := \Pi^{(e)}(Z)$. If $k_1 = -\infty$, Q is $(0, 0, ..., 0)^T$.

Ensemble Representation

- Projection
  - ⋆ The operator $\Pi^{(e)} : Z \to Q$ is a projection
  - ⋆ Information of the absolute position is lost
  - ⋆ Relative distances are preserved
  - ⋆ This projection is still Markov
- The following holds
  - ⋆ Define

$$\lambda_Q(Q', Q) := \sum_{\substack{\Pi^{(e)}(Z')=Q' \\ s(Z)\leq n}} \lambda(Z', Z).$$

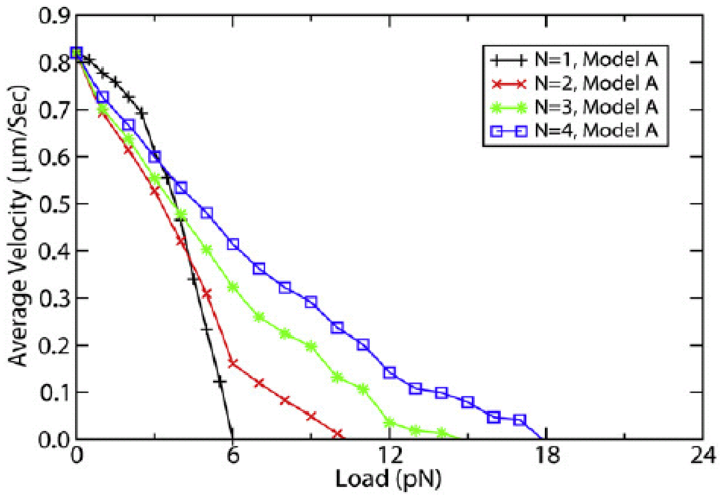
If $s(Z(t_0)) < n$ the dynamics of the projection space follows

$$\frac{\partial}{\partial t} P_Q(Q,t) = -P_Q(Q,t) \sum_{Q' \in \mathcal{Q}} \lambda(Q', Q) + \sum_{Q' \in \mathcal{Q}} \lambda(Q, Q') P_Q(Q', t)$$

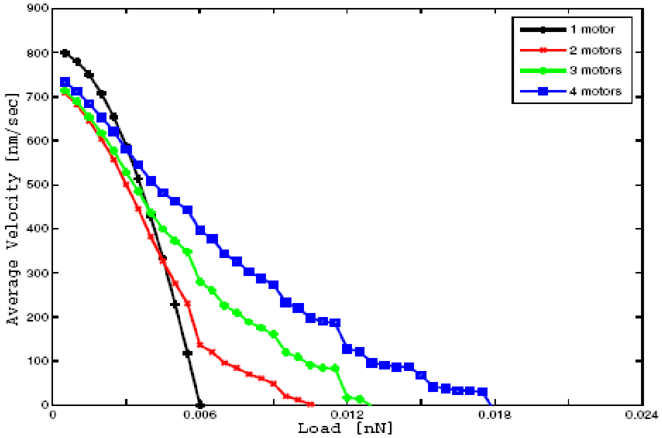
Achievements

- Projection
  - ⋆ The dynamics of the system has no steady state
  - ⋆ The dynamics of the projection has a steady state
  - ⋆ The expected velocity of the ensemble can be determined
  - ⋆ The average runlength can be determined
  - ⋆ The number of engaged/active motors can be determined
  - ⋆ Rare events are captured

Unexpected Behavior



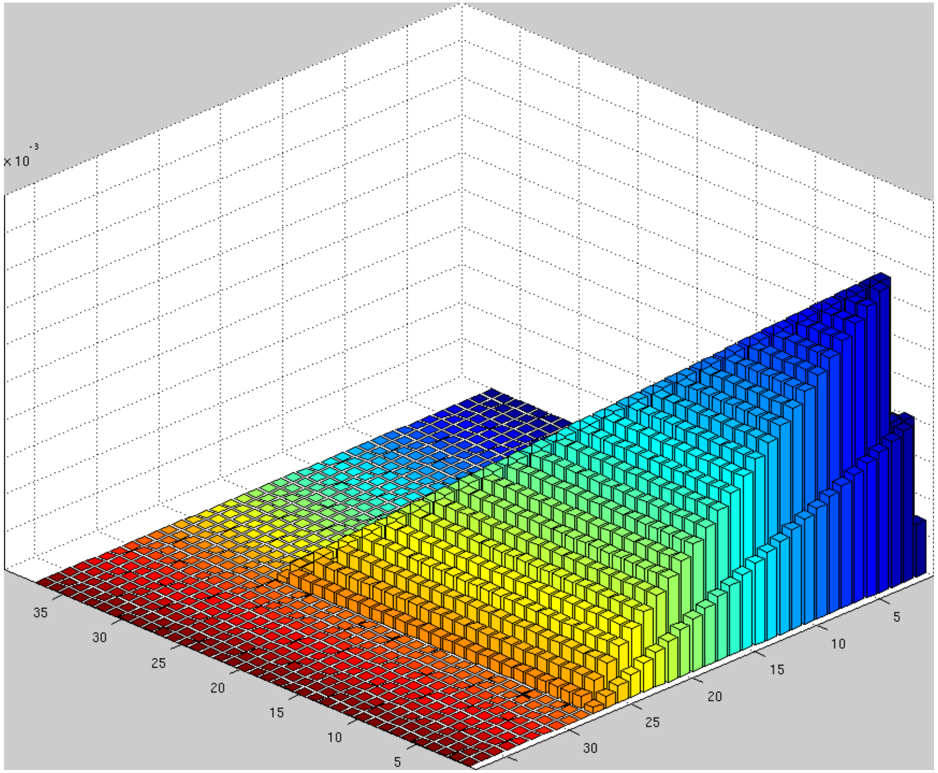
(Gross)



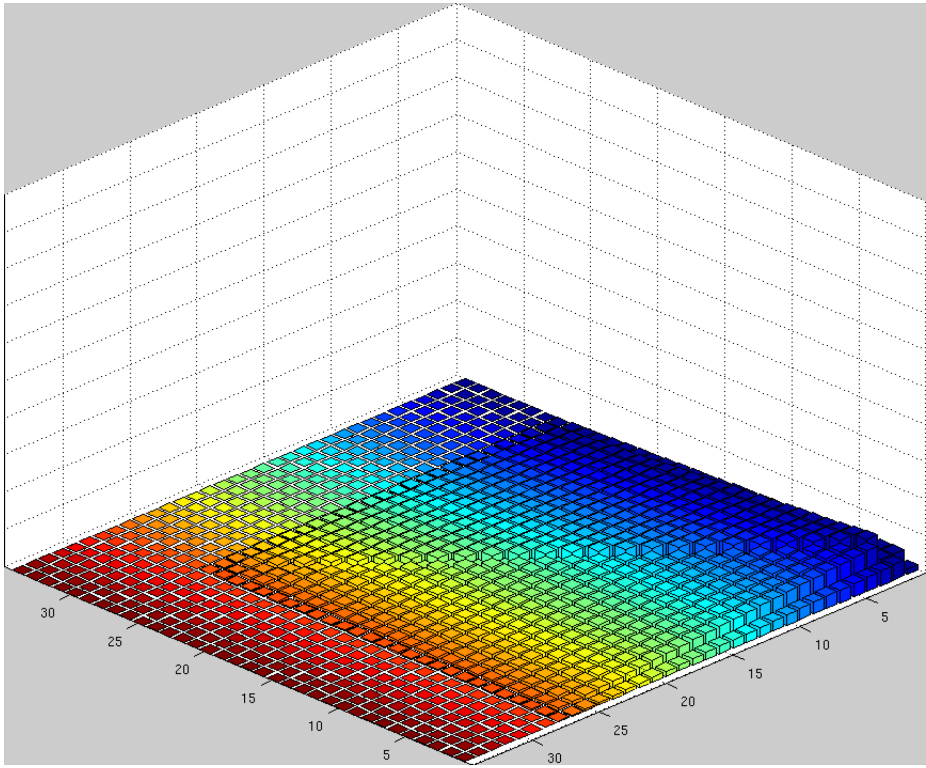
(Master Equation)

- Unexpected behavior
  - ⋆ Issue
    - – If two motors or motors are attached their center of mass is always faster (in average) than a single motor
    - – If all but one motor detach, the one still attached is still as fast as a single motor
    - – Why, for low loads, more motors are slower than one single motor?
  - ⋆ Possible explanation
    - – This can be explained by backward reattachments
    - – The vanguard motors are relatively more likely to detach
    - – The phenomenon does not occur at high loads because at high loads motors tend to clusterize and share the load

Marching or a stroll

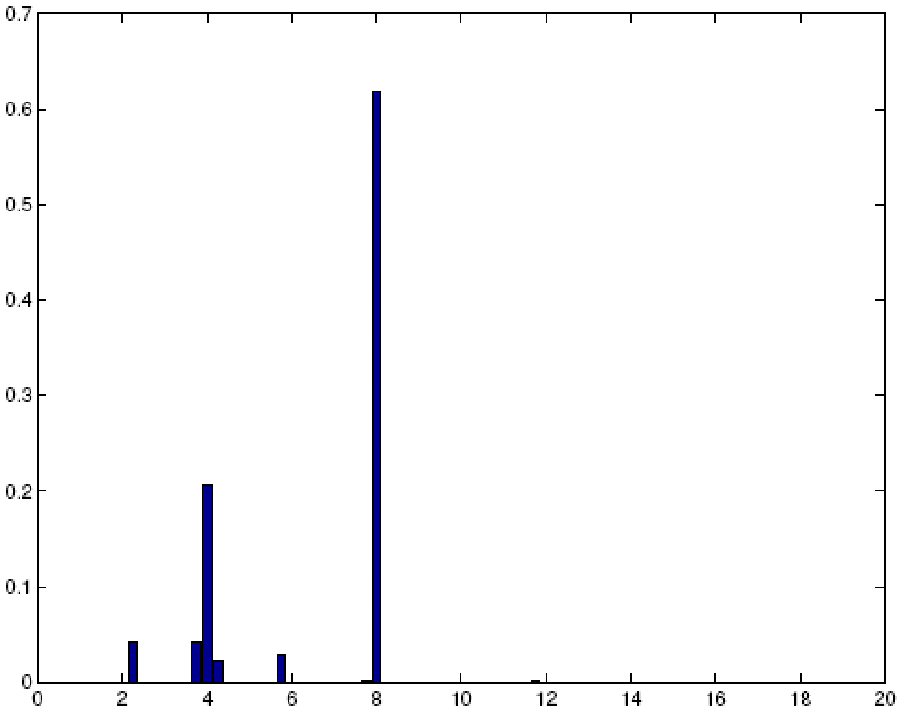


Low Load

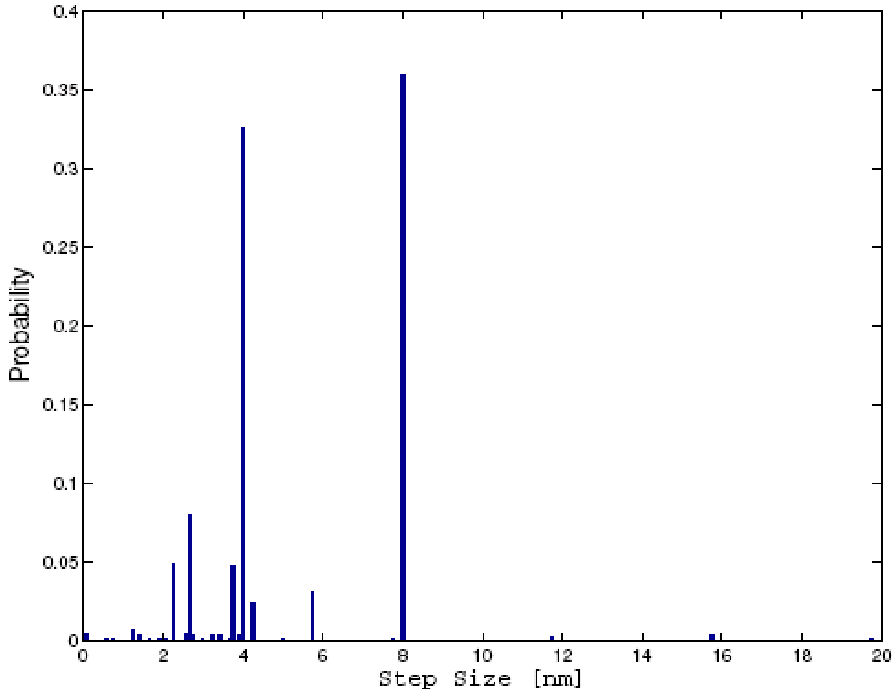


High load

PDF of Step Size



(two motors)



(three motors)

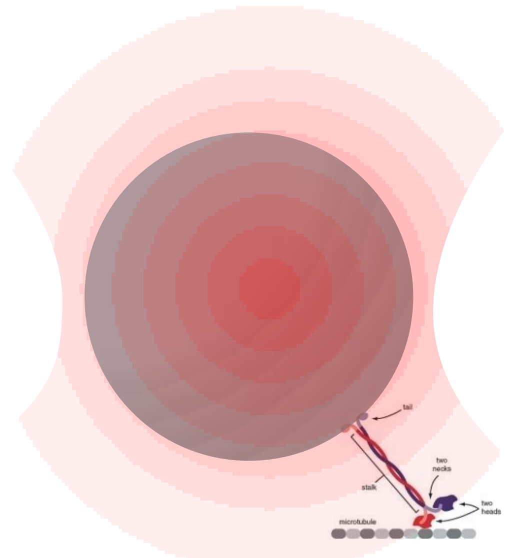
- Small probability of a $11.5nm$ step
- Detachment of a reargard motor

New Insights

- We can obtain insights about the transient dynamics
  - How fast can the motors reach steady state?
  - Attachment/Detachment are less frequent events
  - Is it true that the number of engaged motors defines at each time the behaviour of the system?
- We can obtain insights on **rare events**
  - Rare steps can be found
  - Possible explanation of jumps in [Leduc et. al]?
  - Better runlength when close to stall? Verifiable?

High Bandwidth Step Detection Method

Tanuj Aggarwal



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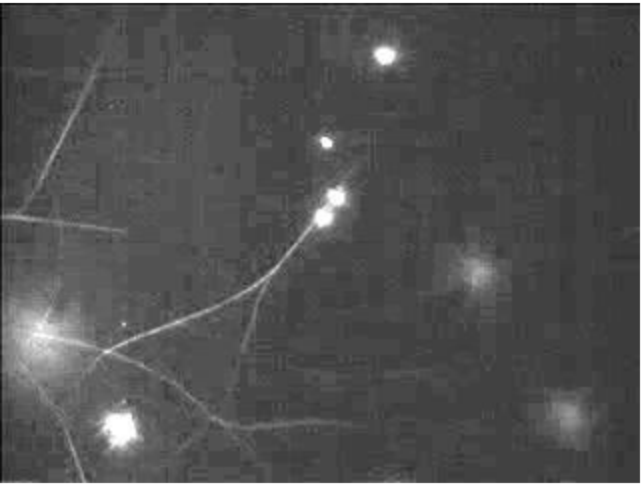
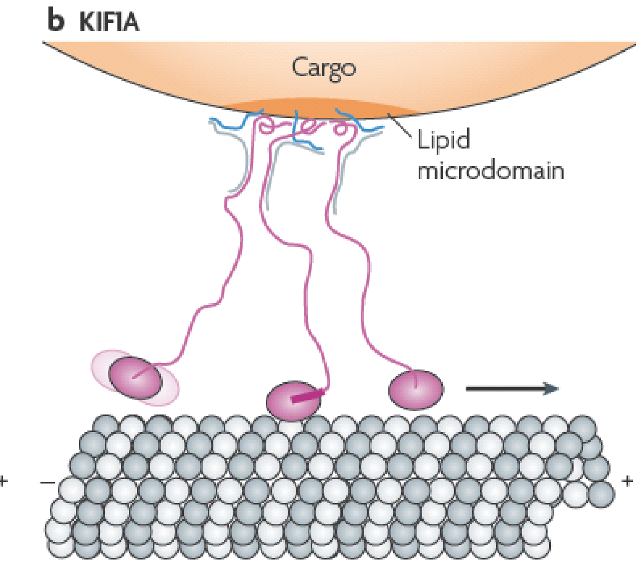
Kinesin Walk





- Kinesin moves in discrete steps
  - ⋆ Steps of 8nm
- Multiple Kinesin can carry the same cargo
  - ⋆ sychronous vs asynchronous behavior
  - ⋆ Phase Transitions
  - ⋆ Rare modalities
  - ⋆ Mutated Kinesin (disease causing)

Challenges

- Higher bandwidth operation
  - ⋆ Motivation
    - – Kinesin walk occurs in vivo often over 1 $\mu m/s$
    - – Kinesin walk deliberately slowed to approximately 25nm/s by lower ATP concentration
    - – Kinesin dynamics remains elusive at higher speeds
- Resolution
  - ⋆ A typical setup achieves $<$ 5 nm at 40-50 Hz
    - – Multiple Kinesin molecules driving a load can take smaller steps (<4 nm) at 1 $\mu m/s$

Challenges

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  - ⋆ A typical setup achieves $<$ 5 nm at 40-50 Hz
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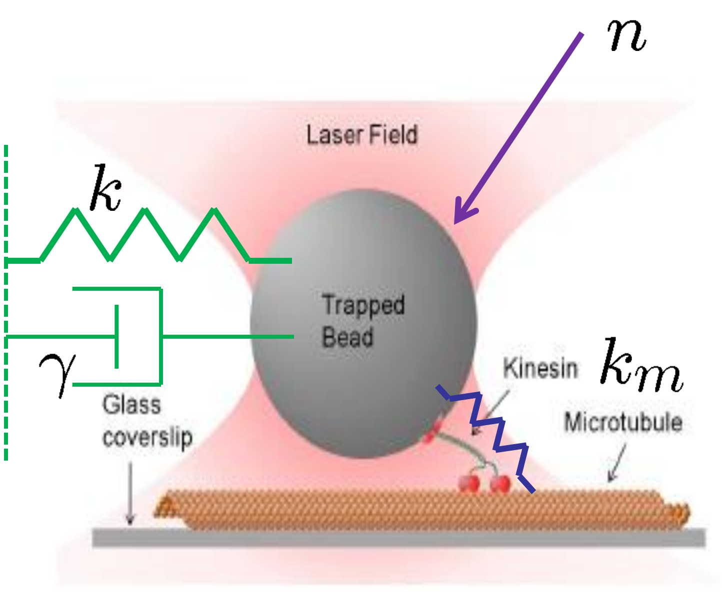
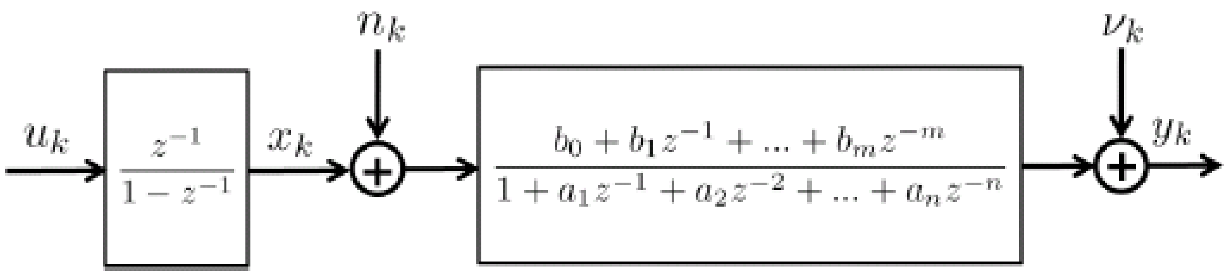
Step Detection



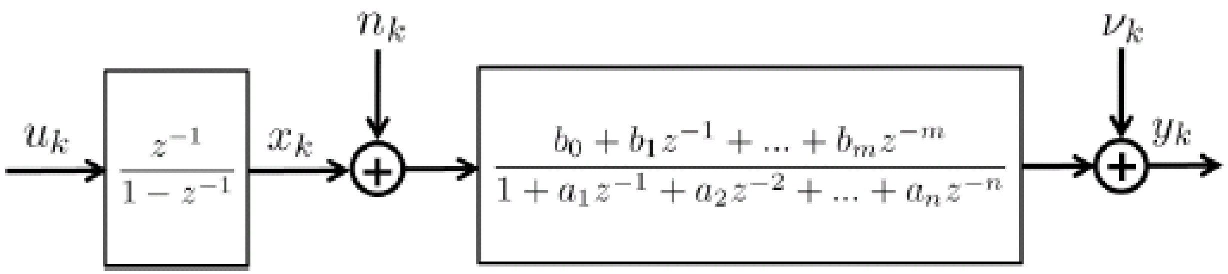
- Model
  - ⋆ Process noise is thermal
  - ⋆ Optical trap modeled as a Hookean Spring
  - ⋆ Damping is substantial; inertial effects negligible
  - ⋆ Kinesin motor modeled as a spring
  - ⋆ Bead position is measured
    - Measurment noise is ν.
  - ⋆ Dynamics
    - $\gamma \dot{x}_b + kx_b = k_m(x_m - x_b) + n$
  - ⋆ Measurement
    - $y = x_b + \nu$.

Step Detection



- Model
  - ⋆ $x_{k+1} = x_k + u_k$. (u_k denotes a step; a prior for u_k is assumed)
  - ⋆ $x_1^N = \{x_1, \ldots, x_N\}$
- Problem: Solve for
$$\hat{x}_1^N = \text{arg max}_{x_1^N} \ [p_{x_1^N|y_1^N}(x_1^N|y_1^N)].$$
- One can show that
$$\hat{x}_1^N = \text{arg max}_{x_1^N} \left[\sum_{i=1}^{N} (y_k - \bar{x}_k + \bar{y}_{k+1})^2 + W(u_k) \right].$$
  Prior
  - ⋆ $\bar{x}_k := \sum_{i=1}^{m} b_i x_{k-i}$, $\bar{y}_k = \sum_{j=1}^{l} a_j y_{k-j}$. $W(u_k) = -2\bar{\sigma}_n^2 \log p(u_k)$.

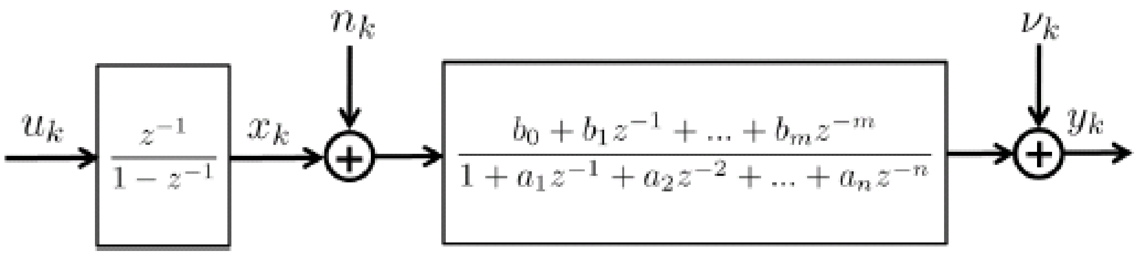
Step Detection



Quadratic term *Step Penalty*

- $$\hat{x}_1^N = \text{arg max}_{x_1^N} \left[\sum_{i=1}^{N} (y_k - \bar{x}_k + \bar{y}_{k+1})^2 + W(u_k) \right].$$
  - ⋆ $\bar{x}_k := \sum_{i=1}^{m} b_i x_{k-i}$, $\bar{y}_k = \sum_{j=1}^{l} a_j y_{k-j}$. $W(u_k) = -2\bar{\sigma}_n^2 \log p(u_k)$.
  - ⋆ Problem is equivalent to minimizing a quadratic cost with penalty for steps
  - ⋆ Relation to "compressive sensing" issues

Step Detection

- 

$$\hat{x}_1^N = \text{arg max}_{x_1^N}\left[\sum_{i=1}^{N}(y_k - \bar{x}_k + \bar{y}_{k+1})^2 + W(u_k)\right].$$

  - ⋆ $\bar{x}_k := \sum_{i=1}^{m} b_i x_{k-i}$, $\bar{y}_k = \sum_{j=1}^{l} a_j y_{k-j}$. $W(u_k) = -2\bar{\sigma}_n^2 \log p(u_k)$.
  - ⋆ Let $s_k := [x_k, \ldots, x_{k-m+1}]'$. Then

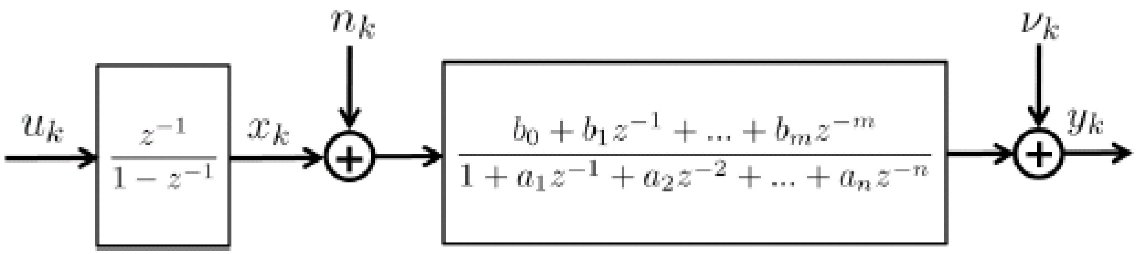
$$\hat{x}_1^N = \text{arg max}_{x_1^N}\sum_{k=0}^{N-1} g_k(s_k, u_k).$$

- All pole model then

$$g_k(s_k, u_k) = (y_{k+1} - b_0 x_k - b_0 u_k + \sum_{j=1}^{\ell} y_{k+1-j})^2$$

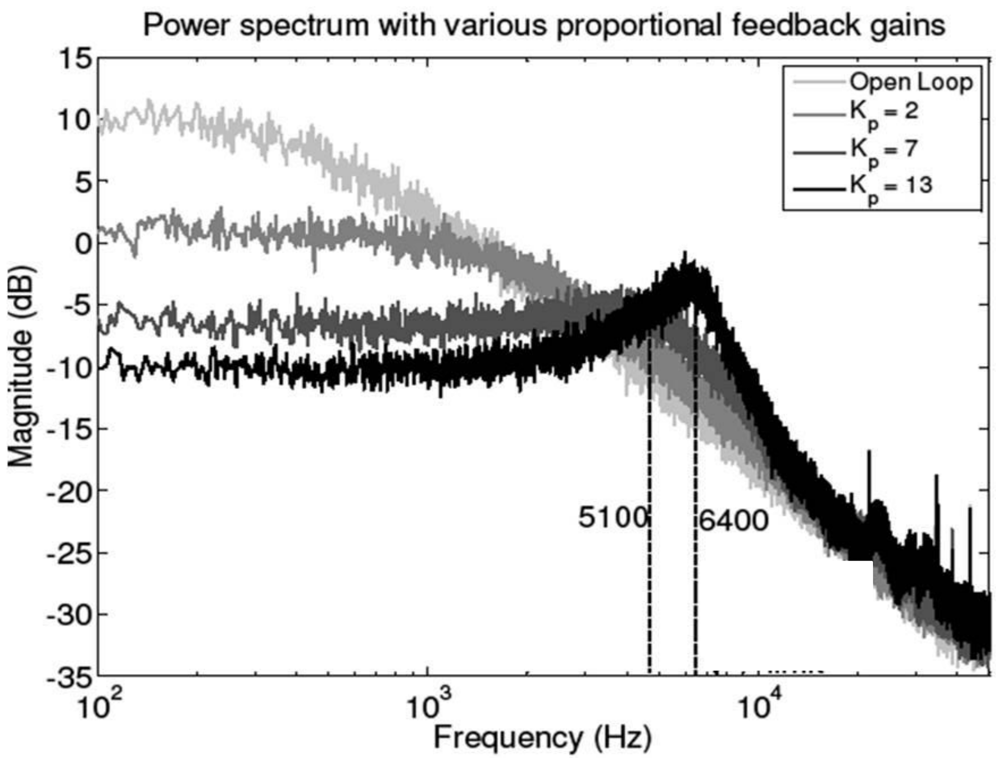
  - ⋆ Tractable Dynamic Programming Problem

Pdf Updation using Priors



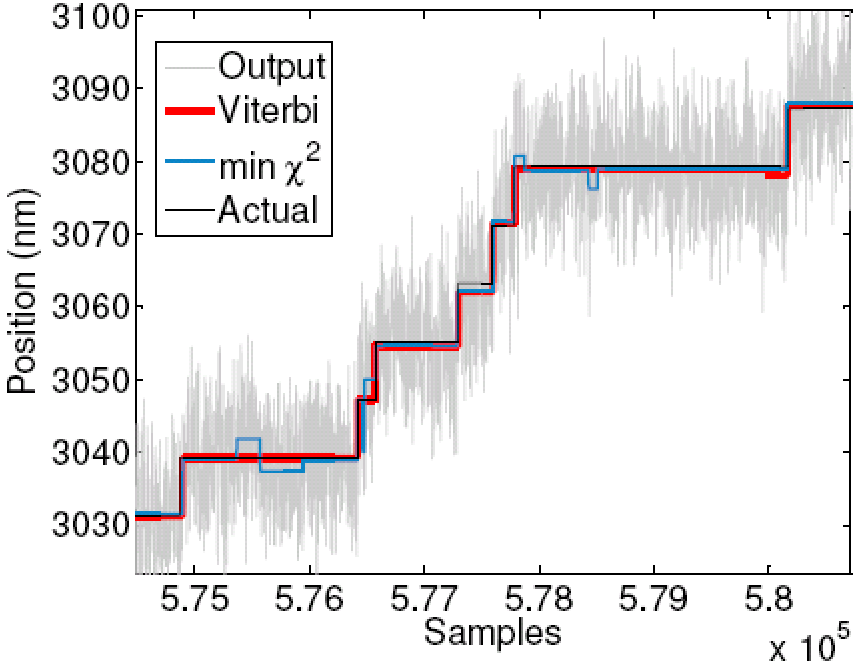
- $$\hat{x}_1^N = \text{arg max}_{x_1^N} \left[\sum_{i=1}^{N} (y_k - \bar{x}_k + \bar{y}_{k+1})^2 + W(u_k) \right].$$
  - ⋆ $\bar{x}_k := \sum_{i=1}^{m} b_i x_{k-i}$, $\bar{y}_k = \sum_{j=1}^{l} a_j y_{k-j}$.
- All pole model then
  $$g_k(s_k, u_k) = (y_{k+1} - b_0 x_k - b_0 u_k + \sum_{j=1}^{\ell} y_{k+1-j})^2$$
- Update Prior based on previous iteration
  - ⋆ $W(u_k) = -2\bar{\sigma}_n^2 \log p(u_k)$.

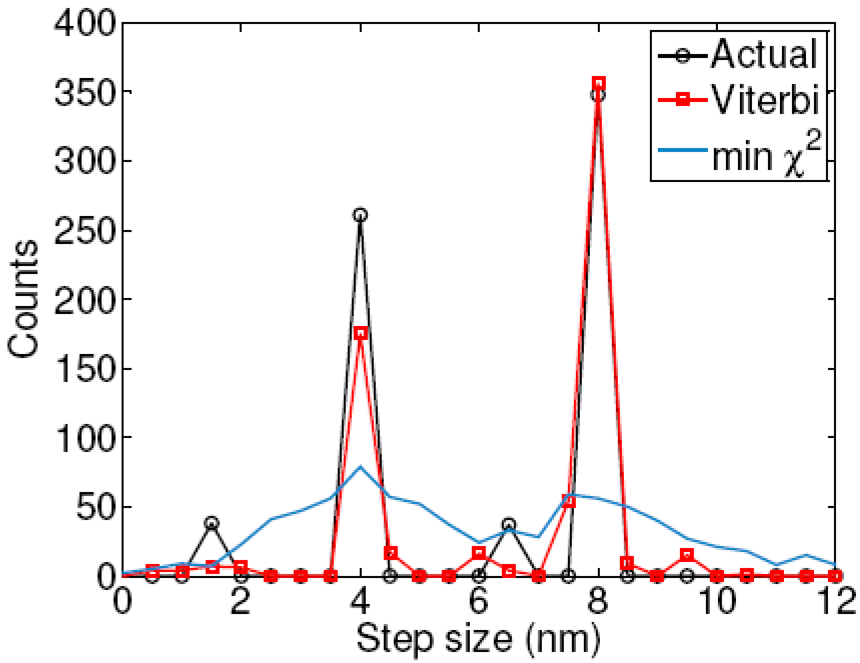
Anomalous Behavior



- $\frac{X(s)}{\eta(s)} = \frac{1}{\beta s + k(1+k_p)}$
  - Model when implemented experimentally does not work
  - Systems model
  - Assess the input-output model
    - Not first principles based

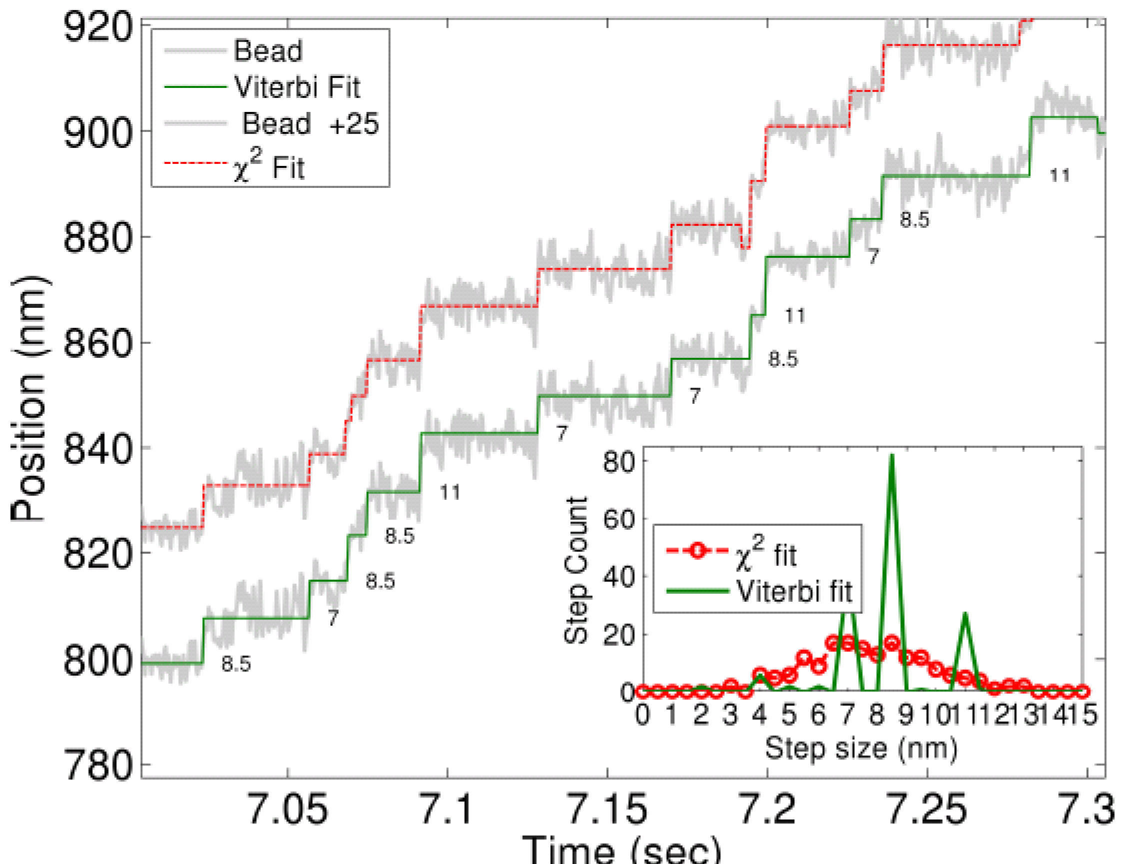
Step Detection





- Advanced step detection in place to hone into 4nm substeps
- Work to asses error probailities being undertaken

Experimental data



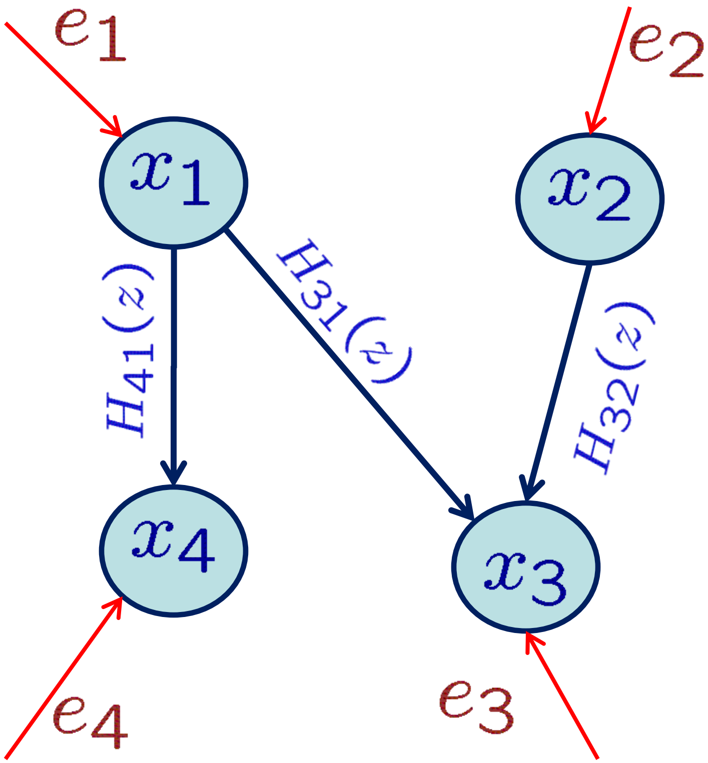
- Advanced step detection in place to hone into 4nm substeps
- Work to asses error probailities being undertaken

Network Topology Reconstruction

NanoDynamics Systems LAboratory

Dr. Donatello Materasski

Framework



$$x_3 = H_{32}x_2 + H_{31}x_1 + e_3$$

- Assumed Underlying Model
  - ⋆ $x_j = \sum_{i=1}^{N} H_{ji}(z)x_i + e_j$.
    - x_j is the node value,
    - e_j is noise affecting each node
- Given the time series data $x_j(t),\ t \in R$ for all $j = 1, \ldots, N$ identify if $H_{ji} = H_{ij} = 0$.
  - ⋆ Reconstruction of the undirected graph
- No assumptions on the graph's edges
  - ⋆ Loops are allowed

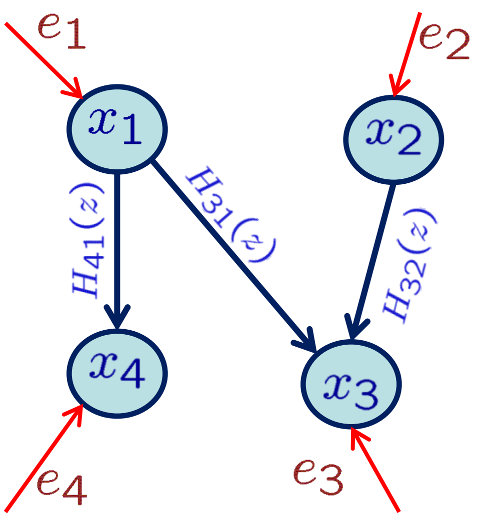
Strategy

- ⋆ Project x_j on the $M_j :=$ tf-span$\{x_1, x_2, \ldots, x_{j-1}, x_{j+1}, \ldots, x_N\} \subset H$.

  ⋆ Find
  - $\widehat{x}_j = \sum_{i \neq j} W_{ji}(z) x_i$
  - $(x_j - \widehat{x}_j) \perp M_j$

  ⋆ It turns out that $W_{ji} \neq 0$ only if i is a kin of j

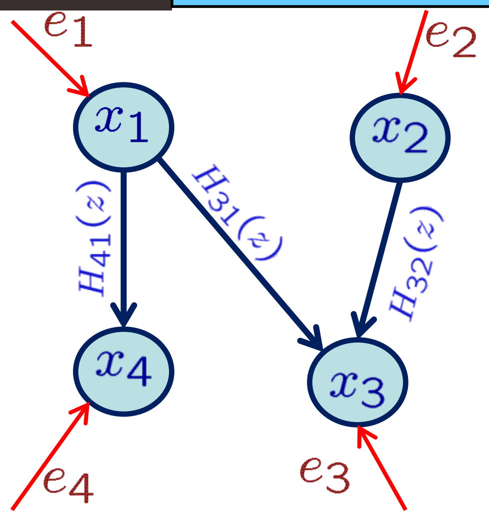
Kins



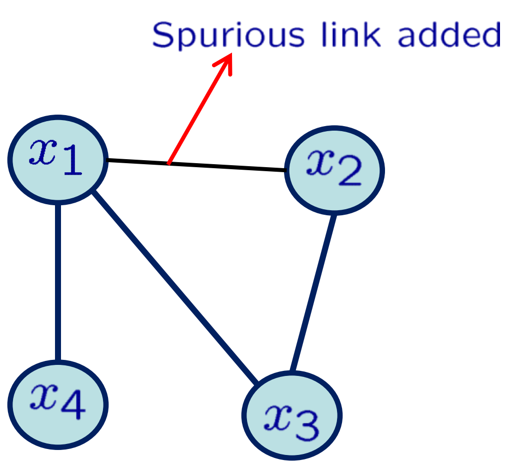
$$x_3 = H_{32}x_2 + H_{31}x_1 + e_3$$

- Assumed Underlying Model
  - ⋆ $x_j = \sum_{i=1}^{N} H_{ji}(z)x_i + e_j$.
    - x_j is the node value,
    - e_j is noise affecting each node
- Kins
  - ⋆ Consists of parents, children and co-parents
    - x_1 is a parent of x_3, x_4 is a child of x_1.
    - x_2 is a co-parent of x_1 (with child x_3).
    - Kins of x_1 are x_3, x_1 and x_2.

Kins have all the information

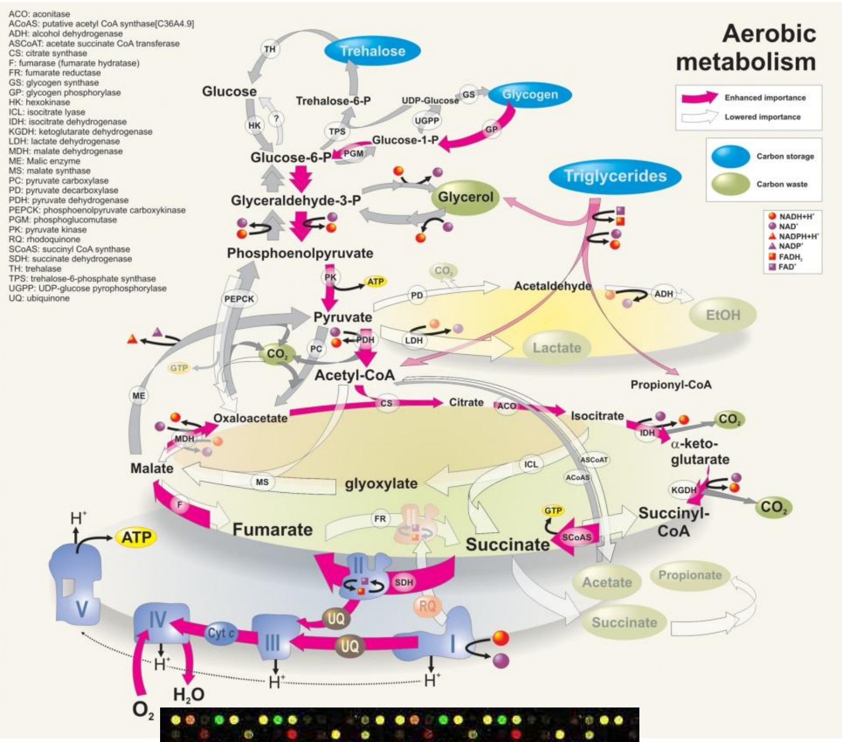


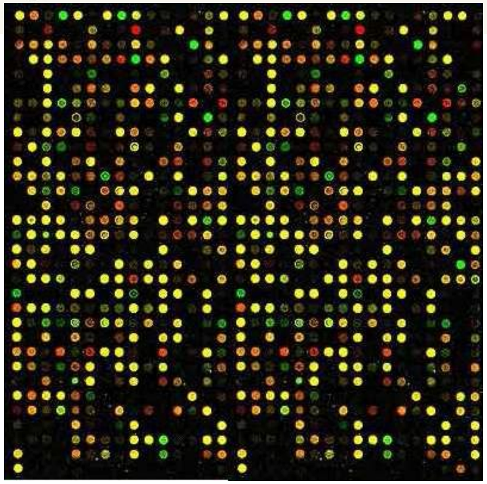
$$x_3 = H_{32}x_2 + H_{31}x_1 + e_3$$



- Projection on Kins
  - ⋆ Let $\mathcal{K}_j$ be the tf-span of kins of j and let $\widehat{x}_j'$ be the projection of x_j on $\mathcal{K}_j$
    - – $(x_j - \widehat{x}_j') \perp \mathcal{K}_j$
  - ⋆ $\widehat{x}_j$ is the projection on tf-span$\{x_i\}_{i \neq j}$.
- Theorem: $\widehat{x}_j' = \widehat{x}_j$
  - ⋆ $\widehat{x}_j = \sum_{i \neq j} W_{ji} x_i = \sum_{i \in K_j} W_{ji} x_i$.
  - ⋆ Projection on all nodal values=Projection on Kins
- Can be used to reconstruct topologies
  - ⋆ $$W_j(\omega) = \phi_{x_j x_{jc}}(\omega)\phi^{-1}_{x_{jc} x_{jc}}(\omega).$$
    - – Can be constructed using time-series data
  - ⋆ Spurious Links remain local

Model is Good!



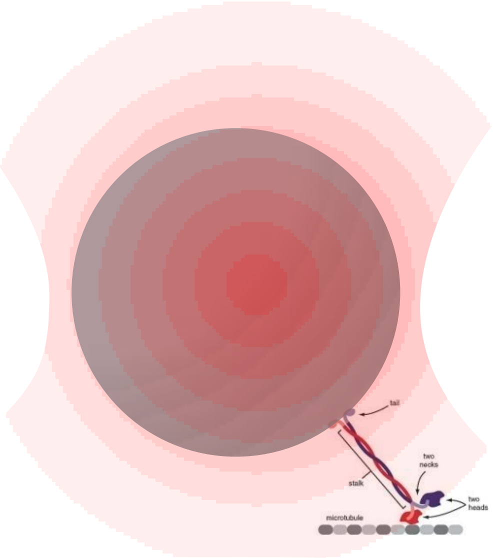


- Assumed Underlying Model
  - ⋆ $x_j = \sum_{i=1}^{N} H_{ji}(z) x_i + e_j.$
    - x_j is the node value,
    - e_j is noise affecting each node
- Above model underlying many systems
  - ⋆ Correctly reconstructs topology in gene-networks, financial data etc

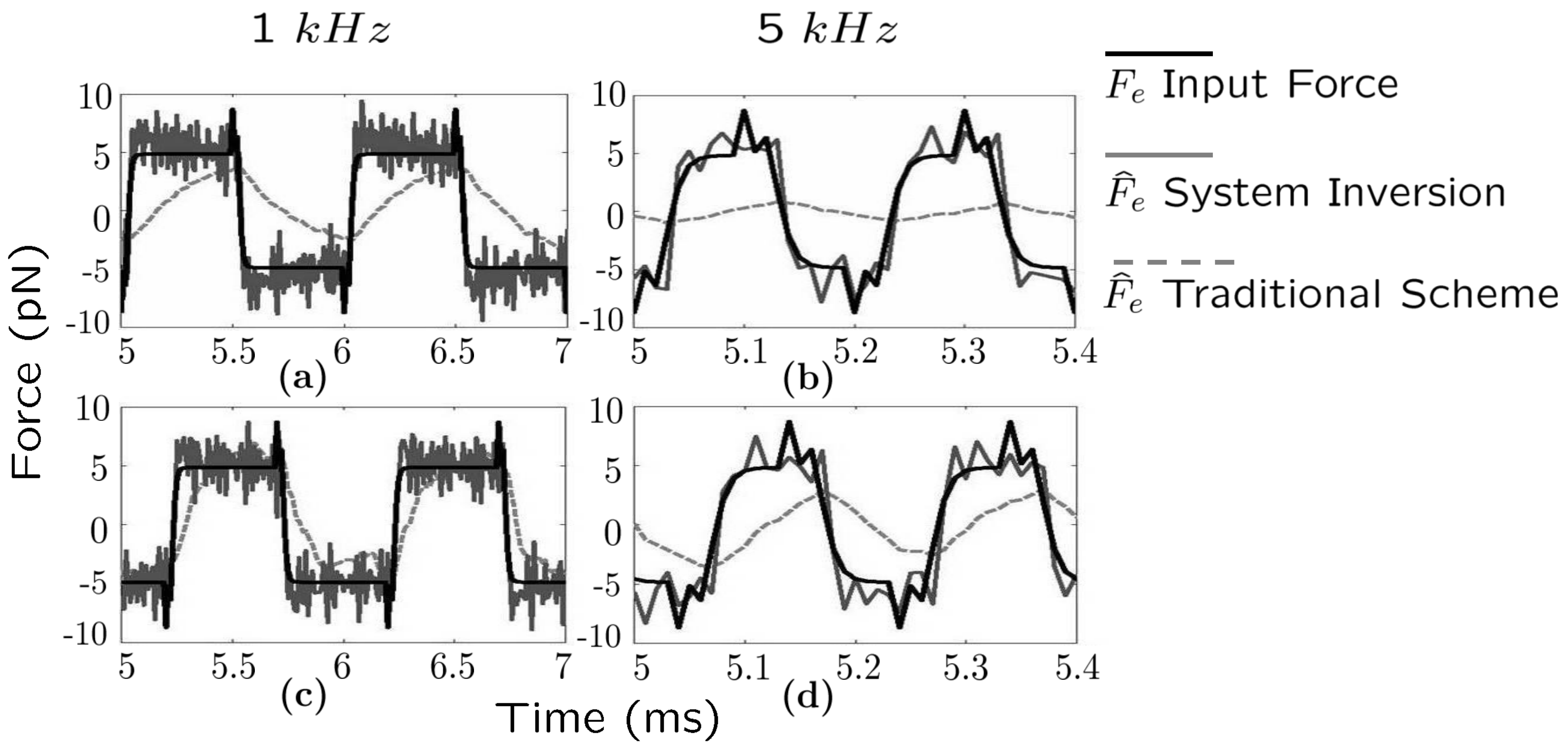
Conclusions

- Exciting times as nanoscale biological functioning units now accessible for probing and engineering
  - ⋆ Huge opportunities to further open up newer temporal and spatial regimes
    - – Robust Control techniques are in place
- Experiments need to be driven by hypothesis to be provided by simulation engines
  - ⋆ Need for innovaton for simulation capabilities of realistic cases
    - – Five to six motor configurations can be simulated in "closed-form" on a PC
- Experiments
  - ⋆ Quote
    - – Do not think too much. Do the experiment. JUST DO IT
  - ⋆ A new technique to probe sub 5 nm steps developed

Systems model and robust control design for isometric clamp

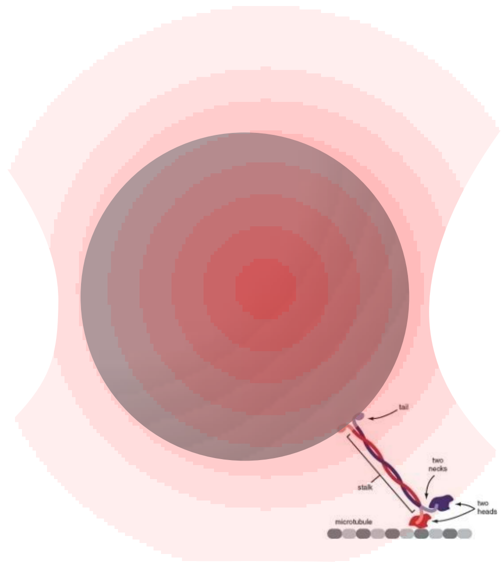


Experimental Results

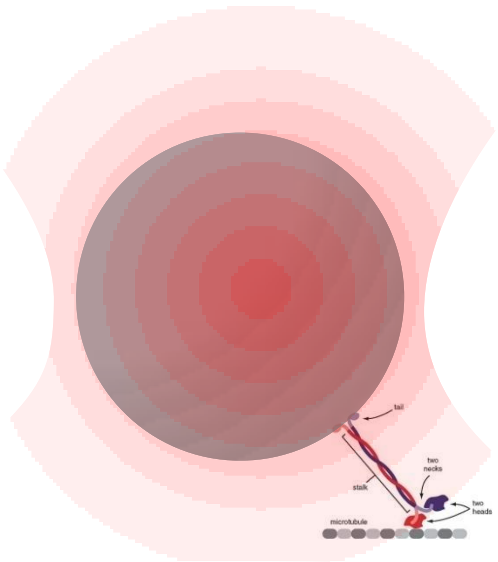


- Open Loop results in (a) and (b). Closed Loop results in (c) and (d).
- More than an order in magnitude of bandwidth increase in force estimation

Stochastic Simulation Engine



Future Objectives



Instrumentation Objectives

- Instrumentation objectives
  - ⋆ Robust control techniques for isotonic clamps of optical tweezers to achieve an order increase in estimation of motor movement
  - ⋆ Simultaneous x,y,z control of the cargo
  - ⋆ Resolution enhancements to detect 1nm steps at 1 Khz bandwidth or more; this will enable experimental corroboration for four kinesin motors attached to the same cargo
  - ⋆ A enhanced FPGA based hardware platform to implement controllers
- Instrumentation objectives
  - ⋆ Enhanced CCD camera
  - ⋆ DIC optics
  - ⋆ Microfluidic channels to induce flows (nutrient supplies)
  - ⋆ Enhanced nanopositioning stage

Biological Objectives

- Modeling
  - ⋆ Enhanced Stochastic Simulation engine and integration with the supercomputing facility
    - Study of cooperative behavior amongst similar motors arraying cargo as well as different species carrying cargo
    - Study of changing parameters of a fraction of attached motors to simulate diseased motor
    - Study of transient behavior and related time scales
    - Links with equilibrium statistical mechanics
    - Study of control actions based on modalities of cargo movement
    - Integration with experimental results
    - Thermal ratchet studies

- Experimental objectives
  - ⋆ Higher bandwidth instrumentation for studying Kinesin stepping for realistic ATP concentrations and other parameters.
  - ⋆ Methods to attach and discern mutiple Kinesin motors
  - ⋆ Methods for mutant Kinesin molecules to be made and to study their processivity
  - ⋆ Develop dynein model system
  - ⋆ Develop methods to attach Dynein and Kinesin to the same cargo
  - ⋆ Study tug-of-war issues on how cargo is transported
  - ⋆ Discern ways to see if the thermal ratchet is the means of movement or is it a power stroke or both
  - ⋆ Controls to increase efficiency of energy conversion

Ensemble Representation

- Projection
  - ⋆ The way of coding the configurations is critical
  - ⋆ Examples with a trivial code
    - – $n = 20$, $m = 4$, detach, then $N = 160,000$
  - ⋆ Examples with a "smart" code
    - – $n = 20$, $m = 4$, no detach, then $N = 1,540$
    - – $n = 20$, $m = 4$, detach, then $N = 1,771$
    - – $n = 30$, $m = 4$, no detach then $N = 4,960$
    - – $n = 40$, $m = 4$, no detach, then $N = 11,480$
    - – $n = 40$, $m = 4$, detach, then $N = 12,302$
  - ⋆ The number m of motor is a smaller concern
  - ⋆ Low stiffness requires high n
  - ⋆ There are techniques to tackle the problem reducing the dimensionality at low cost for the accuracy