Summer 2009

Single-Molecule Biophysical Assays With Electrokinetics

Mark Arsenault
University of Pennsylvania, marsenau@seas.upenn.edu

Follow this and additional works at: http://repository.upenn.edu/edissertations
Part of the Applied Mechanics Commons, and the Biophysics Commons

Recommended Citation

This paper is posted at ScholarlyCommons. http://repository.upenn.edu/edissertations/6
For more information, please contact repository@pobox.upenn.edu.
Single-Molecule Biophysical Assays With Electrokinetics

Abstract
Molecular motors and actin filaments play critical roles in disease processes and, more generally, in cell motility. The thesis developed new techniques to study the mechanical and electrical properties of actin filaments and the motion of motor proteins unhindered by nearby surfaces. Devices consisting of a pair of electrodes separated with a small gap were patterned on a glass wafer. With the application of AC electric fields, rhodamine-phalloidin-labeled actin filaments were polarized, attracted to the gap, and became suspended across the two electrodes. The thermal fluctuations of the suspended actin filaments were imaged, and the filament’s tension was estimated by comparing the experimental observations with theoretical predictions of a linear, Brownian dynamics model for filament thermal vibrations. The filament’s tension increased linearly with the electric field intensity-squared. The Brownian dynamics theory was verified by carrying out a set of experiments in which forces were controllably applied to the filaments with optical traps. The theoretically-estimated forces were compared and agreed with the applied forces. The optical trap experiments highlighted the importance of selecting appropriate exposure times to avoid biasing the experimental position data. Furthermore, optical tweezers were used to bring a motor protein-coated bead into close proximity with a pre-selected, suspended actin filament, facilitating the myosin-mediated bead attachment to the filament. The clearance beneath the filament allowed the bead to move freely along and around its filamentous track. The bead’s three-dimensional position was tracked as a function of time to obtain its trajectory. The combined use of electrical and optical tweezers provides a new and convenient means to study motor motility. Variants of this technique would enable studies of motor motility in the presence of filaments’ networks such as found in cells. Finally, the persistence lengths of free-floating filaments were measured as a function of their solution’s ion concentration. Preliminary observations were made of free floating filaments orienting and straightening in spatially uniform, AC electric fields. The thesis contributes to biophysics by providing new means to manipulate filaments and motor proteins and by contributing to our understanding of the effects of electric fields on actin filaments.

Degree Type
Dissertation

Degree Name
Doctor of Philosophy (PhD)

Graduate Group
Mechanical Engineering & Applied Mechanics

First Advisor
Dr. Haim H. Bau

Second Advisor
Dr. Yale E. Goldman

This dissertation is available at ScholarlyCommons: http://repository.upenn.edu/edissertations/6
SINGLE-MOLECULE BIOPHYSICAL ASSAYS WITH ELECTROKINETICS

Mark Arsenault

A DISSERTATION

in

MECHANICAL ENGINEERING AND APPLIED MECHANICS

Presented to the Faculties of the University of Pennsylvania

in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

2009

Professor Haim H. Bau
Supervisor of Dissertation

Professor Yale E. Goldman
Supervisor of Dissertation

Professor Pedro Ponte Castañeda
Graduate Group Chairperson
Acknowledgements

I would like to thank the University of Pennsylvania and the Mechanical Engineering and Applied Mechanics Department for the great opportunity to undertake this thesis. My advisors Drs. Haim H. Bau and Yale E. Goldman deserve a great deal of credit for giving me encouragement, great scientific insight, and for being excellent examples of scientific mentors.

I would like to thank the other members of my thesis committee: Drs. Dennis Discher and Prashant Purohit. It was a special honor to be the first Ph.D. student for whom Prashant Purohit played a large role advising. Other mentors who I would like to thank are Drs. Henry Shuman, E. Michael Ostap, and Howard H. Hu.

I would like to thank the members of the Bau and Goldman labs and also Dr. Brian Edwards. They were a constant source of support, advice, and camaraderie. Special thanks is reserved for Dr. Hui Zhao with whom, since our first days as fellow Ph.D. students, I have become great friends.

I thank the NSF, the NIH, and UPENN’s NBIC for their financial support.

Finally, I would like to thank and dedicate this thesis to my parents, my sister, and my wonderful wife who have supported me for the past several years. This would not have been possible without them.
ABSTRACT

Single-Molecule Biophysical Assays with Electrokinetics

Mark Arsenault

Advisors: Drs. Haim H. Bau & Yale E. Goldman

Molecular motors and actin filaments play critical roles in disease processes and, more generally, in cell motility. The thesis developed new techniques to study the mechanical and electrical properties of actin filaments and the motion of motor proteins unhindered by nearby surfaces. Devices consisting of a pair of electrodes separated with a small gap were patterned on a glass wafer. With the application of AC electric fields, rhodamine-phalloidin-labeled actin filaments were polarized, attracted to the gap, and became suspended across the two electrodes. The thermal fluctuations of the suspended actin filaments were imaged, and the filament’s tension was estimated by comparing the experimental observations with theoretical predictions of a linear, Brownian dynamics model for filament thermal vibrations. The filament’s tension increased linearly with the electric field intensity-squared. The Brownian dynamics theory was verified by carrying out a set of experiments in which forces were controllably applied to the filaments with optical traps. The theoretically-estimated forces were compared and agreed with the applied forces. The optical trap experiments highlighted the importance of selecting appropriate exposure times to avoid biasing the experimental position data. Furthermore, optical tweezers were used to bring a motor protein-coated bead into close proximity with a pre-selected, suspended actin filament, facilitating the myosin-mediated bead attachment to the filament. The clearance beneath the filament allowed the bead to move
freely along and around its filamentous track. The bead's three-dimensional position was tracked as a function of time to obtain its trajectory. The combined use of electrical and optical tweezers provides a new and convenient means to study motor motility. Variants of this technique would enable studies of motor motility in the presence of filaments’ networks such as found in cells. Finally, the persistence lengths of free-floating filaments were measured as a function of their solution’s ion concentration. Preliminary observations were made of free floating filaments orienting and straightening in spatially uniform, AC electric fields. The thesis contributes to biophysics by providing new means to manipulate filaments and motor proteins and by contributing to our understanding of the effects of electric fields on actin filaments.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>LIST OF TABLES AND FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>Chapter 1. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Motivation</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Actin Filaments</td>
<td>4</td>
</tr>
<tr>
<td>1.2.1 Mechanical Properties</td>
<td>5</td>
</tr>
<tr>
<td>1.2.2 Electrical Properties</td>
<td>7</td>
</tr>
<tr>
<td>1.3 Molecular Motors</td>
<td>11</td>
</tr>
<tr>
<td>1.4 Organization of Dissertation</td>
<td>13</td>
</tr>
<tr>
<td>Chapter 2. Estimating the Force Applied to an Actin Filament by Optical Tweezers with a Brownian Dynamics Model</td>
<td>15</td>
</tr>
<tr>
<td>2.1 Introduction</td>
<td>16</td>
</tr>
<tr>
<td>2.2 Materials and Methods</td>
<td>17</td>
</tr>
<tr>
<td>2.2.1 Optical Tweezers</td>
<td>18</td>
</tr>
<tr>
<td>2.3 Mathematical Model</td>
<td>23</td>
</tr>
<tr>
<td>2.3.1 Filament’s Equilibrium Shape</td>
<td>23</td>
</tr>
<tr>
<td>2.3.2 Filament’s Thermal Fluctuations</td>
<td>27</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

1.1 A eukaryotic cell with the nuclei stained blue, the microtubules stained green, and the actin filaments stained red (http://rsb.info.nih.gov/ij/images/). .. 2

1.2 A cartoon depiction of the three families of molecular motors: kinesin, myosin, and dynein (Vale 2003).......................................................... 3

1.3 A gliding filament assay, where the myosin motors are bound to the surface, and the actin filaments “crowd surf” on top. .................................................. 4

1.4 A cartoon depiction of the electric double layer (EDL) surrounding an ellipsoidal particle......................................................................................... 7

1.5 Two examples of single-molecule biophysical assays (a) fluorescently-labeled myosin motors walk along surface-immobilized actin filaments (b) the Three Bead Assay – an actin “dumbbell” is brought into close proximity to a bead-immobilized myosin motor where it is permitted to interact ............. 11

2.1 A schematic depiction of the experimental set-up and the coordinate system. The beads are ~1 µm in diameter............................................................ 18

2.2 The power spectrum of an optically trapped, 1-µm diameter bead undergoing Brownian motion. The solid line corresponds to equation (2.1) with $\alpha=0.017$ pN/nm, $f_c=280$ Hz. ............................................................. 20

2.3 The position of two optically-trapped beads (solid lines) superimposed on the microscope stage’s position (dashed line) as it oscillates as a triangle wave (reduced by a factor of 20 for clarity). The two signals with different amplitudes correspond to two beads residing in different traps and having different stiffnesses. Upon fitting to equation (2.6), we calculate the two trap stiffnesses $\alpha=0.04$ & $0.1$ pN/nm. A different bead was used in Fig. 2.2........... 22

2.4 The equilibrium shapes of filaments (0<$s$<1) when $m = 0.2$ (dashed line), 2 (solid line), and 20 (dotted line). $a/L = 0.1$. The three circles depict the
position of the bead when \( m = 0.2 \) (dashed line), 2 (solid line), and 20 (dotted line). Symmetric boundary conditions are applied to the filament.

2.5 Comparison between the exact (solid line – equation (2.11)) and approximate (circles – COMSOL) solutions; \( m = 5 \) (squares), \( m = 10 \) (circles), \( m = 20 \) (triangles).

2.6 The discrepancy between the experimentally-observed and the theoretically-estimated variance \( E(\tau) \) as a function of the filament’s tension \( \tau \).

2.7 The correction factor \( S(t_e) \) as a function of the normalized exposure time \( t_e / \hat{t} \) when \( F_1(t) = \sin(\omega t) \) and \( F_{n>1} = 0 \). \( \lambda = 4 \), \( m = 1 \), and \( \omega = 1 \). The solid, dashed, and dotted lines correspond, respectively, to the exact calculation, a fit obtained with equation (2.42), and a fit obtained with equation (2.43).

2.8 A filament’s transverse displacement’s variance depicted as a function of position along the filament for several camera exposure times 75 (solid circles), 200 (hollow circles), and 300 (stars) ms. The various lines correspond to theoretical fits with hinged-hinged boundary conditions.

2.9 The normalized, experimental variance is depicted as a function of the camera exposure time. The upright triangles, solid squares, and solid circles correspond, respectively, to \{ \( L \), \( \tau_{\text{applied}} \), \( \eta \) \} = \{17 \mu m, 0.26 \text{ pN}, 1 \text{ mPa-s} \}, \{12 \mu m, 1.2 \text{ pN}, 10.8 \text{ mPa-s} \}, \{15 \mu m, 0.27 \text{ pN}, 10.8 \text{ mPa-s} \}. In the above, \( L \) represents the filament’s length, \( \tau \) represents the applied tension, and \( \eta \) represents the suspension’s viscosity. The variance was normalized with the variance measured at the smallest exposure time. The solid, dotted, and dashed lines correspond to the best fits of equation (2.43), respectively.

2.10 The average width of a 16.6 \( \mu \text{m} \)-long fluorescent filament’s intensity profile across its transverse axis depicted as a function of exposure time (means ± s.d.).

2.11 A single actin filament’s variance of transverse thermal fluctuations with three different forces being applied (0.22 pN – circles, 0.37 pN – triangles, [...]}
& 0.48 pN - squares) depicted as a function of the position along the filament. The various lines correspond to theoretical fits with hinged-hinged boundary conditions, solved numerically (0.33 pN – solid line, 0.84 pN – dash-dotted line, & 1.5 pN – dashed line). ................................................................. 40

2.12 The force estimated for 24 filaments (in water, \( \eta = 1 \) mPa·s) by the hinged-hinged model, depicted as a function of the applied force. The solid line represents a 1:1 correlation between actual and estimated force values............. 41

2.13 The force estimates using the hinged-hinged model (circles) and the corrected force estimates from fits to variance data adjusted using equation (2.43) (triangles), depicted as a function of the applied force. ......................... 42

2.14 The relative discrepancy between the force estimates and the applied forces as a function of the filaments’ theoretical time constants \((n=1)\). The lines are to guide the eye. ................................................................. 43

2.15 The force estimated for 6 filaments (in glycerin, \( \eta = 10.8 \) mPa·s) by the clamped-clamped model, depicted as a function of the applied force. The solid and hollow circles correspond, respectively, to the prediction of the clamped-clamped and hinged-hinged models. The solid line represents a 1:1 correlation between actual and estimated force values.............................. 44

2.16 The relative discrepancy between the force estimate and the applied force as a function of the filament’s time constant, \( n=1 \) (triangles – in glycerin; circles – in water). The lines are to guide the eye................................. 45

2.17 a) The intensity of a CCD image (symbols) depicted along a line drawn transverse to an actin filament’s long axis. The image’s background was subtracted, and the image was smoothed using a 16-pixel Gaussian smoothing kernel. A Gaussian curve was fitted (line) to obtain the filament’s location and its width (variance - \( \sigma \)). b) The variance of the fitted Gaussians, similar to those depicted in (a), at all of the filaments’ midpoints depicted as a function of the theoretical time constant (squares; red = glycerin, black = water). Also depicted are the midpoint variance values (circles;
red=glycerin, black=water) calculated similarly to the datasets depicted in Fig. 2.11. ................................................................. 46

2.18 The relative discrepancy between the “blur variance” force estimate and the applied force as a function of the filament’s time constant (based on the fundamental mode \( n=1 \)) (squares; red = glycerin; black = water)................. 47

3.1 Schematic depiction of the anodic bonding process. a) a 150 µm-thick glass coverslip was coated with a 100 nm-thick layer of silicon; b) the silicon was patterned in positive resist (Shipley 1813), and the glass was etched further using buffered oxide etch; c) the flow cell was heated to 375 °C, and a 1000 V potential was applied; d) ions in the glass migrated; e) oxygen bound to the silicon................................................................. 52

3.2 Schematic depiction of the electrode fabrication process. a) a 150 µm-thick glass coverslip was coated with a 10 nm-thick layer of nickel-chromium and a 100 nm-thick layer of gold; b) positive resist (Shipley 1813) was spun onto the gold surface; c) the electrodes were patterned, and the metal was removed; d) the glass was etched using buffered oxide etch (for experiments detailed in Chapters 4 & 5); e) the flow chamber is capped either by pressing it into contact with the first coverslip or using double-sided tape (for experiments detailed in Chapters 4 & 5).......................................................... 54

3.3 An actin filament of length \( L \) is described by the curve \( \theta(s) \). Its transverse displacement \( y_f(s) \) can be measured from any arbitrary line defined as the filament’s reference frame. \( \theta(s) \) is the angle formed between the tangent to the filament and the chosen reference line. In this picture, the line connecting the filament’s endpoints is chosen as the reference line. ................. 56

3.4 The first four modes of oscillation, using equation (3.4), depicted as a function of position along the filament. Here the displacement is measured from a line (a) connecting the filament’s endpoints and (b) intersecting the center of mass and parallel to the line connecting the filament’s endpoints. .... 57
3.5  \((<A_{n+1}^2>/<A_n^2>)^{1/4}\) from experiment as a function of \(n\) for one actin filament (symbols). The theoretical ratio \(d_n/d_{n+1}\) is overlaid (solid line). The length of the filament is 6 \(\mu\)m. .......................................................... 58

3.6  Predictions for the variance of a 15 \(\mu\)m-long actin filament, measured from a line connecting its endpoints, using both the beam basis functions (solid line - equation (3.4 and 3.10)) and a cosine basis functions (dotted line - equation (3.11a)). One mode was used to construct each curve.......................... 60

3.7  \((<a_{n+1}^2>/<a_n^2>)^{1/2}\) from experimental measurements of the tangent angle as a function of \(n\) for one actin filament (symbols). The expected scaling \(n/(n+1)\) is overlaid (solid line). The length of the filament is 6 \(\mu\)m. ................. 61

3.8  A single actin filament’s mode amplitudes as a function of time. Each panel represents a single mode. Cosine basis functions were used to perform the Fourier decomposition. The length of the filament is 20 \(\mu\)m. ....................... 63

3.9  The mean-squared mode amplitudes of the transverse displacement as a function of lag time \(\Delta t\) of an 11 \(\mu\)m long filament (\(n = 1\) – asterisks, \(n = 2\) – circles, \(n = 3\) – squares). Fits to each mode’s data (solid lines) are to equation (3.16) with the fitted \(\tau_n = 1400, 230,\) and 73 ms, respectively............ 65

3.10  \(L_p\) depicted as a function of KCl-concentration [KCl], calculated using both the cosine basis functions (triangles, equation (3.12)) and the beam basis functions (circles, equation (3.4)). The horizontal lines are the mean values of the two datasets. The triangles are offset slightly to the right for clarity. Uncertainties are presented as \(\pm 1\) standard deviation. 30 \(\mu\)M (\(n = 8\)), 300 \(\mu\)M (\(n = 6\)), 3 mM (\(n = 6\)), 30 mM (\(n = 3\)), 300 mM (\(n = 13\))................................. 66

3.11  Experimental variance of the transverse displacement (symbols) depicted as a function of position along the filament. Fits are to equation (3.9). The displacement was measured from the line a) connecting the filament’s endpoints (solid line, \(L_p = 15.9\) \(\mu\)m) and b) intersecting the center of mass
and parallel to the line connecting the filament’s endpoints (solid line, \( L_p = 19 \, \mu\text{m} \)).

3.12 \( L_p \) depicted as a function of KCl-concentration [KCl], calculated from minimizing the difference between the theoretical variance estimated by equation (3.9) and the variance of transverse fluctuations from the line connecting the filament’s endpoints (triangles, dashed line = mean) and the line intersecting the filament’s center of mass and parallel to the line connecting the endpoints (circles, dotted line = mean). The triangles are offset just to the right for clarity. Uncertainties are presented as ± 1 standard deviation. 30 \( \mu \text{M} \) (n = 8), 300 \( \mu \text{M} \) (n = 6), 3 mM (n = 6), 30 mM (n = 3), 300 mM (n = 13).

3.13 Experimental root-mean-squared end-to-end distance \( R \) (symbols) depicted as a function of filament length \( L \). Fit is to equation (3.15): (solid line, \( L_p = 29 \, \mu\text{m} \)).

3.14 The predicted change in the total persistence length \( L_t \) (equation (3.18)) as a function of monovalent ion concentration for F-actin (solid line) and DNA (dashed line).

3.15 A time series of a singly-attached fluorescently-labeled actin filament orienting along field lines in a spatially-uniform AC electric field (20 fps).

4.1 The voltage required to trap actin filaments across a 7 \( \mu \text{m} \) gap depicted as a function of solution conductivity according to equation (4.21), \( \sigma_{\text{actin}} = 2 \, \text{S/m} \) (solid line), \( \sigma_{\text{actin}} = 8 \, \text{S/m} \) (dashed line). Gray lines denote \( \sigma_{\text{actin}} \).

4.2 An actin filament’s Clausius-Mossotti factor estimated using equation (4.21): \( \sigma_m = 0.06 \, \text{S/m} \) (solid line), 0.5 S/m (dotted line), 3.25 S/m (dashed line).

4.3 Schematic depiction of AC-Electroosmosis (ACEO) on an electrode pair similar to our geometry.
4.4 Circuit model used to describe our electrode geometry’s polarization when bathed in an electrolyte. $R_E$ – electrolyte resistance, $R_{\text{EDL}}$ – EDL resistance, $C_{\text{EDL}}$ – EDL capacitance, $R_{\text{current}}$ – current measuring resistor, $V_1$ – total voltage, $V_2$ – current, $V_3$ – voltage across electrodes.

4.5 Schematic depiction of the two electrode geometries used to trap actin filaments.

4.6 The average velocity of 880 nm particles being driven by electrothermal flow (from illumination) over a 15 µm distance, in the direction toward the electrode gap.

4.7 The theoretical drag force estimate, obtained using equation (4.27), on a 7 µm-long actin filament by the velocities depicted in Fig. 4.6. The solid line is meant to guide the eye.

4.8 Screenshots of beads being driven by ACEO. The arrows indicate the tracked 880 nm beads. The gray stripe in the center is the gap between the gold electrodes, which are in black.

4.9 The velocity of particles being driven by ACEO in a solution of $\sigma_m = 0.56$ S/m, which is expected to exhibit the double dispersion evident by the experimental data (circles). The curves are fits to equation (4.24): solid line (best fit) – $\sigma_m = 0.01$ S/m; dashed line – $\sigma_m = 0.56$ S/m.

4.10 The theoretical drag force estimate, obtained using equation (4.27), on a 7 µm-long actin filament by the velocities depicted in Fig. 4.9. The solid line is meant to guide the eye.

4.11 (a) Schematic of the experimental setup (b) Electrodes Patterned on a glass substrate for dielectrophoresis experiments. (c) Contrast-enhanced image of an actin filament trapped across the gap between two electrodes. In this case the electrode gap is dark, and the electrodes are gray.

4.12 Depiction of the electric potential (surface plot) and electric field lines (contours) on our electrode geometry. This plot was generated in
COMSOL®, using the electrostatics mode in the AC/DC module. The Laplace equation is solved in the bulk. The electrode surfaces are at a fixed potential, and the other boundaries are insulated.

4.13 Schematic depiction of the conductivity cell used to study the electrode polarization behavior of gold electrodes. The gold pads are 1 cm x 1 cm x 1 cm.

4.14 The ratio $V_E/V_3$, which represents the relative potential drop across the electrolyte compared to the EDLs.

4.15 Contrast-enhanced image of an actin filament trapped across the gap between two electrodes. The image also depicts the discretized position data (*) obtained by using a custom-written algorithm using MatLab®.

4.16 The variance of the filament’s transverse displacement is plotted vs. relative location across the gap at electric field intensities 0.2, 0.3 and 0.5 V/µm. The symbols and lines correspond, respectively, to experimental data and theoretical predictions of a tension-optimized Brownian dynamics-based model for a clamped rod with flexural rigidity $\kappa = 7.3 \times 10^{-26}$ N m².

4.17 The average, standard deviation of the displacement (circles) (± 1 s.e.m.) as a function of the electric field-squared. The solid line is overlaid to guide the eye.

4.18 The apparent tension estimated from the clamped model (circles) as functions of the electric field-squared for 58 filaments (± 1 s.e.m.). The solid line is overlaid to guide the eye.

4.19 The scaled potential [current/conductivity] required to trap actin filaments across a 7 µm gap as a function of the solution conductivity.

5.1 A schematic depiction of a dielectrophoretically positioned and tightened actin filament suspended across a trench between two gold electrodes. A myosin-coated bead is being positioned near the filament with optical tweezers.
5.2  a) A series of bright-field optical micrographs of a myosin V-coated bead traveling along a tightly suspended actin filament. The illuminating condenser is stopped down from its maximum to increase contrast. The viewing objective is located below the sample. The time interval between frames is 850 ms and the exposure time is 80 ms. For clarity, dotted lines are overlaid on the actin filament. b) Images of a stationary bead on the microscope slide surface moved in 150 nm increments along the optical axis, z. Images 1 & 8 are, respectively, closer to and farther from the imaging objective.

5.3  a) & b) Smoothed surface plots of images similar to those in Fig. 5.2. c) & d) Fits of equation (5.1) to images a & b, respectively e) & f) Average radial line profiles from the center of the images shown in a) & b), respectively (symbols), and the corresponding radial slices of the axisymmetric fits shown in c) & d), respectively (solid lines).

5.4  a) The displacement in the transverse direction, x (solid line) and the displacement in the optical axis, z (dashed line) of a myosin V-coated bead, vs. the position along the filament, y. The z-position lags the x-position by roughly 90-degrees, as expected for a left-handed helical path. b) The left-handed, helical motion of the same myosin V-coated bead as it traveled along an actin filament.

5.5  The path taken by a myosin X-coated bead as it travels along an actin filament. The pitch calculated for this bead was 1.2 \( \mu m \).

A.1  A screenshot of the custom-written (Matlab®) GUI “Track_Filament”. This program can determine the position of filaments anchored on either 0 (free-free), 1 (cantilever), or 2 (supported) ends.

A.2  A “supported” actin filament (a) intensity image (b) search grid shown (c) final position overlaid.
A.3  A background subtracted intensity profile taken transverse to an actin filament’s long axis (circles) with a Gaussian fit (dashed line) for position calculation ................................................................................................................................. 136

A.4  A “cantilever” actin filament (a) intensity image (b) search grid shown (c) position overlaid (d) how position may be corrected ................................................................. 137

A.5  A “free-free” actin filament (a) intensity image (b) skeletonized (binary) image (c) corrected binary image (d) search grid shown (e) position overlaid. 138

A.6  The estimated position of two stationary beads along the optical axis [mean ± standard deviation (gray-shaded) as determined from 20 images (10 images/bead) using equation (5.2)] depicted as a function of their actual position set by the position of a piezo-electric stage on the microscope. Negative values of z are closer to the viewing objective, below the focal plane ........................................................................................................................................... 139

A.7  Zeroth moment, $m_0$, of a bead intensity profile, calculated using equation (A.1), plotted vs. z. The origin of the abscissa corresponds approximately to the focal plane. The inset details the range of depth explored by the bead presented in Fig. A.10 (and Figs. 5.2a & 5.4)........................................................................................................................................... 141

A.8  Second moment, $m_2$, calculated using equation (A.2), of the intensity profiles plotted vs. z. Origin and inset as in Fig. A.7 ................................................................. 141

A.9  The radius of the outermost Gaussian ring ($\max(R_1, R_2)$), fitted to a stationary bead’s images using equation (5.1), plotted vs. z. Origin and inset as in Fig. A.7 ........................................................................................................................................... 142

A.10  (top) A myosin-V coated bead’s position along the optical axis, z, depicted as a function of its position along the filament, y, calculated using four different measures: (i) fit comparison (solid line), (ii) zeroth moment, $m_0$ (dashed line), (iii) second moment, $m_2$ (dotted line), and (iv) radius of the outermost Gaussian ring (dash-dotted line). (bottom) the transverse position,
A.11 (a) An intensity image of a myosin thick filament with its endpoints chosen by the user. (b) The search grid overlaid on the filament’s intensity image.

A.12 The intensity values along one of the slices shown in Fig. A.11 depicted as a function of position across the filament.

A.13 The raw intensity (averaged over a transverse slice) at a fixed position along a myosin thick filament’s long axis (circles) depicted as a function of time. The solid line is a fit to equation (A.4).

A.14 (a) The on-rate and b) off-rate of fluorescent intensity along the length of a myosin thick filament calculated from fits of equation (A.4) to fluorescence intensity vs. time data.

A.15 The increase (circles) and decrease (triangles) in fluorescence intensity between two frames depicted as a function of position along a myosin thick filament. The Gaussian curves represent the additions (solid line) and subtractions (dotted line) of fluorescent intensity.
LIST OF TABLES

3.1 Estimates of $L_p$ (in $\mu$m) using different methods and for several monovalent ionic concentrations. 1) mode analysis with cosine basis functions (Gittes et al. 1993), 2) mode analysis with beam basis functions (Liu et al. 1996), 3) variance calculation from the line connecting the endpoints, 4) variance calculation from the line intersecting the center of mass and parallel to the line connecting the endpoints, and 5) end-to-end relationship. Variabilities are $\pm$ one standard deviation. .......................................................... 71

4.1 Resistance values calculated using *equation (4.30) and #from using the current-voltage relationship of the assumed purely resistive electrolyte. $C_{EDL}$ is calculated using equation (4.22)................................................................. 102

5.1 Summary of myosin V-coated bead experiments (variabilities are presented as $\pm$ 1 standard deviation). ........................................................................................................ 128
Chapter 1

Introduction

1.1 Motivation

Cell motility is essential to many biological processes. Without it, cells would not divide, cells would not respond to cell signaling cues, bacteria would not swim, and plant cells would die. Motor proteins and their associated tracks are the primary facilitators of cell motility. The ubiquitous nature of motility in living cells and the structural homologies among motor proteins, many signaling molecules, and metabolic enzymes, gives fundamental research on motor proteins broad impact. Disorders of these proteins are associated with many diseases such as several tumors, abnormal blood cell motility, and immunological syndromes.

The cytoskeleton is responsible for the structural integrity of the cell. There are three cytoskeletal filaments: actin filaments, microtubules, and intermediate filaments. Figure 1.1 depicts a stained image of a eukaryotic cell (http://rsb.info.nih.gov/ij/images/), with the nucleus stained blue, microtubules stained green, and actin filaments stained red. Microtubules support motility of the kinesin and dynein families of molecular motors. They are hollow, ~25 nm in diameter, arranged in a radial pattern in vivo, and are stiff on the length scales found in cells. Microtubule-based motility is involved with chromosomal movement during mitosis and powers the beating of cilia and flagella. Actin filaments support motility of the myosin family of molecular motors and are a
primary subject of this thesis. In their polymerized form, they are a double helix, ~7 nm in diameter, and are rather flexible on the length scales found in vivo. Actin-based motility is involved with muscle contraction and cytokinesis, to name a couple. Intermediate filaments are not known to support motility but do resist tensile forces.

![Image](image.png)

**Figure 1.1:** A eukaryotic cell with the nuclei stained blue, the microtubules stained green, and the actin filaments stained red (http://rsb.info.nih.gov/ij/images/)

Molecular motors, or motor proteins, interact with the filamentous proteins described above and produce force, powering many of the processes our body undertakes on a regular basis. As was previously stated, the kinesin and dynein families of molecular motors are microtubule-based molecular motors, while the myosin family of motors is actin filament-based. Figure 1.2 shows a cartoon depiction of these motors (Vale 2003). These three families of motors are ATPases that convert the chemical energy stored in adenosine triphosphate (ATP) into mechanical energy. Not all ATPases produce work, however, and the chemical energy may be converted into heat or light. One ATP
molecule provides 100 pNnm of energy or $100 \cdot 10^{-21}$ J. An estimate of the energy used by a single myosin V molecular motor, resisting a force of 2 pN and moving the distance of its working stroke, or stride length (~36 nm), puts the value at $80 \cdot 10^{-21}$ J, so motor proteins have evolved to be quite energy efficient!

![Motor Toolbox](image)

**Figure 1.2:** A cartoon depiction of the three families of molecular motors: kinesin, myosin, and dynein (Vale 2003)

Here, we will focus on myosin motors. All myosin motors have a globular head domain and a light chain domain. The head contains the catalytic heavy chain (actin and ATP-binding domains). The light chain domain can vary in length and makeup and serves as the lever arm of the motor. The lever arm stores the chemical energy released by ATP hydrolysis, during the head’s conformational change, as elastic energy. This combination alone supports motility on (or for) actin filaments. Filamentous actin itself
has a polarity owed by the asymmetry of its monomeric subunits. This polarity dictates that all known myosin isoforms, save myosin VI, “walk” in the same direction that often coincides with the direction radial to the cell periphery. This polarity derives from the monomeric actin’s physically-, electrostatically-, and hydrophobically-unique topology being more complimentary to a specific orientation of the myosin head’s actin binding site. A common question of biophysicists asks why some myosin motors have evolved to work either cooperatively or autonomously. An example of a motility assay is depicted in Fig. 1.3. Referred to as a “gliding assay”, myosin heads coat a surface, and actin filaments essentially “crowd surf”. This assay tests motor functionality and kinetics, among other things, of both autonomous and non-autonomous motors. Recently, an exciting subfield of biophysics has focused on single-molecule assays that probe motor function. This will be discussed in section 1.3.

![Figure 1.3: A gliding filament assay, where the myosin motors are bound to the surface, and the actin filaments “crowd surf” on top](image)

1.2 Actin Filaments

Actin is stable in its monomeric form at low salt concentrations and polymerizes into filamentous actin (F-actin) at physiological salt concentrations. Much work has been
performed to determine the mechanical properties of F-actin. The mechanical properties of biological filaments are intimately connected to their electrical properties, so this section will survey both.

1.2.1 Mechanical Properties

A biological filament’s persistence length $L_p$ is a convenient measure of its resistance to bending,

$$L_p = \frac{EI}{k_B T} = \frac{\kappa}{k_B T}, \quad (1.1)$$

where $E$ is the material’s elastic modulus, $k_B$ is the Boltzmann constant, $T$ is the temperature, $\kappa = EI$ is the flexural rigidity, and $I$ is the geometrical moment of inertia of the filament’s cross-section. A filament’s persistence length is the length over which the correlation between two tangent vectors along its arc length decays by a factor of Euler’s number. DNA has a persistence length of ~50 nm, whereas actin has a persistence length of ~18 µm and microtubules ~3 mm.

F-actin’s end-to-end distance was measured to estimate its persistence length (Yanagida et al. 1984), similar to studies performed on DNA (Smith 1992). Tsuda (1996) found that the breaking force of actin filaments under no torsion was about 600 pN (Tsuda et al. 1996). Gittes et al. (1993) monitored the thermal fluctuations of single actin filaments and used the equipartition theorem to determine its flexural rigidity to be 7.3·10^4 pN nm^2 and its corresponding persistence length to be ~18 µm (Gittes et al. 1993). Under similar conditions to those in Gittes et al.’s (1993) experiment, Riveline et al. (1997) attached a bead to one end of an actin filament, oscillated the bead with an optical trap at 0.1-6 Hz with amplitudes of 5-10 µm transverse to the filament’s long axis,
and estimated a persistence length of 7.4 µm based on a linear bending theory for the filament and the hydrodynamics of a slender body. Thermal fluctuations of the actin were neglected in Riveline et al.’s (1997) analysis.

Isambert et al. (1995) obtained the profile of a single actin filament’s instantaneous shapes, extracted cosine correlation functions from the data and fitted them with the equation $\langle C(s) \rangle = \langle \cos[\theta(s) - \theta(0)] \rangle = e^{-s^2/2\ell_p}$, where $s$ is the arc length and $\theta$ is the tangent angle. They calculated a persistence length of 18 µm for phalloidin-stabilized F-actin and 9 µm for F-actin in the absence of phalloidin. Phalloidin is an actin-stabilizing agent, commonly used to preserve long filamentous molecules in vitro. In the presence of troponin and tropomyosin, two proteins involved in muscle function, F-actin’s persistence length was estimated to be 20 µm while in the on state (+Ca$^{2+}$) and 12 µm in the off state (no Ca$^{2+}$) (Isambert et al. 1995).

Most analytical methods used in the study of fluctuating filaments (for instance, Gittes et al. (1993), and Isambert et al. (1995)) extract the local curvature (and not the displacement) along the filament and then use results from semi-flexible polymer models to interpret the experimental data. The reason for doing so is that the bending energy is quadratic in the curvature and therefore the equi-partition theorem is applicable, leading to simple expressions for the variance in the amplitudes of the curvature modes. The root-mean-squared displacement ($D_{rms}$) can also be estimated from experimental data, but analytical solutions are available only for small displacements (linear theory).

Other methods where the $D_{rms}$ is directly determined from experimental data (for instance, to detect change in stiffness as myosin binds to actin (Veigel et al. 1999))
approximate the entire filament as a single spring with a single displacement (the ends or the mid-span) being the kinematic variable whose statistical properties are studied. This method does not take advantage of the detailed data available from the experiments described in this thesis, during which we monitor the position of the entire filament.

1.2.2 Electrical Properties

Since molecules in solution typically carry a net electric charge, this charge attracts counter ions in solution that "screen" the surface charge and form an electric double layer (EDL). The surface charge may be permanent charge, adsorbed/desorbed charges, or induced charges (in the presence of an external electric field). Figure 1.4 depicts the electric double layer in a cartoon.

![Figure 1.4: A cartoon depiction of the electric double layer (EDL) surrounding an ellipsoidal particle](image)

A filament’s EDL can have significant impact on its mechanical properties. DNA’s persistence length has been shown to be a strong function of ionic concentration. For example, in solutions of high ionic concentration, the EDL is very thin, electrostatic...
repulsion forces are screened, and the DNA filament collapses upon itself. In solutions of low ionic strength, the EDL is thick compared to DNA’s characteristic size and charge spacing, and electrostatic interactions along the DNA filament’s contour lead to stiffening of the filamentous molecule (Baumann et al. 1997). Later in the thesis, I investigate the effect of suspending solution’s ionic strength on F-actin’s persistence length (Chapter 3).

Actin filaments have been reported to exhibit anomalous behavior in electric fields. Kobayasi, et al. (1964) reported that in a DC electric field, actin filaments align themselves transverse to the electric field lines and speculated that the filaments have a strong dipole moment transverse to their axis (Kobayasi et al. 1964). In contrast, the application of electric fields caused actin filaments, transiently-attached (via myosin) to a glass surface, to gradually align along the electric field lines (Asokan et al. 2003).

The application of electric fields leads to a variety of electrokinetic phenomena (Adamson & Gast 1997; Hunter 2001; Lyklema 1995). An externally applied electric field will act on the surface charge (Coulombic force) and cause the particle to migrate. This is the well-known phenomenon of electrophoresis. When the charged surface is stationary, the electrical force will cause the ionic cloud to move. The ionic cloud will drag the fluid with it, which will give rise to fluid motion relative to the surface. This is known as electroosmosis. The electrophoretic and electroosmotic forces and the corresponding migration speeds are proportional to the electric field intensity to the first order. When AC fields are applied at sufficiently high frequency (e.g. > kHz), like in some of our experiments, the electric field alternates direction, under perfect symmetric conditions, the electrophoretic force averages to zero, and does not produce any net motion. This is not true, however, with respect to the electroosmotic motion. In the case
of an AC field, the positive ions in the EDL that surrounds the filament migrate in the
direction of the field. Since the electric conductivity of the EDL is much larger than that
of the bulk solution, the ions accumulate next to the filament’s ends and form a dipole.
The interaction between the induced dipole and the externally applied electric field may
lead to net forces that are proportional to the square of the electric field intensity, and
thus independent of the electric field’s direction. We mention all this to emphasize that
the well-known phenomena of electrophoresis does not play any role in our experiments.

As a novel route to manipulating and positioning biological polymers for studying
their biophysical properties, and for potential use of biopolymers in analytical devices,
dielectrophoretic trapping and orientation confer several advantages over other methods.
Dielectrophoresis (DEP) is the migration of dielectric particles in spatially non-uniform
electric fields and will be presented in Chapter 4. In most single molecule investigations,
there is little control over the positioning of individual molecules. Dielectrophoretic
trapping provides a flexible and convenient means for applying remote forces to viruses
(Morgan et al. 1999), DNA (Washizu and Kurosawa 1990; Porath et al. 2000), beads
(Green and Morgan 1999), actin (Asokan et al. 2003), and microtubules (Uppalapati et al.
2008). The electric fields can be readily applied and controlled by judiciously patterning
electrodes on the surface of a glass or quartz slide. Since the electrodes can be patterned
with very small gaps between them, it is possible to apply very large electric fields with
relatively small potential differences between the electrodes. The electric field polarizes
the object, and more importantly, the electrical double layer (EDL) that surrounds the
object.
The process of polarization involves the rearrangement of positive and negative charge centers in an electric field. These charge centers eventually align themselves along the electric field lines. Two forms of polarization, such as atomic and electric polarization involve the rearrangement of charge in an atom’s nucleus or the rearrangement of charge on an atom’s surface, respectively. These processes occur very quickly (ps) and are not of concern in this study. The process by which a molecule rotates to align its polarization axis along the electric field lines is called electro-orientation and is a slower form of polarization. This process occurs slowly enough to be of concern in our study (~1 s, depending on filament length). What is also of paramount importance is the polarization of the EDL surrounding our particles. Without the electric field present, an EDL would still exist, but, with it present, the EDL’s charge distribution rearranges. The movement of the countercharge in the electric field gives rise to a dipole. Particles can be manipulated by applying forces to these induced dipoles.

The very strong perturbation of actin filaments’ apparent stiffness by applied electric field in our experiments may reveal important features of actin's cytoskeletal function and distributed electrical behavior of polyelectrolytes in general. While the magnitude of electric fields used to position the actin filaments in these experiments (2·10^5 V/m) are not commonly present in the cytoplasm of cells, fields approaching this value are present within biological membranes (Pollard and Earnshaw 2002). Thus the influence of electric fields in an experimentally amenable system may reveal novel properties of the protein-field interaction.
1.3 Molecular Motors

Molecular motors are on the order of 10 nm in size. In order to observe them, biophysicists have used traditional biochemical techniques to label the motors with visible labels (i.e. organic, fluorescent dyes, quantum dots, micron-sized beads). This thesis only discusses the subgroup of motors that work autonomously. For an excellent treatment on conventional myosins (Warrick 1987) and the myosin I family (Pollard 1991), two major subgroups of non-autonomous motors, please consult the cited references.

a)

b)

Figure 1.5: Two examples of single-molecule biophysical assays (a) fluorescently-labeled myosin motors walk along surface-immobilized actin filaments (b) the Three Bead Assay – an actin “dumbbell” is brought into close proximity to a bead-immobilized myosin motor where it is permitted to interact
For the most part, mechanical studies of myosin and other molecular motors have utilized surface-immobilized motors (Finer et al. 1994; Mehta et al. 1999; Rock et al. 2001; Veigel et al. 2003; Takagi et al. 2006) or filaments, (Block et al. 1990; Svoboda et al. 1993; Rief et al. 2000; Forkey et al. 2003; Yildiz et al. 2003; Ross et al. 2006; Sun et al. 2007). The proximity to a solid surface impacts a motor’s range of motion. An example of these assays is depicted in Fig. 1.5a, where the yellow object represents a fluorescent beacon. For example, the autonomous, two-headed myosin V has been shown to have an average step size of 36 nm, approximately coinciding with the half-pitch of the actin helix (Mehta et al. 1999; Yildiz et al. 2003). However, in these studies, the actin track was attached to a glass microscope slide that restricts motions to the demicylindrical domain on one side of the filamentous track, possibly constraining the motor to particular binding sites. The classic three-bead optical trap assay - an actin “dumbbell” lowered onto a myosin-coated polymeric or ceramic bead - has yielded valuable information on the force production, step size, and kinetics of molecular motors (Finer et al. 1994; Mehta et al. 1999; Rock et al. 2001; Veigel et al. 2003; Takagi et al. 2006). In this case, immobilization of a motor onto the bead may again adversely impact the motor’s range of motions. This assay is depicted in Fig. 1.5b. To enable greater freedom of motion, it is desirable to develop motility assays that enable the transport activity to take place away from any surfaces. An assay we developed to serve this purpose will be described in Chapter 5.
1.4 Organization of Dissertation

It was inevitable that the quests of investigators in separate scientific disciplines would intersect and that there would be fruitful collaborations resulting in new, exciting technologies and scientific understanding. The questions that need to be answered in the field of biology benefit greatly from the expertise of physicists and engineers. Likewise, engineering design can benefit from inspiration from nature. One of the many exciting fields where this physical ingenuity can benefit the field of biology is molecular motors. When a single molecule's function or structure is in question, the clever physicist or engineer can devise methods by which to facilitate their study.

If we are to explain the fundamental mechanisms of transport in cells and muscle contraction, we must garner a more complete understanding of the electrostatic interactions between molecular motors and their respective tracks. The interaction between these nanoparticles is wholly dependent on the electric double layer (EDL), whose precise structure has not been solved.

The questions to be investigated in this thesis are the following:

1. Can the tension being applied to a filament trapped using optical tweezers be estimated using a linear Brownian dynamics model? (Chapter 2)

2. Does ionic strength affect the bending rigidity of actin filaments? (Chapter 3)

3. Why does an electric field cause an actin filament's thermal fluctuations to decrease in amplitude? The answer to this question may provide insight into the surface conduction properties of actin filaments, because this property is coupled to the force felt in an electric field. (Chapter 4)
4. If we attribute the aforementioned electrical effect to a tension, can this linear Brownian dynamics model estimate this applied tension from the variance of thermal fluctuation amplitude? (Chapter 4)

5. Can dielectrophoretic manipulation be used to facilitate single-molecule biophysical assays? (Chapter 5)
Chapter 2

Applying the Brownian Dynamics Model to an Optically Trapped Filament

Abstract

Brownian dynamics-based estimates of polymer tension are compared with optical traps’ direct force measurements. We suspended phalloidin-stabilized, actin filaments between two beads. The positions of the beads were controlled with optical traps facilitating direct measurement of the forces acting on the beads. The lateral, thermal fluctuations of each filament were visualized with a video camera, and the images were used to obtain the transverse displacement as a function of position along the filament, time, and camera exposure time. The fluctuation’s variance was calculated from the experimental data. When the camera exposure time was increased above a certain threshold, the estimated variance decreased. To obtain an unbiased estimate of the variance, it was necessary to carry out the observations at exposure times shorter than the threshold time interval that was related to the filament’s oscillatory time scale. The variance was estimated as a function of the applied force with a linear Brownian dynamics model. Then, an inverse problem was solved to estimate the filament’s tension. The estimated force was compared and agreed within a factor of 2 with the trap force measurements. The technique described herein can be used for non-contact estimates of polymer’s and fiber’s tension.
2.1 Introduction

We have used electric forces to position actin filaments across gaps between electrodes’ pairs. In the course of the experiments, we observed that the filament’s apparent stiffness increased nearly linearly with the square of the electric field intensity. To estimate the filament’s tension, we measured the transverse thermal fluctuations of the filament as a function of position along the filament and time to estimate the variance of the thermal fluctuation as a function of position along the filament. Assuming that the filament behaves like a slender elastic beam, we used a simple Brownian-dynamics model and the principle of equi-partition of energy to estimate the variance of the thermal fluctuations as a function of position, filament tension, and filament rigidity. Using accepted values of the filament’s rigidity and minimizing the discrepancy between the theoretical estimates and the experimental observations, we estimated the filament’s tension.

The use of Brownian dynamics models and the principle of equipartition of energy to estimate the mechanical properties of polymers are not new. For example, Yanagida et al. (1984) and Gittes et al. (1993) deduced actin filaments’ and microtubules’ flexural rigidities from their individual modes of vibration. Since this is the first time the beam equation has been used to study a doubly anchored biological polymer’s tension, we felt it prudent to verify the method by comparing the Brownian dynamics-based force estimates with direct force measurements.

Optical traps provide a convenient means to simultaneously apply forces in the range of piconewtons and measure subnanometer displacements. Pioneered in the 1970s by Ashkin et al. (1970 and 1986), the trap is commonly employed to study molecular
motors (Svoboda & Block 1994; Mehta et al. 1998, 1999) and biofilaments (Tsuda et al. 1996; Smith et al. 1996; Perkins et al. 1994; Dupuis et al. 1997; Riveline et al. 1997) at the single-molecule level. In these assays, micron-sized particles, trapped by tightly focused laser beams, act as handles to manipulate attached molecules of interest. The interference of the laser light scattered from the bead with unscattered light allows the estimation of the force acting on the bead. When displacements are small, the trap acts like a linear spring.

Wang et al. (1997) used optical tweezers to estimate the bending rigidity of DNA and obtained similar results to those obtained with the application of hydrodynamic and magnetic forces to DNA filaments (Smith et al., 1992). Schnurr et al. (1997) optical tweezers studies of the shear moduli of filamentous networks yielded similar results to earlier rheological techniques (Janmey et al., 1988). Here, we measure the variance of filamentous actin’s transverse fluctuations as a function of the applied axial force applied with optical tweezers and compare the applied force values with a Brownian-dynamics model’s predictions.

2.2 Materials and Methods

A 150-µm high flow cell, confined between two glass coverslips was filled with a solution of 50 nM rhodamine-phalloidin-stabilized actin, polymerized in 2 mM MgCl₂, 150 mM KCl, and 2 mM Hepes (pH = 7.4). The solution also included 1 mg/mL BSA to prevent non-specific adhesion of the actin filaments to the glass surfaces and 50 mM DTT, 7.2 mg/mL glucose, 9 units/mL catalase, and 4 mg/mL glucose oxidase to slow rhodamine’s photo-bleaching. Buffers were prepared with either 0% or 60% (by mass) glycerin to study the effect of suspending medium’s viscosity on the filaments’
oscillatory frequencies. Subsequently, 1 µm-diameter, N-ethylmaleimide (NEM)-myosin-coated beads were infused into the flowcell. These beads were prepared as described in Veigel et al. (1998). The flowcell was sealed with grease to prevent solvent evaporation and was placed on the stage of an inverted microscope (Olympus IX70).

A 1064 nm optical trap (Takagi et al., 2006) was used to attach two 1 µm-diameter beads to a single actin filament, one on each end of the filament (Fig. 2.1). It is likely that the filament is linked to the bead by multiple myosin molecules. Macroscopically, the filament appeared to be firmly attached to the beads.

An electron multiplying CCD camera (Andor Technologies) collected images every 2 s, with exposure times of 10, 20, 30, 50, 75, 100, 200, and 300 ms. A custom-written Matlab™ algorithm tracked the filament’s position.

2.2.1 Optical Tweezers

To verify the direct force measurement, we employed two force calibration methods (Svoboda & Block 1994; Neuman & Block 2004; Dupuis et al. 1997). In one method, we measured the power spectrum of the bead’s thermal fluctuations and compared the measurements with theoretical predictions to obtain the trap’s spring
constant. For our second calibration method, we exerted a known drag force on the bead by oscillating the microscope stage back and forth with a user-defined triangular wave and calculated the spring constant from the drag force. The trap spring constant varies from bead to bead, depending on trap’s laser intensity, and ranged from 0.01 to 0.02 pN/nm. The estimates of the trap spring constants obtained with the two calibration techniques were within 10%.

The power spectrum of the thermal fluctuations of a bead in a trap is given by (Svoboda and Block 1994)

\[
G(f_t) = \frac{k_B T}{\pi^2 \beta (f_c^2 + f_t^2)},
\]

where \(f_t\) is the frequency, \(\beta\) is the bead’s hydrodynamic drag coefficient, \(f_c = \alpha (2\pi \beta)\) is the trap’s characteristic frequency, \(\alpha\) is the trap’s spring constant, \(k_B\) is the Boltzmann constant, and \(T\) is the absolute temperature. Figure 2.2 depicts the power spectrum \(G\) of an optically trapped, 1 \(\mu\)m-diameter bead undergoing Brownian motion, sampled at 20 kHz. When \(f_t \rightarrow 0\), \(G = 4k_B T \beta \alpha^2\). From this low frequency asymptote, we determine the spring constant \(\alpha\) (N/V). This peculiar unit is used since the detector supplies readings in units of voltage. The power spectrum’s dispersion is then fit by equation (2.1) (solid line). The stiffness of the trap whose calibration curve is depicted in Fig. 2.2 was calculated to be 0.017 ± 0.0004 pN/nm, where the standard deviation was calculated from independent measurements on the same bead. The corner frequency \(f_c = 280\) Hz. The advantage of using the power spectrum method is that it eliminates the need to calibrate the photodiode’s signal to known bead displacements out of the trap. For example, the
laser trap could be moved about an area centered on an immobilized bead to determine the detector response (Neuman and Block 2004).

Figure 2.2: The power spectrum of an optically trapped, 1-µm diameter bead undergoing Brownian motion. The solid line corresponds to equation (2.1) with $\alpha = 0.017$ pN/nm, $f_c = 280$ Hz.

As a second calibration technique, we applied hydrodynamic drag to the optically-trapped beads by moving the microscope stage in a triangle wave and measuring the bead’s displacement. Figure 2.3 depicts the quadrant-photodiode signal of two optically-trapped beads (solid lines) superimposed on the microscope stage encoder’s signal (dashed line – reduced by a factor of 20 to fit within the range of the figure). The triangle wave displacement profile of the microscope stage exerts a square wave hydrodynamic drag profile on the bead. The equation of motion of the bead, neglecting the inertial term, is

$$\beta \frac{dx}{dt} + \alpha x = F_z(t), \quad (2.2)$$
where $F_S$ is the stage’s driving force. Taking the Laplace Transform of equation (2.2), we have

$$L \left[ \frac{d^2x}{dt^2} + \frac{\alpha}{\beta} x = \frac{F_s(t)}{\beta} \right] \rightarrow \left[ \tilde{X}_s + \frac{\alpha}{\beta} \tilde{X} = \frac{F_s}{s\beta} \right]. \quad (2.3)$$

where $L$ denotes the Laplace transform. Solving for $\tilde{X}$, we are left with

$$\tilde{X}(s) = \frac{F_s}{s^2 + \frac{\alpha}{\beta} s}. \quad (2.4)$$

Taking the inverse Laplace Transform,

$$L^{-1}[\tilde{X}(s)] = x(t) = \frac{F_s}{\alpha} \left( 1 - e^{-\frac{t}{\beta}} \right). \quad (2.5)$$

Here, $F_s = \beta u$, $u = \frac{A}{T/2}$ is the stage’s velocity, $T = 1/\omega$ is the half-period of the triangle wave, $A$ is the amplitude of the stage’s displacement, and $\omega$ is the frequency of the triangle wave ($s^{-1}$). Therefore, the displacement of a bead attached to a Hookean spring, in response to a constant drag force, can be described as

$$x = \frac{2\beta A \omega}{\alpha} \left( 1 - e^{-\alpha/\beta} \right). \quad (2.6)$$
Figure 2.3: The position of two optically-trapped beads (solid lines) superimposed on the microscope stage’s position (dashed line) as it oscillates as a triangle wave (reduced by a factor of 20 for clarity). The two signals with different amplitudes correspond to two beads residing in different traps and having different stiffnesses. Upon fitting to equation (2.6), we calculate the two trap stiffnesses $\alpha = 0.04 \, \text{&} \, 0.1 \, \text{pN/nm}$. A different bead was used in Fig. 2.2.

By measuring the steady-state displacement of the bead from equilibrium after each half-period, we can estimate the trap stiffness. The two bead signals’ different amplitudes in Fig. 2.3 correspond to them residing in traps having different stiffnesses. Upon fitting to equation (2.6), we calculate the two trap stiffnesses $\alpha = 0.04 \, \text{&} \, 0.1 \, \text{pN/nm}$. A different bead was used in Fig. 2.2. The power spectrum and hydrodynamic drag calibration techniques agreed to within a factor of 2.
2.3 Mathematical Model

We consider the actin filament to behave like a slender, elastic beam with uniform bending rigidity \( EI \) and tension \( \tau \). We first determine the filament’s equilibrium shape and then consider thermal fluctuations about equilibrium.

2.3.1 Filament’s Equilibrium Shape

Consider an inextensible filament of contour length \( 2L \) connected to two beads subjected to opposing forces of magnitude \( \tau \) in the \( x \)-direction (Fig. 2.1). The position of a point along the filament is specified with arc length \( \hat{s} \) having its origin at the filament’s mid length \(( -L \leq \hat{s} \leq L )\). We use the overscript “hat” to denote dimensional quantities that will be rendered dimensionless later in the chapter. The moment acting on the filament at any position \( \hat{s} \) is \( M = EI \frac{d\theta}{d\hat{s}} \), where \( EI \) is the bending rigidity and \( \theta \) is the angle formed between the tangent to the filament and the \( x \)-axis. Force equilibrium requires \( \theta \) to satisfy the dimensionless beam equation (Landau and Lifshitz, 1959)

\[
\frac{d^2\theta}{d\hat{s}^2} - m^2 \sin \theta = 0, \quad (-1 \leq \hat{s} \leq 1)
\]  

(2.7)

where \( m^2 = \frac{\tau L^2}{EI} \) and \( L \) is the length scale, i.e., \( s = \hat{s} / L \). At \( s = \pm 1 \), balance of moments yield

\[
\frac{d\theta(s = \pm 1)}{ds} = m^2 \left( \frac{a_+}{L} \right) \cos(\theta(s = \pm 1)),
\]  

(2.8)

where \( a_+ \) is the radius of the bead at the right end of the filament. We adopt the notation that subscripts “+” and “-” denote, respectively, the bead on the right and the bead on the left. For conciseness, we describe here only the case when both beads have the same
radius \( a_+ = a_- = a \). The derivation, however, can be extended, straightforwardly, for the more general case of \( a_- \neq a_+ \). In the experiment, the filament and the two beads’ centers may not reside in the same plane. For the purpose of this section, we will consider only the planar case. Furthermore, in the planar arrangement, one can distinguish between a symmetric case \( \theta(0) = 0 \) and an antisymmetric case \( \frac{d\theta(0)}{ds} = 0 \). We show results only for the former.

Equation (2.7) can be readily integrated once to yield

\[
\frac{1}{2} \left( \frac{d\theta}{ds} \right)^2 + m^2 \cos \theta = C,
\]

where \( C \) is a constant of integration. It is instructive to consider the order of magnitude of \( m \). The bending rigidity of actin is estimated \( EI \sim 7 \cdot 10^{-26} \text{Nm}^2 \) (Gittes et al., 1993); the bead radius is \( a \sim 0.5 \mu\text{m} \); the actin length \( 2L \sim 10 \mu\text{m} \). When the trap force \( \tau \) is on the order of 1 pN, \( m^2 = 400 \). Thus, in most circumstances encountered in our optical trap experiments, \( m \gg 1 \).

The exact solution of equations (2.7-2.8) is in terms of an elliptic integral, which is cumbersome to use. Instead, we solve the equations numerically with finite elements. Figure 2.4 depicts the equilibrium shape of the filament in the range \( 0 < s < 1 \) when \( m = 0.2, 2, \) and \( 20 \) and \( a/L = 0.1 \) using the Cartesian coordinates \( x \) and \( y \). The \( x \) coordinate goes through the beads’ centers, and its origin is at the filament’s midlength. The coordinates \( x \) and \( y \) were computed together with the solution of equation (2.7), by solving the equations \( \frac{dx}{ds} = \cos(\theta(s)) \) and \( \frac{dy}{ds} = \sin(\theta(s)) \) with the boundary conditions \( x(0) = 0 \) and
\( y(l) = \left( \frac{a}{L} \right) \cos(\theta(l)). \) The right bead’s center is located at \( \{x, y\} = \{ \int_0^1 \cos(\theta(\xi)) d\xi + \frac{a}{L} \sin(\theta(l)), 0 \} \). When \( m>10 \), along a significant fraction of the filament’s length, excluding small regions next to the beads, \( \theta(s) \sim y(s) \sim 0 \). We will take advantage of this fact in the next section.

\[ y(x) = \left( \frac{a}{L} \right) \cos(\theta(x)). \]

\[ \theta(s) = 4 \tan^{-1}\left( \frac{\tan\left( \frac{\theta(1)}{4} \right) e^{m(s-1)}}{1 + m^2 \sin^2(\frac{\theta(1)}{2})} \right), \quad (2.11) \]

**Figure 2.4:** The equilibrium shapes of filaments \((0<s<1)\) when \( m = 0.2 \) (dashed line), 2 (solid line), and 20 (dotted line). \( a/L = 0.1 \). The three circles depict the position of the bead when \( m = 0.2 \) (dashed line), 2 (solid line), and 20 (dotted line). Symmetric boundary conditions are applied to the filament.

When \( m>>1 \), one can obtain a closed form, approximate solution for \( \theta \). When \( |s|<<1 \), we expect \( \theta \sim 0 \). Thus, from equation (2.9), we conclude that the integration constant \( C \sim m^2 \), and the equation can be re-written as

\[ \frac{d\theta}{ds} = 2m \sin\left( \frac{\theta}{2} \right). \quad (2.10) \]

Equation (2.10) can be readily integrated to yield

\[ \theta(s) = 4 \tan^{-1}\left( \frac{\tan\left( \frac{\theta(1)}{4} \right) e^{m(s-1)}}{1 + m^2 \sin^2(\frac{\theta(1)}{2})} \right), \quad (2.11) \]
where the integration constant is expressed in terms of the angle \( \theta(1) \), which is then determined from the boundary condition (2.8). More explicitly, equation (2.8) leads to

\[
\theta(1) = 2 \sin^{-1}\left( \frac{\sqrt{2}}{2} \sqrt{1 + \frac{1}{2m^2} \left( \frac{L}{a} \right)^2} - \frac{L}{2ma} \right).
\]  

(2.12)

When \( m \gg 1 \), Taylor series expansion yields:

\[
\theta(1) \sim \frac{\pi}{2} - \sqrt{2} \left( \frac{L}{ma} \right) + \left( \frac{L}{ma} \right)^2 + O\left( \frac{L}{ma} \right)^3.
\]  

(2.13)

When \( m \frac{a}{L} \to \infty \), \( \theta(1) \to \pi / 2 \). Figure 2.5 depicts \( \theta \) as a function of \( s \) when \( a/L=0.1 \) and \( m = 5 \) (squares), \( m = 10 \) (circles), \( m = 20 \) (triangles). The symbols and solid line correspond, respectively, to the approximate (numerical) and exact (equation 2.11) solutions. Witness the excellent agreement between the approximation and the exact solution. As \( m \) increases, the discrepancy between the exact and approximate solutions decreases.

\[\text{Figure 2.5: Comparison between the exact (solid line – equation (2.11)) and approximate (circles – COMSOL) solutions; m = 5 (squares), m = 10 (circles), m = 20 (triangles).}\]  

- 26 -
2.3.2 Filament’s Thermal Fluctuations

Next, we consider small fluctuations of the filament about its equilibrium state. Based on the analysis of section 2.3.1, at equilibrium and subject to the typical forces applied in the experiments, \( y(s) = 0 \) along most of the filament’s length. The transverse, dimensionless displacement \( \zeta(s,t) \) (normalized with \( L \)) of a beam with uniform flexural rigidity \( EI \) (Nm\(^2\)), linear density \( \rho \) (kg/m), and tension \( \tau \) (N), vibrating in a viscous medium with viscous resistance \( f_\perp \) (Ns/m\(^2\)) per unit length, is given by the dimensionless beam equation (Landau & Lifshitz 1959)

\[
\left( \frac{\rho EI}{f_\perp L^4} \right) \frac{\partial^2 \zeta}{\partial t^2} + \frac{\partial \zeta}{\partial t} = m^2 \left( \frac{\partial^2 \zeta}{\partial s^2} \right) - \frac{\partial^4 \zeta}{\partial s^4} + \frac{L^3}{EI} F(s,t) \quad (-1 \leq s \leq 1). \tag{2.14}
\]

In the above, the time \( t \) is scaled with \( \Lambda = \frac{fL^4}{EI} \) and \( f = 4\pi \eta / (\ln(2L/r) + 0.5) \) (Brennen & Winet 1977); \( \eta \) is the suspending fluid viscosity. \( F(s,t) \) (N/m) is a white, stochastic force per unit length of the filament. The ensemble average of \( F \) satisfies

\[
\langle F(\zeta, t) \rangle = 0 \tag{2.15}
\]

and the correlation

\[
\langle F(s_i,t_i)F(s_j,t_j) \rangle = \phi(s_i - s_j, t_i - t_j), \tag{2.15}
\]

where \( \phi \) is approximated with the Dirac-Delta function, and <> denotes an ensemble average. Bokaian (1990) studied the natural frequencies of vibrating beams under tension with various boundary conditions.
The boundary conditions require some elaboration. Since we are focusing on the straight segment of the filament, we approximate the filament’s ends as if they were free to rotate and specify zero moment conditions at both ends

\[ \frac{\partial^2 \xi(\pm 1, t)}{\partial s^2} = 0. \] (2.17)

When analyzing the experimental data, we measured the displacements relative to a line that connects the filament’s ends. Hence,

\[ \xi(\pm 1, t) = 0. \] (2.18)

Boundary conditions (2.17) and (2.18) correspond to a hinged-hinged beam. Since the boundary conditions are not precisely known, it is constructive to consider also the other extreme case of a clamped beam

\[ \frac{\partial \xi(\pm 1, t)}{\partial s} = 0, \] (2.19)

which will provide a low bound for the estimated tension.

2.3.3 Solution of the Beam Equation

We seek a solution of the form

\[ \xi(s, t) = \sum_{n=1}^{\infty} A_n(t) Y_n(s), \] (2.20)

where \( A_n(t) \) and \( A_m(t) \) are uncorrelated for any \( n \neq m \), i.e., \( \langle A_n(t) A_m(t) \rangle = 0 \). The thermal force is expanded in a similar fashion, i.e., \( F(s, t) = \sum_{n=1}^{m} F_n(t) Y_n(s) \). Substituting (2.20) into equation (2.14) and separating variables, we obtain the self-adjoint, eigenvalue problem

\[ \frac{\partial^4 Y_n}{\partial s^4} - m^2 \frac{\partial^2 Y_n}{\partial s^2} - \lambda_n m^4 Y_n = 0 \quad (-1 \leq s \leq 1) \] (2.21)
with the boundary conditions

\[ Y_n^H (\pm 1) = \frac{\partial^2 Y_n^H (\pm 1)}{\partial s^2} = 0 \]  

(2.22)

in the hinged case and

\[ Y_n^C (\pm 1) = \frac{\partial Y_n^C (\pm 1)}{\partial s} = 0 \]  

(2.23)

in the clamped case. In the above, we used the superscripts \( H \) and \( C \) to denote, respectively, the hinged-hinged and clamped-clamped cases. We will use the above superscripts only when it is necessary to distinguish between the hinged and clamped cases. The factor \( m^4 \) was introduced in \( \lambda_n m^4 \) for notational convenience.

For the specified boundary conditions, the eigenmodes \( Y_n(s) \) are orthogonal and can be normalized so that

\[ \int_{-1}^{1} Y_n(s)Y_m(s) = \delta_{nm} . \]  

(2.24)

Equation (2.21) admits a general solution of the form

\[ Y_n(s) = C_{n,1} \cosh(z_{1,n}s) + C_{n,2} \sinh(z_{1,n}s) + C_{n,3} \cos(z_{2,n}s) + C_{n,4} \sin(z_{2,n}s) , \]  

(2.25)

where

\[ z_{1,n} = \frac{m}{\sqrt{2}} \left( \sqrt{4\lambda_n + 1} + 1 \right)^{\frac{1}{2}} \]  

and \[ z_{2,n} = \frac{m}{\sqrt{2}} \left( \sqrt{4\lambda_n + 1} - 1 \right)^{\frac{1}{2}} . \]  

(2.26)

In the case of the hinged-hinged beam,

\[ Y_n^H(s) = \sin \left( \frac{n\pi}{2} (s + 1) \right) , \]  

(2.27)

\[ \lambda_n^H = \left( \frac{n\pi}{2m} \right)^2 \left( 1 + \left( \frac{n\pi}{2m} \right)^2 \right) , \]  

and \( n \) is an integer \((n=1, 2, \ldots)\).
In the case of the clamped-clamped beam, the eigenvalues are determined by the solution of the characteristic equation

\[ 4\sqrt{\lambda_n} \left( \cosh(z_{1,n}) \cos(2z_{2,n}) - \cos^2(z_{2,n}) \right) - \sinh(2z_{1,n}) \sin(2z_{2,n}) = 0. \] (2.28)

For moderate values of \( \lambda_n \), equation (2.28) simplifies to \( \tan(2z_{2,n}) = 4\sqrt{\lambda_n} \). When \( \lambda_n \) is even larger, \( \lambda_n = \left( \frac{2n+1}{4m} \right)^2 \left( \frac{(2n+1)\pi}{4m} \right)^2 + 1 \), where \( n = 1, 2, \ldots \) is an integer. For example, when \( m = 1.25 \), the first few exact eigenvalues are \( \{14.78, 104.73, 390.03, \ldots \} \).

The first implicit approximation gives \( \{15.41, 106.02, 391.79, \ldots \} \) and the second explicit approximation yields \( \{16.18, 107.23, 393.55, \ldots \} \). The eigenfunctions are

\[ Y_n^C(s) = C^* \left( \cosh(z_{1,n}(s+1)) - \cos(z_{2,n}(s+1)) \right) \frac{z_{2,n} \sinh(z_{1,n}(s+1)) - z_{1,n} \sin(z_{2,n}(s+1))}{\cosh(2z_{1,n}) - \cos(2z_{2,n})} \] (2.29)

where the constant \( C^* \) is selected so as to render \( \|Y_n^C(s)\| = 1 \).

According to the principle of equipartition of energy, the elastic energy associated with each mode

\[ U_n = \frac{<A_n^2(t)>}{2} \int_0^L \left( \frac{EI}{L} \frac{d^2Y_n}{ds^2} \right)^2 + m^2 \left( \frac{dY_n}{ds} \right)^2 \, ds = \frac{EI}{2L} <A_n^2(t)> \int_0^L \left( \frac{d^2Y_n}{ds^2} \right)^2 + m^2 \left( \frac{dY_n}{ds} \right)^2 \, ds \] (2.30)

equals \( k_B T / 2 \), where \( k_B \) is the Boltzmann constant and \( T \) is the absolute temperature. The variance of the transverse fluctuations is

\[ V_{th}(s) = \left\{ \left( \xi'(s,t) - \xi'(s,t) \right) \right\}^2 = \sum_{n=1}^\infty <A_n(t)^2> \left( Y_n(s) \right)^2. \] (2.31)

In the hinged-hinged case, we have

\[ <A_n^H(t)^2> = \frac{16k_B TL}{EI(n\pi)^2 \left( 4m^2 + (n\pi)^2 \right)} \] (2.32)
and

\[ V^H_{wh}(s) = \frac{16k_BT L}{EI} \sum_{n=1}^{\infty} \frac{1}{(n\pi)^2} \left( 4m^2 + (n\pi)^2 \right) \sin^2 \left( \frac{n\pi}{2}(s+1) \right). \]  

The latter expression can be summed-up in closed form to give

\[ V^H_{wh}(s) = \frac{k_BT L}{2m^2EI} \left( 1 - s^2 \right) \left( 1 - \frac{\cosh(2m) - \cosh(2ms)}{m \sinh(2m)} \right). \] 

The first term on the right in the above is the variance of a fluctuating string with tension \( \tau \). The second term represents the reduction in the variance due to the flexural rigidity. The second term decays as \( m \) increases. When \( m \gg 1 \), equation (2.34) has the asymptotic approximation

\[ V^H_{wh}(s) \sim \frac{k_BT L}{2\pi L} \left( 1 - s^2 \right) \left( 1 - \frac{1}{m} \left( 1 - e^{-2m(1-s)} \right) \right) \quad (m \gg 1). \] 

The corresponding expressions for the clamped-clamped case are considerably lengthier, were computed numerically, and are not reproduced here.

**2.3.4 An Inverse Problem: Estimation of the filament’s tension**

We used the experimentally observed transverse variance to estimate the filament’s tension. To this end, we consider the theoretical variance to be a function of the filament’s tension, and we seek the tension \( \tau \) that minimizes the discrepancy

\[ E(\tau) = \int_{-1}^{1} \left( V_{wh}(s, \tau, \kappa) - V_{exp}(s, \tau, \kappa) \right)^2 ds, \] 

between theoretical predictions \( V_{wh}(\tau) \) and experimental observations \( V_{exp} \). In the minimization process, we used the accepted value of the flexural rigidity \( EI = 7.3 \cdot 10^{-26} \) Nm² of actin filaments (Gittes et al., 1993). Figure 2.6 depicts an example of \( E(\tau) \) as a
function of $\tau$. Witness the relatively deep, global minimum which allows us to estimate the tension force within ±10%.

![Graph](image)

**Figure 2.6**: The discrepancy between the experimentally-observed and the theoretically-estimated variance $E(\tau)$ as a function of the filament’s tension $\tau$.

2.4 Results and Discussion

2.4.1 Theoretical Considerations

In the course of the experiments, the filament vibrated relative to the camera. As a result, blurring of the image was unavoidable. The impact of this phenomenon depended on the relative magnitude of the camera’s exposure time $t_e$ compared with the characteristic time of the filament’s fluctuations $\hat{t}$. As $t_e/\hat{t}$ increased, the variance estimated from the experimental data decreased and the estimated filament tension $\tau$ increased.
Recently, Towles et al. (2008) demonstrated that the measured variance of a tethered bead undergoing Brownian motion decreases as the camera’s exposure time increases. In other words, the bead’s position is biased toward its equilibrium state. Here, we extend Towles et al. (2008) analysis to the case of the vibrating filament. From equation (2.14), neglecting inertia,

\[ \frac{dA_n}{dt} = -\lambda_n m^4 A_n + \frac{L^3}{EI} F_n(t), \]  

(2.37)

which admits the solution

\[ A_n(t) = A_n(0)e^{-\lambda_n m^4 t} + \frac{L^3}{EI} \int_0^t F_n(u)e^{\lambda_n m^4 (t-u)} du. \]  

(2.38)

Below, we will assume that the effects of the initial data have decayed. The amplitude observed by the camera is the average amplitude over the exposure time \( t_e \).

\[ A_n^{\text{CAMERA}}(t) = \frac{L^3}{t_e EI} \int_0^{t_e} \int (\int_0^t F_n(u)e^{\lambda_n m^4 (t-u)} du) dv. \]  

(2.39)

The time constant associated with mode \( n \) is \( \hat{\tau}_n = \frac{1}{\lambda_n m^4} \). When \( n \) is small and \( m \) is moderate, the “string modes” dominate, \( \hat{\tau} \propto \tau^{-1} \), and the vibration’s time constant decreases as the filament’s tension increases. When \( n \gg 1 \), the flexural modes dominate and the time constant is independent of the filament’s tension.

To compute the variance, we calculate the integral

\[ V_X = \lim_{t \to \infty} \frac{1}{t} \int_0^t X^2(z) dz. \]  

(2.40)

We define the ratio between the variance constructed using camera images and the true variance as
\[ S(t_e) = \frac{V_A^{\text{CAMERA}}}{V_A^{\text{TRUE}}}. \]  

(2.41)

Unfortunately, the correction \( S(t_e) \) depends on the thermal force and does not lend itself to a simple expression. Figure 2.7 illustrates the behavior of the correction \( S(t_e) \) as a function of the normalized exposure time \( t_e / \hat{t} \) when \( F_1(t) = \sin(\omega t) \) and \( F_{n>1} = 0 \) (solid line).

Towles et al. (2008) proposed a correction factor of the form

\[ S(t_e) = \left( \frac{\hat{t}}{t_e} \left(1 - e^{-\frac{t_e}{\hat{t}}} \right) \right)^2, \]  

(2.42)

where the time constant \( \hat{t} \) is determined empirically. Although expression (2.42) provides qualitatively correct behavior, the quantitative predictions do not match well the computed data of Fig. 2.7. We find, however, that the computed data of Fig. 2.7 correlates well with an expression of the form

\[ S(t_e) = \frac{1}{\left(1 + \left(\frac{t_e}{\hat{t}}\right)^2 \right)^2}. \]  

(2.43)

In Fig. 2.7, the dashed and dotted lines correspond to fits of equations (2.42) and (2.43), respectively, to \( S(t_e) \).
Figure 2.7: The correction factor $S(t_e)$ as a function of the normalized exposure time $t_e / \hat{t}$ when $F_1(t) = \sin(\omega t)$ and $F_{\infty} = 0$. $\lambda = 4$, $m = 1$, and $\omega \approx 1$. The solid, dashed, and dotted lines correspond, respectively, to the exact calculation, a fit obtained with equation (2.42), and a fit obtained with equation (2.43).

2.4.2 Experimental Observations

To examine the effect of the camera exposure time on the variance, we carried out a sequence of measurements of filaments’ vibrations while varying the camera exposure time. Figure 2.8 depicts the variance of the filament’s transverse vibrations (symbols) as a function of position along the filament for several camera exposure times $t_e = 10, 75, 300$ ms. The filament was suspended in water and had a length of 17 µm. The trap’s applied force was 0.3 pN. To guide the eye, we fitted the experimental data with theoretical fits based on the hinged-hinged model (lines). As the exposure time $t_e$ increased, the variance decreased, and the estimate for the tension force increased.
Figure 2.8: A filament’s transverse displacement’s variance depicted as a function of position along the filament for several camera exposure times 75 (solid circles), 200 (hollow circles), and 300 (stars) ms. The various lines correspond to theoretical fits with hinged-hinged boundary conditions.

The effect of the camera exposure time on the variance is summarized in Figure 2.9. The figure depicts the normalized variance integrated along the filament’s length (symbols) as a function of the camera exposure time for three different filaments. The normalization factor is the variance measured at the shortest available exposure time. In other words, the figure depicts the experimental estimate of $S$ (equation (2.41)) as a function of the camera exposure time. The solid circles correspond to a 17 μm long filament suspended in water and subjected to 0.3 pN of trap force. The solid triangles and squares correspond to filaments suspended in 60% (by mass) glycerin ($\eta = 10.8$ mPa·s). The glycerin was used to reduce the filament’s frequency of vibrations (increase filament’s oscillatory time constant). The solid triangles correspond to a 12 μm long
filament subjected to 1.2 pN tension force. The solid squares correspond to a 15 µm long filament subjected to a 0.3 pN force. The various lines correspond to best fits of equation (2.43). Figure 2.9 illustrates that the variance decreases as the exposure time increases. Indeed, the plots feature plateaus only in the glycerin experiments, indicating that the camera exposure time used in the water experiments was not sufficiently short to obtain the true variance.

Figure 2.9: The normalized, experimental variance is depicted as a function of the camera exposure time. The upright triangles, solid squares, and solid circles correspond, respectively, to \( \{L, \tau_{\text{applied}}, \eta\} = \{17 \, \mu\text{m}, 0.26 \, \text{pN}, 1 \, \text{mPa}\cdot\text{s}\}, \{ 12 \, \mu\text{m}, 1.2 \, \text{pN}, 10.8 \, \text{mPa}\cdot\text{s}\} \) and \( \{ 15 \, \mu\text{m}, 0.27 \, \text{pN}, 10.8 \, \text{mPa}\cdot\text{s}\} \). In the above, \( L \) represents the filament’s length, \( \tau \) represents the applied tension, and \( \eta \) represents the suspension’s viscosity. The variance was normalized with the variance measured at the smallest exposure time. The solid, dotted, and dashed lines correspond to the best fits of equation (2.43), respectively.
The theoretical time constants (based on the fundamental mode $n=1$) for the three scenarios presented in Fig. 2.9, calculated using both hinged and (clamped) boundary conditions, were 130 (58), 160 (72), and 1200 (510) ms, respectively. The solid, dotted, and dashed lines correspond to best fits of equation (2.43) with the fitted time constants 130, 150, and 570 ms, respectively. Unfortunately, it was not always possible to obtain either the short or long exposure time plateaus due to camera sensitivity limitations or fluorophore bleaching, respectively. Despite these practical limitations, the experimental trends of the exposure time-driven variance biasing qualitatively agree with the theoretical timescales.

Qualitative evidence of the importance of exposure time emerges when the width of a fluorescent filament’s intensity profile across its transverse axis is examined as a function of exposure time. The width is defined as the standard deviation of a fitted Gaussian curve and increases with $t_e$, which is indicative of the filament moving during an exposure. The average width ($\pm$ s.d.) of individual snapshots is depicted in Fig. 2.10 as a function of $t_e$.

![Figure 2.10](image)

**Figure 2.10:** The average width of a 16.6 μm-long fluorescent filament’s intensity profile across its transverse axis depicted as a function of exposure time (means ± s.d.).
This may seem confusing, given what has been discussed. To reintroduce the phenomenon, longer exposure times lead to blurred images and thus wider (larger variance) transverse profiles. This biases our calculation of the filament’s position, leading to an underestimate of the transverse displacement’s variance about equilibrium.

2.4.3 Estimates of the filament’s tension based on the variance of the transverse fluctuations in water

24 filaments were observed in water with exposure times of 30 (n=8), 50 ms (n=5), and 100 (n=11) ms. The filaments’ lengths ranged from 6 to 20 µm. The data processing included the measurement of the variance of the thermal fluctuations as a function of position along the filament to obtain curves similar to the ones depicted in Fig. 2.8. The experimental data was then fitted with the theoretical curves of the hinged-hinged and clamped-clamped models to obtain estimates of the filament tension. An example of the experimental variance data for one filament, being subjected to different trap forces, is depicted in Fig. 2.11. The actual forces applied with the optical tweezers were \( \tau_{\text{applied}} = 0.22, 0.37, \) and \( 0.48 \) pN. The symbols and lines correspond, respectively, to experimental data \( (t_e = 100 \text{ ms}) \) and theoretical predictions for a hinged-hinged filament. The estimated forces obtained for the data in Fig. 2.11 were \( \tau_{\text{estimated}} = 0.33, 0.84, \) and \( 1.5 \) pN, respectively.
Figure 2.11: A single actin filament’s variance of transverse thermal fluctuations with three different forces being applied (0.22 pN – circles, 0.37 pN – triangles, & 0.48 pN – squares) depicted as a function of the position along the filament. The various lines correspond to theoretical fits with hinged-hinged boundary conditions, solved numerically (0.33 pN – dash-dotted line, 0.84 pN – dashed line, & 1.5 pN – solid line).

To ensure that the inverse problem had only one solution, we depicted $E(\tau)$ as a function of $\tau$ in Fig. 2.6 and observed the presence of a single minimum. Figure 2.12 depicts the forces estimated (from the hinged-hinged model) for all 24 filaments as a function of the applied forces (symbols). The solid line represents a 1:1 correlation between actual and estimated force values. There is variability in the applied trap force on the order of 5%. The filament tracking algorithm’s uncertainty is on the order of 50 nm.
Figure 2.12: The force estimated for 24 filaments (in water, $\eta = 1$ mPa·s) by the hinged-hinged model, depicted as a function of the applied force. The solid line represents a 1:1 correlation between actual and estimated force values.

At low trap forces, the model-based force estimates agree well (within 20%) with the forces applied by the optical trap. However, as the applied force increased, the Brownian dynamics–based estimate began to overestimate the force with increasing severity. This cannot be explained by the inherent uncertainty of the optical tweezers alone (see section 2.2.1). The data, however, were collected on filaments with different lengths and with different camera exposure times. The explanation for this divergence with applied force is the growing mismatch between $t_e$ and $\hat{t}$. If we apply the correction to the experimental variance (equation 2.43), using the applied force and the filament length to calculate the theoretical time constant, the fitted, estimated force data come closer to the expected values. This result is depicted in Fig. 2.13. Here, the estimated force data from the
hinged-hinged model are reproduced (circles) along with corrected force estimates (triangles). Unfortunately, we never reached a sufficiently short exposure time, horizontal asymptote with the water data (see Fig. 2.9), leaving us without an accurate “correction formula”.

Figure 2.13: The force estimates using the hinged-hinged model (circles) and the corrected force estimates from fits to variance data adjusted using equation (2.43) (triangles), depicted as a function of the applied force.

To illustrate the importance of the change in fundamental frequency with both tension and length, Fig. 2.14 depicts the relative discrepancy between the estimated force and the applied force as a function of the filament’s time constant. The figure clearly illustrates the importance of minimizing the mismatch between experimental observation times and the time constant of the filament’s fluctuations.
Figure 2.14: The relative discrepancy between the force estimates and the applied forces as a function of the filaments’ theoretical time constants \((n=1)\). The lines are to guide the eye.

2.4.4 Estimates of the filament’s tension based on the variance of the transverse fluctuations in glycerin

To obtain more accurate measurements of the filament’s displacement as a function of time, it is necessary to reduce the exposure time of the camera below the time constant of the filament’s fluctuations. Unfortunately, as the exposure time is reduced so is the amount of light collected by the camera and the signal to noise ratio. Since we were not able to sufficiently reduce the camera exposure time for the experiments carried out in water, we report in this section on results of experiments carried out with filaments suspended in a glycerin-water mixture. Since the viscosity of the glycerin-water mixture was about 10 times larger than that of the water, the time constant of the fluctuations was
increased by approximately a factor of 10 as well. All the measurements were carried out with a camera exposure time of 30 ms.

Figure 2.15 depicts the Brownian dynamics-based estimates of the filament tension (in glycerin) as a function of the applied optical trap force. The solid and hollow circles correspond, respectively, to the prediction of the clamped-clamped and hinged-hinged models. The solid line is a 45° line. At relatively low forces (<1 pN), the estimates of the filament tension are in excellent agreement with the known, applied trap forces. As the magnitude of the force increases, so does the discrepancy between the estimated force and applied trap force. Just as before (in water), the increase in the discrepancy is attributed to a reduction of the filament’s time constant as the force increases.

**Figure 2.15:** The force estimated for 6 filaments (in glycerin, η = 10.8 mPa·s) by the clamped-clamped model, depicted as a function of the applied force. The solid and hollow circles correspond, respectively, to the prediction of the clamped-clamped and hinged-hinged models. The solid line represents a 1:1 correlation between actual and estimated force values.
Figure 2.16 depicts the relative discrepancy between the force estimate and the applied force as a function of the filament’s theoretical time constant $\hat{t}$ (triangles). The circles correspond to the same discrepancy data (in water) depicted in Fig. 2.14.

**Figure 2.16**: The relative discrepancy between the force estimate and the applied force as a function of the filament’s time constant, $n=1$ (triangles – in glycerin; circles – in water). The lines are to guide the eye.

The extent of a filament’s transverse fluctuations serves as an additional indicator of the applied force affecting a filament’s oscillatory variance. First, all the filament’s snapshots were averaged. Second, the image’s background was subtracted, and the image was smoothed using a 16-pixel Gaussian smoothing kernel. Third, intensity profiles taken transverse to the filament’s long axis at its midpoint were fitted with Gaussian profiles. This fitting process is depicted in Fig. 2.17a with the Gaussian curve’s width $\sigma$ denoted.
The variances $\sigma^2$ of the fitted Gaussian curves for all the filaments’ are depicted as a function of the theoretical time constant in Fig. 2.17b (squares; red=glycerin, black=water). Also depicted are the midpoint variance values (circles; red=glycerin, black=water) calculated similarly to the datasets depicted in Fig. 2.11. The trends of the two datasets are a bit different. The blur variance remains relatively constant as a function of the theoretical time constant, whereas the variance calculated from tracking the filament’s displacement over time decreases as a function of theoretical time constant.

**Figure 2.17:** a) The intensity of a CCD image (symbols) depicted along a line drawn transverse to an actin filament’s long axis. The image’s background was subtracted, and the image was smoothed using a 16-pixel Gaussian smoothing kernel. A Gaussian curve was fitted (line) to obtain the filament’s location and its width (variance - $\sigma$). b) The variance of the fitted Gaussians, similar to those depicted in (a), at all of the filaments’ midpoints depicted as a function of the theoretical time constant (squares; red = glycerin, black = water). Also depicted are the midpoint variance values (circles; red=glycerin, black=water) calculated similarly to the datasets depicted in Fig. 2.11.
Figure 2.18 depicts the relative discrepancy between the “blur variance” force estimate and the applied force as a function of the filament’s time constant (based on the fundamental mode \( n=1 \)) (squares; red = glycerin; black = water). Applying the hinged-hinged model to the experimental blur variance consistently yields an underestimate of the force applied to the filament. This is not unexpected as due to experimental errors (independent of the exposure time), we are likely to overestimate the true variance of the filament. Contrary to the force estimates obtained from the variances calculated from observing the filament over time, however, the blur variance dataset’s accuracy does not have as severe a dependence on the theoretical time constants. A theoretical basis for extracting a true variance from the “width” of a filament’s average position is necessary to complete this analysis.

\[
\frac{\tau_{\text{est}} - \tau_{\text{applied}}}{\tau_{\text{applied}}}
\]

\textbf{Figure 2.18:} The relative discrepancy between the “blur variance” force estimate and the applied force as a function of the filament’s time constant (based on the fundamental mode \( n=1 \)) (squares; red = glycerin; black = water).
2.5 Conclusions

Snapshots of actin filaments fluctuating in both water and glycerin were collected as a function of time and optically applied force, analyzed, and used to calculate transverse displacement variances as functions of position along the filament. By comparing the experimentally measured variance with predictions of a linear Brownian dynamics model, we estimated the filament’s tension. The estimated filament’s tension was compared and favorably agreed with the known applied force. The experiments demonstrate that the methods of statistical mechanics can be used to estimate the filament’s tension. Our experiments provide yet additional piece of evidence to a growing body of literature demonstrating that the methods of statistical mechanics and the principle of equipartition of energy are applicable to mesoscopic structures.

The experiments highlight the importance of adjusting the camera exposure time to reduce blurring effects. When the exposure time is significantly larger than the time constant of the filament’s fluctuations, a significant error would occur in estimating the filament’s position, which, in turn, results in underestimating the variance of the transverse fluctuations. This effect was minimized by observing the filaments in glycerin.
Chapter 3

Estimating the Rigidity of Free-Free Actin Filaments as a Function of Solution’s Ionic Strength

Abstract

This chapter introduces new techniques for estimating the persistence length $L_p$ of freely-fluctuating actin filaments (F-actin) and compares them with existing techniques. Additionally, we study the effect of the ionic environment on $L_p$. Phalloidin-stabilized F-actin filaments were confined in a narrow space between two anodically-bonded coverslips, and their thermally-driven oscillations were tracked. $L_p$ was calculated using several statistical mechanics methods: mode analysis, variance of the transverse fluctuations, and the variance of the end-to-end distance. The solution’s ionic strength in the range 60 µM – 250 mM had little or no effect on $L_p$. Finally, preliminary observations were made of free filaments orienting and straightening in spatially uniform, AC electric fields.
3.1 Introduction

F-actin resists and applies force to accomplish the tasks described in Chapter 1. Accordingly, much work has focused on studying its mechanical properties using statistical mechanics (Yanagida et al. 1984; Gittes et al. 1993; Isambert et al. 1995) and mathematical models that capture its transient dynamics (Riveline et al. 1997). Electron micrographs of F-actin were used to extract a temperature-dependent elastic modulus at 4, 22, & 40 °C (Takebayashi et al. 1977), finding that it increased with temperature.

Filaments’ electrical properties are also of great interest. For example, a DNA molecule’s counterion cloud, or electric double layer (EDL), regulates the binding of its associated proteins during transcription and its packaging into viral capsids (Manning 1978). A DNA molecule’s flexural rigidity (persistence length) decreases as its environment’s ionic concentration increases (Baumann et al. 1997). Both DNA and F-actin form aggregates in high ionic strength solutions due to charge condensation and counterion sharing (Tang and Janmey 1996). The torsional rigidity of F-Ca²⁺-actin is three times larger than F-Mg²⁺-actin (Yasuda et al. 1996), whereas the flexural rigidity remains nearly the same (Yasuda et al. 1996; Isambert et al. 1995). These electrical properties are intimately coupled to mechanical properties through electrostatic interactions, which is made especially evident when filaments are subjected to electric fields.

F-actin can be specifically positioned by electric fields using dielectrophoresis (Asokan et al. 2003). This technique takes advantage of the strong dipole moment created by polarizing the filament’s EDL. The EDL contributes to a particle’s total conductivity, and changes to its structure will affect a particle’s polarizability (O’Konski 1960). For
example, the thickness of a particle’s EDL is inversely proportional to the ionic strength of its suspending medium. Chapter 4 discusses the strong effect on the apparent stiffness of F-actin as a function of electric field strength. We seek to observe the thermal fluctuations of free-free F-actin over a larger range of ionic strengths (60 µM - 250 mM) than previously investigated. We used three statistical mechanics techniques: mode analysis, variance of transverse fluctuations, and end-to-end distance to estimate $L_p$.

Additionally, this chapter presents preliminary observations of free filaments orienting and straightening in spatially uniform, AC electric fields. To investigate the origin of this apparent stiffening, we first sought to discover the effect of ionic strength on the persistence length of F-actin in the absence of electric field.

### 3.2 Materials and Methods

Our experimental apparatus consisted of an anodically-bonded (Berthold et al. 2000) flow cell confined between two glass coverslips. The anodic bonding process lets us consistently make flow cells of the same height. The process is schematically depicted in Fig. 3.1. A 100 nm-thick layer of silicon was thermally evaporated on a sulfuric-peroxide (H$_2$SO$_4$:H$_2$O$_2$, 3:1)-cleaned 150 µm-thick glass coverslip to act as a diffusion barrier (Fig. 3.1a). 1 mm-wide channels were defined in positive photoresist (Shipley 1813). The exposed silicon was etched using SF$_6$ plasma. The exposed glass was etched another 500 nm using buffered oxide etch (Fig. 3.1b). A second glass coverslip was laid across the remaining silicon, and the entire flow cell was heated to 375 °C. A 1000 V potential was applied across the flow cell between a finely-tipped steel probe on the upper surface and a grounded plate on the lower surface (Fig. 3.1c). The electric field...
caused ions in the glass to migrate (Fig. 3.1d). Without the silicon barrier, the ions would migrate through the two glass slides. Finally, the oxygen in the glass bound to the silicon diffusion barrier (Fig. 3.1e).

**Figure 3.1**: Schematic depiction of the anodic bonding process. a) a 150 μm-thick glass coverslip was coated with a 100 nm-thick layer of silicon; b) the silicon was patterned in positive resist (Shipley 1813), and the glass was etched further using buffered oxide etch; c) the flow cell was heated to 375 °C, and a 1000 V potential was applied; d) ions in the glass migrated; e) oxygen bound to the silicon.

The flow cell’s depth was estimated by focusing on the bottom surface and the top surface with a nanostage (Mad City Labs Inc.). The flow cell was filled with a solution of 50 nM rhodamine-phalloidin-stabilized actin, polymerized (in 2 mM MgCl₂, 150 mM
KCl, 2 mM Hepes, pH=7.4), and diluted in DI water to various ionic concentrations (see Table 3.1). 1 mg/mL BSA was included in the final solutions to prevent non specific adhesion of the F-actin on the glass surfaces. The following anti-bleaching system was also included: 50 mM DTT, 7.2 mg/mL glucose, 9 units/mL catalase, and 4 mg/mL glucose oxidase. The flow cell was placed on the stage of an inverted microscope. An electron multiplying CCD camera (Photometrics Cascade II) collected images every 2 s, with an exposure time of 10 ms. For all the filaments examined in this study, this exposure time is much shorter than the first fundamental modes’ timescales. A custom-written Matlab™ algorithm calculated the filament’s position.

The apparatus for the electric field experiments consisted of a set of gold electrodes sandwiched between two glass slides. Using standard microfabrication techniques, a pair of gold electrodes with a gap of ~7 µm between them was patterned on one of the slides. The process is depicted in Fig. 3.2. First, a 150 µm-thick glass coverslip was coated with a 10 nm-thick layer of nickel-chromium and a 100 nm-thick layer of gold (Fig. 3.2a). A layer of positive resist (Shipley 1813) was spun onto the gold surface, and the electrodes were patterned (Fig. 3.2b). The metal was then removed, leaving the electrodes (Fig. 3.2c). The glass was etched using buffered oxide etch to create a trench (Fig. 3.2d), though this was only used in the experiments described in Chapters 4 & 5. A second glass coverslip was attached to the first coverslip either by simply pressing it into contact with the first coverslip or with double-sided tape (for experiments detailed in Chapters 4 & 5). The flow cell was filled with 50 nM phalloidin-stabilized, rhodamine-labeled actin suspended in solutions with KCl as the predominant electrolyte. The openings were sealed with grease to prevent evaporation. The electric field was produced
by applying an AC voltage across the electrodes. We strove to apply a predominantly uniform electric field in this confined geometry, thus removing any effects due to dielectrophoresis or electrokinetic flows. These phenomena will be discussed in Chapter 4. A nonuniform field was likely experienced by some filaments, because the sealed flowcell was an order of magnitude taller (~2 µm) than the 100 nm-tall gold electrode edge.

**Figure 3.2:** Schematic depiction of the electrode fabrication process. a) a 150 µm-thick glass coverslip was coated with a 10 nm-thick layer of nickel-chromium and a 100 nm-thick layer of gold; b) positive resist (Shipley 1813) was spun onto the gold surface; c) the electrodes were patterned, and the metal was removed; d) the glass was etched using buffered oxide etch (for experiments detailed in Chapters 4 & 5); e) the flow chamber is capped either by pressing it into contact with the first coverslip or using double-sided tape (for experiments detailed in Chapters 4 & 5).

### 3.3 Beam Functions

We decompose the fluctuations of the filament into the normal modes
\[ y_i(s) = (a(t)s + b(t)) + \sum_{n=1}^{\infty} A_n(t) y_n(s) \quad (0 \leq s \leq L), \]  

(3.1)

where the subscript \( l \) denotes measurement in the laboratory reference frame, \( L \) is the filament contour length, \( \kappa \) is the flexural rigidity \((EI)\), \( E \) is Young’s modulus, \( I \) is the filament’s moment of inertia, and \( \lambda_n \) are the eigenvalues of mode \( n \). The first term on the RHS of equation (3.1) represents rigid body translation and rotation. The sum on the RHS of equation (3.1) represents flexural modes. The eigenmodes \( y_n(s) \) are the solutions of the 4th-order eigenvalue problem

\[ \kappa \frac{d^4 y_n}{ds^4} = \lambda_n y_n \quad (0 \leq s \leq L) \]  

(3.2)

and satisfy four boundary conditions

\[ \frac{\partial^2 y_n(0)}{\partial s^2} = \frac{\partial^2 y_n(L)}{\partial s^2} = \frac{\partial^3 y_n(0)}{\partial s^3} = \frac{\partial^3 y_n(L)}{\partial s^3} = 0, \]  

(3.3)

as appropriate for a free-free rod. Figure 3.3 depicts an actin filament of length \( L \) described by the curve \( \theta(s) \). Its transverse displacement \( y_f(s) \) can be measured from any arbitrary line defined as the filament’s reference frame. The only requirement is that the reference line used to process the experimental data is the same as the one used in the theoretical analysis. The line connecting the filament’s endpoints was chosen and is depicted in Fig. 3.3. \( \theta(s) \) is the angle formed between the tangent to the filament and the chosen reference line. The above boundary conditions correspond to zero moment and zero shear force at both ends of the filament (Liu et al. 1996). Equations (3.2) and (3.3) admit solutions consisting of the eigenmodes

\[ y_n(s) = \sqrt{\frac{1}{L}} \sinh \left( \frac{d_n s}{L} \right) - \sinh \left( \frac{d_n s}{L} \right) - \sinh \left( \frac{d_n s}{L} \right) - \sinh \left( \frac{d_n s}{L} \right) + \cosh \left( \frac{d_n s}{L} \right) + \cos \left( \frac{d_n s}{L} \right) \]  

(3.4)
which satisfy the orthonormality condition

\[ \int_{0}^{L} y_n(s)y_m(s)ds = \delta_{nm}. \quad (3.5) \]

In the above, \( d_n^4 = (\lambda L^4/\kappa) \) are the roots of the equation

\[ \cos(d_n)\cosh(d_n) = 1. \quad (3.6) \]

The first few roots are: \( d_n = 4.73, 7.8532, 10.9956, 14.1373 \ldots, (2n+1)\pi/2. \)

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure3.png}
\caption{An actin filament of length \( L \) is described by the curve \( \theta(s) \). Its transverse displacement \( y_f(s) \) can be measured from any arbitrary line defined as the filament’s reference frame. \( \theta(s) \) is the angle formed between the tangent to the filament and the chosen reference line. In this picture, the line connecting the filament’s endpoints is chosen as the reference line.}
\end{figure}

Figure 3.4 depicts the first four modes of oscillation (equation 3.4) as a function of position along the filament. Here, the displacement is measured from a line a)
connecting the filament’s endpoints (Fig. 3.4a) and b) a line intersecting the center of mass and parallel to the line connecting the filament’s endpoints (Fig. 3.4b).

![Figure 3.4](image)

**Figure 3.4**: The first four modes of oscillation, using equation (3.4), depicted as a function of position along the filament. Here the displacement is measured from a line (a) connecting the filament’s endpoints and (b) intersecting the center of mass and parallel to the line connecting the filament’s endpoints.

The flexural rigidity of an oscillating filament can be estimated from the variance of its thermal fluctuations (Purohit et al. 2008). Starting with a rod’s energy due to bending,

\[
U = \frac{\kappa}{2} \int_0^L \left( \frac{d\theta}{ds} \right)^2 ds = \frac{\kappa}{2} \int_0^L \left( \frac{d^2y}{ds^2} \right)^2 ds ,
\]

(3.7)

we can insert equation (3.4) into equation (3.7) and assign each vibrational mode a mean energy of \( k_B T / 2 \), where the brackets \( \langle \cdot \rangle \) denote ensemble averaging,

\[
\frac{k_B T}{2} \left\langle A_n^2 \right\rangle \frac{\kappa}{2} \left( \frac{d_n}{L} \right)^4 .
\]

(3.8)
The above equation allows us to estimate $<A_n^2>$.

Witness that $(<A_{n+1}^2>/ <A_n^2>)^{1/4} = d_n / d_{n+1}$. We calculate the experimental $A_n$ by decomposing the filament’s displacement from the reference line into the basis functions given in equation (3.4). Figure 3.5 depicts the experimental $(<A_{n+1}^2>/ <A_n^2>)^{1/4}$ as a function of $n$ for one actin filament (symbols). The theoretical ratio $d_n / d_{n+1}$ is overlaid (solid line). This good agreement between theory and experiment for the first ten modes ($n<10$) is consistent with Gittes et al. (1993) observations. Beyond $n = 10$, experimental noise precluded us from comparing the experimental data with theoretical predictions.

Figure 3.5: $(<A_{n+1}^2>/ <A_n^2>)^{1/4}$ from experiment as a function of $n$ for one actin filament (symbols). The theoretical ratio $d_n / d_{n+1}$ is overlaid (solid line). The length of the filament is 6 µm.

We construct the variance of the transverse displacement both by directly measuring the transverse displacement and by measuring the tangent angle.
The expressions for the variance of the transverse displacement based on the
tangent angle and the eigenmodes are, respectively,

\[
\langle \left| y_{\text{ref}}(s) \right|^2 \rangle = \int_0^s \theta(u) \cdot \theta(v) du dv .
\] (3.9a)

and

\[
\langle \left| y_{\text{ref}}(s) \right|^2 \rangle = \sum_{n=1}^{\infty} \left( A_n^2 \right) \left( y_n(s) - \langle y_n(s) \rangle \right)^2 .
\] (3.9b)

We determine the variance of the displacement with respect to the reference line 
\((a(t)s + b(t))\) as a function of position along the filament using the more general
expression for the displacement

\[
y_{\text{ref}}(s) = \sum_{n=1}^{\infty} A_n \left[ (y_n(0) - y_n(L)) \frac{s}{L} - (y_n(0) - y_n(s)) \right] .
\] (3.10)

The variations of the reference line’s position and inclination, from one frame to another,
represent rigid body translation and rotation.

We wish to examine how the choice of base functions affects the estimate of the
persistence length. For example, we may construct the variance as a function of \(s\) using a
cosine series for \(\theta\). Inserting this choice of basis set into equation (3.9) and integrating,
we obtain

\[
\langle y_{\text{ref}}^2(s) \rangle = \frac{k_B T}{\kappa} \sum_{n=1}^{\infty} \frac{2L^3 \sin \left( \frac{\pi n}{L} s \right)^2}{\pi^4 n^4} .
\] (3.11a)

The above expression can be summed-up in a closed form to obtain

\[
\langle y_{\text{ref}}^2(s) \rangle = \frac{k_B T L^3}{3\kappa} \left( \frac{s}{L} \right)^2 \left( 1 - \frac{s}{L} \right)^2 .
\] (3.11b)
Figure 3.6 depicts predictions for the variance of a 15 µm-long actin filament, measured from a line connecting its endpoints, using both the beam basis functions (solid line - equations 3.4 and 3.10) and a cosine basis functions (dotted line - equation (3.11a)). In both cases, only the first term in the series was used. The two theoretical predictions are nearly identical and agree even more closely after higher modes are included.

Figure 3.6: Predictions for the variance of a 15 µm-long actin filament, measured from a line connecting its endpoints, using both the beam basis functions (solid line - equation (3.4 and 3.10)) and a cosine basis functions (dotted line - equation (3.11a)). One mode was used to construct each curve.

Instead of measuring the transverse displacement, Gittes et al. (1993) measured the tangent angle and used a cosine series to conduct a mode analysis on freely-fluctuating filaments, where

$$\theta(s) = \sum_{n=0}^{\infty} \theta_n(s) = \sqrt{\frac{2}{L}} \sum_{n=0}^{\infty} a_n \cos\left(\frac{n \pi s}{L}\right). \quad (3.12)$$

Substituting equation (3.12) into equation (3.7), we have
\[ U = \frac{\kappa}{2} \sum_{n=1}^{\infty} \left( \frac{n\pi}{L} \right)^2 (a_n)^2. \] (3.13)

As before, we assign \( k_B T / 2 \) of average energy to each vibrational mode. After calculating the mode amplitudes for each image, we can relate their variance to the persistence length \( L_p = \kappa k_B T \),

\[
\text{var}(a_n) = \left\langle (a_n)^2 \right\rangle = \frac{k_B T}{\kappa} \left( \frac{L}{n\pi} \right)^2
\]

\[ \rightarrow L_p = \frac{L^2}{n^2\pi^2 \text{var}(a_n)} \] (3.14)

obtaining an independent estimate of \( L_p \) from each mode. Figure 3.7 depicts \( (\langle a_{n+1}^2 \rangle/\langle a_n^2 \rangle)^{1/2} \) obtained from experimental data as a function of \( n \) for one actin filament (symbols). The theoretically expected scaling \( n/(n+1) \) is overlaid (solid line). As in Fig. 3.5, the experimental estimates are in good agreement with theoretical predictions for the first 10 modes.

**Figure 3.7:** \( (\langle a_{n+1}^2 \rangle/\langle a_n^2 \rangle)^{1/2} \) from experimental measurements of the tangent angle as a function of \( n \) for one actin filament (symbols). The expected scaling \( n/(n+1) \) is overlaid (solid line). The length of the filament is 6 \( \mu \)m.
Yet another method to estimate the persistence length $L_p$ is based on the end-to-end length. The root-mean-squared end-to-end distance $R$ for a long, worm-like-chain (Landau and Lifshitz, 1958) is

$$R = \sqrt{2L_p L \left[ 1 - \frac{L_p}{L} \left( 1 - e^{-L/L_p} \right) \right]}. \quad (3.15)$$

It is important to note that the above expression applies only when $L/L_p >> 1$, which may not be the case in our experiments.

### 3.4 Results and Discussion

#### 3.4.1 No Electric Field

Figure 3.8 depicts a single actin filament’s mode amplitudes as functions of time. Each panel represents one mode. The mode number is indicated by the number at the bottom. In this case, cosine basis functions from equation (3.12) were used to perform the Fourier decomposition of the filament’s tangent angle. Notice that the variance of the mode amplitudes decreases with mode number. Each mode’s variance provides an independent estimate of the filament’s persistence length.
Figure 3.8: A single actin filament’s mode amplitudes as a function of time. Each panel represents a single mode. Cosine basis functions were used to perform the Fourier decomposition. The length of the filament is 20 µm.

When carrying out the mode analysis, we used images separated by time intervals $\Delta t$, larger than the relaxation time $\tau_m$ of mode $m$. In other words, the shapes in the images were uncorrelated so as to minimize the required sample size of images for calculating the variance. More importantly, minimizing the sample size prevented the premature bleaching of the filament. (Incidentally, when no oxygen scavenging system was used, the mode amplitude variance would visibly increase with time when depicted on graphs similar to Fig. 3.8.) In most cases, $\Delta t = 2$ s was appropriate. However, the longest filaments’ 1st modes may not have decayed over this time scale. In the cases of long
filaments, only the 2nd – 4th modes’ estimates were used to calculate an average value of $L_{p}$. Conversely, the 1st mode’s estimate for $L_{p}$ was sufficient for the shortest filaments, because noise in the digitization of a filament’s shape begins to dominate as $\tau_n$ decreases and becomes comparable to the exposure time.

To estimate the appropriate time constant $\tau_n$, Fig. 3.9 depicts the mean-squared mode amplitudes of the transverse displacement $(a_n(t+\Delta t) - a_n(t))^2$ of an 11 µm-long filament’s first three modes’ amplitudes ($n = 1$ – asterisks, $n = 2$ – circles, $n = 3$ – squares) as functions of the time lag $\Delta t$. The experimental data was fitted with the theoretical expression (Brangwynne et al. 2007)

$$\langle (a_n(t+\Delta t) - a_n(t))^2 \rangle = \left(1 - e^{-\Delta t/\tau_n}\right) \frac{2}{L_p} \left(\frac{L}{n\pi}\right)^2,$$  

(3.16)

where $\tau_n$ is defined as (Gittes et al. 1993)

$$\tau_n \approx \frac{\gamma}{L_p k_B T} \left[\frac{L}{\pi(n+1/2)}\right]^{1/4},$$  

(3.17)

$\gamma \equiv 4\pi\eta / \ln(2h/r)$ (Brennen and Winet 1977), $\eta$ is the suspending medium’s viscosity, $h$ is the height of the cylinder above the surface (1 µm), $r$ is the cylinder’s radius (3.5 nm), and $L_p$ is the fitting parameter. Notice that $L_p$ is lumped into both the timescale and the expression’s amplitude.

For our shortest filament (~5 µm), $\tau_1 = 32$ ms and $\tau_2 = 4$ ms. For our longest filament (~22 µm), $\tau_1 = 12$ s and $\tau_2 = 1.5$ s. Notice in Fig. 3.9 how the fluctuation correlation times for the three modes decrease as the mode number increases. The three datasets were fit with a variant of equation (3.16), where the multiplicative factor on the
right side was fixed using a value for $L_p$ from the literature (17.7 µm). Instead, only $\tau_n$ was optimized and yielded time constants for the first three modes, 1400, 230, and 73 ms, respectively. The theoretical estimates of the first three modes’ time constants are 810, 100, and 27 ms, respectively. Aside from any uncertainty in the precise value of the drag coefficient, the time constant is highly sensitive to the length of the filament. Error in the filament’s digitization from frame to frame may explain the discrepancy between theory and experiment.

![Graph showing mean-squared mode amplitudes vs. lag time](image)

**Figure 3.9:** The mean-squared mode amplitudes of the transverse displacement as a function of lag time $\Delta t$ of an 11 µm long filament ($n = 1$ – asterisks, $n = 2$ – circles, $n = 3$ – squares). Fits to each mode’s data (solid lines) are to equation (3.16) with the fitted $\tau_n = 1400, 230, \text{and } 73 \text{ ms, respectively.}$

**Estimation of the persistence length based on mode amplitude**

Next, we estimate $L_p$ using the variance of the mode amplitudes from either the cosine basis functions or the beam basis functions (equations 3.8 and 3.14, respectively).
Figure 3.10 depicts $L_p$ as a function of KCl-concentration [KCl] calculated using both the cosine basis functions (triangles) and the beam basis functions (circles); 30 μM (n = 8 filaments), 300 μM (n = 6), 3 mM (n = 6), 30 mM (n = 3), 300 mM (n = 13). The triangles are offset just to the right for clarity. Uncertainties are presented as ± 1 standard deviation. The estimates of $L_p$ agree with the results of previous studies.

**Figure 3.10:** $L_p$ depicted as a function of KCl-concentration [KCl], calculated using both the cosine basis functions (triangles, equation (3.12)) and the beam basis functions (circles, equation (3.4)). The horizontal lines are the mean values of the two datasets. The triangles are offset slightly to the right for clarity. Uncertainties are presented as ± 1 standard deviation. 30 μM (n = 8), 300 μM (n = 6), 3 mM (n = 6), 30 mM (n = 3), 300 mM (n = 13)

**Estimation of the persistence length based on the variance of the transverse fluctuations**

Figure 3.11a depicts the variance of an actin filament’s transverse displacement from the reference line connecting its endpoints (symbols). The best fit line (solid line) is the solution to equation (3.9) with $L_p = 15.9$ μm. The variance of the same actin
filament’s transverse displacement from the line that intersects its center of mass and is parallel to the line connecting its endpoints yielded a somewhat larger estimate of $L_p$ (19 µm). These data (symbols) and fit (solid line) to equation (3.9) are depicted in Fig. 3.11b. The variance at the filament’s far end decreases due to variability in the filament’s measured length over time. An explanation for why the choice of reference line consistently yields different results has not been discovered, but this variability in filament length over time may play a role. Only three modes were summed during the fitting process ($L_p$ changing by < 1%, independent of $L$).

**Figure 3.11**: Experimental variance of the transverse displacement (symbols) depicted as a function of position along the filament. Fits are to equation (3.9). The displacement was measured from the line a) connecting the filament’s endpoints (solid line, $L_p = 15.9$ µm) and b) intersecting the center of mass and parallel to the line connecting the filament’s endpoints (solid line, $L_p = 19$ µm).

Figure 3.12 depicts $L_p$ as a function of [KCl], calculated by minimizing the difference between the theoretical variance estimated by equation (3.9) and the
experimental variance of transverse fluctuations from the line connecting the filament’s endpoints (triangles, dashed line = mean) and the line intersecting the filament’s center of mass and parallel to the line connecting the endpoints (circles, dotted line = mean). Uncertainties are presented as ± 1 standard deviation. The results obtained when using these beam basis functions that satisfy four boundary conditions are the same as those obtained when using the cosine basis functions. These results are also in agreement (within 10%) with those obtained when performing a mode analysis.

Figure 3.12: $L_p$ depicted as a function of KCl-concentration [KCl], calculated from minimizing the difference between the theoretical variance estimated by equation (3.9) and the variance of transverse fluctuations from the line connecting the filament’s endpoints (triangles, dashed line = mean) and the line intersecting the filament’s center of mass and parallel to the line connecting the endpoints (circles, dotted line = mean). The triangles are offset just to the right for clarity. Uncertainties are presented as ± 1 standard deviation. 30 μM (n = 8), 300 μM (n = 6), 3 mM (n = 6), 30 mM (n = 3), 300 mM (n = 13)
Figure 3.13 depicts the root-mean-squared end-to-end distance $R$ as a function of $L$ (symbols, $n = 40$ filaments) with the best fit to equation (3.15) (solid line, $L_p = 29 \, \mu m$). This measure of filament rigidity has been commonly used to calculate the rigidity of DNA molecules (Baumann et al. 1997) and F-actin (Takebayashi et al. 1977; Yanagida et al. 1984) in the past and serves as a robust validation of our method relating the variance of thermal fluctuations to the solution to the beam equation. All of the results are tabulated in Table 3.1.

![Figure 3.13: Experimental root-mean-squared end-to-end distance $R$ (symbols) depicted as a function of filament length $L$. Fit is to equation (3.15): (solid line, $L_p = 29 \, \mu m$).](image)

The absence of any effect from the monovalent ionic concentration on the rigidity of F-actin in the range 60 $\mu M$ - 250 mM is not altogether surprising. It was shown that F-actin retained the same rigidity in two distinct ionic environments: 1 mM MgCl$_2$ (or...
CaCl$_2$) and 1 mM MgCl$_2$ (or CaCl$_2$) + 100 mM KCl (Isambert et al. 1995). Also, the nonlinear Poisson-Boltzmann theory for uniformly charged cylinders predicts little change in the total persistence length $L_t = L_p + L_{el}$ over this range, where $L_{el}$ is the electrostatic contribution to $L_t$ (Odijk 1977; Baumann et al. 1997)

$$L_t = L_p + L_{el} = L_p + \frac{\lambda^2}{4l_B}, \tag{3.18}$$

where $\lambda$ is the Debye screening length and $l_B = e^2/4\pi \varepsilon_0 \varepsilon kT$ is the Bjerrum length (7.1 Å when $\varepsilon = 80$ at 20°C). Figure 3.14 depicts the predicted change in $L_t$ (equation (3.18)) as a function of monovalent ion concentration for both F-actin (solid line) and DNA (dashed line). Notice that F-actin is expected to begin stiffening at an ionic concentration much lower than the point at which DNA has been shown to stiffen.

**Figure 3.14**: The predicted change in the total persistence length $L_t$ (equation (3.18)) as a function of monovalent ion concentration for F-actin (solid line) and DNA (dashed line).
### Table 3.1: Estimates of $L_p$ (in µm) using different methods and for several monovalent ionic concentrations.

<table>
<thead>
<tr>
<th>[KCl] $\rightarrow$ Method ↓</th>
<th>60 µM, $\sigma = 0.67$ mS/m ($n = 8$)</th>
<th>120 µM, $\sigma = 1.7$ mS/m ($n = 6$)</th>
<th>850 µM, $\sigma = 11$ mS/m ($n = 6$)</th>
<th>25 mM, $\sigma = 0.44$ S/m ($n = 3$)</th>
<th>240 mM, $\sigma = 3.0$ S/m ($n = 13$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mode analysis – cosine</td>
<td>15 ± 0.61</td>
<td>16 ± 0.89</td>
<td>15 ± 1.0</td>
<td>15 ± 1.2</td>
<td>16 ± 1.7</td>
</tr>
<tr>
<td>mode analysis – beam</td>
<td>16 ± 1.1</td>
<td>17 ± 1.1</td>
<td>16 ± 2.1</td>
<td>18 ± 0.75</td>
<td>17 ± 2.2</td>
</tr>
<tr>
<td>variance – endpoint</td>
<td>17 ± 0.82</td>
<td>19 ± 2.0</td>
<td>17 ± 3.3</td>
<td>20 ± 1.2</td>
<td>18 ± 2.0</td>
</tr>
<tr>
<td>variance – center of mass</td>
<td>19 ± 0.90</td>
<td>20 ± 1.6</td>
<td>18 ± 2.8</td>
<td>20 ± 0.99</td>
<td>20 ± 2.1</td>
</tr>
<tr>
<td>end-to-end distance</td>
<td>31 ± 1.6</td>
<td>34 ± 3.8</td>
<td>30 ± 3.6</td>
<td>31 ± 2.5</td>
<td>34 ± 5.5</td>
</tr>
</tbody>
</table>

1) mode analysis with cosine basis functions (Gittes et al. 1993), 2) mode analysis with beam basis functions (Liu et al. 1996), 3) variance calculation from the line connecting the endpoints, 4) variance calculation from the line intersecting the center of mass and parallel to the line connecting the endpoints, and 5) end-to-end relationship. Variabilities are ± one standard deviation.

#### 3.4.2 Electric Field

To test whether a spatially uniform AC electric field has any effect on the orientation or apparent rigidity of F-actin, we applied an AC potential between two planar electrodes with a similar geometry to that depicted in Fig. 3.2. In contrast to experiments described later in Chapter 4 & 5, however, we are not interested in the gradient force, but only the DC component of the polarization. To achieve a spatially uniform field, the filaments were confined to two dimensions, similar to that discussed earlier in this
chapter. When filaments were observed to linger between the electrodes, the potential was turned on, and the filaments aligned their long axis to and then stiffened in the electric field. The dataset for this experiment is still small and has not yet been quantified.

Upon comparing the electrical energy to thermal energy and considering the smallest electrode gaps used (5 µm), we can estimate a minimum electric field required. The theory behind this estimate can be found in Chapter 4. Considering an actin filament with length comparable to the electrode gap size and a nominal estimate of the polarization factor in the electric field, we estimate ~7 V as a minimum required peak-peak voltage. This is consistent with our experimental findings in the nonuniform field case (Chapter 4), but a larger voltage was required to see a stiffening effect in the uniform field case (~15 V pk-pk).

When a uniform field was applied, the filaments aligned in the field and migrated to either electrode at random. This migration occurred much faster than would be expected from diffusion alone. Indeed, the migration occurred too quickly to record with our CCD camera. It would be interesting to discover a correlation between filament shape and its trajectory in the electric field. As an alternative to observing free filaments, we captured the motion of filaments attached at one end somewhere between the electrode gaps. Figure 3.15 shows a time series of a singly-attached fluorescently-labeled actin filament orienting along field lines in a spatially-uniform AC electric field (20 fps). Upon removal of the electric field, the singly-attached filament returned to its original orientation. This electric-field-dependent orientation/relaxation could be repeated several times.
Figure 3.15: A time series of a singly-attached fluorescently-labeled actin filament orienting along field lines in a spatially-uniform AC electric field (20 fps).

3.5 Conclusions

We have used three statistical mechanics methods to calculate the persistence length $L_p$ of freely-fluctuating F-actin. Mode analyses yielded similar values to those found in the literature, $\sim 17 \, \mu m$. Comparing experimental data to theoretical expressions for the variance of the transverse fluctuations yielded a value for the persistence length of $\sim 18 \, \mu m$. Using the average end-to-end distance relationship yielded a persistence length of $\sim 30 \, \mu m$. We showed that monovalent ionic concentration does not affect the persistence length of F-actin over the range 60 $\mu M$ – 250 mM. This is consistent with the predictions of the Poisson-Boltzmann theory of uniformly charged rods. When studying the stiffening phenomenon in an AC electric field, in solutions of varying ionic concentration, we can rule out there being changes to the intrinsic elastic modulus. There is, however, a clear effect on the apparent tension applied to a filament being subjected to a spatially uniform, AC electric field. Future studies will explain this behavior and may provide insight into the mechanism of surface conduction in the filament’s electric double layer.
Chapter 4

Positioning Actin Filaments and Estimating the Apparent Tension of Suspended Actin Filament by an Electric Field

Abstract

When an AC electric field was applied across a small gap between two metal electrodes elevated above a surface, rhodamine-phalloidin-labeled actin filaments were attracted to the gap and became suspended between the two electrodes. The variance $<s^2(x)>$ of each filament’s horizontal, lateral displacement was measured as a function of electric field intensity and position along the filament. $<s^2(x)>$ markedly decreased as the electric field intensity increased. Hypothesizing that the electric field induces tension in the filament, we estimated the tension using a linear, Brownian dynamic model. Our experimental method provides a novel means for trapping and manipulating biological filaments and for probing the surface conductance and mechanical properties of single polymers.
4.1 Introduction

Actin filaments are polymers whose ATP-driven assembly in the cell cytoplasm propels cell shape changes, locomotion, and division. They are also the cytoskeletal tracks for the myosin family of molecular motors, including those for muscle contraction. Given actin filaments’ ubiquity, it is not surprising that much work has focused on studying their mechanical (Riveline et al. 1997; Tsuda et al. 1996; Gittes et al. 1993) and electrical (Kobayasi et al. 1964) properties and the motility of myosin motors along them. Here we present a study in which actin filaments are manipulated using electric fields and studied using the linear Brownian dynamics model presented in Chapter 2. From their thermal fluctuations we can estimate the tension being applied either directly, or indirectly, by the electric field.

4.2 Basic Electrokinetics

To manipulate actin filaments and study their mechanical and electrical characteristics, as well as to facilitate biophysical assays, we used a technique called dielectrophoresis. The introductory chapter already mentioned the unique nature of AC electric fields and the importance of electric double layer. To further introduce this topic, this section will discuss the subject of electrokinetics.

4.2.1 The Electric Double Layer

The electric double layer forms as a result of Coulombic interactions between the particle’s surface charge and the counterions and coions in solution. The surface charge may be free charge, adsorbed/desorbed charge, or induced charge. The Coulombic force
decreases with the square of the distance from the surface, so the effect of the surface charge quickly diminishes.

In order to devise a model for the structure of the outer (diffuse) layer of the EDL, we start with Poisson’s equation relating the charge density to the electric potential \( \Phi \)

\[
\varepsilon_r \varepsilon_0 \nabla^2 \Phi = -\rho = -e \sum_i z_i c_i ,
\]

(4.1)

where \( e \) is the charge on an electron (1.6\( \times \)10\(^{-19} \) C), \( \rho \) is the charge density, \( c_i \) is the local ionic concentration of species \( i \), and \( z \) is the valence. At equilibrium, the counter and coions assume the Boltzmann distribution and equation (4.1) becomes

\[
\frac{d^2 \Phi}{dz^2} = -\frac{e}{\varepsilon_r \varepsilon_0} \sum_i z_i c_i \exp \left( \frac{-z_i e \Phi}{k_B T} \right) .
\]

(4.2)

After applying the far-field boundary conditions and the capacitive boundary condition on the interface and assuming that \( \Phi < k_B T / e \), equation (4.2) simplifies to

\[
\frac{d^2 \Phi}{dz^2} = \frac{\Phi}{\lambda^2} ,
\]

(4.3)

where

\[
\lambda = \sqrt{\frac{e_e \varepsilon_0 k_B T}{\sum_i (z_i e)^2 c_i}}
\]

(4.4)

is the Debye screening length. Notice that \( \lambda \) is the length scale over which the potential will exponentially decay as one moves away from the surface. For example, in a physiological 200 mM salt solution, \( \lambda = 1 \) nm.

The Gouy-Chapman model of the electric double later was extended to incorporate an additional layer called the Stern Layer. This layer treats the countercharge
species as objects of finite size. It is believed that a closely packed layer of counterions collects close to the particle’s surface due to the large Coulombic attraction in this region. Steric hindrance will impose a limit on the number of counterions allowed to adsorb or attach to the surface. Many times, water molecules will attach to a particle’s surface. This will do nothing to neutralize the total charge since water is neutral, so the potential will not necessarily decay over the length scale of the Stern layer. Figure 1.4 depicts the electric double layer in a cartoon. The properties of the Stern Layer are up to debate, including the conduction properties that will be shown to be important later on. Below we discuss conduction in the EDL.

Surface conduction is the conduction that takes place due to the presence of EDLs. Tangentially applied electric fields apply forces to the excess charge present in the EDL. Just as current density is quantified by the bulk conductivity, the surface conduction is quantified by the surface conductivity. The SI units of bulk conductivity are Sm$^{-1} = CV^{-1}m^{-1}s^{-1}$, whereas the units of surface conductivity are S.

The total conductivity $\sigma_p$ of a spherical particle with radius $a$ can be defined as the sum of both the intrinsic (bulk) and surface conductivity $K_s$ (O’Konski 1960)

$$\sigma_p = \sigma_{\text{intrinsic}} + \frac{2K_s}{a}. \quad (4.5)$$

For large $a$, the surface conduction can be neglected. This is further illustrated by the dimensionless Dukhin number

$$Du = \frac{K_s}{aK_L} = \frac{\sigma_i\mu_i^t}{2az_iF c_i\mu_i^L}, \quad (4.6)$$
which is the ratio of the surface conductance and the bulk conductance $K_L$. Here, $F$ is Faraday’s constant and $\mu$ is the charged species mobility.

For proteins, the intrinsic conductivity can be neglected, so the total conductivity of an actin filament is dominated by its surface conductance. The surface conductance can be considered to have two contributions, one from charge movement in the Stern layer and one from charge movement in the diffuse layer of the electric double layer (Lyklema 1995).

$$K_s = K_s^i + K_s^d \quad (4.7)$$

The Stern layer conductance is the product of the charge density, $\sigma^i$, and the ion mobility, $\mu^i$,

$$K_s^i = \sigma^i \mu^i \quad (4.8)$$

In the diffuse layer, the conductance has three contributions, migration in electric fields, diffusion, and convection. The last contribution is absent in the Stern layer part of the double layer, because the fluid is considered to be immobile.

When the diffusion coefficients of the cation and the anion are equal, the conductance of the diffuse layer can be written as (Lyklema 1995)

$$K_s^d = \frac{4F^2cz^2D^d(1 + 3m/z^2)}{RT\kappa} \left( \cosh \left[ \frac{ze\zeta}{2kT} \right] - 1 \right) = \sigma^d \mu^d \quad (4.9)$$

where $D$ is the ion diffusion coefficient, $R$ is the gas constant, $\zeta$ is the zeta-potential, $\sigma^d$ is the counter charge density in the diffuse layer, and $\mu^d$ is the ion mobility in the diffuse layer. The dimensionless parameter, $m$, describes the contribution of electroosmosis to ion flux by
where $\eta$ is the viscosity.

We may use these theoretical estimates of the EDL conductivity to better understand the behavior of proteins in electric fields. A useful application of electric fields to micro- and nano-sized particles is presented in the next section.

### 4.2.2 Dielectrophoresis

Dielectrophoresis (DEP) is the migration of particles under the influence of a spatially non-uniform electric field due to the polarization of both the particle itself and its EDL. Dielectrophoresis of particles in dielectric media is relatively well understood and was discussed extensively in monographs (Pohl 1978; Jones 1995; Hughes 2003) and in numerous research articles. In prior work, our group used dielectrophoresis to position carbon nanotubes at predetermined locations (Riegelman et al. 2006). This manipulation technique effective in both DC and AC fields, AC fields are preferred to minimize electrodes' reactions (electrochemistry) and to reduce the potential drop at the electrodes’ surfaces.

When a particle is suspended in a perfectly dielectric medium whose dielectric constant is smaller than that of the particle, the particle is attracted to the location of the maximum electric field intensity (positive dielectrophoresis). When the dielectric constant of the particle is smaller than that of the surrounding medium, the particle is repelled from the location of the maximum electric field intensity (negative dielectrophoresis). The real part of the dielectric permittivity of proteins has been estimated to range from 10 to 36 $\varepsilon_0$, where $\varepsilon_0$ is the permittivity of free space (King et al. 

\[
m = \left( \frac{RT}{F} \right)^2 \frac{2\varepsilon_m}{3\eta D^2}
\]
1991; Smith et al. 1993). The permittivity of water is approximately 78 $\varepsilon_0$. Thus, one would expect proteins to undergo negative dielectrophoresis. In fact, when the electric field frequency is not too high ( <~100MHz) the opposite is true, due to polarization of the EDL.

The process of polarization involves the rearrangement of positive and negative charge centers in an electric field. These charge centers eventually align themselves along the electric field lines. Two forms of polarization, such as electronic and atomic polarization involve the rearrangement of charge in an atom’s nucleus or the rearrangement of charge on an atom’s surface, respectively. These processes occur very quickly and are not of concern in this study.

The process by which a molecule rotates to align its polarization axis along the electric field lines is called electro-orientation and is the slowest form of polarization. This process occurs slowly enough to be of concern in our study. What is also of paramount importance is the polarization of the EDL surrounding our particles. Without the electric field present, an EDL would still exist, but, with it present, the EDL’s charge distribution rearranges. The movement of the countercharge in the electric field gives rise to a dipole. The previous section discussed surface conduction in the EDL, which augments the particle's polarizability. Below, we present an approximate theory that accounts only for some of the processes taking place in the EDL and the surrounding bulk solution. The theory provides a qualitative picture of the processes in play.

As long as the electric field acting on the dipole has no contributions from the dipole itself, the force on an infinitesimal dipole is (Jones 1995)

$$\vec{F}_{\text{dipole}} = \vec{p} \cdot \nabla \vec{E},$$

(4.11)
where \( \overline{p} \equiv q\overline{d} \) is the dipole moment, \( d \) is the distance between the charges, and \( q \) is the charge. The torque on the dipole is

\[
\overline{T} = \overline{p} \times \overline{E}.
\] (4.12)

The potential due to effective dipole moment \( p_{\text{eff}} \) is

\[
\Phi_{\text{dipole}} = \frac{p_{\text{eff}} \cos \theta}{4\pi\varepsilon r^2},
\] (4.13)

where \( \theta \) and \( r \) are the polar angle and radial position in spherical coordinates, respectively, and \( \varepsilon \) is the dielectric permittivity. The origin of the coordinate system is centered at the dipole’s center. The electric fields outside \( (\Phi_1) \) and inside \( (\Phi_2) \) of a lossy sphere are, respectively,

\[
\Phi_1 = -E_0 r \cos \theta + \frac{A \cos \theta}{r^2} \quad (r > R)
\]
(4.14a)

and

\[
\Phi_2 = -B r \cos \theta \quad (r < R).
\]
(4.14b)

Applying the boundary conditions at the interface between the particle and the suspending medium and comparing the external electric field to equation (4.13), we get an expression for \( p_{\text{eff}} \),

\[
p_{\text{eff}} = 4\pi\varepsilon_1 K R^{-3} E_0,
\] (4.15)

where \( K = \left( \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2 \varepsilon_m^*} \right) \) is the Clausius-Mossotti factor and \( \varepsilon_m^* \) and \( \varepsilon_p^* \) are, respectively, the complex permittivities of the medium and particle. In the above,

\[
\varepsilon_m^* = \varepsilon_0 \varepsilon_0 + j \frac{\sigma}{\omega}
\] (4.16)
and $\sigma$ is the (total) conductivity.

If the particle is an ellipsoid with semi-axes $a_1$, $a_2$, and $a_3$, the Clausius-Mossotti factor in the direction of the $a_i$ axis is (Stratton 1941)

$$K_i = \left( \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_m^* + (\epsilon_p^* - \epsilon_m^*)L_i} \right),$$

(4.17)

where $L_i$ is the depolarization factor. In the case of a prolate ellipsoid ($a_1 \gg a_2 = a_3$), which is a reasonable approximation of actin filaments ($a_2/a_1 \approx S \sim 0.001$),

$$L_1 \sim S^3 \left[ \ln \left( \frac{2}{S} \right) - 1 \right] + O(S^4)$$

(4.18a)

and

$$L_2 \sim \frac{1}{2} + \frac{1}{2} (1 - \ln 2 + \ln S)S^2 + O(S^4).$$

(4.18b)

The Clausius-Mossotti factor for a prolate ellipsoid simplifies to

$$K = \left( \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_m^*} \right).$$

(4.19)

Accordingly, the force acting on our actin filaments

$$F_{DEP} = \frac{2\pi a^2}{3} L \epsilon_m \text{Re} \left( \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_m^*} \right) \nabla \epsilon_{rms}^2,$$

(4.20)

where $L$ is the filament length. Upon multiplying both sides by $dx$ and comparing the result to the thermal energy $1.5 k_B T$, we obtain a criterion for the minimal force needed to overcome thermal fluctuations and for trapping filamentous molecules,

$$\frac{\pi}{6} r^2 \epsilon_m \text{Re}[K(w)] E_{rms}^2 \geq 1.5 k_B T.$$
The electric field needed to overcome the thermal energy depends on the actin’s surface conductivity (Clausius-Mossotti factor). If we assume that the total (surface) conductivity of the actin is 2 S/m (Asokan et al. 2003), which was estimated using linear charge density (Tang et al. 1996), we can generate a plot using equation 4.21 predicting the electric field that would be required to trap an actin filament (Fig. 4.1). The electric field is being approximated by the voltage drop divided by the gap size. It shows that at least 5 \( V_{\text{rms}} \) is required to trap the actin across a 7 \( \mu \text{m} \) gap when the buffer conductivity is 0.5 S/m. We were able, however, to trap actin filaments with lower electric field strengths than theoretically predicted, which suggests that the surface conductivity of the actin may be larger than 2 S/m. For comparison the dashed curve in Fig 4.1 corresponds to an actin filament having a surface conductivity of 8 S/m.

**Figure 4.1**: The voltage required to trap actin filaments across a 7 \( \mu \text{m} \) gap depicted as a function of solution conductivity according to equation (4.21), \( \sigma_{\text{actin}} = 2 \) S/m (solid line), \( \sigma_{\text{actin}} = 8 \) S/m (dashed line). Gray lines denote \( \sigma_{\text{actin}} \).
The gray lines denote the simulated surface conductivity of the actin filaments. The more conductive the protein (colloid) being trapped, the more easily trapping is accomplished. As was previously stated, our experimental findings imply a larger surface conductivity than the one estimated using linear charge density. It should be noted that the surface conductivity of a colloid in solution may be coupled to the bathing medium’s conductivity through the structure of the filament’s EDL, so the relationship depicted in Fig. 4.1 is not entirely accurate. As the solution conductivity increases, the thickness of the EDL decreases. The effect of this structural change on the surface conductivity is still unknown.

Using equation (4.19), Fig. 4.2 depicts the Clausius-Mossotti factor as a function of the AC field frequency (Hz). Here, the actin surface conductivity is assumed to be 2 S/m; $\sigma_m = 0.06$ S/m (solid line), 0.5 S/m (dotted line), 3.25 S/m (dashed line). The complex permittivity is calculated using equation (4.16), and the radius is assumed to be 3.5 nm. Witness that the crossover frequency (the frequency at which the DEP force is zero) increases as the surface conductivity of the actin increases. Also, the actin is expected to be repelled from the region of highest electric field when it is suspended in the high conductivity medium (dashed line). This is not the case in our experiments, which is yet another indication that the current theoretical models require modification.
Figure 4.2: An actin filament’s Clausius-Mossotti factor estimated using equation (4.21):

\[ \sigma_m = 0.06 \text{ S/m (solid line), 0.5 S/m (dotted line), 3.25 S/m (dashed line)} \]

4.2.3 Electrokinetic Flows

AC Electroosmosis

Since we used alternating (AC) electric fields, the electroosmotic and electrophoretic motions resulting from the filament's and the electrodes' free surface charges average to zero. Nevertheless, the applied electric field polarizes the fluid layer next to the electrodes to form an induced EDL, which in turn (depending on solution concentration and field frequency), will induce fluid circulation in the flow cell. The magnitudes of the electric forces and the induced electroosmotic velocity are proportional to the square of the electric field intensity \( (E^2) \). The induced electroosmotic flow is depicted schematically with red lines in Figure 4.3. The electric forces and fluid motion
around the particle have been dubbed induced-charge electroosmosis (ICEO) (Squires and Bazant 2004).

![Schematic depiction of AC-Electroosmosis (ACEO) on an electrode pair similar to our geometry.](image)

**Figure 4.3:** Schematic depiction of AC-Electroosmosis (ACEO) on an electrode pair similar to our geometry.

The phenomenon of induced electroosmosis was discussed in the Russian literature (Dukhin 1986; Gamayunov et al. 1986; Dukhin and Murtskovkin 1986; Gamayunov et al. 1992; Murtskovkin 1996), and only recently has attracted attention in the west (Ramos et al. 1999; Squires and Bazant 2004; Levitan et al. 2005; Squires and Bazant 2006). All these studies focus, however, on the limiting case when the thickness of the EDL (the Debye length $\lambda_D$) is much smaller than the characteristic diameter ($a$) of the particle. This approximation is valid when the particle has a characteristic diameter of about 1µm or larger, but not when the characteristic diameter is a few nanometers, as in the case of macromolecules. The above approximation also fails to predict some of the phenomena observed in experiments such as the cessation of induced electroosmotic flows at salt concentrations of about 30-50 mM.
Ramos et al. (1999), Green et al. (2000), Gonzales et al. (2000), and Green et al. (2002) performed a circuit analysis on an electrode system similar to the one depicted in Fig. 4.3. They performed experiments on planar electrodes with 25 um gaps and an 8.6 mS/m KCl solution. They applied an AC field up to $5 V_{pk-pk}$ and watched the bead-seeded (200 and 600 nm in diameter), frequency-dependent flow.

Assuming the EDL can be modeled as a capacitor and the electrolyte as a resistor, the EDL/electrolyte/EDL system can be approximated as impedances in series, where $R_E$ is the electrolyte resistance, $R_{EDL}$ is the EDL resistance, $C_{EDL}$ is the EDL capacitance, $R_{current}$ is the current measuring resistor, $V_1$ is the total voltage, $V_2$ is essentially the current, and $V_3$ is the voltage across electrodes. This is depicted in Figure 4.4. One can estimate the EDL’s incremental capacitance to be

$$C_{EDL} = \frac{\varepsilon d(\Delta z)}{\lambda},$$  \hspace{1cm} (4.22)

where $d$ is the width of the electrode, measured along the gap’s edge, $\lambda$ is the EDL thickness, and $\Delta z$ is an incremental distance along the electrode, measured from the gap.

The impedance of the electrolyte can be modeled as purely resistive current flux tubes, roughly coinciding with the electric field lines, of approximate length $\pi z$,

$$R_E(z) = \frac{\pi z}{\sigma d(\Delta z)},$$  \hspace{1cm} (4.23)

where $\sigma$ is the solution conductivity. This is true only as long as $\omega << \sigma \varepsilon$, the charge relaxation time. This implies that each differential capacitor will charge with a different time constant. The voltage across the double layer, as a function of $z$, can be
approximated as such: \( V_d(z) = \frac{V_0}{2 + j \omega \pi z \frac{\varepsilon}{\sigma \lambda}} \). If we assume \( E_i = -\partial V_d / \partial z \), one can estimate the time-averaged velocity as a function of the AC frequency \( \omega \) and position \( z \):

\[
\langle v \rangle = \frac{1}{2} \mathrm{Re} \left\{ \frac{\Delta \sigma_q E_i^{*} \lambda}{\eta} \right\} = \frac{1}{8} \frac{\varepsilon V_0^2 \Omega^2}{\eta z(1 + \Omega^2)^2},
\]

where \( \eta \) is the viscosity, \( \Delta \sigma_q \) is the excess charge in the EDL, and \( \Omega = \frac{\omega \varepsilon \pi}{\sigma 2 \frac{1}{\lambda}} \).

**Figure 4.4**: Circuit model used to describe our electrode geometry’s polarization when bathed in an electrolyte. \( R_E \) – electrolyte resistance, \( R_{EDL} \) – EDL resistance, \( C_{EDL} \) – EDL capacitance, \( R_{current} \) – current measuring resistor, \( V_1 \) – total voltage, \( V_2 \) – current, \( V_3 \) – voltage across electrodes.

Upon measuring the complex impedance of the system, Ramos et al. (1999) determined that the voltage across the electrolyte tended to zero at low frequencies (~100 Hz). As the electrode surface becomes shielded by counterions, and the tangential electric
field on the electrode surface goes to zero, the AC Electroosmosis (ACEO) should cease. At high frequencies (~10 kHz), the EDL cannot form, and all of the potential is dropped across the electrolyte. Without an excess of counterions present in the EDL, this should also cause ACEO to cease. Both of these assertions were verified experimentally. Our circuit model is described in Section 4.3.2.

Joule Heating

Joule heating due to electric current causes a temperature gradient, with the highest temperature existing in the electrode gap. The joule heating enters into the heat equation as a source term. The temperature field at steady state is governed by the following relation (Ramos et al. 1998),

$$k \nabla^2 T = -\sigma E^2. \quad (4.25)$$

The temperature gradient creates a conductivity gradient and an electrical permittivity gradient in the fluid. The body force exerted on the fluid by the electric field is

$$\langle f_E \rangle = \frac{1}{2} \text{Re} \left[ \left( \frac{\sigma \nabla \epsilon - \epsilon \nabla \sigma}{\sigma + i \omega \epsilon} \right) \cdot E_0 \right] E_0^* - \frac{1}{4} E_0 \cdot E_0^* \nabla \epsilon. \quad (4.26)$$

The conductivity and permittivity of an electrolyte change ~2 % and ~-0.4 % per degree Kelvin, respectively. The force creates a flow pattern similar to the AC electroosmotic flow, out across the surface of the electrodes (Fig. 4.3), with a velocity on the order of 0.1 \( \mu \text{m/s} \). This flow is proportional to \( V^4 \), because the temperature increase is proportional to \( V^2 \) and the electrical stress is proportional to \( T^2 \). The flow reverses direction when \( \omega \gg \sigma / \epsilon \).
Illumination-Induced

Another interesting electrothermal effect appears when the energy absorbed by the electrodes from the illumination source causes them to be at a higher temperature than the ambient. This causes a gradient of the fluid's conductivity and permittivity in the direction normal to the electrodes’ surfaces, and results in a flow pattern of opposite direction compared to that caused by joule heating. The speed of the flow induced this way is roughly an order of magnitude larger than that created by joule heating and is proportional to $V^2$ (Green et al. 2001). In order to better understand the role of any electrokinetic flows on actin filament positioning and tension, a sequence of experiments was carried out to study the flow patterns as functions of solution conductivity and electric field frequency and magnitude.

PIV Experiments

Induced electro-osmotic flow and electrothermal flows were previously investigated experimentally and theoretically (with the zero-thickness electric double layer approximation) (Ramos et al. 1998; Ramos et al. 1999; Ramos et al. 2003; Gonzalez et al. 2000). The previous investigations suggest that the intensity of the induced electroosmotic flow decreases as the solution conductivity increases, and that above ~ 10 mM, it may cease altogether (Squires and Bazant 2004). We found this to not be the case.

To map the flow pattern, we used fluorescent polystyrene particle-seeded fluid (880 nm diameter), and the motion of these particles was tracked with a video camera mounted on a microscope. These particles were large enough to dampen the effects of Brownian motion, but small enough to still avoid disrupting the flow field in the vicinity
of our microscopic features. The small particles were illuminated by epifluorescence, and the velocity was determined by monitoring the particles' displacement between two subsequent video frames (Meinhart et al. 1999 and 2000). A Graphical User Interface (GUI) was written using MatLab® to facilitate this task. Only a focal plane at ~ 1µm from the surface of the electrodes was used for measurements.

A specific particle was identified by eye in every frame. Its instantaneous velocity was dotted with a unit vector normal to the gap’s edge and averaged over the time it took the particle to travel about 15 µm. The total distance traveled by the particles was very large compared to the particle size, so limited error was introduced by this manual method when calculating the average velocity.

![Diagram of electrode geometries](image)

**Figure 4.5**: Schematic depiction of the two electrode geometries used to trap actin filaments.
I experimented with two sets of electrodes. The first of the two electrode geometries studied is shown in Figure 4.5a. The gap’s length (~ 50 µm) was about one order of magnitude larger than its width (~ 7 µm). When a potential was applied, the particle moved towards the electrode gap. Figure 4.6 depicts the average speed of particles traveling 15 µm toward the gap, measured from ~20 µm out on the electrode surface (30 mM KCl solution being exposed to a 2 MHz signal).

![Graph showing velocity vs. electric field squared](image)

**Figure 4.6**: The average velocity of 880 nm particles being driven by electrothermal flow (from illumination) over a 15 µm distance, in the direction toward the electrode gap.

The direction indicates that the flow was not ICEO, nor was it dominated by the effects of Joule heating or dielectrophoresis. At 2 MHz, these particles should be repelled from the gap, not moving towards it. The velocity scales linearly with $V^2$, which indicates it was due to the illumination of the system (Green et al. 2000). Adjusting the position of the illumination altered the flow pattern on a very short timescale, allowing me to drive
the flow unidirectionally by positioning the illumination source above either electrode. This time scale is predicted to be on the order of ms \( t_{\text{diff}} = \left( \frac{\rho c_p l^2}{k} \right) \), where \( \rho \) is the fluid density, \( c_p \) is the specific heat constant, \( l \) is the relevant length scale, and \( k \) is the thermal conductivity.

Any drag force that would be imparted to the filaments due to electrothermal flow can be estimated using this expression from Happel and Brenner (1983)

\[
F_z = \frac{4 \pi \eta L U}{\ln \frac{L}{r} + 0.19315},
\]

where \( \eta \) = viscosity, \( L \) = length, \( U \) = velocity, and \( r \) = radius. Figure 4.7 depicts these force estimates on a 7 \( \mu \)m-long filament using the velocity data depicted in Fig. 4.6.

![Figure 4.7](image_url)

**Figure 4.7**: The theoretical drag force estimate, obtained using equation (4.27), on a 7 \( \mu \)m-long actin filament by the velocities depicted in Fig. 4.6. The solid line is meant to guide the eye.
The solid line is meant to guide the eye. The low speed of this flow pattern made it negligible when measuring the apparent tension on actin filaments using electric fields of varying potential at a frequency of 2 MHz. For the sake of comparison, the dielectrophoretic force estimated under these same conditions, estimated from the filaments’ thermal fluctuations was on the order of 1 pN, which will be discussed later in this chapter. Additionally, any force from electrothermal flow would be felt as compression, not tension.

Figure 4.5b depicts the second electrode geometry. The gap’s length (~ 1 cm) is >2 orders of magnitude larger than its width (~ 7 μm). In this case, which mimics more closely the experimental setup of Ramos et al. (1999) more closely, a different flow field was observed. The flow field was out across the electrodes from the gap’s center, indicative of ICEO. Four frames of a movie at 0, ~1, ~1.5, and 2 s show the trajectories of two beads away from the electrode gap (gray strip) in either direction (Figure 4.8). The arrows follow the beads’ trajectories across the gold electrodes (shown in black).

![Figure 4.8: Screenshots of beads being driven by ACEO. The arrows indicate the tracked 880 nm beads. The gray stripe in the center is the gap between the gold electrodes, which are in black.](image)
With this electrode geometry, there was no illumination-induced flow toward the gap. We are not sure why this geometry exhibited one flow phenomenon and not another, especially since the first geometry’s aspect ratio was not exceedingly small (~7). The flow depicted in Fig. 4.8 was absent at high frequencies ($\omega = 2$ MHz), dominated at low frequencies ($\omega = 20$ kHz), and again was absent at lower frequencies ($\omega = 2$ kHz). These characteristics indeed suggest ICEO, and Fig. 4.9 depicts the experimental velocity (circles) as a function of frequency in the case of a solution of conductivity $\sigma_m = 0.56$ S/m and constant potential $V_{\text{rms}} = 4$.

![Figure 4.9](image)

**Figure 4.9**: The velocity of particles being driven by ACEO in a solution of $\sigma_m = 0.56$ S/m, which is expected to exhibit the double dispersion evident by the experimental data (circles). The curves are fits to equation (4.24): solid line (best fit) – $\sigma_m = 0.01$ S/m; dashed line – $\sigma_m = 0.56$ S/m.

The best fit theoretical estimate using equation (4.24) to this experimental data is overlaid in Fig. 4.9 (solid line, $\sigma = 0.01$ S/m). The velocity estimate is scaled down by
three orders of magnitude, and the critical frequency does not agree with the theoretical estimate, calculated using the experimental solution conductivity. Ramos et al. (2003) also observed the velocity to scale down dramatically as a consequence of the electrodes’ EDLs polarizing. This factor, which changes with solution conductivity, would need to be calculated empirically. Ramos et al. (2003) used solutions in the mS/m range and thus did not use more conductive solutions similar to our case. For the sake of comparison, in Fig. 4.9, the dashed line corresponds to $\sigma = 0.56$ S/m, which corresponds to the experimental solution conductivity.

There are several sources of uncertainty here. The EDL polarization was already mentioned. Also, we must remember that the frequency corresponding to the maximum velocity is sensitive to location along the electrode. In other words, the critical frequency may be biased by the locations over which the beads’ velocities were averaged. This is a consequence of the electrode’s EDL charging at different time scales in different locations. ACEO experiments using solutions similar to those used by Ramos et al. (1999) were carried out. These velocities were not quantified but clearly show the dual dispersion, whose critical frequency characteristically increases with solution conductivity. Figure 4.10 depicts the theoretical force estimates, using equation (4.27), on an actin filament being subjected to flow fields of the magnitudes depicted in Fig. 4.9.
Figure 4.10: The theoretical drag force estimate, obtained using equation (4.27), on a 7 µm-long actin filament by the velocities depicted in Fig. 4.9. The solid line is meant to guide the eye.

The solid line is meant to guide the eye. In our DEP experiments, there is no ACEO present, but Fig. 4.10 shows how significant the flow can be. Chapter 5 discusses an experiment that harnesses ACEO for the purposes of tensing actin filaments.

4.3 Materials and Methods

4.3.1 Device Construction

Our experimental apparatus consisted of a 75 µm-high flow cell sandwiched between two quartz slides. Using standard microfabrication techniques, a pair of gold electrodes (10 nm-thick adhesion layer of NiCr and 100 nm-thick gold layer) with a gap of ~7 µm between them was patterned on one of the slides and a 1 µm-deep trench was etched between the two electrodes using buffered oxide etch. This process is depicted in Fig. 3.2. The purpose of the trench is to allow the actin filaments to fluctuate without
hitting the glass surface to which they would adhere and to allow myosin motors to walk freely without interference from nearby surfaces. Figure 4.11a depicts schematically the cross-section of the electrodes’ arrangement. Figure 4.11b is a brightfield image of a dielectrophoretic electrode arrangement. Figure 4.11c shows actin filaments being trapped across the patterned electrodes. The flow cell was filled with 50 nM phalloidin-stabilized, rhodamine-labeled actin suspended in solutions with KCl as the predominant electrolyte. Observations were made from below with epifluorescent illumination.

**Figure 4.11:** (a) Schematic of the experimental setup (b) Electrodes Patterned on a glass substrate for dielectrophoresis experiments. (c) Contrast-enhanced image of an actin filament trapped across the gap between two electrodes. In this case the electrode gap is dark, and the electrodes are gray.
The electric field produced by applying an AC voltage across the electrodes is shown in Fig. 4.12. The electric potential is shown as a surface plot, and the normalized electric field is shown as contours. Notice the large electric field strength near the corners of the electrodes. This plot was generated in COMSOL®, using the electrostatics mode in the AC/DC module. The Laplace equation is solved in the bulk. The electrode surfaces are at a fixed potential, and the other boundaries are insulated.

**Figure 4.12:** Depiction of the electric potential (surface plot) and electric field lines (contours) on our electrode geometry. This plot was generated in COMSOL®, using the electrostatics mode in the AC/DC module. The Laplace equation is solved in the bulk. The electrode surfaces are at a fixed potential, and the other boundaries are insulated.

### 4.3.2 Electrode Polarization

To better understand the electric field felt by the actin filaments, we must understand the polarization undergone by the electrodes themselves. The most basic experiment by which to study electrode polarization is with an electrochemical cell, where the electrode is the electronic conductor, and the electrolyte is the ionic conductor. The change in potential when moving from one conducting phase to another mostly...
occurs at the interface, producing large electric fields in this transition region. The following is a description of the experiments used to estimate the electrode polarization in our experimental setup.

A 1 cm x 1 cm x 1 cm cube conductivity cell was made out of plastic with aluminum contacts. This setup is shown below in Fig. 4.13. The aluminum was coated with a 100 nm-thick gold layer on the face bordering the solution to more closely mimic our trapping electrodes’ surfaces. This setup allows for a more controlled, predictable experimental platform and one in which the electrolyte voltage can be probed directly. Solutions of KCL were tested.

Figure 4.13: Schematic depiction of the conductivity cell used to study the electrode polarization behavior of gold electrodes. The gold pads are 1 cm x 1 cm x 1 cm.

Figure 4.4 shows the equivalent electric circuit. To measure the current across the electrode system, we place a resistor of known value, $R_{\text{current}}$, in series with the cell. $V_1$ is the voltage across the entire setup. $V_3$ is the voltage across the electrode-solvent junction. $V_2$ is the voltage across the known resistor, which is obtained by subtracting waveforms...
$V_1 - V_3$. Several resistors of different magnitudes were used in order to obtain a measurable signal, depending on the relative impedances across $V_2$ and $V_3$.

Here, we model the electrode system as an electrolyte (resistor) in series with the EDL. This scheme is shown in Fig. 4.4. At the frequencies used in the experiment, the electrolyte can be modeled as being purely resistive. The EDL is modeled as a pure capacitor. In parallel with the EDL, there is a faradaic impedance. The diode in the EDL was found to be inconsequential. In our experiment’s range of voltages and frequencies, the current vs. voltage relationship was linear at any frequency. The EDL charging time scale in this macroscopic experiment is much longer than in our microscopic electrode case, so we were always operating above the critical charging time.

Knowing that the current through the system is constant, we arrive at the following expression for the voltage ratio between the electrolyte $V_E$ and the total system $V_3$,

$$\frac{|V_E|}{|V_3|} = \frac{R_E}{R_E + \left( \frac{1}{R_{EDL}^2 + i\omega C_{EDL}} \right)^{-1}}. \quad (4.28)$$

When $\omega \rightarrow 0$, the EDL capacitor does not transmit any current. When $\omega \rightarrow \infty$, the EDL capacitor is shorted.

$$\frac{|V_E|}{|V_3|}(\omega \rightarrow 0) = \frac{R_E}{R_E + R_{EDL}}. \quad (4.29)$$

$$\frac{|V_E|}{|V_3|}(\omega \rightarrow \infty) = 1$$

Two Ag/AgCl reference electrodes were placed in the 1 cm gap of the conductivity cell (Fig. 4.13), across which a voltage smaller than one volt was applied.
The potential difference $V_E$ between the two reference electrodes was monitored. Figure 4.14 depicts the ratio $V_E/V_3$. If we overlay equation (4.28) on the experimental data, we notice the absence of the low frequency plateau. Again, this is to be expected, because the characteristic charging frequency is likely well below the limit at which we would generate bubbles. By calculating the resistance across the electrolyte in the plateau region, we compared these values to the theoretical resistance values calculated using

$$R = \frac{L}{A\sigma^*},$$

(4.30)

where $A = 1 \text{ cm}^2$, $L = 0.6 \text{ cm}$, and $\sigma$ is the solution’s conductivity, measured using a conductivity meter. Table 4.1 presents these results.

<table>
<thead>
<tr>
<th>Solution</th>
<th>$\sigma$ (S/m)</th>
<th>$R_E^*$ (Ω)</th>
<th>$R_E^#$ (Ω)</th>
<th>$\lambda$ (nm)</th>
<th>$C_{EDL}$ (µF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 µM KCl</td>
<td>0.0079</td>
<td>7600</td>
<td>8600</td>
<td>13</td>
<td>5.3</td>
</tr>
<tr>
<td>~3 mM KCl</td>
<td>0.06</td>
<td>1000</td>
<td>786</td>
<td>4.8</td>
<td>15</td>
</tr>
<tr>
<td>~30 mM KCl</td>
<td>0.56</td>
<td>107</td>
<td>92</td>
<td>1.6</td>
<td>45</td>
</tr>
<tr>
<td>~300 mM</td>
<td>3.53</td>
<td>17</td>
<td>20</td>
<td>0.6</td>
<td>110</td>
</tr>
</tbody>
</table>

Table 4.1: Resistance values calculated using *equation (4.30) and #from using the current-voltage relationship of the assumed purely resistive electrolyte. $C_{EDL}$ is calculated using equation (4.22).

The resistances calculated using the reference electrodes matched the estimated values within ~15%, and the voltage dropped between the reference electrodes was comparable to the voltage dropped across the entire electrode system. This discrepancy could be due to inaccurately measuring the distance between the two reference electrodes.
Figure 4.14: The ratio $V_E/V_3$, which represents the relative potential drop across the electrolyte compared to the EDLs.

Analogously, we can estimate the resistance of the flow cell used in our experiments (Fig. 9a) after bathing them with an electrolyte by adapting equation (4.23)

$$ R_E(\sigma) = \left( \int_{0.5d}^{1\text{centimeter}} \frac{\sigma_m \ast d}{\pi z} \, dz \right)^{-1}, \quad (4.31) $$

where $d$ is the electrode gap width ($\sim 50 \mu m$) and integrating over 1 centimeter of the electrode in the direction away from the gap, $z$. This expression takes into account the semi-circular resistors formed by the current paths of the same shape (resistors in parallel). This probably overestimates the resistance as most of the current flows through the region of relatively uniform electric field very close to the gap, thus distorting this image of semi-circular current tubes. We were not able to compare the estimated values from equation (4.31) with experimental values, because we did not measure the potential drop between the electrodes, excluding the EDLs.
The conductivity cell exercise showed that very little voltage was dropped across the EDLs at the frequencies used in our experiments. However, in our actin trapping experiments, the charging time scale is shorter due to the small electrode gap, so the critical frequencies will be higher. The critical frequencies in Fig. 4.14 correspond to the frequencies at which one would expect any ACEO to peak. Our PIV experiments showed that ACEO had diminished at low frequencies and ceased at our experimental frequencies on the electrode geometry with small gap size. We are confident, therefore, that the electrodes’ EDLs were essentially shorted during our actin trapping experiments. We did not take this for granted, however, and still measured the current by unbiased means.

4.4 Results and Discussion

When an AC (2 MHz) potential difference was applied across the electrodes, the electric field caused the actin to align parallel to the field lines, migrate towards the maximum electric field intensity, and bridge the gap across the electrodes. This observation is consistent with Asokan et al. (1993). In Asokan et al. (1993), the filaments, once trapped, were attached to the surface. In contrast, in our setup the filaments were suspended over a trench.

In contrast to our results, Kobayasi et al. (1964) reported optical birefringence measurements suggesting that in a DC field, actin filaments align transverse to the electric field. They speculated that the filaments have a strong, intrinsic dipole moment (of unknown origin) transverse to their long axis. This effect is unlikely to manifest itself in AC fields.

In the absence of the electric field, the filaments settled randomly with just a few filaments anchoring firmly to the gold electrodes. The contour lengths ($L$) of the
filaments’ segments confined between the electrodes’ edges were typically much larger than the gap’s width \((G)\). When the electric field was present, a greater number of filaments bridged the gap between the electrodes, and \(L \geq G\).

Once a filament was observed to bridge the gap across the electrodes, an electron multiplying CCD camera (Photometrics Cascade II) took images at 10 Hz. A custom-written Matlab\textsuperscript{TM} program was used to obtain the filament’s transverse displacement \(s(x)\) (Fig. 4.15) as a function of the distance \(x\) from one of the anchoring points. The data was then used to compute the variance of the displacement \(<s^2(x)>\) as a function of \(x/G\).

![Figure 4.15](image_url)

**Figure 4.15:** Contrast-enhanced image of an actin filament trapped across the gap between two electrodes. The image also depicts the discretized position data (*) obtained by using a custom-written algorithm using MatLab®.

A sample of our experimental data obtained with different filaments having similar \(L/G\) (<1.0) ratios is given in Fig. 4.16 at various field intensities. The magnitude of \(E_{rms}\) is calculated as the root-mean-square potential difference \(V_{rms}\) across the electrodes divided by the gap’s width \(G\). As the electric field intensity increases, the variance decreases. Figure 4.17 depicts the normalized standard deviation averaged over the gap’s width,
\[ \hat{s} = \frac{1}{G_0} \int_0^G \sqrt{\langle s^2(x) \rangle} \, dx, \]

as a function of the electric field intensity squared, \( E_{rms}^2 \). The amplitude of the thermal fluctuations decreases markedly as the electric field intensity increases.

Figure 4.16: The variance of the filament’s transverse displacement is plotted vs. relative location across the gap at electric field intensities 0.2, 0.3 and 0.5 V/µm. The symbols and lines correspond, respectively, to experimental data and theoretical predictions of a tension-optimized Brownian dynamics-based model for a clamped rod with flexural rigidity \( \kappa = 7.3 \cdot 10^{-26} \text{ N m}^2 \).

This “straightening” effect may result from electric field induced tension that causes torque opposing the filament’s bending. To estimate the magnitude of this tension, we modified linear, Brownian dynamics theories of vibrating beams (Van Lear and Uhlenbeck 1931) to accommodate beams with uniform effective tension. Using the equipartition principle, we calculated the asymptotic (long time) value of the variance for a
simply supported beam (zero displacement and zero moment at the edges) and for a clamped beam (zero displacement and zero slope at the edges). This expression was derived in Chapter 2.

\[
\langle s^2(x) \rangle = \sum_{n=1}^{\infty} \frac{2k_B T L^3 \sin^2 \left( \frac{n\pi x}{L} \right)}{n^4 \pi^4 L^2 + \kappa n^2 \pi^2} 
\]  

(4.33)

**Figure 4.17**: The average, standard deviation of the displacement (circles) (± 1 s.e.m.) as a function of the electric field-squared. The solid line is overlaid to guide the eye.

In equation (4.33), $\kappa$ is the flexural rigidity and $\tau$ is the apparent tension. Equation (4.33) is based on a linear theory that assumes $s \ll L$ and $L \equiv G$, as observed for filaments that were trapped in the presence of the electric field. The linear theory applies separately to the vertical and horizontal modes of vibrations. The expression for the variance of the clamped beam is lengthy and is given in the on-line Supplementary Information along with the derivation of equation (4.33). The inclinations in the variance
data curves at \( x = 0 \) and \( x = G \) suggest that the filaments’ boundary conditions were somewhere between clamped and simply supported. This is not surprising, because the protein-gold linkage was likely made up of several actin monomers non-specifically attaching to the surface. One interaction would intuitively constitute a simply-supported boundary condition, but many would certainly resist torque, resulting in a partially clamped boundary condition.

Many filaments that settled across the gap in the absence of an electric field significantly deviated from the small-deflection assumption of the linear theory, precluding us from using it to obtain an accurate estimate of \( \kappa \).

4.4.1 Varied Voltage

Using a value for the flexural rigidity previously estimated under similar environmental conditions as in our experiments, \( \kappa = 7.3 \cdot 10^{-26} \) Nm\(^2\) (Gittes et al. 1993), we calculated the apparent tension \( \tau \) by minimizing the squared difference between the predictions of the beam theories and experimental observations of the variance (Fig. 4.16). An example of the error \( E(\tau) \) (equation (2.32)) as a function of \( \tau \) when optimizing the solution to equation (2.19) to experimental data is depicted in Fig. 2.5. Figure 4.18 depicts the apparent tension estimated from the clamped model (circles) as functions of \( E_{\text{rms}}^2 \) for 58 filaments. The estimated tension ranged from 0.5 to 5 pN, was insensitive to the choice of \( \kappa \), and varied nearly linearly with \( E_{\text{rms}}^2 \), consistent with forces caused by polarization of the filament and its adjacent electric double layer (EDL). The solid line is overlaid to guide the eye. The error bars are ± one standard error of the mean.
**Figure 4.18:** The apparent tension estimated from the clamped model (circles) as functions of the electric field-squared for 58 filaments (± 1 s.e.m.). The solid line is overlaid to guide the eye.

### 4.4.2 The Effects of Solution Conductivity

The EDL is central to understanding the behavior of colloidal particles in an electric field. To better understand the actin filament’s EDL, we sought to trap filaments in aqueous media of different conductivities. The goal was to measure the forces on the filaments, at constant electric field intensity, in different solutions. To accomplish this task, the complicated nature of the electrodes’ polarization was ignored. Instead, the current was monitored as previously described. By adjusting the current being passed through the electrolyte to account for the change in electrolyte resistivity, the potential drop was kept constant. For example, if one solution was ten times more conductive than a second solution, the first solution would require ten times more current passed through
it to maintain the same potential as that across the second solution. The actin filaments were incubated with 1 mg/mL BSA to prevent any aggregation, which was monitored by comparing the intensity of the trapped filaments to the known intensity of single filaments. The length of the trapped filaments did not vary with the solution’s conductivity.

Figure 4.19 depicts the minimum potential required to achieve trapping in solutions having different conductivities. The pH values of these solutions are difficult to accurately measure due to the extremely low conductivity solution’s interaction with the pH meter. DI water, of course, registers a reading of pH=5.5. In other words, the pH of these solutions may play a role in the phenomena discussed below. The scaled potentials plotted along the y-axis were calculated by dividing the currents passed through the circuit during the experiment by the solutions’ conductivities. This is analogous to multiplying the currents by the solutions’ resistances. Consequently, the value plotted on the y-axis is the relative potential drop seen by the actin in the different cases. In other words, we do not explicitly know the magnitude of the potential seen by the actin filament. These results are counterintuitive, because as the solution conductivity increases, the relative polarizability of the filament should decrease (see Fig. 4.1). We see the opposite here, however. As the solution conductivity increases, the potential required for trapping decreases. It is possible that we do not yet have a sufficient enough understanding of the EDL. Perhaps the surface conduction in the electric double layer actually increases as it shrinks in size.
Figure 4.19: The scaled potential [current/conductivity] required to trap actin filaments across a 7 µm gap as a function of the solution conductivity.

4.4.3 Discussion

By what mechanism is the apparent tension generated? One possible contributor to the apparent tension is the polarization of the filament, which induces an effective dipole moment (Jones 1995). The resulting apparent tension

$$
\tau = \chi \frac{2\pi a^2}{3} \varepsilon_m \text{Re} \left( \frac{\varepsilon_f^* - \varepsilon_m^*}{\varepsilon_m^*} \right) E_{\text{rms}}^2
$$

(4.34)

where subscripts \(m\) and \(f\) denote, respectively, the medium and the filament; \(a \sim 4\) nm is the filament’s radius; \(\varepsilon^* = \varepsilon - j\cdot\sigma/\omega\) is the complex dielectric permittivity; \(\varepsilon\) is the dielectric permittivity; \(\sigma\) is the conductivity, and \(\chi\) is a constant to adjust for the non-uniformity of the electric field along the filament’s length. Through numerical simulations, we estimate \(\chi \sim 3\). When \(\varepsilon_m = 78\cdot\varepsilon_0\), \(\varepsilon_f = 36\cdot\varepsilon_0\) (at the frequency of our experiments, the magnitude of \(\varepsilon_f\) has an insignificant effect), and \(\sigma_m = 0.56\) S/m, we
estimate $\tau \equiv 1$ pN at $E_{rms} = 0.3$ V/µm. According to equation (4.34), the corresponding $\sigma_f \equiv 85$ S/m. In the case of a thin EDL (O’Konski 1960), $\sigma_f$ can be decomposed into three components: (i) the intrinsic conductivity of the filament; (ii) the diffuse layer’s conductivity estimated as 0.95 S/m (surface charge of $6.4\times10^{-19}$ C/nm = $4e$/nm, pH = 7.2, and potassium counterions) (Asokan et al. 2003); and (iii) the stagnant layer’s conductivity (for which data is not available). The stagnant layer’s conductivity is typically assumed to have a similar magnitude to that of the diffuse layer. Thus, to obtain the estimated tension with equation (4.34), $\sigma_f$ would need to be nearly two orders of magnitude larger than the estimated contribution of the diffuse layer to the (surface) conductivity of actin filaments.

The EDL in our experiment ([KCl] ~ 40 mM) is quite thick relative to the filament’s radius, $\lambda_D/a \sim 0.4$, where $\lambda_D$ is the Debye screening length. To account for a thick EDL, we calculated the shear stress induced by the flow of ions in the EDL enveloping the filament. The alternating electric field causes the ions in the EDL to migrate from the filament’s center towards its ends. The resulting hydrodynamic flow imposes a shear stress along the filament’s surface, manifesting itself as apparent tension. This phenomenon also leads to electric current flow along the filament’s surface. This current is occasionally approximated as the diffuse layer’s contribution to the surface conductivity (O’Konski 1960). We computed the ion transport around the filament when the filament’s linear charge density is $6.4\times10^{-19}$ C/nm, the stagnant layer’s conductivity is negligible, and $E_{rms} = 0.3$ V/µm, to find that the corresponding viscous forces contribute about 0.01 pN or two orders of magnitude less than the estimated tension. In order to obtain the apparent tension estimated from the experiments, a charge density of $\sim 190\times10^{-19}$ C/nm.
19 C/nm would be needed, which is about two orders of magnitude larger than the value calculated from the protein net charge (Tang et al. 1996). We expected the force to increase as the solution conductivity decreased (or as the EDL thickness increased), but Fig. 4.19 implies this is not the case.

Of course, there are other induced charges, such as in the EDL along the electrodes (AC electroosmosis, (Squires and Bazant 2004)) and in the bulk of the solution (resulting from temperature gradients caused by the microscope’s illumination (Green et al. 2000)), that will migrate in the electric field and induce fluid motion. At the electric field frequency and solution conductivity of our experiments, AC electroosmosis is negligible. As was previously discussed, by seeding the fluid with small particles, we visualized the electrothermal convection and found it to be directed upwards in the gap between the electrodes, which would induce compression forces rather than tension. In Chapter 2, we explored the effect of exposure time on calculating the position of our filaments. The forces estimated here and depicted in Fig. 4.16 are surely an overestimate, on the order of a factor of 3-5. This still does not compensate, however, for the discrepancy between our experimental findings and our computational estimates.

4.5 Conclusion

In summary, we report for the first time on the effect of electric fields on the thermal fluctuations of actin filaments. As the electric field’s intensity increases, the amplitude of the filament’s lateral fluctuations decreases, and the apparent tension increases. These features are consistent with the electric field affecting the filament’s behavior through the polarization of the filament and its surrounding electric double layer. The magnitude of the reduction of the filament’s vibrations by the electric field is
not quantitatively explained using established values for actin filament properties. According to our analysis, either the intrinsic or the surface conductivity of the filament would need to be several orders of magnitude larger than expected. However there might be other, yet unknown, unaccounted effects that may explain our observations. The voltage required for trapping as a function of solution conductivity results are also difficult to explain. The simplicity of trapping, observing, and manipulating filaments by electric fields may make this approach useful for further study of the surface properties of polyelectrolytes, for directed assembly of biopolymers, and for motility-based biophysical experiments. Finally, the technique described here provides a novel means to estimate the surface conductivity of single molecules.
Chapter 5

Using Electric and Optical Tweezers to Study the Helical Path of Molecular Motors

Abstract

Dielectrophoresis was used to stretch and suspend actin filaments across a trench etched between two electrodes patterned on a glass slide. Optical tweezers were used to bring a motor protein-coated bead into close proximity to a pre-selected, suspended actin filament, facilitating the myosin-coated bead’s attachment to the filament. The clearance beneath the filament allowed the bead to move freely along and around its filamentous track, unhindered by solid surfaces. Using defocused images, the bead’s three-dimensional position was tracked as a function of time to obtain its trajectory. Experiments were carried out with myosin V and myosin X. Both motor proteins followed left-handed helical paths with the myosin X motor exhibiting a shorter pitch than the myosin V. The combined use of electrostatic and optical tweezers facilitates the preparation of motility assays with suspended tracks. Variants of this technique will enable higher complexity experiments in vitro to better understand the behavior of motors in cells.
5.1 Introduction

Molecular motors are cellular energy transducers involved in determining cell shape and motions. They power muscle contraction, transport cargo along tracks of cytoskeletal actin or microtubule filaments, and are implicated in many disease processes (Schliwa 2003; Vale 2003; Hasson et al. 1997; Hirokawa et al. 2004).

As was previously stated in the introductory chapter, for the most part, mechanical studies of myosin and other molecular motors have utilized surface-immobilized motors (Finer et al. 1994; Mehta et al. 1999; Rock et al. 2001; Veigel et al. 2003; Takagi et al. 2006) or filaments, (Block et al. 1990; Svoboda et al. 1993; Rief et al. 2000; Forkey et al. 2003; Yildiz et al. 2003; Ross et al. 2006; Sun et al. 2007). These assays, and those that use a surface-immobilized motor (Finer et al. 1994; Mehta et al. 1999; Rock et al. 2001; Veigel et al. 2003; Takagi et al. 2006), may impact a motor’s range of motion.

One way of avoiding surface immobilization of the filament and motor is to suspend filaments from fixed supports, giving the motor or the motor-coated bead unimpeded freedom of motion about its filamentous track. To this end, Ali et al. suspended actin filaments (randomly) between two immobilized 4.5 µm-diameter beads and, using optical tweezers, brought 1 µm diameter, myosin-coated beads into close proximity to the filament (Ali et al. 2002; Ali et al. 2004). They were able to detect the myosin-coated bead’s path along and around actin for two myosin isoforms in the absence of constraints imposed by surface attachment. This technique is laborious since just a small fraction of the filaments falls in or next to a horizontal plane and few are
sufficiently stretched between the two supporting beads to facilitate accurate tracking of the attached bead.

Here, we describe an attractive alternative that facilitates control of actin filaments’ or microtubules’ placement and tightness. The method consistently yields tightly suspended filaments positioned in a single horizontal plane, allowing us to keep a filament’s entire length in focus during motility experiments. Dielectrophoresis, which enables positioning the filaments at predetermined locations and controlling their tautness, is combined with an optical trap (laser tweezer), which is used to bring a motor protein-coated bead near the filament. Once the bead is positioned next to the filament and a motor protein binds to the filament, the optical trap is turned off, and the bead is carried along the filament by the molecular motors. The coordinates of the bead’s center, $\bar{x}$, $\bar{y}$, and $\bar{z}$, are then tracked by processing images obtained with an optical microscope. Although the use of electric fields to trap and position actin filaments and microtubules is not new (Asokan et al. 2003), the combined use of dielectrophoresis and optical traps, to the best of our knowledge, has not been previously reported.

5.2 Materials and Methods

The electrode arrangement, a filament, and a bead are depicted schematically in Fig. 5.1. Electrodes with a 7 μm gap and a 2 μm-deep trench were patterned on a glass coverslip using standard photolithography techniques. The coverslip containing the electrodes was assembled into a flow cell using a second coverslip and double-sided adhesive tape. Electrodes were fabricated on sulfuric-peroxide (H$_2$SO$_4$:H$_2$O$_2$, 3:1)-cleaned, 150 μm-thick glass coverslips. 10 nm of NiCr was evaporated to act as an
adhesion layer, upon which 100 nm of gold was evaporated. The electrode pattern was defined in positive photoresist (Shipley 1813), and the gold and NiCr were wet-etched using a potassium-iodide-based etchant and nichrome etchant, respectively. Hydrofluoric acid (HF) was used to etch a ~2 µm-deep trench in the glass between the electrodes, using the patterned electrodes as a mask. Electric fields (20 kHz, 2 V\text{rms} AC) were applied across the electrodes, and the signals (and currents) were monitored using an oscilloscope (Tektronix 3036-B).

![Figure 5.1](image_url): A schematic depiction of a dielectrophoretically positioned and tightened actin filament suspended across a trench between two gold electrodes. A myosin-coated bead is being positioned near the filament with optical tweezers.

G-actin was obtained from rabbit skeletal muscle and purified according to Pardee et al. (1982). F-actin was prepared from G-actin, at 1 µM total actin monomer concentration, and stabilized with 1.1 µM rhodamine-phalloidin (Molecular Probes, Carlsbad, CA). F-actin was suspended in 37 mM KCl, 2 mM MgCl₂, 1 mM K-EGTA, 20
mM Hepes, and 1 mM DTT. Polystyrene beads (1 µm diameter, Polysciences, Inc.) were incubated in 30 mM KCl, 2 mM MgCl₂, 1 mM K-EGTA, 20 mM Hepes, 10 mM DTT, 6 µM CaM (expressed in bacteria according to Putkey et al. (1985)), 100 ng/mL tetramethyl rhodamine (TMR) BSA, and then with myosin V or X at a molar ratio of ~10⁴ myosin molecules per bead. The TMR-coated beads were easily observable under low-light conditions before attachment to actin. Chick brain myosin V was purified from tissue according to Cheney (1998). Myosin X was a kind gift of Drs. Mitsuo Ikebe and Osamu Sato, University of Massachusetts. The motility buffer (pH = 7.4) also contained 0.2 mg/mL casein, 5 mM phosphocreatine (Sigma P7936), 0.1 mg/mL creatine phosphokinase (Sigma C3755), 0.5 mg/mL unlabeled BSA, 7.2 mg/mL glucose, 9 units/mL catalase, 4 mg/mL glucose oxidase, and various concentrations of MgATP.

A solution of actin filaments was infused into the flow cell. An AC potential was applied across the electrodes. The electric field polarized the suspended filaments and their adjacent electric double layer and induced AC electroosmosis in the cell, which was observed by seeding the solution with 900 nm diameter tracer beads. It consisted of two counter-rotating vortices with the flow at the electrode surfaces directed away from the center of the gap and the flow velocity next to the electrodes’ edges on the order of µm/s.

Thus, the electric field served two purposes. It facilitated the positioning of the filaments across the gap between the electrodes, and it controlled the filaments’ tension (Uppalapati et al. 2008). Once the filaments settled across the gap, their ends adhered non-specifically to the electrodes’ gold surfaces. When several filaments were suspended tightly across
the gap (< ~ 200 nm rms lateral displacement at the gap’s center), the potential was removed, and the free actin was washed out of the flow cell.

The myosin-coated beads were infused into the flow cell, and a 1064 nm optical trap (Takagi et al. 2006) was used to position an individual bead next to the center of a pre-selected actin filament. Single filaments were identified by their relative intensity. Once a motor-coated bead attached to the actin, the bead was released from the trap. The motors traveled along, and rotated around, the actin filament. The bead motion was recorded using bright-field illumination in an inverted microscope and a 60x, 1.2 NA water immersion objective (Olympus plan apo). Fluorescent images of the filament were periodically taken to ensure that, throughout the experiment, the filament remained securely attached to the gold electrodes and remained approximately in focus. The three-dimensional coordinates of the bead’s position were determined using the defocused images and a custom-written algorithm in MatLab™.

5.3 Results and Discussion

Figure 5.2a shows a series of images of a myosin-V-coated bead traveling along a tightly suspended actin filament. The white, dotted lines identify the filament’s location. Bright and dark images of the bead’s center indicate, respectively, that the bead is above and below the focal plane. In other words, the motor followed a helical path around the actin filament. In Fig. 5.2, the path is left-handed as explained below.

When a bead is displaced out of the focal plane of the imaging optics, spherical aberrations and diffraction form ring patterns centered on the bead (Gosse et al. 2002;
Crocker et al. 1996). The unique topologies of these images can be used to estimate the bead’s out-of-plane position.

**Figure 5.2:** a) A series of bright-field optical micrographs of a myosin V-coated bead traveling along a tightly suspended actin filament. The illuminating condenser is stopped down from its maximum to increase contrast. The viewing objective is located below the sample. The time interval between frames is 850 ms and the exposure time is 80 ms. For clarity, dotted lines are overlaid on the actin filament. b) Images of a stationary bead on the microscope slide surface moved in 150 nm increments along the optical axis, \( z \).

Images 1 & 8 are, respectively, closer to and farther from the imaging objective.

A bead fixed to each slide was used to calibrate the \( z \)-position data. These calibration images were taken as the slide was translated at 50 nm increments along the optical axis and were fit to the triple Gaussian function (one Gaussian peak and two Gaussian rings)
\[ A(x, y) = A_B + A_a \exp \left[ -\frac{1}{2} ((x - \bar{x})^2 + (y - \bar{y})^2) / \sigma^2_0 \right] + \]
\[ A_a \exp \left[ -\frac{1}{2} (\sqrt{(x - \bar{x})^2 + (y - \bar{y})^2} - R_i)^2 / \sigma^2_i \right] + A_e \exp \left[ -\frac{1}{2} (\sqrt{(x - \bar{x})^2 + (y - \bar{y})^2} - R_j)^2 / \sigma^2_j \right] \] 

, (5.1)

similar to Gosse et al. (2002). In the above equation, \( A_B \) is the background intensity, \((\bar{x}, \bar{y})\) is the lateral position of the bead’s center, \( \sigma_i \) (\( i = 0, 1, 2 \)) is a width factor, and \( R_i \) (\( i = 1, 2 \)) is the radius of the Gaussian ring. Figure 5.2b shows the defocused images of a stationary, calibration bead at 150 nm increments along the optical axis, where image 1 is closest to the microscope objective lens, located below the focal plane. \( z \)-movement of the stage is equivalent to \( z \)-motion of a bead in solution, because the objective lens is a water immersion type. Figure 5.3 depicts the intensity distributions of the images for a bead located farther from the objective lens (a, above the focal plane) and closer to the objective lens (b, below the focal plane). The images had their background intensities subtracted using a two-dimensional moving average, taken over a square region with sides approximately equal to the outermost Gaussian ring’s diameter, and were smoothed using a Gaussian convolution kernel of half width = 1 pixel (Crocker et al. 1996). Equation 1 was fitted to the smoothed images by minimizing the square of the difference between the fitted function (Figs. 5.3c and d) and the smoothed image. Figures 5.3e and f compare \( A(x, y) \) as a function of \( x \) (solid line) and the experimental data (symbols) of Figs. 5.3a and b.
Figure 5.3: a) & b) Smoothed surface plots of images similar to those in Fig. 5.2. c) & d) Fits of equation (5.1) to images a & b, respectively e) & f) Average radial line profiles from the center of the images shown in a) & b), respectively (symbols), and the corresponding radial slices of the axisymmetric fits shown in c) & d), respectively (solid lines).
The fits of the experimental data were normalized with \( (\text{Max}[A(x,y)]-\text{Min}[A(x,y)]) \). To determine a bead’s \( z \)-position, we compared the normalized fits \( A^\text{norm} \) (equation (5.1)) of the experimental data with the \( k \) normalized fits of the calibration data \( A^\text{cal,norm}(k) \) (Fig. 5.2b). In other words, a bead’s \( z \)-position was determined by minimizing

\[
\chi_k = \sum_{i,j} (A_{i,j} - A^\text{cal}_{i,j}(k))^2
\]

over \( k \), where \( k = 1, 2, 3\ldots \) correspond to the fitted surfaces of the calibration bead intensities that were located at known \( z \)-positions and \( i \) and \( j \) are integers designating individual pixels.

Additionally, as checks of this procedure, estimates of the \( z \) positions were obtained using the outermost Gaussian ring’s fitted radius and using the zeroth and second moments of the light intensity (Crocker et al. 1996). All these techniques yielded similar estimates for \( z \) (Fig. A.10).

After beads were brought into proximity with the suspended actin filament and released from the optical trap, many of the beads remained in contact with and moved along the actin filament with helical paths. The diameter of the helix was approximately the diameter of the bead, 1 \( \mu \text{m} \), as expected, making the helical path readily observable. The trajectory, velocity, pitch, and handedness of the path of each bead were determined from its three-dimensional position as a function of time. Twenty-one myosin V-coated beads were bound to single actin filaments, and all were observed to travel along their filaments. The average pitch was calculated using only beads that rotated for more than half a turn (Table 5.1). Rotations of less than half a turn were a result of short runs. Only one bead was observed to travel an appreciable distance without rotating. The average
velocities in the direction of the filament axis at 500 nM, 2 µM, and 5 µM MgATP were very similar to those reported previously (Forkey et al. 2003).

Displacements in the transverse-direction (x) and along the optical axis (z), depicted as functions of the position along the filament (y) (Fig. 5.4a), showed that the x-position (solid line) is roughly 90-degrees out-of-phase with the z-position (dashed line), as expected for a helical path. The bead moves in the positive y-direction. Since, in the first quadrant (x>0, z>0), \( \frac{dx}{dy} < 0 \) and \( \frac{dz}{dy} > 0 \), the path is left-handed (Fig. 5.4b).

All helical paths taken by myosin V coated beads were left-handed with an average pitch of 1.8 µm without significant dependence on [MgATP] (Table 5.1). The handedness and pitch agree with a previous report that found the average pitch of myosin V-coated beads on suspended actin filaments to be 2.2 µm (Ali et al. 2002).

**Figure 5.4:** a) The displacement in the transverse direction, x (solid line) and the displacement in the optical axis, z (dashed line) of a myosin V-coated bead, vs. the position along the filament, y. The z-position lags the x-position by roughly 90-degrees, as expected for a left-handed helical path. b) The left-handed, helical motion of the same myosin V-coated bead as it traveled along an actin filament.
Similar experiments with myosin X at 50 µM MgATP yielded left-handed helical motion with a pitch of 1.5 µm (calculated from 4 beads – all the beads that were tested rotated). Figure 5.5 depicts the path taken by a myosin X-coated bead as it travels along an actin filament. The path, handedness, and pitch of myosin X have not been previously reported. Our results are compatible with a recent report indicating torque generation by myosin X in gliding filament assays (Nagy et al. 2008). In the latter study, the pitch and the handedness of the motion resulting from this torque were not determined.

**Figure 5.5:** The path taken by a myosin X-coated bead as it travels along an actin filament. The pitch calculated for this bead was 1.2 µm.

With processive myosins, a helical path along actin can be explained by the motor preferentially binding to actin monomers that do not coincide with the pitch of the actin helix. The helical geometry of the actin filament can be defined in several ways. The
tightest helical disposition, termed the ‘genetic’ helix, is a left-handed 5.5 nm pitch helix, with an axial spacing of 2.75 nm per monomer and left-handed rotation angle around the filament axis of 166° per monomer. Because this rotation per subunit along the genetic helix is close to 180°, the structure is often described as two 72-74 nm pitch, right-handed helices that coil around each other.

Actin filaments are often approximated as a 13/6 helix when interpreting the path of myosins on actin (Ali et al. 2002). The 13/6 nomenclature defines an actin filament with monomers located along the left-handed short-pitch (5.5 nm) genetic helix rotating 6 full turns in 13 monomers. Taking the origin as the zeroth actin monomer, the 13th monomer along this short-pitch helix is oriented at the same azimuth around the filament’s axis as the zeroth monomer. A motor binding to successive sites 13 monomers apart would walk straight. Based on the left-handed helical motions of the beads along the suspended actin filaments, it was surmised that myosin V occasionally binds the 11th monomer, resulting in a gradual, left-handed rotation about the actin filament that was insensitive to the number of myosin motors bound to the bead (Ali et al. 2002). On the 13/6 actin helix, myosin V binding to the 11th monomer more often than the 15th would produce left-handed twirling. Since the filament is flexible, other known actin helical indices would also be consistent with left handed rotation (Vilfan 2005). For instance, on a 28/13 helix (generated by a slight untwist of the 13/6 helix), monomer 13 is not disposed on a straight path, but azimuthally 13° to the left of monomer zero. Monomer 15 is disposed 13° to the right. Thus myosin binding to monomer 13 more often than monomer 15 would still result in a left-handed path. We have also found that beads
coated densely with myosin X track a left-handed helix with a shorter pitch than myosin V. In contrast, myosin VI has been shown to twirl with a right-handed pitch according to a different assay (fluorescent labeled actin monomers in a gliding assay) (Sun et al. 2007).

| Table 5.1 Summary of myosin V-coated bead experiments (variabilities are presented as ± 1 standard deviation) |
|---------------------------------------------------------------|-----------------|-----------------|
| [MgATP]            | 500 nM | 2 µM | 5 µM |
| myosin/bead ratio | $10^4$  |      |      |
| # of trials        | 2      | 11   | 8    |
| # of beads that rotated >0.5 turns | 1      | 5    | 5    |
| linear velocity    | 21 ± 3 nm/s | 49 ± 11 nm/s | 116 ± 46 nm/s |
| pitch              | 2 µm   | 1.9 ± 0.9 µm | 1.7 ± 0.4 µm |

Whenever a comparison is possible between data on single molecule trajectories and unconstrained paths of cargos transported by several motors, the paths have been similar in handedness and pitch (Sun et al. 2007; Ali et al. 2002, 2004; Beausang et al. 2008), indicating that the helical paths are generated by intrinsic properties of the motor mechanism. For processive motors, the main determinants of their paths seem to be the step size distribution and the actin monomer indices selected by the stepping molecule. Helical paths, possibly resulting from off-axis forces or attachment biased towards sites before or after the azimuthally optimal binding site, have also been observed with myosin II, a non-processive motor (Beausang et al. 2008; Nishizaka et al. 1993). The techniques presented here may help to better our understanding of the various factors determining the functional mechanisms of molecular motions.
5.4 Conclusions

In summary, we have developed an improved method for studying the path and azimuthal rotation of molecular motors in vitro that uses two non-contact techniques: dielectrophoresis and optical trapping. Although both methods have been previously used independently, their combined application is new. The ability to place tracks for molecular motors at predetermined locations and to control their tension with electrostatic tweezers overcomes a significant technical hurdle – searching for tracks that are fortuitously suspended between fixed beads with a sufficient degree of tension.

We describe an assay that facilitates the study of the motility of motors unhindered by solid surfaces, enabling the motors to rotate around their tracks as they may be able to do in vivo. The study indicates that when they are afforded the opportunity, myosin V and myosin X motors, indeed, rotate.

The left-handedness of myosin V reported earlier was reproduced, and we found an average pitch of 1.8 µm without significant dependence on [MgATP]. Additionally, we found that myosin X also follows a left-handed helical path, with an average pitch of 1.5 µm, as it proceeds along actin.

Our method makes progress toward the goal of generating in vitro assays that reproduce some of the complexity present in cells. Using other electrode and flow arrangements will allow more complex filamentous networks to be generated for molecular motor studies.
Chapter 6

Conclusion

This dissertation described work toward a single molecule biophysical assay using electrokinetics. The behavior of actin filaments in electric fields and under the application of tensile forces was characterized. This work is important for understanding the electric double layer, polymer mechanics, and how to manipulate nano-sized particles.

We used dielectrophoresis (DEP) to manipulate actin filaments and place them in predetermined locations. There are several advantages to using AC electric fields when it comes to the experimental design such as minimizing electrodes’ reactions (electrochemistry) and reducing the potential drop at the electrodes’ surfaces, so this technique will likely prove to be advantageous for incorporation into man made diagnostic, lab-on-a-chip devices. While exploring the possibility of using this non-contact manipulation technique to facilitate biophysical assays, it was observed that the thermal fluctuations of the trapped actin filaments decreased their amplitude as the electric field increased. We endeavored to quantify this behavior as a tool for estimating the tension acting on the filament.

We tracked the position of the trapped, fluctuating filaments using a custom-written MatLab™ image analysis routine, which allowed us to calculate the equilibrium variance of the oscillations. A linear Brownian dynamics-based model related the thermal fluctuations’ variance to an applied tension. We estimated the force exerted by the electric field to be on the order of (pN). This force scaled, as expected for the force on an
induced dipole, with the field magnitude-squared. This model was validated using optical tweezers, which allows one to controllably apply forces on the pN-scale. By matching the estimated force to the applied force, we were able to establish confidence in our mathematical model.

Subsequent computational modeling in our lab that attempted to attribute this force to a surface conduction-aided dipole formation estimated tensions that were two orders of magnitude lower than our mechanical models estimated. This discrepancy may be attributed to a number of possibilities: surface conduction in the Stern layer, which was not treated; geometrical constraints that were simplified by modeling the filament as an ellipsoid; hydrophobic patches along the actin filament that contribute to anomalous surface conduction; inadequacies of our mechanical model; undetectable electrokinetic flows near the electrode edges. There is agreement in the electrokinetics community that the Stern layer requires extensive investigation. The underlying physics that govern dipole formation of colloidal particles are not well understood, so the complex nature of the protein’s topology, for example, make computational models that much more difficult to derive.

Our mechanical model was validated using optical tweezers, during which we explored the effect of observation time on the filament’s position calculation. At most, our published force estimates are overestimated by a factor of two. Electrokinetic flows were studied using bead-seeded electrolytes, and we made sure to operate in frequency regimes and with solutions that nullified these phenomena. It is possible, however, that small, as of yet undetected AC electroosmotic convection rolls exist at the electrode edges that contribute to tensing the filaments. Filaments confined to two-dimensions,
however, were observed to orient and tense in an electric field that was spatially-uniform, while residing several microns away from the electrodes’ edges. Joule heating was not the dominant forcing mechanism, because it scales with the field magnitude to the fourth power. Finally, illumination-induced flow would apply compressive force, not tensile.

To determine the effect of ionic strength on actin filament intrinsic rigidity, we built on the existing literature and measured the persistence length $L_P$ of actin filaments fluctuating on a surface. We calculated $L_P$ using three statistical mechanics techniques: mode analysis transverse fluctuation variance, and the root-mean-squared end-to-end distance. Discovering there was no ionic strength dependence, we applied a spatially-uniform AC electric field to these free-free filaments. Here, the filaments oriented their long axis along the electric field lines and appeared to stiffen, similarly to in the DEP case. As was previously stated, this adds confidence to our belief that electrokinetic flows are not responsible for the electric field-dependent stiffening, because the filaments are confined to a $\sim 2 \, \mu m$ plane that would discourage the formation of convection rolls. Although these experiments were not performed under varying ionic strength conditions, they emphasize the importance of studying surface conduction in the electric double layer when it relates to dipole formation. Varying the ionic strength (and the structure of the electric double layer) will be an important experiment.

Having explored a number of interesting topics working toward our goal of a DEP-assisted biophysical assay, we finally used our DEP manipulation apparatus to suspend actin filaments for studies involving myosin motors. We attached myosin-coated beads to the trapped, suspended filaments using optical tweezers and observed the helical paths taken by the cargo-laden motors. The left-handed path of myosin V that has been
previously studied was observed. We also observed, for the first time, the left-handed, shorter-pitched path of myosin X. This technique advances the field in the sense that the motors are afforded more freedom than in traditional biophysical assays that incorporate surface-immobilized motors or filaments. By observing the helical path (handedness and pitch) taken by a molecular motor, we can garner a fuller understanding of that motor’s mechanics and precise function \textit{in vivo}. Variants of this technique will enable types of higher complexity found in cells to be addressed with \textit{in vitro} experiments.
Appendix

Image Analysis

A.1 Suspended Filament Tracking

This section describes the image processing technique used to analyze the thermal fluctuations of actin filaments suspended across gaps or with both ends attached to beads. A Graphical User Interface (GUI) was created to make the data analysis more user-friendly. It was configured to determine the position of filaments anchored on either 2 (supported), 1 (cantilever), or 0 (free-free) ends. Figure A.1 depicts a screenshot of the GUI.

![Track_Filament GUI](image)

**Figure A.1:** A screenshot of the custom-written (Matlab®) GUI “Track_Filament”. This program can determine the position of filaments anchored on either 0 (free-free), 1 (cantilever), or 2 (supported) ends.
Movies were collected using an electron multiplying CCD camera (either the Photometrics® Cascade II or the Andor™ iXon). The filaments’ displacements were monitored and digitized using a custom MatLab® algorithm. Each frame was inspected to ensure that no glitches occurred in the fitting process. Frames representing poor fits were thrown out and skewed points were occasionally corrected by linearly interpolating between the two neighboring points. A skewed point could be caused by a bright contaminant coming into view or by a neighboring filament breaking and flapping in the vicinity of the filament being analyzed. 96% of the total frames analyzed were retained. About 8.6% of retained frames had points corrected in the interior of the filament, which excludes the two points neighboring the fixed endpoints. Figure A.2 shows the step-by-step process used to analyze filaments with both ends fixed.

Here is a step-by-step summary of how the filaments are analyzed:

1. The end points are chosen manually (Fig. A.2a). For a filament attached to electrodes, this task is made quite simple. However, for a filament attached at either end to freely-rotating beads, this task is made less straightforward.

2. The number of segments to divide the filament into must be defined. Lines transverse to the line connecting the filament’s endpoints are drawn (Fig. A.2b). The algorithm searches for the filament along these lines. These lines are fixed (i.e. the segment sizes are not)

3. The algorithm generates a line profile of the image’s intensity along each of these lines, averaging neighboring pixels along its length to reduce noise. Gaussian curves are fit to the curves, and the centroids are defined as the actin filament’s location. Brangwynne et al. (2007) also used this centroids refinement technique
to improve their spatial resolution. An example of this data and the fitted result is depicted in Fig. A.3. A screenshot of an actin filament being tracked is depicted in Fig. A.2c.

**Figure A.2:** A “supported” actin filament (a) intensity image (b) search grid shown (c) final position overlaid

**Figure A.3:** A background subtracted intensity profile taken transverse to an actin filament’s long axis (circles) with a Gaussian fit (dashed line) for position calculation
From here, the position data was utilized by theoretical tools at our disposal. Incidentally, singly-anchored filaments were treated as a special case. Figure A.4 depicts the step-by-step process by which they were analyzed.

Figure A.4: A “cantilever” actin filament (a) intensity image (b) search grid shown (c) position overlaid (d) how position may be corrected

A.2 Free-free Filament Tracking

Tracking freely oscillating (free-free) filaments was accomplished on the same basic premise, where transverse lines were drawn across the filaments’ long axes. In the case of free-free filaments, however, the curvature was much greater than in the case of doubly- or even singly-anchored filaments. In other words, we could not approximate the filament shape as a straight line and simply draw transverse cuts. Below is a summary of the steps:

1. Subtract the background (Fig. A.5a), threshold, identify the filament to analyze (Fig. A.5b) and skeletonize the image until each non-endpoint pixel had only two...
nearest neighbors (including diagonals) (Fig. A.5c). (The pixels’ numbers of nearest neighbors identified the filament’s endpoints.)

2. Move along the filament, fitting a polynomial function to bundles of pixels, thus approximating the slope.

3. Draw transverse cuts to obtain line profiles (Fig. A.5d). The filament position is found along these lines, similarly to the doubly- and singly-anchored filament cases (Fig. A.5e).

![Figure A.5: A “free-free” actin filament (a) intensity image (b) skeletonized (binary) image (c) corrected binary image (d) search grid shown (e) position overlaid](image)

**A.3 3-D Bead Tracking**

Figure A.6 depicts the mean positions of two stationary beads along the optical axis, \( z \), estimated using the method described in Chapter 5 (equation (5.2)), as a function of their actual positions. The calibration (comparison) bead was a third bead in the same 50 \( \mu m \times 50 \mu m \) field of view. The typical variability in the estimated bead \( z \)-position was
~ 200 nm, which is larger than the incremental steps taken when collecting calibration data. Accordingly, we did not interpolate between successive calibration images when estimating a bead’s z-position. The typical uncertainty in a bead’s x- or y-position was ~50 nm, which increased as the brightfield lamp intensity was decreased. The lamp intensity was kept low enough to monitor an actin filament’s position and stiffness by fluorescence but was still sufficient to calculate a bead’s rotational pitch.

![Figure A.6](image)

**Figure A.6:** The estimated position of two stationary beads along the optical axis [mean ± standard deviation (gray-shaded) as determined from 20 images (10 images/bead) using equation (5.2)] depicted as a function of their actual position set by the position of a piezo-electric stage on the microscope. Negative values of z are closer to the viewing objective, below the focal plane.

To verify the results obtained for the bead’s position along the optical axis, we explored three other methods: (i) the radius of the outermost fitted Gaussian ring,
max([R_1 \ R_2]) \text{ (equation (5.1)) (ii) the zeroth moment of the light intensity’s absolute deviation from background, } m_0, \text{ and (iii) its second moment, } m_2 \text{ (Crocker et al. 1996),}

\[
m_0 = \sum_{i^2 + j^2 \leq w^2} |A(\bar{x} + i, \bar{y} + j)|
\]

\[
m_2 = \frac{1}{m_0} \sum_{i^2 + j^2 \leq w^2} |A(\bar{x} + i, \bar{y} + j)|(i^2 + j^2).
\]

In the above equations, \(A\) is a pixel’s intensity, \(i\) and \(j\) are integers designating individual pixels, \(\bar{x}\) and \(\bar{y}\) are the pixel coordinates of the bead’s center, and \(w\) is the outer radius of the bead’s image.

\(m_0\) corresponds to the absolute ‘volume’ of the intensity profile and \(m_2\) it’s radius of gyration-squared. For example, \(m_2\) would approximate the standard deviation-squared of a single Gaussian peak. Figs. A.7 and A.8 depict, respectively, \(m_0\) and \(m_2\) of a stationary bead’s images as functions of its \(z\) location. Negative \(z\) is closer to the viewing objective (positioned below the focal plane). If a bead’s excursions are restricted to either side of the extrema in Figs. A.7 & A.8, an accurate, single-valued determination of its \(z\)-position can be made from these moments. Notice that the \(z\)-range in Figs. A.7 and A.8 span 10 \(\mu\)m, and a 1 \(\mu\)m-diameter bead is only expected to explore \(~1 \mu\)m along the optical axis when traveling along an actin filament that lies in the focal plane. The insets in Figs. A.7-A.9 expand the \(~1 \mu\)m range that is explored by the bead of Fig. A.10 (and Figs. 5.2a & 5.4). The exact location of the focal plane is uncertain, so \(z = 0\) was chosen to approximately coincide with the minimum in Fig. A.7.
Figure A.7: Zeroth moment, $m_0$, of a bead intensity profile, calculated using equation (A.1), plotted vs. $z$. The origin of the abscissa corresponds approximately to the focal plane. The inset details the range of depth explored by the bead presented in Fig. A.10 (and Figs. 5.2a & 5.4).

Figure A.8: Second moment, $m_2$, calculated using equation (A.2), of the intensity profiles plotted vs. $z$. Origin and inset as in Fig. A.7.
The outermost fitted Gaussian ring radius (equation (5.1)) of a stationary bead is not as robust a measure of the $z$-position as $m_0$ and $m_2$. The diffraction patterns weaken near the focal plane, so fitting the radii becomes poorly constrained and sensitive to the initial guess (Fig. A.9) (Speidel et al. 2003). However, if a bead is restricted to locations corresponding to either side of the minimum, similarly to the cases of $m_0$ and $m_2$, the $z$-position can be readily determined.

**Figure A.9:** The radius of the outermost Gaussian ring ($\max([R_1, R_2])$), fitted to a stationary bead's images using equation (5.1), plotted vs. $z$. Origin and inset as in Fig. A.7.

These alternative methods' calibration curves pose ambiguities for beads near the focal plane. For the data presented in the main article, the same trends in $z$ are produced using all four different methods (Fig A.10 (top)). Figure A.10 (bottom) shows the
transverse position, $x$, as a function of its position along the filament, $y$, leading the $z$ position data by approximately $90^\circ$.

**Figure A.10:** (top) A myosin-V coated bead’s position along the optical axis, $z$, depicted as a function of its position along the filament, $y$, calculated using four different measures: (i) fit comparison (solid line), (ii) zeroth moment, $m_0$ (dashed line), (iii) second moment, $m_2$ (dotted line), and (iv) radius of the outermost Gaussian ring (dash-dotted line). (bottom) the transverse position, $x$, depicted as a function of its position along the filament. Its phase leads all four of the $z$-trajectories.

**A.4 Myosin II Polymerization**

This section describes the application of filament tracking techniques for analyzing the polymerization of myosin thick filaments. The raw data comes in the form
of .tif stacks of \( n \) frames showing the bleaching, association, and dissociation of fluorescent myosin monomers, dimers, trimers, etc. from myosin thick filaments. Both the association and dissociation of molecules are assumed to be much slower than the bleaching rate.

The filaments are on the order of 33 nm (< 1 pixel) in width. For the purposes of the analysis, they are considered one-dimensional (i.e. they have no width). The endpoints of the filament are chosen by eye, using the filament’s intensity image (Fig. A.11a). Thresholding later refines this position. A line is drawn along the length of the filament (\( k \) pixels), and \( k + 1 \) slices are drawn perpendicular to the line joining the endpoints, equally spaced along the length of the filament. The slices extend a sufficient number of pixels (>10) to either side of the filament (Fig. A.11b). Drift in the setup can be accounted for by locating a fiducial point at the start and finish of the movie. The use of this process is prompted to the user. (NOTE: This only accounts for constant, linear drift)

![Figure A.11](image)

**Figure A.11:** (a) An intensity image of a myosin thick filament with its endpoints chosen by the user. (b) The search grid overlaid on the filament’s intensity image.

A calibration is performed on every movie by slicing single molecules in the observation region with the same size slices as those shown in Fig. A.11b (length \( k + 1 \),
obtaining the intensity from before and after a single-step bleaching. This is fit to a single Gaussian curve. The area under the curve is used for calculating the size of molecules being added and subtracted from the thick filament. When analyzing an actual thick filament, the pixel intensity values are plotted similarly to that depicted in Fig. A.12. Here, the intensity values along one of the slices shown in Fig. A.11 are depicted as a function of position across the filament. The data collected in these plots are averaged for every instance in time. This maps the two-dimensional, intensity surface of the filament into a one-dimensional vector of length $k + 1$.

![Image](image.png)

**Figure A.12**: The intensity values along one of the slices shown in Fig. A.11 depicted as a function of position across the filament.

An example of one slice’s average intensity as a function of time is depicted in Fig. A.13. We can extract rate constants from this data by using a simple kinetic model. The following differential equation describes the intensity as a function of time
\begin{align}
\frac{dI}{dt} &= k_{on} - k_{off} I. \quad (A.3)
\end{align}

The solution to equation (A.3) is
\begin{align}
I(t) &= I_0 e^{-k_{off}t} + k_{on} / k_{off} \left(1 - e^{-k_{off}t}\right). \quad (A.4)
\end{align}

Each slice’s intensity data is fit to equation (A.4). Figure A.13 has a solution overlaid (solid line).

**Figure A.13:** The raw intensity (averaged over a transverse slice) at a fixed position along a myosin thick filament’s long axis (circles) depicted as a function of time. The solid line is a fit to equation (A.4).

Figure A.14 depicts the values of $k_{on}$ and $k_{off}$ extracted from each position along the filament using equation (A.4). In the case of this filament, one can see that the rates of association and dissociation are less in the filament’s central region, possibly indicating the presence of the thick filament’s bare zone.
Figure A.14: (a) The on-rate and b) off-rate of fluorescent intensity along the length of a myosin thick filament calculated from fits of equation (A.4) to fluorescence intensity vs. time data.

After the rate constants are extracted, we quantify the number of molecules that fell and their locations for each frame. Each frame's intensity vector has its preceding frame's intensity vector subtracted, resulting in \( n - 1 \) intensity difference vectors. We then create two vectors: a subtraction vector (positive values are set to zero) and an addition vector (negative values are set to zero). An example of these two vectors is depicted in Fig. A.15. The algorithm asks the user to decide how many Gaussians (additions (subtractions) of arbitrary size) to fit to the addition (subtraction) curve. It then prompts the user to supply a guess for the Gaussian curves’ centroids. The Newton-Raphson method can deliver poor results if adequate guesses are not supplied. The fits are overlaid after the optimization completes, and the user can repeat the fitting process if desired.
**Figure A.15** The increase (circles) and decrease (triangles) in fluorescence intensity between two frames depicted as a function of position along a myosin thick filament. The Gaussian curves represent the additions (solid line) and subtractions (dotted line) of fluorescent intensity.

After completing this analysis, the program supplies information on where proteins fell, at what frequency, and the proteins’ sizes.


(1997).


N. G. Green, A. Ramos, A. Gonzalez, A. Castellanos, and H. Morgan. Electrothermally


J. A. Putkey, G. R. Slaughter, A. R. Means, Bacterial expression and characterization of


M. Schliwa, Molecular Motors, 2003 Wiley-VCH.


M. Speidel, A. Jonas, and E. Florin, Three-dimensional tracking of fluorescent


A. Yildiz, J. N. Forkey, S. A. McKinney, T. Ha, Y. E. Goldman, P. R. Selvin, Myosin V walks hand-over-hand: Single fluorophore imaging with 1.5-nm localization.