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Interplay of Micro-scale Flow and Fluid Micro/Nanostructure: Solutions of DNA and Suspensions of Single Walled Carbon Nanotubes

by

Rajat Duggal

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APPROVED, THESIS COMMITTEE:

Matteo Pasquali, Assistant Professor, Chair
Chemical and Biomolecular Engineering

Clarence A. Miller, Louis Calder Professor
Chemical and Biomolecular Engineering

Michael S. Wong, Assistant Professor
Chemical and Biomolecular Engineering

Robert M. Raphael, T. N. Law Assistant Professor, Bioengineering

Fazle Hussain, Cullen Distinguished Professor, Mechanical Engineering
U. Houston

Houston, Texas
June, 2005
UMI Number: 3216698

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ABSTRACT

The dynamics of dilute solutions of DNA flowing in a scaled down roll-knife free surface coating flow are investigated on multiple scales. The flow is generated between a rotating roll and a stationary glass knife. Extension of fluorescently stained DNA molecules is measured at the minimum gap at low roll speeds. The macroscopic flow is computed and microscopic predictions are obtained by simulating the DNA by Brownian dynamics combined with successive fine-graining (Sunthar and Ravi Prakash 2005). The simulations predict that the DNA should stretch almost to full extension near the roll surface in the region of minimum gap; this does not agree with experimental measurements. The assumption of linear velocity across the chains fails near free surfaces and is the likely cause of the discrepancy.

At high roll speed two separation surfaces arise in the coating bead. The distribution of DNA extension is measured at the separation surface upstream of minimum gap. Slow nodular recirculations are present under the upstream and downstream free surfaces; unexpectedly, DNA molecules are stretched axially in these regions.

Individual single-walled carbon nanotubes (SWNTs) in aqueous suspension are visualized directly by fluorescence video-microscopy. The fluorescent tagging is simple, biocompatible, and allows observation of the dynamics of SWNTs in water. The rotational diffusion coefficient in confinement is measured and the critical concentration at which SWNTs in suspensions start interacting is determined. By analyzing the fluctuating shape of SWNTs, the persistence length of SWNTs is found to range between 32 and 174 μm, in agreement with theoretical estimates; thus, common SWNTs in liquids can be considered as rigid Brownian rods in the absence of imposed external fields.

Drying microscopic drops of a suspension of individual SWNTs in aqueous solution of F68 pluronic surfactant exhibit complex dynamics. The drops dry on glass substrates forming a “crust” at the free surface. The crust is thin (~ 100 nm) and consists of an entangled mesh of nanotubes and pluronic. The self-assembled crust envelopes the drying drop and leads to a free surface inversion as evaporation proceeds. The convective flow associated with the drying preferentially assembles the micelles into hexagonal arrangement. This technique is promising for developing thin, optically transparent coatings and films consisting of SWNTs.
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# Contents

1 Introduction ................................. 1

1.1 Complex fluids ......................... 1

1.1.1 Polymer solutions ................... 2

1.1.2 Carbon nanotube solutions .......... 2

1.2 Complex flows .......................... 3

1.3 Studying DNA conformation in coating flows by multi-scale methods .... 4

1.4 Real-time fluorescence visualization of individual single-walled carbon nanotubes ......................... 9

1.5 Drop drying of surfactant stabilized aqueous dispersion of single-walled carbon nanotubes ................. 11

2 DNA Visualization in Coating Flows 12

2.1 Introduction ............................. 13

2.1.1 Polymers ............................. 13

2.1.2 Free surface flows .................. 16

2.1.3 Coating Flows ....................... 18

2.1.4 Visualization of flowing DNA molecules ................. 21

2.2 Experimental section .................. 23

2.2.1 Apparatus ........................... 23

2.2.2 Materials ............................ 25

2.2.3 Fluorescence microscopy setup ........ 29

2.2.4 Image analysis ....................... 30

2.3 Results and discussion ................ 31
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3.1</td>
<td>Low capillary number flow</td>
<td>32</td>
</tr>
<tr>
<td>2.3.2</td>
<td>High capillary number flow</td>
<td>44</td>
</tr>
<tr>
<td>2.4</td>
<td>Conclusions</td>
<td>57</td>
</tr>
<tr>
<td>3</td>
<td>Macro-micro Simulation of Dilute DNA in a Roll-knife Coating Flow</td>
<td>60</td>
</tr>
<tr>
<td>3.1</td>
<td>Introduction</td>
<td>61</td>
</tr>
<tr>
<td>3.2</td>
<td>Flow calculation</td>
<td>65</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Results and discussion</td>
<td>69</td>
</tr>
<tr>
<td>3.3</td>
<td>Successive-fine graining (SFG) applied to DNA in complex flow</td>
<td>73</td>
</tr>
<tr>
<td>3.3.1</td>
<td>Streamline tracing</td>
<td>79</td>
</tr>
<tr>
<td>3.3.2</td>
<td>DNA modeling</td>
<td>80</td>
</tr>
<tr>
<td>3.3.3</td>
<td>Results and discussion</td>
<td>84</td>
</tr>
<tr>
<td>3.4</td>
<td>Conclusions</td>
<td>109</td>
</tr>
<tr>
<td>4</td>
<td>Real-time Fluorescence Visualization of Single Walled Carbon Nanotubes</td>
<td>111</td>
</tr>
<tr>
<td>4.1</td>
<td>Introduction</td>
<td>111</td>
</tr>
<tr>
<td>4.2</td>
<td>Visualizing individual SWNTs</td>
<td>114</td>
</tr>
<tr>
<td>4.3</td>
<td>Brownian motion of SWNTs</td>
<td>120</td>
</tr>
<tr>
<td>4.3.1</td>
<td>Introduction</td>
<td>120</td>
</tr>
<tr>
<td>4.3.2</td>
<td>Experimental section</td>
<td>123</td>
</tr>
<tr>
<td>4.3.3</td>
<td>Results and discussion</td>
<td>124</td>
</tr>
<tr>
<td>4.4</td>
<td>Rigidity of individual SWNTs</td>
<td>130</td>
</tr>
<tr>
<td>4.4.1</td>
<td>Introduction</td>
<td>130</td>
</tr>
<tr>
<td>4.4.2</td>
<td>Thermal fluctuations in a rigid-rod</td>
<td>134</td>
</tr>
<tr>
<td>4.4.3</td>
<td>Shape parametrization</td>
<td>137</td>
</tr>
<tr>
<td>4.4.4</td>
<td>Procedure validation</td>
<td>141</td>
</tr>
<tr>
<td>4.4.5</td>
<td>Results and discussion</td>
<td>145</td>
</tr>
<tr>
<td>4.5</td>
<td>Conclusions</td>
<td>152</td>
</tr>
</tbody>
</table>
5 Drop Drying of Surfactant Stabilized Aqueous Dispersion of Single
Walled Carbon Nanotubes 154

5.1 Introduction ........................................ 155
5.2 Experimental ...................................... 159
  5.2.1 Materials ...................................... 159
  5.2.2 Solution preparation and characterization ..... 159
  5.2.3 Microscopy .................................... 160
  5.2.4 Weight measurement ............................ 162
  5.2.5 Rheology ...................................... 162
  5.2.6 Differential scanning calorimetry .......... 163
5.3 Results and discussion ............................ 163
5.4 Conclusions ....................................... 178

A Image analysis 181
  A.1 Thresholding .................................... 181
  A.2 Molecular properties ............................ 182

B Atomic force microscopy of fluorescent SWNTs 184

C Fluorescence visualization of SWNTs 186

D SWNTs in Water 188
  D.1 Exfoliation of SWNT bundles using light .... 188
  D.2 SWNT at a free surface ....................... 188
List of Figures

1.1 Coupling between flow and microstructure. ............................................. 6
1.2 Effect of flow on polymer conformation. ................................................. 7
1.3 Multi-scale approach to study the interplay of flow and polymer microstructure. ................................................................. 8

2.1 Overlap concentration in polymer solutions. ........................................... 16
2.2 Design drawing of the roller coating flowcell. ......................................... 24
2.3 Photograph of the roller coating flowcell. .................................................. 26
2.4 Speed calibration for the roller ................................................................. 26
2.5 Schematic of the roll-and-knife coating flowcell and the epifluorescence microscopy setup. ................................................................. 27
2.6 Images of deformed and undeformed fluorescent DNA. .......................... 28
2.7 Flow in the coating bead at low capillary number ................................... 34
2.8 DNA molecules flowing below the coverslip at low capillary number ....... 35
2.9 DNA molecule in the zero-velocity horizontal plane at the minimum gap between the roll and the coverslip. ................................. 37
2.10 Flow strength parameter in low capillary number flows. ....................... 38
2.11 Mean fractional extension of molecules in the pure shear flow at the minimum gap location. ................................................................. 40
2.12 Normalized distribution of molecular extension in the shear flow region at minimum gap position. ................................................................. 41
2.13 Yo-yo motion of a DNA molecule .......................................................... 42
2.14 In-plane average conformation of DNA at the minimum gap computed with the ellipsoid approximation. ..................................................... 43
2.15 Images of DNA molecules near contact lines at low capillary number. 45
2.16 Trapping of DNA at the contact line. 46
2.17 Flow in the coating bead at high capillary number. 46
2.18 Streamlines showing stagnation flow near the Upstream Separation Surface. 48
2.19 DNA flowing at the Upstream Separation Surface. 49
2.20 Probability distribution of molecular extension at the Upstream Separation Surface. 52
2.21 DNA configurations at the Upstream Separation Surface. 53
2.22 DNA Molecules imaged at the Downstream Separation Surface. 54
2.23 Schematic paths of DNA molecules between the upstream stagnation line (US5) and the upstream meniscus (UM). 55
2.24 Axially flowing DNA near the Upstream Meniscus. 56
2.25 Axially flowing DNA near the Downstream Meniscus. 58

3.1 Mapping of the physical domain into the computational domain. 66
3.2 Boundary conditions on the mesh, momentum and continuity equations in the roll-knife coating flow. 68
3.3 Computed mesh of the roll-knife coating flow at low capillary number. 70
3.4 Detail of the computed mesh. 71
3.5 Convergence of free surfaces at low capillary number. 72
3.6 Pressure profile at the coverslip. 73
3.7 Velocity at the minimum gap. 74
3.8 Streamlines at low capillary number. 75
3.9 Measured velocity of DNA in experiments. 76
3.10 Velocity and gradient interpolation in a 9-node quadrilateral element along a streamline trace. 80
3.11 Streamline tracing in the recirculation flow region at low capillary number. 81
3.12 Successive-fine graining approach applied to model DNA molecules. 81
3.13 Time-step convergence in molecular extension for 7 bead chain.  . . .  85
3.14 Effect of starting configuration of ensemble on molecular extension. .  86
3.15 Effect of number of cycles in the recirculation region of the flow on molecular extension. .................................................. 87
3.16 Extension map of N=7 bead-spring chain in the two-dimensional flow. 88
3.17 Extension dominated flow regions at the free-surface at low capillary number. ................................................................. 90
3.18 Images of a single chain of 11 beads moving along a streamline. . . . 91
3.19 Molecular extension of DNA as a function of time along the streamline passing through $x = 0, y = -3.3 \, \mu m$ (E4). ......................... 93
3.20 Molecular extension of DNA as a function of time along the streamline passing through $x = 0, y = -21.8 \, \mu m$ (E8). ......................... 94
3.21 Molecular conformation of chains in experiment E4. ................. 95
3.22 Molecular conformation of chains in experiment E8. .................. 96
3.23 Molecular extension as a function of position in the gap. ............. 98
3.24 Mesh convergence of molecular extension. .............................. 99
3.25 Mixed flow at the minimum gap position. .............................. 101
3.26 Molecular extension in mixed homogeneous flow at different positions in the gap. .................................................. 102
3.27 SFG predictions for molecular extension at different positions in the gap. 103
3.28 Effect of a point force induced due to a finite extension $Q$ in the spring of a simple dumbbell representation of a polymer chain. .............. 106
3.29 Snapshots of 7 bead chain, traversing along streamline corresponding to E4, crossing the liquid domain. ......................... 108

4.1 TEM micrograph of individual and bundles of SWNTs. .............. 112
4.2 Fluorescent tagging of individual SWNTs. ............................. 116
4.3 Raman fluorescence spectrum of SWNTs dispersed in 1 wt % SDS. . 117
4.4 Fluorescence microscopy images of individual single-walled carbon nanotubes tagged with PKH26 in SDS. ....................... 119
4.5 Transition from a dilute concentration regime to semi-dilute concentration regime for a suspension of rod-like molecules. 123
4.6 Confinement of a thin rod between two parallel plates. 125
4.7 Time lapse images of diffusing fluorescent SWNTs. 126
4.8 Orientation of a SWNT as a function of time. 127
4.9 Exponential decay of the first three harmonic orientation correlation functions. 128
4.10 Dilute to semi-dilute transition of SWNTs in solution. 129
4.11 Two dimensional rotational diffusion coefficients of SWNTs of different lengths at different number concentrations. 131
4.12 Shape functions $W(s = s/L)$ of the first three bending modes. 136
4.13 Fluorescent SWNTs and corresponding skeletons. 139
4.14 Simulated filament on a square lattice. 142
4.15 Errors in calculated contour and persistence length on simulated filaments using method 1. 143
4.16 Errors in calculated contour length of simulated filaments using method 2. 144
4.17 Errors in calculated persistence length of simulated filaments using method 2. 146
4.18 Mean square end-to-end distance for simulated filaments of contour length $L = 3 \, \mu m$. 147
4.19 Shape fitting to backbone of a SWNT using shape functions $W$. 148
4.20 Variance of mode amplitudes for a SWNT using method 2. 149
4.21 Persistence length of HiPco SWNTs. 152

5.1 Drying mediated patterns on substrates. 158
5.2 de Gennes model describing crusting in a polymer film on a substrate. 159
5.3 Raman fluorescence spectrum of SWNTs dispersed in 2 wt% F68 Pluronic. 161
5.4 Time-lapse video microscopy images of a 1 $\mu l$ drop of F68-SWNT solution on glass. 163
5.5 Drop radius as a function of time. ............................................. 164
5.6 Contact angle SWNT-F68 drying on a glass substrate. ............. 165
5.7 Variation of drop weight versus time. ................................. 167
5.8 Schematic of the drying process. ......................................... 168
5.9 Drying drop of SWNT-F68 on glass. ..................................... 169
5.10 Images of a dried drop of SWNT-F68 on glass. .................... 170
5.11 Images of a dried drop of F68 and of SWNT-SDS on glass. ...... 171
5.12 AFM and SEM images of surface of dried drops of SWNT-F68 .... 172
5.13 SEM image showing entangled mesh-like morphology of the crust. . 174
5.14 AFM and TEM images showing entangled mesh-like morphology of the crust ................................................................. 175
5.15 Raman spectrum of the crust displaced from a drying drop of SWNT-F68 on glass. ................................................... 176
5.16 Differential Scanning Calorimetry of F68 and F68-SWNT mixture. 178
5.17 Rheology of aqueous solutions of F68. ................................. 179
A.1 Thresholding of image of a stretched DNA molecule .............. 182
B.1 AFM images of SWNTs ...................................................... 185
C.1 Fluorescence microscopy image of SWNTs tagged with PKH67. . 187
D.1 Exfoliation of SWNT bundle by light. .................................. 189
D.2 SWNTs at air-water interface. ............................................ 190
## List of Tables

2.1 Location of the image planes (in depth) at the minimum gap position for the various local Weissenberg numbers, Capillary numbers and the experimental conditions.

3.1 Parameter values used in the Brownian dynamics simulations. The worm-like chain force law was used with $N_k = 250$, $\hat{h}^* = 0.23$ and $K = 1$. The corresponding longest relaxation times obtained from equilibrium simulations are listed for solvent quality $z = 1$ and viscosity $\eta = 44.6$ mPa s. .......................................................... 84

3.2 Location of the image planes (in depth) at the minimum gap position for the various local Weissenberg numbers and the experimental conditions. R: Roll; C: Coverslip; $R_g = 0.7$ $\mu$m; $L = 22$ $\mu$m. ............... 107

4.1 Persistence length $L_p$ of fluorescently tagged SWNTs computed using method 1 for 13 SWNTs of contour length $L$. The results from the first three modes are presented. ............................................. 150

4.2 Persistence length $L_p$ of fluorescently tagged SWNTs computed using method 2 for 13 SWNTs of contour length $L$. The results from the first three modes are presented. ............................................. 151
Chapter 1

Introduction

1.1 Complex fluids

Fluids which at macroscopic scales have a continuous appearance but at microscopic scales have a structure are called complex fluids (Larson 1999). The structure could be suspended particulate, polymers, bubbles, drops, or micelles. Common examples of such fluids are lubricants, paints, liquid crystals, plastics, shampoos, egg white, mayonnaise, ice-cream, mucus, blood, synovial fluid, etc.

Traditional material characterization classify substances as either simple liquids or solids. Simple liquids can be “simply” distinguished from solids by the ability of the former to take the shape of the container they occupy, while the latter maintain their shape indefinitely. More precisely, simple liquids cannot support a deviatory stress in the absence of flow and every configuration is an undistorted state (Truesdell and Noll 1992). Due to the presence of microstructure, complex materials have properties that lie in between the classical definitions of liquids and solids. These fluids are termed “complex” because they display a strong coupling between their microstructure and flow properties. Moreover, the microstructure of complex fluids is dynamically influenced by flow. They encompass materials with a wide variety of properties: viscoelastic materials behave as “solids” at short times and as “liquids” at long times and part of the energy of deformation such materials store is as potential energy while the rest is dissipated through viscous forces (Tschoegl 1989); gels change from solid-like to liquid-like or vice versa when subjected to a large enough deformation.
(Macosko 1994). The understanding of flows involving complex fluids is still incomplete: direct visualization of the evolving microstructure in flows can provide insight into the flow-microstructure coupling. This thesis focusses on two microstructured and nanostructured (structure has length scales in nanometers) fluids: dilute solutions of DNA and suspensions of single-walled carbon nanotubes (SWNTs) in water.

1.1.1 Polymer solutions

Polymer molecules consist of a large number of units connected together to form a single structure (Flory 1953). Polymers can be man-made (e.g., polyethylene, nylon), naturally occurring in plants (e.g., cotton, cellulose) and biological polymers (e.g., DNA, actin). Complex liquids are formed when even minute quantities of polymer are added to a solvent; polymer molecules in liquids provide microstructure to the liquid. The flow behavior of such liquids can vary enormously from the simple linear relationship between stress and rate of strain (Newtonian liquids); for example, the shear viscosity of most polymer solutions such as aqueous solutions of poly-ethylene oxide decrease with shear rate (Macosko 1994). \( \lambda \)-DNA is a long chain flexible polymer of contour length \(16.3 \ \mu \text{m} \) and hydrodynamic diameter \( \sim 2 \ \text{nm} \) (Perkins 1997). Dilute solutions of DNA in water were the focus in this thesis.

1.1.2 Carbon nanotube solutions

Carbon nanotubes, are allotropes of carbon: they can be thought of as rolled up sheets of graphite (Iijima 1991). They have been the focus of extensive materials research during the past decade due to the amazing mechanical, electrical and thermal properties shown by individual and aggregates (called ropes) of nanotubes (Baughman et al. 2002). Carbon nanotubes with a single wall have diameter about 1 nm; the lengths may vary from a few nanometers to tens of microns (Iijima and Ichihashi 1993, Bachilo et al. 2002). They are amphiphobic molecules, i.e., they do not like either water or organic solvents (Wang and Hbbie 2003), and thus have been difficult to disperse in common solvents such as water. Moreover, raw nanotubes exist as ropes that are bound tightly by high van der Waals forces (Thess et al. 1996). The
morphology of the nanotubes in various attempts at dispersing in solvents is uncontrolled, not quantified properly and controversial: thus, the behavior of nanotubes in liquid suspensions is poorly understood. Dissolution in organic solvents has been reported with short length SWNTs at 95 mg/l (Bahr et al. 2001). Chemically modified SWNTs have been dispersed at a concentration ~ 1 mg/l in organic solvents (Chen et al. 1998) and at ~ 1 g/l in water and in methanol (Kahn et al. 2002). Surfactant and polymer facilitated dissolution in water was reported at concentrations 0.5 to 15 wt% (Bandyopadhyaya et al. 2002), at < 100 mg/l (Moore et al. 2003), at 20 g/l (Islam et al. 2003) and by polymer wrapping at a concentration ~ 50 mg/l (O’Connell et al. 2001). Concentrated suspensions of about 8 wt% were obtained in super-acids where the nanotubes transitioned from isotropic phase to liquid crystal phase with increasing SWNT volume fraction (Davis et al. 2004). Surfactant stabilized aqueous suspensions of SWNTs at very low concentrations (~ 20 mg/l) were the focus in this thesis.

1.2 Complex flows

Flows and motions can be decomposed into a rotational component, which does not modify the microstructure nor induces stress, and deformation component called elongation. In general, all flows are such mixed flows (Larson 1999); i.e., all flows combine the characteristics of the two ideal flow types, pure rotation and pure elongation. In cartesian coordinates, if the velocity field is written as \( \mathbf{v} = v_x(x,y)\mathbf{i} + v_y(x,y)\mathbf{j} \), then pure rotation in two-dimensions has vorticity \( \omega = \frac{1}{2} \left( \frac{\partial v_y}{\partial x} - \frac{\partial v_x}{\partial y} \right) \) and flow field \( \mathbf{v} = \omega y\mathbf{i} - \omega x\mathbf{j} \). Here \( \mathbf{i} \) and \( \mathbf{j} \) are the unit vectors along \( x \) and \( y \), respectively. In planar extension flow the strain rate is \( \dot{\epsilon} = \frac{1}{2} \left( \frac{\partial v_y}{\partial x} + \frac{\partial v_x}{\partial y} \right) \) and flow field \( \mathbf{v} = \epsilon x\mathbf{i} + \epsilon y\mathbf{j} \). Another ideal flow type is shear flow. In a simple plane Couette flow in two-dimensions, in general the velocity gradients \( \frac{\partial v_x}{\partial x} = \frac{\partial v_y}{\partial y} = \frac{\partial v_x}{\partial y} = 0 \) and \( \frac{\partial v_x}{\partial x} \) is non-zero.

Flows can be further classified on the basis of the spatial velocity gradient. Simple flows are flows where the velocity gradients are spatially homogeneous, for example, the flow between two parallel sliding plates (Couette flow); complex flows have a
spatially inhomogeneous velocity gradient. Realistic flows observed in nature and in industry such as drying droplets of water on window panes after rain, drying coffee rings on counter tops, coating flows, ink-jet printing, etc., are complex. The "simplest" complex flow that can be thought of is the pressure-driven flow in a straight cylindrical pipe (Poiseuille flow); the velocity gradient along each streamline is constant, but differs across streamlines.

This thesis is focused on complex flows, free surface flows in particular. Free surface flows arise when a flowing liquid layer meets another fluid (gas or liquid) to form an interface. Examples of flows with free surfaces or free boundaries can be found in coating, polymer processing, manufacturing process (e.g., ink-jet printing), micro-fluidics (e.g., DNA arrays), and biology (e.g., deformation of blood cells, air displacement in pulmonary alveoli).

1.3 Studying DNA conformation in coating flows by multi-scale methods

There is a great interest from an economic perspective to simulate complex fluid systems of industrial and technological importance—designing and controlling important processes such as ink-jet printing, spraying, and coatings. Coating operations are an essential part of manufacturing processes for commodity and specialty products—painted metal in automobiles and appliances, photographic films, coatings for memory storage in video tapes and diskettes, coatings of substrates to make bio-sensors and DNA chips and microfluidic devices. Coating liquids are complex and have microstructure in the form of polymers or suspended particulate. In 2003, coated products and coating processes contributed about $18 billion to the US economy (Tullo 2004). Thus the great motivation to study the effect of flow on the microstructure and vice-versa in coating flows. In the present study roll-knife coating, a common coating flow, has been studied.

When microstructured fluids flow, the flow and the configuration (conformation) of the microstructure are coupled (figure 1.1). Together with imposed boundary
conditions, the interplay between the two determines the flow pattern and its stability. The velocity convects the microstructure in the liquid, while the velocity gradients act to deform the microstructure (figure 1.2) which induces stress in the microstructure. When the stress due to the microstructure is comparable or larger than pressure and viscous stress, the flow field changes, sometimes dramatically. For example, the addition of small amount of polymer in a liquid can suppress droplet breakup in spraying which is desirable (Marano et al. 1997); in coating flows, polymer molecules can enhance ribbing which causes non-uniformity in the coated product (Dontula 1999), which is undesirable.

The coupling between the microstructure of the fluids with their flow properties is nonlinear and analytical solutions cannot be obtained. Thus, computer simulations are required to understand this interplay of the flow and the fluid microstructure. Molecular models describing polymer microstructure have been developed and validated using empirical measurements in simple flows (Hur et al. 2000, Hur et al. 2001, Hur et al. 2002, Jendrejack et al. 2002, Sunthar and Ravi Prakash 2005). However, there is need for developing simulation models that capture the essential features of the materials in complex flows such as coating flows. Directly visualizing polymer molecules in complex flows would promote progress in the development of computer models—by displaying how detailed flow features affect polymer configuration, and by providing valuable information to test and validate models of the fluid dynamics of dilute polymer solutions.

Recent advances in measurement of microscopic quantities using single molecule visualization have made it possible to study the effect of the flow on polymer microstructure. The technique has furthered understanding of the dynamics of dilute polymer solutions in simple flows by visualizing directly DNA molecules in simple shear and extension (Perkins et al. 1997, Smith et al. 1999). The observation and measurements on individual molecules provide a test for the predictions from molecular models. Moreover, multiple transient states are not revealed in ensemble averages (measured by traditional methods—light scattering and birefringence—to detect polymer configuration); for example, consider a hypothetical case where half of the
Figure 1.1: Coupling between flow and microstructure. The velocity transverses the microstructure in the liquid flow domain while the velocity gradient deforms the microstructure (conformation). The deformed microstructure develops stress and affects the flow field.

ensemble of polymer molecules attain an equilibrium configuration, i.e., no extension, and the other half get stretched fully, the ensemble average would predict a 50% extension which does not reveal the presence of only two possible states.

The present study has three major goals. First, to assess whether fluorescence microscopy of DNA could be used to track microstructure evolution of polymer solutions in an industrially relevant complex free surface flow. Second, to provide information about polymer conformation in regions of interest like the recirculation, separation surfaces and menisci. Third, to use the microscopic measurements to test a microscopic model that describes the polymer microstructure in a complex flow.

Figure 1.3 shows the scheme followed to study the coupling between flow and microstructure. The evolution of DNA microstructure was investigated in ultra-dilute polymer solutions in small scale coating flows. In ultra-dilute solutions, the polymer molecules stretch and relax under the combined action of velocity gradient, intramolecular (elastic) and Brownian forces as if they were in dilute solutions; however, because the concentration of polymer molecules is so incredibly low, the macroscopic flow is equivalent to that of a Newtonian fluid (Harrison et al. 1998). Thus by using such ultra-dilute solutions the effect of the flow on the polymer microstructure was
Figure 1.2: Effect of flow on polymer conformation. A flexible polymer has an isotropic coiled conformation at equilibrium \((m_1 = m_2 = m_3)\), where \(m_i\) are the eigenvalues of the conformation tensor \(M(x, t) \equiv \int_{Q \in R^n} QQ\psi(Q, x, t) dQ\). \(Q\) representing the principal stretch of the coil; Pasquali (2000)). Under flow the polymer gets deformed; it extends along one direction while it gets compressed in the other directions \((m_1 > m_2, m_3)\). Flexible DNA molecules and bead-spring chain representing DNA molecule are deformed from the coiled conformation under flow.
Figure 1.3: Multi-scale approach to study the interplay of flow and polymer microstructure. The complex free surface flow was generated in a scaled-down complex roll-knife coating flowcell. DNA, a flexible polymer, was used as an elastic tracer in the Newtonian flow field. The concentration of DNA in the solution was ultra-dilute and the effect of the change in DNA microstructure on the flow was eliminated. Single molecule fluorescence microscopy was used to measure DNA conformation. The macroscopic flow was computed using Computational Fluid Dynamics (CFD) and a coarse grained microscopic model was used to describe DNA as a chain of beads connected with springs. The evolution of the microstructure was computed in the macroscopic flow field and compared with experimental measurements.

Separated from the converse effect of polymer microstructure on the flow. DNA was used as an elastic tracer in a Newtonian flow field and the molecular conformation was observed using fluorescence microscopy. The experimental study of roll-knife coating flows is presented in Chapter 2.

Brownian dynamics simulations were used to predict the observed conformational changes experienced by the DNA molecules as they traveled along the streamlines of the small-scale coating flow. The simulation algorithm was a modified version of the algorithm introduced recently by Sunthar and Ravi Prakash (2005), which used a bead-spring chain representation of the DNA molecule within the framework of a
successive fine-graining procedure, in order to predict conformations in homogenous extensional flows. The macroscopic Newtonian flow was solved by a Galerkin/Finite Element scheme using the method developed by Pasquali and Scriven (2002). The simulations of both the macroscopic flow and the microscopic predictions from Brownian dynamics are presented, and compared with the experiments in Chapter 3.

1.4 Real-time fluorescence visualization of individual single-walled carbon nanotubes

The behavior of SWNTs in liquids is still poorly understood. This is perhaps surprising, because SWNTs are commonly described as high aspect ratio rod-like particles, and slender rigid objects in liquids have been studied for decades (Jeffery 1922, Onsager 1949, Kirkwood 1967). The difficulty in dispersing SWNTs in liquids and the lack of viable techniques for observing their dynamics in suspensions have slowed fundamental progress on liquid-phase behavior of SWNTs. SWNTs in liquids are important in the physical, material, and life sciences; real-time visualization of SWNTs in liquids can impact each of these areas of research. Liquid-phase processing is key to developing scalable techniques for directed assembly or self-assembly of SWNTs, e.g., production of SWNT fibers (Vigolo et al. 2000) and films (Wu et al. 2004), length and type separation of SWNTs. The high aspect ratio and stiffness of SWNTs may enable more efficient delivery of genes and drugs through cell membranes (Lu et al. 2004); directly visualizing SWNTs in water would yield detailed information on the interaction of SWNTs with cells (Cherukuri et al. 2004, Lu et al. 2004) and biomolecules—DNA and proteins can provide unique and selective building blocks for directed assembly of SWNTs into functional nanoscale and microscale structures, for example, sensors (Lin et al. 2004). Visualization can also be important for controlled manipulation of SWNTs into nanostructures, e.g., by optical trapping and tweezing (Plewa et al. 2004) and by micro-scale flows. From a fundamental viewpoint, it is not known whether the theoretical predictions of rotational diffusivity and persistence length stemming from the assumption that SWNTs are homogeneous hollow cylinders
(Doi and Edwards 1986, Larson 1999) can be applied to SWNTs in liquids because the diameter of a SWNT (~1 nm) is close to the size of the solvent molecules, and any imperfections in the sidewalls of the nanotubes could affect dynamic properties.

High resolution techniques like transmission electron microscopy and atomic force microscopy yield SWNT length and diameter (Moore et al. 2003, Richard et al. 2003), but do not provide real-time dynamics in liquids and cannot be applied to living systems. The dynamics of small objects, including SWNTs, in liquids have been studied using bulk techniques such as light and neutron scattering (Badaire et al. 2004, Zhou et al. 2004) and birefringence (Maguire et al. 1980); these techniques involve large ensembles of molecules and the interpretation of results is frayed with difficulties when samples are poorly characterized and polydisperse, as is always the case when dealing with SWNTs. Single-molecule visualization using fluorescence video microscopy has provided dynamic information on molecules and small particles like F-actin (Gittes et al. 1993), microtubules (Gittes et al. 1993), DNA (Smith et al. 1992, Perkins, Quake, Smith and Chu 1994), and worm-like micelles (Dalhaimer et al. 2003). Recent progress has been reported on fluorescence visualization of SWNTs. Previous studies visualized bundles of carbon nanotubes by functionalization (chemical modification) with synthetic polymers (Otobe et al. 2002) and bio-polymers (Lu et al. 2004, Rao et al. 2004), by attaching quantum dots (Chaudhary et al. 2004), multi-walled carbon nanotubes by non-covalent labeling with conventional fluorophores (Prakash et al. 2003) and individual SWNTs by near-infrared fluorescence visualization (Tsyboulski et al. 2005). However, these techniques do not have sufficient temporal and spatial resolution to yield dynamical information on SWNTs in liquids. In Chapter 4 a new technique for fluorescent visualization of individual SWNTs is presented. Unlike previous visualization procedures, this new technique is simple, convenient and does not chemically alter the SWNTs. Real-time visualization of the Brownian motion of SWNTs in water allowed measurement of the rotational diffusion coefficient of SWNTs, and also a direct measurement of the persistence length (rigidity) of SWNTs.
1.5 Drop drying of surfactant stabilized aqueous dispersion of single-walled carbon nanotubes

The unique mechanical, electrical and thermal properties of SWNTs make them attractive material for potential electronic applications, for example, in making sensors and field-emission devices (Baughman et al. 2002). Recent efforts toward realizing these devices include preparing fibers and ribbons of SWNTs (Vigolo et al. 2000); thin, transparent and electrically conducting films of pure SWNTs from surfactant stabilized aqueous dispersions (Wu et al. 2004); in-situ growth of SWNT films on a substrate (Murakami et al. 2004); self-assembled films of SWNT bundles on glass near a receding contact line by solvent evaporation (Shimoda et al. 2002); and, homogeneous films of nanotube-polymer composites (Ramasubramaniam et al. 2003, Kim et al. 2003).

Drying of drops on substrates is ubiquitous—coffee stains on counter tops, ring-deposits on drying utensils and window panes. Evaporation of the solvent induces a complex flow inside the drop which could be used as a driving force for the self-assembly of suspended particles onto substrates. Such processes have been used for patterned deposition of solutes onto non-porous substrates—DNA microarrays (Blossey and Bosio 2002), nano-lithography (Fan and Stebe 2004), protein crystallization (Annarelli et al. 1999), and stretching DNA for hybridization studies (Abramchuk et al. 2001, Jing et al. 1998). Similar to these self-assemblies, the moving contact line of a drying drop could be used to form aligned patterns of SWNTs on substrates for making films or for nano-fabrication. In this study (Chapter 5) drying of drops of surfactant stabilized aqueous suspension of SWNTs has been investigated as a potential route to preparing thin films and coatings of carbon nanotubes. Evaporation of dilute dispersions of SWNTs exhibited complex dynamics.
Chapter 2

DNA Visualization in Coating Flows

Free surface flows of dilute polymer solutions are important in various industrial and biological applications. In these flows, the velocity gradient stretches the polymer molecules, and in turn the stretched polymer molecules change the behavior of the flow, sometime dramatically—for example, in the reduction or suppression of unwanted small droplets in spraying. Understanding the interplay of flow and polymer microstructure in complex flows is key for designing and controlling important processes such as ink-jet printing, spraying, and coating. Much progress has been made recently—chiefly, by Chu and co-workers—towards the understanding of the dynamics of dilute polymer solutions in simple flows by visualizing directly polymer molecules (DNA) in simple shear, extension and mixed flows. Visualizing directly polymer molecules in free surface flows would promote similar progress, by displaying how detailed flow features affect polymer configuration, and by providing valuable information to test and validate, in realistic flows, models of the fluid dynamics of dilute polymer solutions. In this chapter fluorescence microscopy of DNA was used to visualize individual molecules in small scale coating flows of dilute solutions.

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2.1 Introduction

Single DNA molecules were used to investigate the behavior of ultra-dilute polymer solutions in a roll-knife coating flow cell. In ultra-dilute solutions, because the concentration of polymer molecules is so low, the macroscopic flow is equivalent to that of a Newtonian fluid (Harrison et al. 1998), and the effect of the flow on the polymer microstructure can be separated from the converse effect of polymer microstructure on the flow (Pasquali 2000). DNA was thus used as an elastic tracer in a Newtonian flow field and its molecular conformation observed in the flow. Ultra-dilute solutions are key for testing in complex flows coarse-grained as well as microscopic models of polymer chains in dilute solutions.

A brief background on polymer solutions, free surface flows and the single molecule fluorescence microscopy technique is presented next.

2.1.1 Polymers

Polymer molecules consist of a large number of units connected together to form a single structure. A linear polymer is formed by repetition of structural units called monomers, end-to-end in a linear sequence (Flory 1953). Some important parameters required to describe a linear polymer molecule are: degree of polymerization, which is the number of repeat units in the molecule and is related through the molecular weight; end to end distance $\mathbf{R}$, which is the vector connecting the two ends of the molecule; contour length $L$, which is the length of a fully extended polymer chain; radius of gyration $R_g$, which is the square root of the mean-squared distance of all atoms from the center of mass of the molecule; and persistence length $L_p$, which is the distance along the contour over which two unit tangent vectors lose correlation. The ratio of the persistence length and the contour length measures the flexibility of a polymer chain. Based on this parameter, three regimes are defined to differentiate polymers: stiff rods, $L_p \gg L$, e.g., microtubules; semiflexible polymers, $L_p \approx L$, e.g., F-actin; flexible polymers, $L_p \ll L$, e.g., DNA; (Larson 1999).
Flory (1953) considered a polymer to be a freely jointed chain, i.e., a set of \( n \) connectors (bonds) that can freely rotate in space. The statistical properties of such a chain at equilibrium parallel those of a three-dimensional random walk of \( n \) equal steps of length \( l_n \), where \( n \) is the number of covalent bonds along the chain and \( l_n \) is the average bond length. The mean squared end-to-end distance is \( \langle R_e^2 \rangle = n l_n^2 \). The mean-squared radius of gyration of flexible linear polymers is related to the mean squared end-to-end distance as (Flory 1953)

\[
\langle R_g^2 \rangle = \frac{\langle R_e^2 \rangle}{6}.
\]  

(2.1)

The polymer molecule can also be approximated as an equivalent freely jointed chain. The elements instead of being the chemical bonds, as considered by Flory, can be a sequence of links called Kuhn segments (Flory 1953). The length of the sequence has to be large in order to neglect the effect of one element on the next. In the bead-rod model the polymer chain is modeled as a sequence of beads connected by rigid rods with the rods equivalent to the Kuhn segments while the beads offer hydrodynamic resistance to the flowing solvent (Flory 1953). The rods can be replaced with springs that account for bond fluctuations, e.g., Frenkel springs, or the chains can be coarse-grained with springs replacing a number of rods (Kuhn segments) in the bead-spring model, e.g. entropic springs (Bird et al. 1987).

Flexible polymers can assume a large number of configurations due to the rotation of the chemical bonds (Doi and Edwards 1986). The chain configuration depends upon the interactions between the chain segments and the solvent. In \( \theta \) solvents the polymer-solvent and polymer-polymer interactions are equal. In average, polymer coils have the radius of gyration equal to that in a melt. In good solvents, the interaction energy between a polymer segment and a solvent molecule exceeds the interaction energy between two polymer segments (Flory 1953); thus, the coiled molecule swells to reduce polymer-polymer contacts. Conversely, in poor solvents the polymer shrinks to increase the number of polymer-polymer contacts and eventually forms globuli that phase-separate.
The conformation and position of polymer in solution fluctuates continuously due to Brownian motion. This random motion affects various time dependent properties like birefringence, viscoelasticity, etc (Doi and Edwards 1986). Solutions in which the molecules do not interact with each other are called dilute solutions. As the number of molecules per unit volume is increased and reaches the overlap concentration \( c^* \), the coils begin to touch in solution (figure 2.1). Note that long-range hydrodynamic interactions, motion and deformation of a part of the polymer chain that get propagated to another portion of the chain through the solvent, occur well below the overlap concentration (Larson 1999). The overlap concentration is related to the chain dimensions and the molecular weight as (Macosko 1994):

\[
    c^* \equiv \frac{M_w}{N_A \frac{1}{3} \pi R_g^3} 
\]

\[
    \nu^* = \frac{3}{4\pi R_g^3} 
\]

where \( N_A \) is the Avogadro number and \( \nu^* \) is the number concentration. On increasing the concentration of polymer molecules in a good solvent above the overlap concentration, the polymer coils start shrinking because of the increasing number of unfavorable polymer-polymer interactions. This solution concentration regime is termed semi-dilute. Above a critical concentration, further increase in the polymer concentration does not result in chain contraction and the coil reaches \( \theta \) dimensions; such solutions are called concentrated (Graessley 1980). In high molecular weight polymers, as the number of molecules per unit volume grows the polymer molecules may loop around each other and form entangled solutions (Graessley 1980). In such solutions the motion of the chain along the contour is favored as compared to motion transverse to it (de Gennes 1979).

In a dilute solution of flexible and semiflexible polymers in a good solvent, each molecule and segment tends to exclude all other molecules and segments from the volume it occupies. This is called as the excluded volume effect (Flory 1953, Doi and Edwards 1986). The short-range interactions are due to steric effects: any polymer segment has a finite volume and cannot intersect any other segment; the polymer coil
Figure 2.1: Concentration regime in good solvents for flexible polymers (Macosko 1994). In dilute solutions the coiled molecules are far apart. At the overlap concentration $c^*$ the coils begin to touch.

swells (Flory 1949). In the bead-spring representation, the coarse-graining of large, flexible chain segments creates local non-intersecting volumes which become a “bad” solvent for other polymer segments and swell the chain (Flory 1953). As an example, λ-DNA (hydrodynamic diameter 2 nm, contour length 16.3 µm and radius of gyration 0.7 µm in water) occupies only $\sim 0.004\%$ of the volume of the effective sphere of radius equal to the radius of gyration in its undeformed random coil configuration.

### 2.1.2 Free surface flows

Free surface flows arise when a flowing liquid layer meets another fluid (gas or liquid) to form an interface. Examples of flows with free surfaces or free boundaries can be found in coating, polymer processing, manufacturing process (e.g., ink-jet printing), micro-fluidics (e.g., DNA arrays), and biology (e.g., deformation of blood cells, air displacement in pulmonary alveoli).

In coating processes, one or more thin layers of liquid are delivered to and deposited on a fast-moving web (Kistler and Schweizer 1997a). The coating liquid is simultaneously sheared and extended in the coating bead, and the shear deformation rates are typically as high as $10^4$ to $10^5$ s$^{-1}$ and the extension deformation rates are about $10^3$ to $10^4$ s$^{-1}$ (Pasquali 2000). The polymer molecules in the coating liquid are
distorted from their equilibrium configuration by the velocity gradient; this alters the behavior of the liquid, affecting the flow itself. Free surfaces, contact lines, boundary and internal layers, flow separations, and micro-recirculations complicate coating flows. The understanding of the flow behavior of polymer solutions could benefit from experimental information on polymer microstructure in coating flows (Pasquali and Scriven 2002). Such information would guide and validate models of viscoelastic free surface flows.

Flow-induced structural changes in polymer conformation in solutions has been traditionally obtained by two methods—light scattering (Flory 1953, chapter 7) and flow birefringence (Janeschitz-Kriegl 1983, Fuller 1995). Both these methods are bulk methods that rely on interaction of light with inhomogeneities in matter (Larson 1999). Instead of a direct measurement of the polymer conformation an average molecular behavior is inferred—angle at which light scatters (light scattering) is related to the size of the microstructure producing the scattering (Macosko 1994); the index of refraction (birefringence) is related to the stress, which depends on the anisotropy in the microstructure (Larson 1999). The need to precisely measure the scattered light over a wide range of angles around the sample makes light scattering a poor candidate technique for studying complex free surface flows, where free surfaces and glass and metal walls reflect light which interferes with the scattering signal. The lack of a water-based polymer solution with a stress-optic coefficient large enough to permit the detection of birefringence in dilute solutions is the main hurdle in using birefringence to study coating flows (Pasquali 2000).

The small dimensions of ordinary synthetic polymer molecules (10 to 100 nm) make direct visualization difficult because their small dimensions are well below the resolution of the best light microscopes (~300 nm). DNA molecules behave qualitatively as long-chain ordinary synthetic polymers (Perkins, Smith and Chu 1994, Perkins, Quake, Smith and Chu 1994) and can be dissolved in water. DNA molecules of appropriate length can be marked with fluorescent stains and visualized by optical microscopy; therefore, they constitute an attractive model system to study how polymer molecules deform in a coating bead. As new staining techniques develop, synthetic
polymers will also lend themselves to single-molecule visualization studies—e.g., fluorescent ultra-high molecular weight polyacrylamide (Wang et al. 2002).

2.1.3 Coating Flows

Coating operations are an essential part of manufacturing processes for commodity and specialty products—painted metal in automobiles and appliances, photographic films, coatings for printed circuit boards, coatings of magnetic materials for memory storage in computers and video tapes, coatings of substrates to make bio-sensors and DNA chips.

Coating literally means to deposit a layer over another material or a substrate. In liquid film coating, usually a continuous, uniform layer of liquid is deposited onto a solid surface by displacing a gas (Kistler and Schweizer 1997a). The aim is to generate a film of perfectly uniform thickness; in order to get such a film the coating liquid has to be supplied in a measured or regulated amount. This is called metering. Metering can be achieved either by pre-metering or by self-metering of the liquid flow. In pre-metered flows, the amount of the liquid is regulated by controlling flowrates mechanically, e.g., by a displacement pump. Thus all the material metered through the coating die gets transferred on to the solid substrate. Such coating flows are used in precision and patterned operations. In self-metering flows, a excess of the coating liquid is applied and the excess is removed by the surface tension, elastic and viscous forces generated in the flowing liquid. In knife coating, forward roll coating, and reverse roll coating, the coating layer is self-metered by the flow generated in the region between the two solid surfaces close to each other; in slot or extrusion coating, slide coating and curtain coating the liquid flowrate is pre-metered (Kistler and Schweizer 1997b). The type of coating process used for manufacturing varies with the product. For example, curtain coating is used to coat paper with a mixture of water, clay, pigments, starch and polymer latex to make it printable (Gutoff et al. 1995). Photographic films are coated using slot coaters and multilayered films are coated using curtain and slide coating. Some relevant numbers for coating operations to keep in mind are—coating dies are typically ~10 cm–10 m wide; gaps are usually
\( \sim 100 \ \mu m \) or less; viscosity of the coating liquids is in the range \( 0.001-5000 \ \text{Pa.s} \); coating speeds are \( \sim 100-1500 \ \text{m/min} \); the thickness of coating films are \( \sim \text{tens of } \mu \text{m} \) or less (Gutoff et al. 1995).

The basic physics of coating processes involves replacing the gas in contact with the solid surface by a process called dynamic wetting. The curve or the interface on the solid surface at which this happens is termed the dynamic contact line (Blake and Ruschak 1997). The liquid in contact with the solid surface at this interface appears to slip locally. Static contact lines are formed at the edges of the coating die and the interface appears stationary. All coating processes have two free surfaces in the liquid layer being deposited— one that becomes the free surface of the coated layer and the other terminating at the dynamic contact line. For most operating conditions, the region next to the free surface of the coated layer is of interest. The flow is essentially two-dimensional except at the edges.

Coating flows have mixed kinematics, a combination of shear and extension. Extension dominates at the free surfaces while shear kinematics are important in regions where two solid surfaces are close to each other. Such complex flow behavior is not free of three-dimensional flow instabilities such as vortices and eddies formed in the coating bead in slide coating (Gutoff et al. 1995). Defects like streaks, bands and sharkskin are formed in the coat if the material degrades over time because the liquid has long residence times in re-circulating regions. Another common defect seen in processes running at high coating speed is the breaking of the coated layer from the solid substrate surface. This is caused by the increase in the shear stress in the liquid at high speed, which increases the tensile force inside the coating bead, that pulls the bead off the solid substrate (Gutoff et al. 1995). A fluid mechanical instability termed ribbing is particularly important in roll coating. It appears as evenly spaced downstream-lines on the coated surface (Pearson 1960). The streamlines become helical with liquid circulating inside these ribs. Surface waves can also be formed as the liquid flows onto the solid substrate. The occurrence of such ribs destroys the uniformity of the coated film, which is an important requirement in industrial coatings.
Coating at fast rates the required thickness of the liquid material is the key goal, but fast rates of coating can lead to flow instabilities in the coating bead. Though in slot coating at high capillary and Reynolds numbers the operating window of the coating process becomes large (Carvalho and Kheshgi 2000). Polymers are an important ingredient of coating solutions. The coating material contains polymers either as the major component or as a binding additive (Dontula 1999). Polymer solutions due to their viscoelasticity and shear thinning property (lower stresses at higher shear rates) are often easier to coat than simple liquids, even at high speeds (Gutoff et al. 1995). Strong viscoelasticity (high molecular weight polymer solutions) of the coating solution is thought to exacerbate defects like ribbing, filament formation and bridging, although such instability can occur even in Newtonian flows (Dontula 1999, Zevallos et al. 2005).

The flow behavior of polymers in coating flows is still not understood to the point of designing from first principles processes that avoid defects in the coated end-product. Single molecule visualization can be an effective tool in understanding polymer coating flows. Previously flow visualization using tracers has been used to study coating flows (Dontula 1999 and references therein; Schweizer (1997)) but single molecule polymer conformation will provide experimental data on polymer microstructure near the free surfaces and in the coating bead for better modeling of such complex flows.

**Roll-Knife coating**

In a roll coating process a thin film is deposited on a continuous web using two or more rolls. The liquid is carried by one or both the rolls into the gap between the rolls, and the film thickness and its uniformity on the web is controlled by the gap between the rolls and their relative speeds. The film thickness depends on the width and the shape of the gap between the knife and the web (Coyle 1997). A roll-knife coating system combines the two coating processes—the roll feeds the liquid and the knife meters it to form a film on the web. In this study, a stationary glass plate next to a roll is used to create a roll-knife coating process. The flat glass plate acts as a knife that meters the flow and allows optical access to the coating bead. Savage (1977), Bauman et al.
(1982). Coyle et al. (1986) and Adachi et al. (1988) have used a plate-roll system and found that the stability and the behavior of the flow is similar to that of forward roll coating but the operability window is smaller. Gaskell et al. (1998) measured the pressure profile in the coating bead at different roll speeds in a plate-roll apparatus. At low flowrates the pressure at the downstream free surface was greater than that at the upstream free surface, and the profile had a linear gradient similar to that in roll coating. They also confirmed the theoretical prediction that, on increasing the flowrate the slope of the streamwise pressure profile in the bead decreases and has a local maximum (upstream of the minimum gap) and a local minimum (downstream of the minimum gap). This behavior is also observed in forward roll coating (Greener and Middleman 1975, Coyle et al. 1986).

2.1.4 Visualization of flowing DNA molecules

Morikawa and Yanagida (1981) first visualized DNA molecules by fluorescence light microscopy. They used DAPI fluorescent stain and several different types of DNA, including T4 and λ-DNA. The cross section of the DNA (2 nm) was well below the resolution of the optical microscope, so the DNA appeared over 100 times thicker; however, the conformation of the stained molecules was clearly visible. Yanagida and Hiroaka (1983) showed that the instantaneous shape of equilibrated DNA molecules resembles an ellipsoid rather than a sphere. This result has been confirmed recently by Haber et al. (2000).

A considerable amount of work has been done recently in visualizing and manipulating single biomolecules like DNA and actin (Bustamante et al. 2000). Both of these biomolecules are natural polymers and have been used as models of synthetic polymers. Double stranded DNA has a hydrodynamic diameter of 2 nm (Pecora 1991, Einer and Pecora 1991) and a persistence length $L_p$ of 50 nm (Hagerman 1988). Once a DNA molecule has a contour length much larger than its persistence length, it acts like a flexible chain—DNA oligonucleotides with lengths greater than 500 nm or 1500 bp in buffer solution (pH 8). Bustamante et al. (1994) have shown that single DNA molecules follow the typical force-extension relationship of Worm-Like Chains (Marko
and Siggia 1995). The non-linear elastic model that fits the experimental data for such chains is (Bustamante et al. 1994)

\[
F \left( \frac{R}{L} \right) = \frac{k_B T}{L_p} \left\{ \frac{R}{L} + \frac{1}{4} \left( 1 - \frac{R}{L} \right)^{-2} - \frac{1}{4} \right\}
\]

(2.4)

where \( R \) is the chain extension (end-to-end distance) due to force \( F \) applied to a chain of length \( L \) at temperature \( T \). This evidence confirms that DNA molecules behave as long chain synthetic polymers. The other fundamental piece of evidence are the experiments of Chu and co-workers (Perkins, Quake, Smith and Chu 1994) that show that the DNA chain relaxes qualitatively according to the Zimm model. The lack of quantitative agreement between the experiment and the model is because Zimm model describes the chains near equilibrium, whereas Chu and co-workers studied the relaxation of from a highly extended state.

The double helix \( \lambda \)-DNA has a contour length of 16.3 \( \mu \)m and a radius of gyration of 0.7 \( \mu \)m (Smith et al. 1995). DNA molecules can be tagged efficiently with fluorescent dyes like DAPI, i.e. 4',6-diamidino-2-phenylindole (Smith et al. 1992), YOYO-1 (Perkins 1997), enabling their visualization by fluorescent optical microscopy. Another important reason for choosing DNA as a model polymer is the monodispersity of the DNA population of a particular species (like \( \lambda \) bacteria phage). All molecules are exact replicas of one another. This avoids dealing with polydisperse chain length in experiments.

Biochemistry of DNA has been studied extensively. The numerous biological studies of interactions of DNA with various enzymes and proteins provide tools to manipulate DNA molecules. Enzymes and proteins can be used to cleave and bond DNA molecules and oligonucleotides to get chains of required lengths. The overhanging complementary ends on a \( \lambda \)-DNA allows the molecule to be tethered to a surface or to beads (Smith et al. 1992, Perkins et al. 1995, Haber and Wirtz 2000).

Perkins, Smith and Chu (1994) used fluorescent DNA molecules in concentrated entangled aqueous solution to visualize reptating molecules. Although the images show directly only the conformation of the DNA molecules, they provide information
on the approximate location of active entanglement points. (Perkins, Quake, Smith and Chu 1994) measured the relaxation time of DNA molecules of different contour lengths. Fluorescence microscopy of DNA was then used to study dilute DNA molecules in uniform flow (Perkins et al. 1995, Perkins 1997), in planar extensional flow (Perkins et al. 1997, Perkins 1997, Smith and Chu 1998, Smith 1999, Perkins et al. 1999), in shear flow (Smith et al. 1999, Smith 1999, Le Duc et al. 1999, Hur et al. 2001, Teixeira et al. 2005), and more recently in mixed flows (Babcock et al. 2003). In all these experiments the flow field was simple, i.e., the velocity gradient was homogeneous. Recently, the conformation of DNA molecules was studied in inhomogeneous flow in rectangular microchannels (Shrewsbury et al. 2001, Shrewsbury et al. 2002, Shrewsbury 2000, Fang et al. 2005).

Both single-molecule conformation and average conformation of the flowing DNA are important experimental information about the behavior of flowing linear polymers, and can be used to test the predictions of theoretical models of polymer dynamics in viscometric as well as complex flows. Larson et al. (1997), Hur et al. (2000), Jendrejack et al. (2002), and Sunthar and Ravi Prakash (2005) have pioneered comparison between experiments and molecular models of DNA conformation in uniform flow, extensional flow and shear flow. Such modeling studies are now being extended to non-homogeneous and confined flows (Chopra et al. 2003, Jendrejack et al. 2003, Jendrejack et al. 2004, Woo et al. 2004a).

2.2 Experimental section

2.2.1 Apparatus

A small roll-and-knife coating flowcell was designed to fit under a microscope. The flow cell consisted of a steel roll of 12.7 mm diameter mounted on high precision ball bearings (7900CTYDULP4, NSK Bearings, Japan) inside a 22 mm ID steel tube (figure 2.2)\(^2\). Figure 2.3 shows a photograph of the flowcell. The top of the enclosing tube was cut off and a microscope coverslip (0.13 mm thickness) was used to cover

\(^2\)Design by Torrazzi (1998) and Pasquali (2000)
Figure 2.2: Design of the roller coating flow cell. (a) Section (AA) view of the flowcell. (b) Top view (c) Side elevation. Note the drawing is not to scale. Redrawn from Torazzi (1998) and Pasquali (2000). The dimensions are in millimeters.
the cell, leaving an adjustable gap of 25–60 μm between the top of the roll and the bottom of the coverslip. The coverslip was clamped using two teflon sleeves. The gap was measured in each experiment. The bottom of the enclosing tube was flattened and mounted on an aluminium base. The steel roll was connected with a telescopic coupling (UJT-4M; DBL, W M Berg Inc., East Rockaway, NY) to a AC high-resolution microstepping motor Compumotor S/SX 57-51 (Parker Hannifin Corporation, Compumotor Division, Rohnert Park, CA) mounted on an ordinary lab jack. The maximum angular velocity of the motor was 50 rps. Angular velocity of 0.01 to 0.5 rps were used in the study. The angular velocity and acceleration of the motor were controlled by a personal computer using XWare (Compumotor, CA). The roller shaft speed was calibrated with respect to the controlled speed of the driving motor. A mark was put on the shaft, and using a stop watch time required for a fixed number of revolutions was measured at different motor speeds. The calibration results are shown in figure 2.4. The aluminum base was attached to the microscope xyz-positioning stage (Prior ProScan 0.1 μm resolution and 1 μm repeatability). The x-y motion of the stage is compensated by the flexible coupling (telescopic joint). Different regions of the flow were imaged by moving the flowcell with the positioning device.

The cell was filled with approximately 5 ml of liquid. A thin layer of liquid was picked up by the rotating roll and transferred to the coverslip, where it formed the coating bead (figure 2.5). The DNA in the coating bead was imaged from the top; therefore, the images of flowing DNA are two-dimensional projections on a horizontal plane of three-dimensional molecules that are sometimes stretched by the velocity gradient in a vertical plane and hence often straddle the plane of focus.

2.2.2 Materials

Fluorescently labeled λ-bacteriophage DNA was used as model polymer. The λ-DNA molecule (New England Biolabs Inc., MA) has 48, 502 base pairs and has a molecular weight of 31.5 Mg/mol. The DNA molecules were stained with the dye YOYO-1 (Molecular Probes) at 1:4 dye-to-base pair ratio. YOYO-1 is an intercalating dye, and
Figure 2.3: Photograph of the roll-knife coating flowcell. The flowcell consists of two coaxially mounted cylinders; the top of the outer cylinder is cut and a glass coverslip is clamped to cover the cell using teflon sleeves. The inner cylinder is rotated using a microstepping motor (not shown) which is connected to the cylinder by a telescopic coupling. The flowcell is mounted to an aluminium base that fits on the microscope stage.

Figure 2.4: Speed calibration for roller flowcell showing roller speed is 1:1 with the controlled motor speed. The linear fit is $\omega_y = 0.9913 \omega_x + 0.002$. The offset is due to human error in measurement of the time with a stop watch.
Figure 2.5: Schematic of the roll-and-knife coating flowcell and the epifluorescence microscopy setup used to visualize stained DNA molecules in the coating flow cell. Redrawn from Pasquali (2000).

At this staining ratio a DNA molecule has a contour length of 22 μm (Perkins 1997); its persistence length is about 70 nm (5 mM NaCl) and its hydrodynamic diameter is roughly 2 nm (Kam et al. 1981, Hagerman 1988). Figure 2.6a shows images of fluorescent DNA in coiled conformation at equilibrium. Partially stretched DNA molecules stuck on glass are shown in figure 2.6b (following the procedure by Taylor et al. (2000). DNA molecules were stretched in a squeeze flow and attached to the poly-l-lysine coated glass slide). The instantaneous shape of DNA at equilibrium is ellipsoidal (figure 2.6a), this was first observed by Yanagida and Hiroaka (1983) and later confirmed by Haber et al. (2000).
Figure 2.6: Fluorescence microscopy images of \( \lambda \)-DNA stained with YOYO-1. (a) Undeformed, coil-like state at equilibrium. (b) Stretched DNA stuck on glass. The equilibrium radius of gyration of \( \lambda \)-DNA is \( R_g \sim 0.7 \mu m \) and the contour length of fully stretched \( \lambda \)-DNA is \( L \sim 22 \mu m \).

A stock solution with DNA concentration 0.65 \( \mu g/ml \) was prepared in a TBE buffer. The stock DNA was diluted and suspended in high viscosity (\( \sim 40 \) mPa s and \( \sim 100 \) mPa s) sugar solutions. The viscosity of the solution was measured using a strain controlled rheometer ARES 100 FRT (Rheometrics Scientific Inc., NJ). The measurements were done in a Couette geometry (bob diameter = 32 mm, cup diameter = 34 mm). The dimensionless flow numbers were based on the viscosity of each microscopy solution. The stock solution was diluted to achieve a DNA concentration in the coating liquids well below the overlap concentration (10^{-5}c^*, c^* \approx 37 \mu g/ml). At this concentration the DNA molecules are always well-spaced (approximately 3 to 4 molecules in the field of view); thus, single molecules can be visualized. Because of the large molecular spacing, the DNA molecules behave as isolated coils in the dilute liquids. A buffer solution was prepared by diluting to 50% TBE buffer (Sigma Chemical Co., St. Luis, MO) in deionized water, with 5 mM NaCl and 1 % vol of 2-mercaptoethanol (Sigma Chemical Co., St. Luis, MO). Mercaptoethanol slows the photobleaching of the dye by scavenging oxygen free radicals (Yamagida and Hiroaka 1983). Sugar solutions, \( \sim 40 \) to 45 % wt. Sucrose and \( \sim 20 \) % wt. Glucose, prepared
in the buffer were used to enhance the viscosity of the DNA solutions. The static contact angle between the 41 mPa s (≈ 60 % wt. sugar) solution and the coverslip was measured to be 35.5° by video imaging of liquid drops on the glass surface.

The relaxation times of λ-DNA were determined by using the data of Perkins et al. (1997), who reported that the relaxation time of λ-DNA in a 41 mPa s solution of water and sugar is 3.9 s. The relaxation time of λ-DNA is proportional to the viscosity of the solution (Smith and Chu 1998); thus, in our experiments with dilute solutions of viscosity 40 mPa s and 100 mPa s, the relaxation times were approximately 3.9 s and 10 s, respectively.

It is extremely important to have the right salt (NaCl) concentration in the DNA solutions for single molecule flow experiments. DNA is a polyelectrolyte; Hagerman (1988) reviewed that the persistence length \( L_p \) (i.e., flexibility) of DNA is highly sensitive to the salt concentration—\( L_p \) changes from 80-100 nm in 1 mM NaCl to 45-50 nm in 10 mM NaCl. Initial microscopy solutions were prepared at 1 mM NaCl concentration and the deformation of the DNA in flow could not be detected. The molecules appeared coiled even in flow. Also, the salt concentration of the sugar solutions should not be adjusted by adding flakes of NaCl because the solutions thus prepared do not have a homogeneous salt concentration. Instead, a small amount of 1 M NaCl solution in water was added to the sugar solutions to get the required salt concentration. The solutions were left overnight to mix on a bottle roller.

### 2.2.3 Fluorescence microscopy setup

The visualization experiments were performed with a Nikon Eclipse E600 epifluorescence microscope equipped with a 100 W mercury lamp and a Nikon Plan-Apo oil immersion objective with 60X magnification and 1.4 numerical aperture.

YOYO-1 bound to DNA has an absorption peak at 491 nm and an emission peak at 509 nm (Haugland 1996). The filter set XF100-2 (Omega Optical, VT), used to image stained DNA, included a bandpass excitation filter (450-500 nm), a dichroic mirror (500 nm cutoff) and a longpass emission filter (520-560 nm).

Images of flowing DNA were collected at 30 frames per second with an Elec-
tron Bombarded CCD (EBCCD) Camera C7190 (Hamamatsu Photonics, Japan) controlled by the software Metamorph (Universal Imaging Inc.). The images were acquired directly on a PC through Metamorph. Streak-free images of fast-moving molecules (∼ 100 μm/s) were obtained by de-interlacing the videocamera output, i.e., the video output was split into frames corresponding to odd and even-scan lines and the missing alternate lines were filled with previous scan lines.

2.2.4 Image analysis

A custom software is required to measure the orientation and extension of single molecules in different flow conditions. The image acquisition software Metamorph (Universal Imaging) provides the processing features. Molecular size and orientation can be measured by using a computer-generated cursor; however, such measurements are tedious and introduce inconsistencies due to subjective human factors. A software was written in Matlab to processes raw data automatically according to objective procedures to give orientation, centroid, and maximum length of the molecule.

The images obtained using the CCD camera are obtained in a compressed .tif format. The images are 8-bit i.e., with 256 gray-levels. The image is pixelated, i.e., it is divided into spatially discrete regions, which ideally are squares (Inoue and Spring 1997). Each pixel has its own gray scale value, which is in the range from 0 (black) to 255 (white). The pixel dimensions of the image acquired through Metamorph are 480 × 640.

The DNA molecule in the stretched condition was imaged as a region closely resembling an ellipse in shape, with a “corona”, a very bright region, with a fading “halo” around it. The size and shape of the image depended on the molecules conformation. In a no-flow case the shape was almost circular. For a reasonably accurate measurement of the length of the stretched molecule, the image has to be thresholded. This technique isolates objects of interest from the background. Each pixel is classified as either belonging to an object (pixel value 1) or to the background (pixel value 255). Pixels below a certain threshold gray scale are set to 0 and those above

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See Appendix A
it to 255. The proper threshold is usually selected by trial and error, by visualizing
the result (Inoue and Spring 1997). An automatic thresholding technique can also be
used, where a threshold is derived as a linear combination of the mean gray level μ
and the standard deviation σ of the gray levels of the source image, \( \tau = k_1 \mu + k_2 \sigma \)
(Ritter and Wilson 1996). For low resolution images \( k_1 = k_2 = 1 \) and for high res-
olution images \( k_1 = 1 \) or \( k_1 = 1.5 \) and \( k_2 = 2 \) (Ritter and Wilson 1996). Such an
automatic thresholding technique was used where the appropriate \( k_1 \) and \( k_2 \) were
chosen by inspection of the first few images. Converting a 8-bit image to a binary
image by thresholding erodes an object. This means that some pixels belonging to
an object may become disconnected from it. In order to smoothen such edges and
gaps, pixels were re-grown where the gaps were up to 1 pixels. If two white pixels
were separated by one black pixels then, the middle pixels were made white. Also,
isolated black pixels in the interior of a region of white pixels were made white. The
thresholded image obtained by processing was used for size measurements. Standard
image analyzing functions in Matlab were used to compute the centroid, the major,
the minor axis, the orientation, and the maximum length of each selected molecule.

2.3 Results and discussion

Two dimensionless numbers were used to describe the intensity of the flow. A local
Weissenberg number \( Wi \equiv \lambda \dot{\gamma} \), where \( \lambda \) is the relaxation time of the DNA, and \( \dot{\gamma} \) is
the local strain rate and a characteristic Weissenberg number \( Wi^* \equiv \lambda V/H \), where \( V \)
is the roll speed and \( H \) is the minimum gap between the glass coverslip and the roll.
The local strain rate is defined as \( \dot{\gamma} = \sqrt{|D_{2D}|} \), where \( D \) is the rate of strain (the
symmetric part of the gradient of velocity) and \( D_{2D} \) is the second invariant of \( 2D \). In
the experiments described in this section, \( Wi^* \) ranged from 38 to 3500, the Reynolds
number \( Re \equiv \rho V H/\mu \) ranged from \( 4.4 \times 10^{-4} \) to \( 2.5 \times 10^{-2} \) and the capillary number
\( Ca \equiv \eta V/\sigma \) (\( \sigma \) is the surface tension) was \( 2 \times 10^{-4} \) to \( 3 \times 10^{-2} \). The ratio of the gap
to the roll radius ranged from \( 4.2 \times 10^{-3} \) to \( 8.9 \times 10^{-3} \).

The Deborah number in this flow is \( De \equiv \lambda/\tau_0 \equiv \lambda V/L \), where \( \tau_0 \) is the average
residence time of the liquid in the bead computed through the roll’s tangential velocity and the bead length \( L \); the Deborah number is related to the Weissenberg number through the ratio of gap width to bead length, \( \text{De} = \text{Wi} \times \frac{H}{L} \). The length of the bead ranged from approximately 4 mm to 8 mm, depending on the viscosity of the liquid and on the roll’s speed; therefore, the Deborah number in the flow was approximately two orders of magnitude lower than the Weissenberg number.

### 2.3.1 Low capillary number flow

The liquid layer picked up by the rotating roll was thinner than the gap between the coverslip and the top of the roll when the roll velocity was lower than a critical velocity. Wilson (1982) showed that the film thickness at the top of a roll dragging liquid out of a pool is

\[
H^* \sim \frac{(\eta \omega)^{2/3}}{(\rho g)^{1/2} \sigma^{1/6} (1 - \cos \theta)^{1/2}}
\]

where, \( \eta \) is the solution viscosity, \( \omega \) is the roll speed, \( \rho \) is the density of the solution, \( \sigma \) is the surface tension and \( \theta \) is the angle up to which the roll is dipping in the liquid pool. In this study \( \theta = 90^\circ \), i.e., the roll is half submerged in the liquid pool. Table 1 lists the film thickness (computed with equation 2.5) in each of the experiments performed. In all the experiments in this velocity range (capillary number) the minimum gap is greater than the calculated film thickness; thus, the coating bead was established by depositing a drop of liquid on the roll surface before the coverslip was placed on the device. In all the experiments the measured film thickness \( H_0 \) (calculated from the measurement of the flowrate in Section ) was lower than the gap and \( H^* \). The capillary pressure across the free surfaces dominates the viscous pressure and causes a back-flow under the coverslip. A big recirculation is present under the cover slip (figure 2.7a,b). Images were acquired in different planes along the optical axis at the position of minimum gap between the roll and the glass bar. To minimize edge effects the molecules were visualized in the middle of the flowcell at equal distance from the two bearings.
Table 2.1: Location of the image planes (in depth) at the minimum gap position for the various local Weissenberg numbers, Capillary numbers and the experimental conditions.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Wi</th>
<th>Ca</th>
<th>y</th>
<th>Minimum Gap</th>
<th>$y/H$</th>
<th>$H_0$</th>
<th>Drag Film Thickness $H^*/H$</th>
<th>Roll Speed (rps)</th>
<th>Viscosity (mPa s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>×10⁻³ (µm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1</td>
<td>84</td>
<td>2.1</td>
<td>30</td>
<td>41.7</td>
<td>0.72</td>
<td>0.9</td>
<td>7.8</td>
<td>0.19</td>
<td>0.01</td>
</tr>
<tr>
<td>E2</td>
<td>106</td>
<td>2.3</td>
<td>32.9</td>
<td>42.1</td>
<td>0.78</td>
<td>-</td>
<td>8.1</td>
<td>0.19</td>
<td>0.01</td>
</tr>
<tr>
<td>E3</td>
<td>147</td>
<td>8.1</td>
<td>31.8</td>
<td>55.5</td>
<td>0.57</td>
<td>4.1</td>
<td>18.7</td>
<td>0.34</td>
<td>0.015</td>
</tr>
<tr>
<td>E4</td>
<td>176</td>
<td>1.1</td>
<td>3.3</td>
<td>35.3</td>
<td>0.1</td>
<td>5.1</td>
<td>23.6</td>
<td>0.67</td>
<td>0.05</td>
</tr>
<tr>
<td>E5</td>
<td>195</td>
<td>1.1</td>
<td>16.6</td>
<td>35.3</td>
<td>0.47</td>
<td>6.6</td>
<td>22.4</td>
<td>0.63</td>
<td>0.05</td>
</tr>
<tr>
<td>E6</td>
<td>217</td>
<td>1.1</td>
<td>26.3</td>
<td>45.8</td>
<td>0.57</td>
<td>9.2</td>
<td>22.5</td>
<td>0.49</td>
<td>0.02</td>
</tr>
<tr>
<td>E7</td>
<td>302</td>
<td>1.6</td>
<td>25.4</td>
<td>45.8</td>
<td>0.55</td>
<td>10.6</td>
<td>29.3</td>
<td>0.64</td>
<td>0.03</td>
</tr>
<tr>
<td>E8</td>
<td>361</td>
<td>1.1</td>
<td>21.8</td>
<td>35.3</td>
<td>0.62</td>
<td>5.1</td>
<td>23.6</td>
<td>0.67</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Figure 2.7: (a) Schematic diagram of the flow at low roll velocity. The zero velocity surface (ZVS) is shaded. Images of flowing DNA molecules were captured in different planes at the position of minimum gap between the coverslip and the roll. (b) Streamlines computed using lubrication approximation (equation 2.6) at low roll speed. These streamlines correspond to experiment E4 and $Q = 10209 \mu m^2/s$. 
Figure 2.8: Time-lapse images of λ-DNA molecules flowing below the coverslip. Time (in seconds) is indicated at the bottom right corner of each frame. The image plane is located at $y = -3.3 \, \mu m$ and $x = 0$ (above the zero velocity plane and close to the coverslip). The molecules (A, B, C) move opposite to the direction of motion of the roll (arrow).
Flow under the coverslip

The DNA molecules were nearly stationary close to the coverslip. As the focal plane was lowered toward the roll, the DNA moved opposite to the roll (figure 2.8) with growing velocity initially, then slower until a plane was found where the DNA was nearly stationary. As the focus was lowered further, the DNA moved with growing velocity in the same direction of the roll. The velocity profile in the gap can be estimated by lubrication approximation with no-slip boundary condition at the bottom of the coverslip and the top of the roller

\[ v(x, y) = 3\omega R \left( 1 - \frac{x^2}{2R^2} \right) \frac{y^2}{h(x)^2} - \frac{6Q}{h(x)^2} y^2 + \frac{6Q}{h(x)^2} y - 2\omega R \left( 1 - \frac{x^2}{2R^2} \right) \frac{y}{h(x)}. \]  

(2.6)

where \( y = 0 \) is the bottom of the coverslip and \( y = h(x) = -(H + R - \sqrt{R^2 - x^2}) \) is the roll, \( \omega \) is the roll speed, \( x = 0 \) is the minimum gap position and \( Q \) is the flowrate per unit width (figure 2.7). \( Q \) was calculated by measuring the position of the zero velocity plane at \( x = 0 \). Figure 2.7b shows the streamlines computed by lubrication approximation. The streamlines show that there is a recirculation below the coverslip and, a zero velocity plane exits at the minimum gap position. In the zero velocity plane molecules were observed stretching in the direction of the velocity (and possibly of the velocity gradient), whereas no size change in the direction of the vorticity could be detected (figure 2.9). The zero-velocity plane moved closer to the coverslip as the roll velocity was increased. By tracking molecules flowing just below the coverslip, the recirculation was found to extend from the free surface downstream of the minimum gap to the free surface upstream of the minimum gap.

Schunk and Scriven (1990) have modeled complex mixed extensional and shear flows based on the flow classification criterion of Astarita (1979), using the frame invariant quantities \( D \), the rate of strain, and \( w_{\text{rel}} \), the relative rate of rotation of the unit eigenvectors \( e_i \) of \( D \) with respect to the vorticity,

\[ D \equiv \frac{1}{2} \left( \nabla v + \nabla v^T \right) \]  

(2.7)

\[ w_{\text{rel}} \equiv e_i \times \left( \frac{\partial e_i}{\partial t} + v \cdot \nabla e_i \right) - \frac{1}{2} \nabla \times v \]  

(2.8)
Figure 2.9: DNA molecule in the zero-velocity horizontal plane at the minimum gap between the roll and the coverslip. Wi = 195. Time is in seconds. Images in pseudocolor.
Figure 2.10: The flow strength parameter $\|w_{rel}\|/S$ as a function of position in the coating bead at different depths corresponding to local Weissenberg numbers. The minimum gap position is at $x = 0$. The inset shows the flow strength parameter for experiment E3.

The flow is pure shear when $\|w_{rel}\|/S = 1$ and pure extension when $\|w_{rel}\|/S = 0$. $S = \sqrt{\|\mathbf{D}\|}$.

Planes with different velocity gradients ($\sqrt{\|\mathbf{D}(x,z)\|}$) were chosen to investigate molecular conformations in a range of local Weissenberg numbers. Figure 2.10 shows the flow strength parameter ($\|w_{rel}\|/S$) as a function of position $x$ in the coating bead, on streamlines corresponding to the image planes. Near the minimum gap position (400 $\mu$m on either side) clearly the flow is shear dominated ($\|w_{rel}\|/S - 1 \lesssim 3 \times 10^{-3}$). In experiment E3, the velocity gradient nearly vanishes at $x \sim 680 \mu$m; there the flow changes rapidly from shear-dominated, to almost purely rotational ($\|w_{rel}\|/S \gg 1$), to extensional ($\|w_{rel}\|/S \approx 0$), back to shear-dominated.

Images of molecules moving at high speed ($\sim 100$ to 230 $\mu$m/s) were de-interlaced to reduce streaking and processed frame by frame in an image analysis program.
written in Matlab. The major and minor axes of ellipses of equivalent second moment were computed for each thresholded molecule. Molecular extension was measured as the maximum distance between the two farthest points on the molecule. 1000 to 2000 measurements were made at each local Weissenberg number.

Figure 2.11 shows the mean fractional extension of DNA at the minimum gap location. As expected, the polymer extension increases with Weissenberg number due to higher hydrodynamic drag experienced across the molecule. Also plotted for comparison are the results of Smith et al. (1999) at low Wi in pure homogeneous shear flow. There is a slight trend in figure 2.11 and the mean fractional extension grows gradually and asymptotes to 0.5. In pure shear flow such a trend is expected (Smith et al. 1999), and has been confirmed by simulations of Kramers chains (Hur et al. 2000). Figure 2.12 shows the distributions of fractional extension at the different local Weissenberg numbers. The distributions become broader with increasing Wi. Shear flow is a combination of equal parts of extension and rotation (figure 2.13b). The rotational component of the flow destabilizes the extended molecule, which rotates and folds back into a coiled configuration. The molecules fluctuate from an extended state to a coiled state and back into an extended state, like a yo-yo (figure 2.13a). With increasing Wi the molecules experience higher extensions while fluctuating between extended and coiled states. The fluctuations (standard deviation) in the extension were measured to be 13-28% of the contour length. The minimum measured extension is close to the equilibrium size of the molecule (~1.5 \( \mu \text{m} \)). Some measurements (< 1.5%) were 1-2 \( \mu \text{m} \) above the contour length of a DNA molecule because of small errors introduced by the image thresholding algorithm.

Figure 2.14 shows the average shape and orientation of the DNA in the flowing dilute solutions at the minimum gap. The size and orientation were obtained by calculating the ellipse with second moment equal to that of the image (2-D projection) of the molecule. The orientation of the average conformations show that the molecules are completely aligned with the velocity direction. The spanwise dimension (along the roll axis) does not change considerably with local Weissenberg number, in agreement with the results of Smith et al. (1999). Similar to figure 2.11, the stretch
Figure 2.11: Mean fractional extension of molecules (along $x$, the flow direction) in the pure shear flow at the minimum gap location (solid symbols). The mean extension has a trend and asymptotes to 0.5 at high Wi. Open symbols are data on pure shear by Smith et al (1999). Line is a guide to the eye and the error bars are standard deviations.
Figure 2.12: Normalized distribution of molecular extension in the shear flow region at minimum gap position for different values of local Wi. 1 μm bins are used to calculate the histograms which are offset from zero for clarity. Solid and dashed lines represent distributions obtained with $\eta \sim 40$ mPa s and 103 mPa s solutions, respectively.
Figure 2.13: (a) A molecule undergoing oscillations from extended to coiled configurations (Yo-Yo motion) for local $Wi = 361$. The snapshots are at intervals $1/30$ s. Images are in pseudocolor. (b) A simple shear flow can be expressed by superimposing pure rotation and pure extension. The extension component causes the molecule to extend but rotation destabilizes the extended molecule and the chain recoils. The molecules undergo a periodic stretch-tumble dynamics like a yo-yo.
along the velocity direction increases and plateaus within error bars, with increasing Weissenberg numbers. But it should be noted here that simulations combining Finite elements and Brownian dynamics (Chapter 3) show that the behavior (e.g., molecular extension) of DNA above and below the ZVP at the minimum gap is very different. The image planes corresponding to E3 and E4 were located above ZVP, E5 was located at ZVP and the rest were located below the ZVP.

Flow near contact lines

Very few molecules were found at the menisci just below the coverslip, both upstream and downstream of the minimum gap position, even when the concentration of DNA in these solutions was raised to $10^{-4}c^*$. Near the contact lines, the molecules remain coiled while traversing the field of view with a very low axial velocity. Figure 2.15
shows snapshots of molecules taken at 1 s intervals at two different characteristic \( Wi \) each, at the upstream and downstream menisci. The velocity gradient close to the contact lines appears to be too weak to stretch the DNA even though the characteristic Weissenberg number of the flow is high (38 to 247). This may be due to the acute contact angle (the liquid wets the coverslip) at the free surface, which leads to trapping of molecules (figure 2.16). These molecules have high residence time in this region.

### 2.3.2 High capillary number flow

At high roll speeds (high capillary number), the capillary pressure across the two free surfaces is not high enough with respect to the viscous forces to cause a back flow. The big recirculation under the coverslip, present at low roll velocity, breaks into two smaller recirculations near the upstream and downstream menisci (figure 2.17). As the capillary number is increased further, the layer picked up by the roll is thicker than the gap; part of it is rejected by the metering action of the coverslip, and eventually a run-back flow arises of liquid from the coating bead to the pan.

On increasing the roll speed, the upstream contact line was observed to move away from the minimum gap and the downstream contact line moved toward the minimum gap. Images were acquired at the upstream and downstream menisci, at the upstream separation surface demarking the boundary between recirculating liquid and metered liquid, and at the downstream separation surface between the metered liquid and the recirculation at the downstream meniscus. The menisci were located by focusing the objective immediately under the coverslip inside the coating bead, then moving the microscope stage horizontally until a dark region with no DNA molecules appeared. The separation surfaces were found by focusing the objective under the coverslip inside the coating bead, then moving the microscope stage horizontally until a region was found with some DNA molecules moving with the roll and others moving opposite to the roll. Deeper regions of the separation surfaces were found by repeatedly lowering slightly the focal plane and adjusting the horizontal position to keep the separation surface in the center of the field of view.
Figure 2.15: Images of DNA molecules near contact lines. The images of the three molecules (A, B, C) are at 1 s intervals. (a): Near Upstream Meniscus (6 μm below coverslip), \( W_i = 38 \); (b): Near Upstream Meniscus (12 μm below coverslip), \( W_i = 194 \); (c): Near Downstream Meniscus (1.5 μm below coverslip), \( W_i = 44 \); (d): Near Downstream Meniscus (1.6 μm below coverslip), \( W_i = 247 \).
Figure 2.16: The acute contact angle between the free surface and the coverslip causes the DNA to be trapped. These molecules have a high residence time and experience very little flow.

Figure 2.17: Schematic diagram of the flow at high roll velocity. The locations of the visualized regions are shaded: USS Upstream separation surface; DSS Downstream separation surface; UM Upstream meniscus; DM Downstream meniscus. The diagram is not to scale; in particular, the gap is much narrower, and the separation lines are much closer to the location of the minimum gap.
**Upstream separation surface**

The Upstream Separation Surface was 1.1 ±0.05 mm and 1.4 ±0.05 mm upstream of the location of the minimum gap in experiments at average \(Wi = 240\) \((Ca = 0.009)\) and 265 \((Ca = 0.027)\), respectively. The sequence of images in figure 2.19 was taken at characteristic \(Wi^e = 2031\) \((Wi = 240)\). The molecules are moving toward the coverslip. The separation surface is marked in the four images. The molecule above the Upstream Separation Surface \((A)\) moves against the roll (bottom to top) and is in the recirculating liquid. The molecule below the Upstream Separation Surface \((B)\) is in the metered liquid and moves with the roll (top to bottom). The final snapshot shows a molecule \((C)\) that comes into focus stretched at the Upstream Separation Surface.

The DNA molecules in figure 2.19 lie mainly in the plane of focus. Near the separation surface by the stagnation line the flow is chiefly extensional; the principal direction of extension of the rate of strain is essentially parallel to the coverslip, and the principal direction of compression is normal to the coverslip, and the DNA tends to align along the principal direction of extension.

The stagnation flow at the separation surface (near the coverslip) can be described by the stream function \((Batchelor 1967)\)

\[
\psi(X, Y) = AY^2X \sin \theta_0 - AY^3 \cos \theta_0. \tag{2.9} 
\]

Here, \((X, Y) = (0, 0)\) is the stagnation point on the coverslip and, \(X\) and \(Y\) are coordinates parallel and perpendicular to the coverslip (figure 2.17). The stagnation line forms an angle \(\theta_0\) with the coverslip. An approximate value of \(\theta_0\) was measured by moving the stage horizontally (along \(X\)) to position the Upstream Separation Surface in the middle of the field of view at different depths \((Y)\). Figure 2.18 shows the streamlines near the upstream separation surface computed using equation 2.9 in the flow with characteristic \(Wi^e = 2031\). The angle that the stagnation line made with the coverslip was measured at \(\theta_0 = 3.4^\circ\). The characteristic flow number in the field of view is best given as the average over the imaging volume (volume formed by
Figure 2.18: Streamlines showing stagnation flow near the upstream separation surface (USSS) computed using equation 2.9. \( \theta_0 = 3.4^\circ \). The dashed line at \( Y = 3.6 \mu m \) is the plane where images were acquired. The characteristic \( Wi = 2031 \), \( \langle Wi \rangle = 240 \), and \( Ca = 0.009 \).
Figure 2.19: Time-lapse images of λ-DNA molecules moving with and against the roll at the Upstream Separation Surface (USS) in a high capillary number flow with characteristic $Wi^* = 2031$ and $Ca = 0.009$. Time (in seconds) is indicated at the bottom right corner of each frame. Molecule A moves against the roll (bottom to top) and is in the recirculating liquid. B moves with the roll (top to bottom) and is in the metered liquid. Both A & B move away from the USS while moving toward the glass coverslip. Molecule C comes into focus stretched right at the USS. Images are shown in pseudocolor.
the field of view and the depth of field). \( \langle Wi \rangle \equiv \lambda \sqrt{\frac{\langle \|w_{\text{rel}}\|/S \rangle}{V}} dV \). \( \lambda \) was determined by calculating the average number of frames the molecules remained in the imaging volume in each experiment; the molecules moved at speeds \( \sim 10 \) to \( 12 \) \( \mu m/s \) toward the glass slide and stayed in the field of view for a few frames.

Molecular conformation was observed in two flows with \( \langle \|w_{\text{rel}}\|/S \rangle = 0.3728 \), \( \langle Wi \rangle = 240 \) \( (y = 3.6 \mu m, \theta_0 = 3.4^\circ) \) and \( \langle \|w_{\text{rel}}\|/S \rangle = 0.4137 \), \( \langle Wi \rangle = 265 \) \( (y = 6.3 \mu m, \theta_0 = 4.6^\circ) \). Figure 2.20a shows the distributions of extensions measured at the two average \( \langle Wi \rangle \). The two distributions are qualitatively similar, with a large number of measurements near full extension. Various molecular configurations were observed which had been reported previously in pure extensional flow (Perkins et al. 1997): folded, half-dumbbell (molecule A in figure 2.19), stretched (molecule B in figure 2.19), dumbbell (molecule C in figure 2.19) (figure 2.20b). Figure 2.21 shows images of the various polymer configurations and the corresponding backbone structures observed near the upstream separation surface. The mean fractional extension increases with Weissenberg number and is \( 50 \pm 3\% \) and \( 56 \pm 3\% \) for average \( \langle Wi \rangle = 240 \& 265 \), respectively. The major peak, corresponding to stretched molecules, is at fractional extension of \( 0.76 \pm 0.03 \) for average \( \langle Wi \rangle = 240 \) and at \( 0.85 \pm 0.01 \) for average \( \langle Wi \rangle = 265 \) (the distributions were fitted with four Gaussians capturing the four major configurations observed). The extension that the molecules experience is lower than the expected value of about \( 90\% \) observed in the steady mixed flow studies by Babcock et al. (2003). The extension is lower because the molecules experience a strain (product of extension rate and residence time in the stagnation flow region) \( \dot{\epsilon} t_{\text{res}} \sim 1 \) as they traverse the stagnation flow. At such small strain, many molecules are still stretching and have not reached the steady state extensions. This can also be seen in the probability distributions which resemble a combination of distributions of molecules with different residence times in a pure extensional flow (Perkins et al. 1997). In Perkins’ work, the primary peak corresponding to stretched configurations grows as the residence time of the molecules in the flow increases, while the secondary peaks (folded and coiled configurations) diminish as the molecules reach steady state extension. Here I find peaks corresponding to
stretched, folded, and coiled configurations, implying that not all the molecules have reached steady state extension.

**Downstream separation surface**

The DNA enters the stagnation flow region in a distorted conformation and then stretches along the streamline into the focal plane. The principal direction of extension is into the field of view, and the principal direction of compression is in the plane of focus; therefore, the DNA aligns predominantly along the optical axis as it approaches the separation surface. The DNA goes into the plane of focus as it nears the separation surface; thus, the images in figure 2.22 (frames 3-4) are projections of the shape of the molecule onto the field of view. Molecules that are extended along the objective axis as well as undistorted molecules give circular images; therefore, it is not possible to determine whether the molecule imaged at the Downstream Separation Surface are coiled or partially stretched along the objective axis. For this reason, the distribution of measured molecular extension at the DSS is not quantified in this study.

**Upstream and Downstream menisci**

The DNA molecules near the upstream meniscus arrive from the upstream separation surface (figure 2.23), where they are stretched by the extension dominated flow near the stagnation line. The molecules then traverse a region of slow, shear-dominated flow below the coverslip, where the velocity gradient has a large rotational component and a stretching component too weak to further extend the molecules. The DNA molecules therefore start to relax before reaching the upstream meniscus. Molecular relaxation at and near the free surface was not observed in our experiments due to presence of a nodal recirculation (along the roll axis) in which extended molecules were observed moving axially. Figure 2.24 shows a typical DNA molecule at the upstream meniscus at \( \text{Wi}^* = 2031 \) and \( \text{Ca} = 0.009 \). The molecule is moving axially and, unexpectedly, is strongly extended. While molecules in the two-dimensional flow (at minimum gap position in the low capillary number flows) moved in the direction
Figure 2.20: (a) Measured distribution of fractional extension at the Upstream Separation Surface for $\langle \| \mathbf{w} \| / \| \mathbf{S} \| \rangle = 0.3728$, $\langle \mathbf{W} \rangle = 240$ (dashed line) and $\langle \| \mathbf{w} \| / \| \mathbf{S} \| \rangle = 0.4137$, $\langle \mathbf{W} \rangle = 265$ (solid line). (b) Distribution of polymer configurations at the Upstream Separation Surface at $\langle \mathbf{W} \rangle = 240$ (left) and 265 (right).
Figure 2.21: Images in pseudocolor of different DNA configurations—(A) Coiled (B) Folded (C) Kinked (D) Dumbbell (E) Half-dumbbell and (F) Stretched—observed at the upstream separation surface. Schematics of the backbones are also shown (Perkins et al. 1997). The configurations (backbones) can be inferred from the relative light intensity along the image backbones. The light intensity is indicative of the number of dye molecules in an image pixel. Higher intensity indicates larger concentration of chain segments (tagged by dye molecules) in an image pixel. For example, in configuration D the ends of the DNA image shows high intensity (red color) with respect to the rest of the molecule hence the DNA chain is coiled up at the ends and looks like a dumbbell.
Figure 2.22: DNA Molecules imaged at the Downstream Separation Surface (DSS) in the high capillary number flow with characteristic $Wi^* = 1086$. The DNA molecule is moving against the roll (bottom to top). The molecule comes into the focal plane extended. In the first snapshot part of the DNA is not fully in focus. The molecule lies in the focal plane as it moves toward the DSS (frame 2). It moves into the separation surface and goes out of focus leaving behind a blurred trace. (frames 3 and 4).
Figure 2.23: Schematic paths of DNA molecules between the upstream stagnation line (USS) and the upstream meniscus (UM). The DNA approaches the stagnation region at the USS in a nearly coiled conformation (A), then stretches approximately parallel to the coverslip while it traverses the stagnation region (B). In the shear-dominated velocity field it rotates and relaxes (C) while moving towards the free surface. Due to the axial flow observed at the meniscus we cannot comment surely about conformation D. In a flow without nodal recirculation the DNA may completely recoil (D) by the time it reaches the upstream meniscus. The figure is not drawn to scale.

of motion of the roll or against it.

Similarly at the downstream contact line extended molecules were observed moving axially (figure 2.25). The molecules were instead expected to be moving slowly and mostly coiled close to the downstream meniscus even at high Weissenberg number, because a slow recirculation is present near the free surface. Interestingly, the gradients present due to the slow nodal recirculation are strong enough to stretch the molecules along the velocity direction as they move across the field of view. The DNA molecules leave the region of the downstream meniscus in a slightly distorted conformation, traverse a region of shear-dominated flow under the coverslip where little stretching occurs, and reach the downstream separation surface in a conformation that is slightly extended along the flow direction.

Using a low magnification (10X) and long working distance objective the flow near the contact line was investigated to determine if the axial motion is due to the presence of a ribbing instability. The contact line was observed to remain straight
Figure 2.24: DNA Molecules imaged in the high capillary number flow with characteristic Wi* = 2031 near the upstream meniscus (UM). The molecule is moving axially. While the expected two-dimensional flow would be in the direction of motion of the roll or against it. Images are at 0.3 s intervals (a) to (d). The axial motion is due to presence of a nodal recirculation along the roll axis. The contact line/free surface is near the top of the images.
and stationary. The free surface below the contact line was wavy and was oscillating at \( \sim 1 \text{ Hz} \). At the gap to radius ratio in our experiments the critical capillary number for the onset of ribbing instability computed by stability analysis of a simplified lubrication flow (Savage 1984) is \( \sim 0.1 \) and that inferred from experiments (Adachi et al. 1988) is \( \sim 0.14 \). This is approximately tenfold higher than the capillary number in our experiments (\( \text{Ca} = 0.009 \)). Coyle et al. (1990) showed that in symmetric forward roll coating flows the critical value of the capillary number was sensitive to the detection technique: by using a sensitive low angle reflection technique he found that the ribbing instability set in at a capillary number that was five-fold lower than what had been established previously by naked-eye measurements (Pitts and Greiller 1961, Greener et al. 1980). Thus, it is possible that in a plate-roll system the ribbing instability occurs at a lower capillary number than previously measured.

2.4 Conclusions

This study has shown single molecule visualization of DNA using fluorescence microscopy is a viable and useful technique to investigate the behavior of ultra-dilute polymer solutions in scaled-down process flows. A common, relevant and complex roll-knife coating flow was studied. DNA was used as an elastic tracer in a Newtonian flow field and the molecular conformation was observed. Experimental data on molecular conformation was obtained in a flow which is more complex than the spatially homogeneous flows which have appeared in the literature so far. At low speeds, a large recirculation was present below the coverslip. At the minimum gap, where the molecules experience a shear dominated flow, the mean fractional extension increases with local Weissenberg number. At high roll speeds the molecules experience an extension-dominated flow at the two separation surfaces. Interestingly, a weak axial flow was present near the contact lines at a capillary number one order of magnitude lower than the critical literature value for the onset of ribbing; the DNA molecules stretched axially there. The optical axis here was aligned with the velocity gradient and hence in certain regions the DNA straddled the focal plain; more revealing im-
Figure 2.25: DNA Molecules imaged in the high capillary number flow with characteristic $Wi^* = 2532$ near the downstream meniscus (DM). Images are at 1 s intervals (a) to (d). The axial motion is due to presence of a nodal recirculation along the roll axis. The contact line/free surface is at the bottom of the images.
ages may be obtained in future by aligning the optical axis of the microscope with the vorticity.

The results provided here give researchers who are trying to model DNA and, in general, polymers an opportunity to check whether the models which have been developed and tested in homogeneous flows do well enough in more complex flows (e.g., coating flows). Because ultra-dilute solutions (Newtonian solutions) were used, the models of the polymer dynamics can be tested independently of the model of the coupling of the stress to the polymer conformation. In the next chapter (Chapter 3) the flow at low capillary number is compared with macroscopic flow field calculations using Finite element method, and the measured DNA conformation at the minimum gap is compared with predictions from a molecular model. Validating and testing models in an ultra-dilute regime, where the DNA does not affect the flow field, is the first step towards studying fully coupled problems where the DNA concentration is higher and the molecules affect the flow regime.
Chapter 3

Macro-micro Simulation of Dilute DNA in a Roll-knife Coating Flow

Molecular models that approximate a polymer molecule as a chain of beads connected with rods or springs have been successful in predicting the dynamic behavior of dilute polymer solutions in simple homogeneous flows (Larson 2005). However, until two years ago there were no studies to test and validate these approaches in complex inhomogeneous flows; since then, a few experimental (Chopra et al. 2003) and modeling (Chopra et al. 2003, Jendrejacek et al. 2003, Woo et al. 2004b, Woo et al. 2004a) studies have appeared. In this thesis, the stretch and orientation of DNA molecules was measured in a complex, free surface flow using single molecule visualization (Chapter 2). Experimental data have been used to test the rescaling molecular approach developed by Sunthar and Ravi Prakash (2005). The rescaling approach has been validated in simple flows of DNA (Sunthar and Ravi Prakash 2005); here it has been applied and tested in a complex flow. The macroscopic two-dimensional flow field in the experimental roll-knife coating flow cell was first computed with the Galerkin/Finite Element method. The microscopic conformation of DNA was simulated by Brownian dynamics as it evolved along the flow streamlines. The dynamic molecular properties are obtained by successively refining the DNA molecule modeled as a chain of beads connected with springs; and subsequently extrapolating data to the limit of the number of Kuhn segments in the DNA molecule, and then compared with experimental results.
3.1 Introduction

The understanding of the behavior of flowing dilute polymer solutions has progressed tremendously over the past decade. Until recently, the statistical theories of polymer solution dynamics (Flory 1953, Rouse 1953, Zimm 1956) could be compared with only bulk rheological and optical measurements. Direct video-microscopy of individual DNA molecules provided the first direct experimental test of theoretical predictions at the single molecule level. The success of the single molecule visualization technique in studying simple homogeneous shear (Smith and Chu 1998), elongational (Perkins et al. 1995) and mixed (Babcock et al. 2003) flows has paved the way for studying more complex and relevant inhomogeneous flows.

The most common coarse-grained molecular models represent polymer chains as a sequence of beads connected with rods (bead-rod model) or beads connected with springs (bead-spring model) (Bird et al. 1987). In the bead-rod model for flexible polymers, the beads are connected in series by rigid rods of length equal to the Kuhn length—the characteristic step size of a random walk (Flory 1953). Only the beads experience frictional drag from the solvent and the flow does not affect the configuration of the chain at scales below the Kuhn length. Bead-rod models are computationally expensive because the number of beads becomes large with increasing molecular weight (increasing the number of variables). For example, a typical λ-DNA molecule has about 250 Kuhn steps, and a 1.95 Mg/mol polystyrene molecule has 2627 Kuhn steps (Prabhalak et al. 2004). Both these numbers exceed the current capabilities for extensive simulation studies of such polymer molecules (about 100 Kuhn steps). Instead, in bead-spring models many Kuhn lengths are grouped together and represented by springs with an appropriate force law; the effective spring constant of the connector springs can be suitably modified to model larger groups of Kuhn steps without increasing the number of beads.

To describe the non-equilibrium behavior of dilute solutions of flexible polymers using the coarse-grained molecular theories, it is essential to consider the various forces and interactions that determine molecular conformations in flows. The thermally
agitating solvent molecules bombard the polymer and impart a random (fluctuating) Brownian force. The flowing solvent exerts a frictional drag force on the chain because of momentum exchange due to the (average) velocity difference between a portion of the chain and the surrounding solvent (Bird et al. 1987). Motion and deformation of a part of the polymer chain is propagated to another portion of the chain through the solvent. The altered velocity field due to these "hydrodynamic interactions" affects the drag force that any part of the chain experiences (Larson 1999). Another important interaction is the "excluded volume interaction". There are two types of excluded volume interactions. One is the short-range interactions that are due to steric constraints—any polymer segment has a finite volume and cannot intersect any other segment (Flory 1949)—and is appropriate for bead-rod models. This self-avoiding nature of the polymer chain results in a swelling of the polymer coil (Doi and Edwards 1986). The other is related to the coarse-graining of long chain sequences into "beads", and is appropriate for bead-spring models. There are long-range interactions between such local non-intersecting volumes (beads) leading to swelling of the chain. These long-range interactions are also affected by thermodynamics: interactions between the chain segments and chain-solvent interactions; these interactions are due to effective repulsive forces between chain segments that prevent their overlap and cause the chain to expand in solvents where the chain-solvent interactions are energetically favorable (Flory 1953). The entropic spring force (entropic elasticity) between the chain segments also become important as the chain deforms from its equilibrium conformation (Woo et al. 2004b).

The molecular properties of DNA in simple homogeneous flows have been studied computationally using the bead-rod and bead-spring molecular models. Larson et al. (1999) used the bead-spring model with the Worm-Like Chain spring force (Marko and Siggia 1995), taking into account only the Brownian force and the drag force to describe the dynamics of DNA molecules subjected to a planar extensional flow. The quantitative predictions of the ensemble average stretch and rate of stretch from the Brownian dynamics simulations agreed with the single molecule DNA experiments performed by Perkins et al. (1997) and Smith and Chu (1998). Molecular individu-
alism, i.e., dependence on initial configuration (Perkins et al. 1997, de Gennes 1997), observed in the experiments was also captured by the simulations, and various molecular configurations such as folds, kinks, dumbbells and half-dumbbells were predicted by the coarse-grained model. Hur et al. (2000) performed Brownian dynamics of bead-rod and bead-spring chains to study molecular behavior in pure shear flow. A constraint force for inextensibility was used in a bead-rod model and finitely extensible non-linear springs (FENE) and Worm-Like Chains were used in the bead-spring model. The models captured the stretch-tumble dynamics observed in the experiments and the measured average extension of DNA molecules (Smith et al. 1999) was accurately predicted by the simulations. These simulations were extended to study the transient response of molecular extension in start-up shear flows by Babcock et al. (2000). Hur et al. (2001) performed an elaborate study on the dynamics of dilute and semi-dilute DNA solutions in shear flows where they combined fluorescence microscopy, bulk rheological measurements, and Brownian dynamics simulations, to understand the coupling of the flow with the polymer (DNA) microstructure. They found no effect of concentration (ultra-dilute to 6 times the overlap concentration) on the individual dynamics of the DNA molecules. In all these simulations the chains deformed under the action of only Brownian and drag forces. Jendrejaj et al. (2002) also used a bead-spring chain model with a Worm-Like Chain spring force and predicted the DNA extension in shear flows; they also incorporated excluded volume and hydrodynamic interactions in the simulations. Dynamics of single DNA molecules were also studied in homogeneous linear mixed flows using Brownian dynamics by Hur et al. (2002). They predicted that when the strain rate exceeds the vorticity (extension dominated flows) the molecules deform to almost fully stretched state and when the vorticity exceeds the strain rate (rotation dominated flows) the molecules deform in a periodic motion with an average extension lower than that observed in simple shear flows. These predictions were confirmed experimentally by Babcock et al. (2003). In all the aforementioned studies on DNA dynamics in flows the parameters accounting for the spring forces, hydrodynamic interactions and excluded volume interactions were obtained by best-fits to the experimental data. Recently, Sunthar and
Ravi Prakash (2005) used Brownian dynamics simulations to predict the evolution of DNA conformations in elongational flow using a rescaling approach where parameter-free predictions of the molecular properties were obtained. The DNA molecule was represented by a bead-spring model, and excluded-volume and hydrodynamic interactions between the beads were taken into account. A successive fine graining of the polymer chain was performed by progressively increasing the number of beads, and predictions of the microscopic extension were computed in the limit of large number of beads, where they became independent of chain discretization. The theoretical predictions compared well with the experimental observations of DNA stretch by Smith and Chu (1998).

Recently, single molecule DNA visualization has complemented Brownian dynamics simulation in studying complex flows generated in drying drops of DNA solutions (Chopra et al. 2003). The stretch and orientation of DNA on substrates were predicted by using a convection flow computed from lubrication approximation. Wall-hydrodynamics were neglected in these simulations. However, these are important because the molecules flow very close to the substrate and adsorb on the surface; thus the study, though significant, is controversial. Jendrejack et al. (2003) extended the Brownian dynamics simulation method to incorporate long-range hydrodynamic interactions with walls in a confined geometry. They studied the dynamics of long DNA molecules (up to 100 \(\mu\text{m}\)) in a pressure-driven flow in rectangular micro-channels and found that wall hydrodynamics cause a depletion in the concentration of DNA near the wall due to cross-stream migration. This discovery was confirmed experimentally by Fang et al. (2005), who found a depletion layer of 7 \(\mu\text{m}\) next to the walls. Similar results have also been observed by Smith et al. (2005).

It is clear that single molecule visualization of DNA using fluorescence microscopy has contributed significantly to the development of molecular modeling of polymers in flows—mostly homogeneous flows up to now. Understanding molecular behavior in more complex inhomogeneous flows is the next challenge.
3.2 Flow calculation

The liquid in our microscopy experiment was sugar solution with trace amounts of DNA molecules. The solution was so extremely dilute that it behaved as Newtonian. In such an ultra-dilute polymer solution the molecules stretch and relax under the combined actions of the velocity gradient and intramolecular (elastic) and Brownian forces as if they were in dilute solution. Harrison et al. (1998) and Pasquali (2000) have argued that in ultra-dilute solutions, the concentration of polymer molecules is so low that the macroscopic flow would be equivalent to that of a Newtonian liquid. Experiments with ultra-dilute solutions (Pasquali 2000, Duggal and Pasquali 2004) thus allow the separation of the effect of flow on the polymer microstructure from the converse effect of the polymer microstructure on the flow.

The two-dimensional Newtonian free surface flow in the roll-knife coating flowcell was computed using the method and software developed by ?. Numerical solutions are required for complex flows involving complex geometries, even when the fluid is Newtonian fluids. Macroscopic flows involving free surfaces requires solving free boundary problems where the domain of definition of the differential equations describing the flow is unknown and its location must be part of the solution of the problem. The method is based on mapping the unknown flow domain (physical domain) into a computational domain by using the elliptic mesh generation method—the mapping satisfies an elliptic differential equation (Christodoulou 1990). The mapping $\xi = \xi(x)$ between the computational domain $\xi$ and the physical domain $x$, obeys the elliptic differential equation

$$\nabla \cdot \mathbf{D} \cdot \nabla \xi = 0$$

(3.1)

where $\nabla \equiv \frac{\partial}{\partial x}$ denotes differentiation in physical space, and the dyadic $\mathbf{D}$ controls the spacing of the $\xi_1$ and $\xi_2$ coordinate lines (Pasquali 2000, de Santos 1991). Because of the complex geometry of the flow setup, the physical domain was divided into simpler, connected, quadrangular subdomains, each of which was then mapped onto a separate subdomain of the computational domain.

The domain boundaries were described by the geometry of the experimental flowcell
Figure 3.1: Mapping of the physical domain into the computational domain.

(experiments E4 and E8 in Chapter 2). A schematic of the physical domain and the corresponding computational domain is shown in figure 3.1. The radius of the roll was $R = 6.35$ mm, and the minimum gap between the top of the roll and the coverslip was $H = 35.3$ μm.

The transport equations of mass and momentum in a steady, isothermal, incompressible liquid are:

$$0 = \nabla \cdot \mathbf{v}$$

$$0 = \rho \mathbf{v} \cdot \nabla \mathbf{v} - \nabla : \mathbf{T} - \rho \mathbf{g}$$

where $\mathbf{v}$ is the liquid velocity, $\rho$ is the liquid density, $\mathbf{T}$ is the stress tensor (Cauchy) and $\mathbf{g}$ is the gravitational acceleration. $\mathbf{T}$ is split into isotropic and viscous components,

$$\mathbf{T} = -p \mathbf{I} + \mathbf{\tau}$$

where the mechanical pressure $p$ is constitutively indeterminate because the liquid is incompressible, and the viscous stress $\mathbf{\tau}$ obeys Newton’s law of viscosity,

$$\mathbf{\tau} = 2\eta \mathbf{D}$$.
The mesh was computed by solving equation 3.1 coupled with the continuity and momentum equations (equations 3.2 and 3.3) and appropriate boundary conditions using the Galerkin/Finite element method. The solution (velocity, pressure, and mapping fields) was approximated in terms of simple polynomial basis functions. The differential equations were converted to a set of nonlinear algebraic equations by forming their weighted residuals (in the Galerkin method the weighting functions are the same basis functions) and applying boundary conditions. These were solved by Newton's method with analytical Jacobian.

At low capillary number the flow was metered and the incoming film thickness was smaller than the gap between the plate and the roll. The liquid domain in experiments extended from the coating bead into films clinging to the roll, upstream and downstream, into the cylindrical pool. For simplicity, the physical domain in the computations extended only into the film along the roll. The film thickness at the inflow and the outflow was unknown. Thus, the element nodes on the free surface at the inflow and the outflow boundary were allowed to move in the radial direction with respect to the center of the roll.

The location of the contact lines at the coverslip were also unknown. The contact lines were allowed to slide freely over the solid boundary corresponding to the coverslip. At the two contact lines the angle $\theta$ between the coordinate line $\xi_i$ and the boundary was prescribed based on independent empirical measurements, $\mathbf{n} \cdot \nabla \xi_i = |\nabla \xi_i| \cos \theta$, $i = 1$ or 2. The contact angle that the liquid (sugar solution) made with the glass surface was measured using video-microscopy. The solution wetted the glass surface at $\theta = 35.5^\circ$. The liquid cannot cross the free surfaces; the no-penetration boundary condition $\mathbf{n} \cdot \mathbf{v} = 0$ was used there. The position of the element nodes at the minimum gap on both the solid boundaries (roll and the coverslip) were fixed. Because the radius of curvature of the free surfaces was high and the geometry of the fluid domain was complex, the nodes were distributed on the boundary of the physical domain according to different stretching functions $g$ that controlled their spacing, $\xi_i = g(s)$, $i = 1$ or 2. This allowed better control (avoiding folding of the mesh onto itself) over the resulting mesh in the physical domain.
Figure 3.2: Boundary conditions on the mesh, momentum and continuity equations. At the inflow a free boundary condition is used. At the outflow the flow is fully developed. No slip and no-penetration condition was applied at the solid boundaries (roll and the coverslip). The position of the contact lines that the free surfaces make with the coverslip was not fixed, but the contact angle was fixed. A capillary force balance was used at the free surfaces.

At the solid impermeable surfaces (coverslip and the roll surface) a no-slip and no-penetration condition was used. The velocity of the liquid equaled that of the solid, \( \mathbf{v} = \mathbf{v}_s \) (\( v_s = 0 \) at the coverslip and \( v_s = \omega R \) at the roll). A force balance was used at the free surfaces where the shear stress exerted by the gas on the liquid was negligible and the curvature of the free surface induces a capillary pressure in the normal direction

\[
\mathbf{n} \cdot \mathbf{T} = \frac{2\mathcal{H}}{\text{Ca}} \mathbf{n} + p_0 \mathbf{n}
\]  

(3.6)

where \( \mathcal{H} \) is the mean curvature of the interface, \( \text{Ca} = \eta V / \sigma \) is the capillary number, \( V \) is the tangential roll speed, \( \eta \) is the liquid viscosity, \( \sigma \) is the surface tension of the liquid, and \( p_0 \) is the ambient pressure.

At the outflow boundary the flow was assumed to be fully developed \( \mathbf{n} \cdot \nabla \mathbf{v} = 0 \). An appropriate boundary condition is thus required at the synthetic incoming boundary. Because the flowrate and the velocity profile at the inflow were unknown, a free boundary condition was used at the inflow boundary (Papanastasiou et al. 1992). Papanastasiou et al. (1992) and Carvalho (1996) found that such a boundary condition yields realistic flow states by allowing the finite-element weighted residual equation to be satisfied in a weak sense, i.e., over the interior of the boundary. All the boundary conditions are shown in figure 3.2.

Other experimental parameters that were used in the formulation were the prop-
erties of the liquid (sugar solution): viscosity $\eta = 44.61$ m Pa s, surface tension $\sigma = 76.55$ N/m and density $\rho = 1400$ kg/m$^3$. Gravity was included in the calculation, though its effect was found to be negligible.

Computing a solution required following a systematic approach, and convergence of the solution depended on the initial guess (Christodoulou et al. 1997). The coupling of the mesh and the flow was allowed in parts. The inertial flow was first computed on a fixed domain, i.e., the free surfaces were treated as slippery boundaries. This flow field (velocity and pressure) was used as an initial guess for solving the coupled (mesh and flow) problem upstream of the minimum gap position $x < 0$; the mesh downstream $x > 0$ was kept fixed. Once the upstream free surface did not change significantly the entire mesh was allowed to deform as part of the solution. The location of the inflow and outflow sections—which are artificial boundaries—was moved away from the gap repeatedly until the solution (free surface profile, film thickness, etc.) proved insensitive to further lengthening of the inflow and outflow sections.

### 3.2.1 Results and discussion

Figure 3.3 shows the computed free surface liquid domain at $Ca = 0.0011$ (E4 and E8). Mesh 1 had 700 elements and Mesh 2 had 2600 elements. The number of elements at $x = 0$ in Mesh 2 (fine mesh) was twice that in Mesh 1 (coarse mesh). The meshes were computed as part of the flow solution. The details of the fine mesh are shown in figure 3.4. Note that because of the complex diverging and converging geometry the elements upstream and downstream of the minimum gap are distorted.

The size of the coating bead was computed to be $l = 4.3$ mm. Note that the contact angle that the free surfaces made with the solid coverslip was fixed but the position of the contact lines was not. Figure 3.5 shows that shape of the free surfaces computed with the two meshes: clearly, the results are independent of the discretization of the physical domain.

In a similar but large scale roll-plate set-up, Gaskell et al. (1998) found that at small flow rates (low capillary numbers) the pressure in the coating bead was entirely sub-ambient with an almost linear gradient typical of meniscus roll coating. Figure 3.6
Figure 3.3: Finite element mesh solution. (a) Mesh 1 (700 elements, 3264 degrees of freedom) and (b) Mesh 2 (2600 elements, 50844 degrees of freedom) of the roll-knife coating flowcell. The dimensions are in mm. The radius of the roll is $R = 6.35$ mm and the gap is $H = 35.3$ $\mu$m. The capillary number of the flow was $Ca = 0.0011$. The results correspond to the low capillary number experiments E4 and E8 (Chapter 2).
Figure 3.4: Detail of Mesh 2 (a) upstream of the minimum gap, (b) around the minimum gap, and (c) downstream of the minimum gap. The dimensions are in mm.
Figure 3.5: The free surface profiles, (a) upstream and (b) downstream, computed on the two meshes Mesh 1 and Mesh 2.

shows the pressure along the coverslip ($y = 0$) in the coating bead, delimited by the contact lines: the pressure is sub-ambient, with a linear gradient in the middle of the bead ($-0.5 \leq x \leq 0.5$ mm). Because the downstream free surface is less curved as compared to the upstream free surface, the pressure downstream was higher than that upstream causing a back-flow which yielded a Couette-Poiseuille flow in the bead (figure 3.7a). In the experiments at minimum gap ($x = 0$), the DNA molecules right next to the coverslip were observed flowing opposite to the direction of motion of the roll. The molecules started moving faster as the focal plane was lowered into the gap. On further lowering the focal plane, the molecules were observed traversing at slower speeds and a plane was found where the molecules were stationary (the zero velocity plane ZVP). On moving further down into the gap the molecules started moving along with the roll with increasing speeds. Tracking of the molecules next to the coverslip showed that a recirculation existed from the downstream to the upstream free surface. In agreement with experimental observations, streamlines computed in the flow field show this large recirculation below the coverslip (figure 3.8).

The computed velocity agreed well with the lubrication approximation solution (figure 3.7b); the difference in the computed flow rate and that measured experimen-
Figure 3.6: Pressure profile at the coverslip $y = 0$.

tally is 2.12%. The average velocity at the two observation planes E4 $y = -3.3 \, \mu m$ and E8 $y = -21.8 \, \mu m$ was measured by tracking the center of mass of the DNA molecules as they traversed the field of view (figures 3.9a and 3.9b). These velocities are also plotted in figure 3.7b for comparison.

On increasing the capillary number, the recirculation was experimentally observed to break into two smaller recirculations – upstream and downstream of the minimum gap. At higher capillary number the incoming film thickness becomes greater than the gap. The downstream free surface moves toward the minimum gap and the upstream free surface moves away from the minimum gap. The pressure profile inside the bead changes with the pressure upstream becoming greater than that downstream. The pressure difference becomes lower than the viscous drag and there is no backflow.

### 3.3 Successive-fine graining (SFG) applied to DNA in complex flow

In this section the Brownian dynamic simulations using the successive-fine graining (SFG) approach developed by Sunthar and Ravi Prakash (2005) is briefly reviewed.

To describe the dynamics of the polymer molecule, coarse grained models must include excluded volume interactions, hydrodynamic interactions and the finite size
Figure 3.7: (a) The velocity profile at the minimum gap $x = 0$ computed on Mesh 1 and Mesh 2. Mesh 2 has twice the number of elements at minimum gap ($x = 0$) than Mesh 1. Convergence in the velocity solution is clearly seen. (b) Comparison of the velocity profile at minimum gap ($x = 0$) computed by finite elements $\circ$ and that computed by lubrication approximation (solid line). The experimentally measured velocities at the various image planes (zero velocity plane ZVP, E4 and E8) are also plotted $\circ$. 
Figure 3.8: Streamlines in the low capillary number flow $Ca = 0.0011$ in the roll-knife coating flowcell. (a) color contour (b) streamlines showing the large recirculation below the coverslip (c) detail of the streamlines near the minimum gap. The dimensions are in mm.
Figure 3.9: Streamwise $v_x$ and transverse velocity $v_z$ of the ensemble of DNA molecules in experiments (a) E4 and (b) E8 (low capillary number flow, Chapter 2). The solid line is the average velocity and the broken lines are the upper and lower bounds using the standard deviations. The velocity computed by lubrication approximation and finite elements was (a) $-173 \, \mu\text{m/s}$ and $-174 \, \mu\text{m/s}$, respectively; (b) $231 \, \mu\text{m/s}$ and $236 \, \mu\text{m/s}$, respectively.
of the polymer chain. Empirically determined parameters are required to incorporate each of these phenomena into the theory. A polymer molecule is approximated with chains of different number of beads $N$. The solvent-chain segment interactions determine the size of a chain at equilibrium and is represented in the model by the parameter $z^*$, which defines the excluded volume interactions between two beads in a bead-spring chain. In good solvents the chains swell due to favorable solvent-chain interactions as compared to chain-chain interactions (Sunthar and Ravi Prakash 2005). The hydrodynamic interactions are measured in terms of $h^*$, the non-dimensional bead radius. The finite size of the polymer chain introduces the fully stretched length of each spring $b$. The parameters are a function of the choice $N$. It has been shown analytically (Yamakawa 1971) and using Brownian dynamics simulations (Kröger et al. 2000, Schäfer 1999) that the magnitude of the hydrodynamic interactions and the excluded volume interactions in polymer solutions near equilibrium can be expressed as $h \equiv h^*\sqrt{N}$ and $z \equiv z^*\sqrt{N}$, respectively. The various linear viscoelastic properties become universal in the limit $N \rightarrow \infty$. Sunthar and Ravi Prakash (2005) found that the universal behavior was obtained also in dynamic properties such as molecular extension in flows where the polymer chains deviated far from equilibrium. By using this approach, the observed molecular extension in the single molecule DNA experiments in elongational flow by Smith and Chu (1998) were predicted accurately without resorting to fitting the empirical parameters.

A dilute solution of DNA molecules is modeled as an ensemble of noninteracting chains, each of which has $N$ beads connected by massless springs representing an entropic force between two points on the polymer chain. If $\psi(\mathbf{r}_1, \ldots, \mathbf{r}_N)$ is the configurational distribution function of the bead positions $\mathbf{r}_i$, then the evolution of the distribution function is expressed by the Fokker-Planck equation

$$\frac{\partial \psi}{\partial t} = - \sum_{i=1}^{N} \frac{\partial}{\partial \mathbf{r}_i} \cdot [\mathbf{\kappa} \cdot \mathbf{r}_i + \frac{1}{\zeta} \sum_{j=1}^{N} \mathbf{\Gamma}_{ij} \cdot (\mathbf{F}_{ij}^S + \mathbf{F}_{ij}^{int})] \psi + \frac{k_B T}{\zeta} \sum_{i,j=1}^{N} \frac{\partial}{\partial \mathbf{r}_i} \cdot \mathbf{\Gamma}_{ij} \cdot \frac{\partial \psi}{\partial \mathbf{r}_i}. \quad (3.7)$$

Here, $\mathbf{\kappa} \equiv \nabla \mathbf{v}^T$ is a time-dependent, homogeneous, velocity gradient tensor of the fluid surrounding the chain’s center of mass. The assumption is that the velocity field across the size of the molecule is approximately linear. $\zeta$ is the hydrodynamic friction
(drag) coefficient associated with the bead, $k_B$ is Boltzmann constant, and $\Gamma_{ij}$ is the hydrodynamic interaction tensor, representing the effect of the motion of a bead $i$ on another bead $j$ through the disturbances carried by the surrounding fluid. $F_i^S$ is the entropic spring force on bead $i$ due to adjacent beads, $F_i^S = F^c(Q_i) - F^c(Q_{i-1})$, where $F^c(Q_{i-1})$ is the force between the beads $i-1$ and $i$, acting in the direction of the connector vector between the two beads $Q_i = r_i - r_{i-1}$. The spring force used for modeling DNA is a worm-like chain (WLC) force that was experimentally verified by Smith et al. (1992)

$$F_{WLC}^c(Q) = KQ \frac{1}{6q} \left\{ 4q + \frac{1}{(1-q)^2} - 1 \right\}$$

(3.8)

where $q = Q/Q_o$ the ratio of the magnitudes of the connector vector $Q$ and the fully stretched spring length $Q_o$. $K$ is the spring constant.

The quantity $F_i^{int}$ is the sum total of the remaining interaction forces on the bead $i$ due to all other beads and here is equal to the excluded volume interactions. A narrow-Gaussian potential is used to define the excluded volume interactions between a pair of beads separated by a distance $d$

$$F^e(p) = k_BT \frac{\nu}{(2\pi \lambda_H^3)^{3/2}} e^{-r^2 / 2d^2} p$$

(3.9)

where $\nu \equiv z^*(2\pi \lambda_H^3)^{3/2}$ is the excluded volume parameter, $\lambda_H \equiv \sqrt{k_BT / K}$ is the reference length scale and $d$ is the range of interaction of the potential function.

Equation 3.7 was non-dimensionalized using length scale $l_H$ and time scale $\lambda_H \equiv \zeta/(4K)$,

$$\frac{\partial \psi}{\partial t^*} = -\sum_{i=1}^N \frac{\partial}{\partial r_i^*} \left[ \kappa^* \cdot r_i^* + \sum_{j=1}^N \Gamma_{ij}(h^*) \cdot (F_j^S + F_j^{int}(z^*, d^*)) \right] \psi + \sum_{i,j=1}^N \frac{\partial}{\partial r_i^*} \Gamma_{ij}(h^*) \cdot \frac{\partial \psi}{\partial r_i^*}$$

(3.10)

Brownian dynamics simulations were performed by integrating a stochastic differential equation (equation 3.11) equivalent to the Fokker-Plank equation (equation 3.10) using the procedure described by Prabha B. Prakash (2004)

$$dR = \left[ K \cdot R + \frac{1}{2} D \cdot F \right] dt^* + B \cdot dW$$

(3.11)
Here, $R$ (3N entries) contains the position vectors of the $N$ beads. $K$ contains $N \times N$ blocks of $3 \times 3$ matrices with diagonal blocks equal to $\kappa^*$ and off diagonal blocks equal to 0. $F$ contains $3N$ components of the spring force $F^S$ and the excluded volume force $F^{nt}$. $D$ is the diffusion matrix that contains $N \times N$ blocks consisting of $\Gamma_{ij}$, $W$ is a 3N-dimensional Wiener process, and $D = B \cdot B^T$.

### 3.3.1 Streamline tracing

The complex roll-knife coating flow is inhomogeneous, i.e., $\kappa$ is different at each position. The algorithm used by Sunthar and Ravi Prakash (2005) was suitably modified such that in a Lagrangian frame of reference (i.e., a frame moving with the fluid velocity) $\kappa$ was time-dependent. The velocity gradient tensor $\kappa$ was computed along the streamline which enabled computation of the DNA conformation. In the Brownian dynamics simulations the stochastic differential equation is solved at equal time-steps, which require $\kappa$ input at each time-step. The velocity gradient was computed from the macroscopic flow solution obtained by finite-element method by the following procedure:

- A streamline passing through the point of interest was computed from the post-processor developed by Pasquali (2000). The point of interest was located in the computational domain and the streamfunction was evaluated. The contour line with this streamfunction value was computed in the mesh and served as the coarse trace along which the DNA molecule moved in the flow field.

- The tracer (DNA molecule) was constrained to move in equal time-steps along the coarse trace by computing the displacement using the velocity at the previous point. The velocity at each point was interpolated using the finite-element solution. Figure 3.10 shows the schematic of the streamline tracing methodology

\[
x_{n+1} = x_n + v_n \Delta t
\]

(3.12)

where $n$ is the number of trace point, and $x_n$ and $x_{n+1}$ are positions of trace point at time $t_n$ and $t_{n+1} = t_n + \Delta t$, respectively. Figure 3.10 shows the trace points at
time $t_n$, $t_{n+1}$ and $t_{n+2}$, inside a 9-node element formed of the mesh. The element was subdivided into 8 triangles and the velocity $v_n$ and the velocity gradients $\kappa_n$ were interpolated at location $x_n$ using piecewise linear shape functions and the macroscopic flow solution (velocity and velocity gradients) at the 9 nodes.

Figure 3.11 shows an example of the computed trajectory, at equal time steps, along which the DNA molecules move on a streamline. The DNA molecules traverse the minimum gap position ($x = 0$) at $y = -20 \mu m$.

### 3.3.2 DNA modeling

In the SFG approach only three equilibrium properties are required to describe the polymer molecule: the radius of gyration in $\theta$-solvent, the solvent quality $\zeta$ and the contour length $L$. The measured length $L$ of $\lambda$-DNA stained with YOYO-1 is 22 $\mu m$ (Perkins 1997). Because in this study the ratio of the dye molecules to the DNA base-pairs was the same, the contour length was $L = 22 \mu m$. The number of Kuhn steps $N_k$ required to represent a DNA molecule can be found using a number of empirical measurements such as diffusivity, persistence length and radius of gyration. In literature $N_k$ values for YOYO stained DNA vary from 150 to 300. Sunthar and
Figure 3.11: Streamline tracing at $y = -0.0020$ cm and $x = 0$. The solid line is the coarse streamline contour. The symbols are the trace points computed using equation 3.12 at equal time steps $\Delta t = 6.9 \times 10^{-2}$ s. Note that the time steps used in the computation are much smaller than shown here.

Figure 3.12: Successive-fine graining approach applied to model DNA molecules. A bead-spring chain is successively fine grained and results are extrapolated in the limit of a bead-rod chain with $N_k$ Kuhn steps.
Ravi Prakash (2005) suggested using $N_k \sim 250$ in the simulations. Otherwise, $N_k$ can be calculated using experimentally measured contour length and the equilibrium radius of gyration in $\theta$-solvent ($R_g \sim 0.53 \mu m$; Sunthar and Ravi Prakash (2005)) from
\[ N_k \equiv \frac{L^2}{6R_g^2} = 287. \quad (3.13) \]

Sugar solutions are good-solvents, and the DNA molecule is swollen at equilibrium. The solvent quality could be determined empirically by performing swelling experiments but no such study is available for $\lambda$-DNA. Sunthar and Ravi Prakash (2005) found that solvent quality $z = 1$ gave quantitative agreement of the molecular stretch with DNA experiments by Chu and co-workers.

DNA is modeled as a chain of $N$ beads. The number of beads is progressively increased and predictions are made by extrapolating to the underlying bead-rod chain, $N \to N_k$ (figure 3.12). The two basic units used in the model are the length scale $l_H$ and the time scale $\lambda_H$. In order to obtain dimensional quantities, model variables must be multiplied by an appropriate combination of $l_H$ and $\lambda_H$. For example, the dimensional stretch along the x-axis of the polymer molecule is given by
\[ x = x^*l_H \quad (3.14) \]
and the time step of integration
\[ \Delta t = \Delta t^*\lambda_H. \quad (3.15) \]

The velocity gradient tensor $\kappa$ was made dimensionless by
\[ \kappa^* = \kappa \frac{\lambda_1}{\lambda^*_1} \quad (3.16) \]

where $\lambda_1$ is the experimentally determined longest relaxation time for DNA in the sugar solvent ($\lambda_1 = 4.24$ s) and $\lambda_1^*(N, b, h^*, z^*, d^*)$ is the dimensionless relaxation time of the bead-spring chain. Sunthar and Ravi Prakash (2005) have shown that the predictions of the non-dimensional relaxation time is insensitive to $N_k$ in the range 150 to 300. Parameter values were calculated for worm-like chains with $N_k = 250$ in
solvent with viscosity $\eta = 44.6$ mPa s using

$$ h^* = \chi \tilde{h}^* $$
$$ z^* = \chi^2 z N^{-1/2} $$
$$ d^* = K z^{*1/5} $$

where $\tilde{h}^*$ and $K$ are arbitrary parameters from the model for hydrodynamic interactions and excluded volume interactions. Note that since the molecular approach gives parameter free predictions, any value of $\tilde{h}^*$ an $K$ can be used. Here $\tilde{h}^* = 0.23$ and $K = 1$ were used (Sunthar and Ravi Prakash 2005). $\chi(b)$ is a function in the model that depends on the force law and was computed numerically. $b = Q_o^2/\ell^2_H$ is the square of the non-dimensional spring length at full stretch and is a function of $N$. All the parameters used in these simulations are listed in table 3.1.

The following properties are considered for discussion in this thesis. The instantaneous stretch or the extension of the chain along $x$ and $y$ direction is defined as

$$ L_x = \max_i (r_{i,x}^*) - \min_i (r_{i,x}^*) $$
$$ L_y = \max_i (r_{i,y}^*) - \min_i (r_{i,y}^*) $$

where $r_{i,x}^*$ and $r_{i,y}^*$ are the $x$ and $y$ components of the position vector $\mathbf{r}_i^*$ of bead $i$. The ensemble ($N_{\text{traj}}$ is the number of chains) average stretch or the mean stretch is defined as

$$ \langle L_x \rangle = \frac{1}{N_{\text{traj}}} \sum_{n=1}^{N_{\text{traj}}} L_x^n $$
$$ \langle L_y \rangle = \frac{1}{N_{\text{traj}}} \sum_{n=1}^{N_{\text{traj}}} L_y^n $$

The overall stretch of the chain in the two-dimensional plane ($x - y$) is defined as

$$ \langle R_c \rangle = \sqrt{\langle L_x \rangle^2 + \langle L_y \rangle^2} $$

and the mean square end-to-end distance of a chain is defined as

$$ \langle R_e^2 \rangle = (\mathbf{r}_N^* - \mathbf{r}_1^*) \cdot (\mathbf{r}_N^* - \mathbf{r}_1^*) $$
Table 3.1: Parameter values used in the Brownian dynamics simulations. The worm-like chain force law was used with $N_k = 250$. $\tilde{h}^* = 0.23$ and $K = 1$. The corresponding longest relaxation times obtained from equilibrium simulations are listed for solvent quality $z = 1$ and viscosity $\eta = 44.6$ mPa s.

<table>
<thead>
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<th>$N$</th>
<th>$b$</th>
<th>$\chi(b)$</th>
<th>$\lambda_p^*$</th>
<th>$\lambda_H$ (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>127.26</td>
<td>0.945</td>
<td>8</td>
<td>0.69</td>
</tr>
<tr>
<td>11</td>
<td>69.10</td>
<td>0.922</td>
<td>16.3</td>
<td>0.39</td>
</tr>
<tr>
<td>19</td>
<td>33.59</td>
<td>0.880</td>
<td>34.5</td>
<td>0.21</td>
</tr>
<tr>
<td>27</td>
<td>20.83</td>
<td>0.840</td>
<td>55.4</td>
<td>0.15</td>
</tr>
</tbody>
</table>

The (ensemble average) orientation $\theta$ of the chain is computed from the eigenvectors of the radius of gyration tensor $\mathbf{R}_g$ which is equivalent to the second moment of the position of the beads in the chain with respect to the center of mass $\mathbf{r}_c$.

$$R_g^2 = \langle \frac{1}{N} \sum_{i=1}^{N} |\mathbf{r}_i - \mathbf{r}_c|^2 \rangle_{\mathbf{r}_c, \mathbf{r}_i}. \quad (3.26)$$

### 3.3.3 Results and discussion

The DNA molecule was described by a bead-spring chain with $N = 7$, 11, and 19 beads. Experimental results on DNA stretching were available at the minimum gap ($x = 0$); the two image planes were located at $y = -3.3$ $\mu$m and $y = -21.8$ $\mu$m (termed E4 and E8 respectively).

#### Time step convergence

A non-dimensional time step for the simulation was chosen as $\Delta t^* = 10^{-3}$. This dimensionless time was converted to real time (dependent on $N$) and used to obtain $\mathbf{k}(t)$ along a flow streamline. A smaller time step $\Delta t^* = 10^{-4}$ required more computation time, and it was found that the microscopic quantities predicted were converged in time. Figure 3.13 shows the mean fractional extension for $N = 7$ chains for $\Delta t^* = 10^{-3}$ and $\Delta t^* = 10^{-4}$. The two curves overlap within error bars; thus, the larger time step $\Delta t^* = 10^{-3}$ was used in subsequent simulations.
Figure 3.13: Mean fractional extension $(x)/L$ as a function of time of an ensemble of 1000 chains with $N = 7$ flowing along the streamline passing through $x = 0, y = -3.3 \mu m$. Two traces with time step $\Delta t = 6.9 \times 10^{-4}$ s and $\Delta t = 6.9 \times 10^{-5}$ s are plotted.
Figure 3.14: Mean fractional extension \( \langle x \rangle / L \) as a function of time of an ensemble of 100 chains with \( N = 7 \) flowing along the streamline passing through \( x = 0, y = -21.8 \) \( \mu \)m. The starting configuration, equilibrium (red) and steady shear at \( \partial v / \partial y = (\partial v / \partial y)_{y=-21.8} \) (blue), did not affect the fractional extension at the end of the revolution.

**Initial configuration**

The DNA molecules moving along the two experimentally investigated image planes were trapped in a recirculation. In the simulations, the initial configuration of the chain was unknown. It was found that the initial configuration of the ensemble at the starting location along the streamline in the recirculation had no effect on the chain conformation at the end of one complete circulation. Figure 3.14 shows mean fractional extension of an equilibrium ensemble of 100 chains (\( N = 7 \)) and another ensemble of 100 chains initialized to steady state under the action of pure shear flow (\( \dot{\gamma} = \partial v / \partial y \)), evolving along the recirculating streamline. Note that at the end of one cycle, which was the point of observation \( x = 0 \), both the ensembles predict equal extensions (within the error bars).
Figure 3.15: Mean fractional extension $<x>/L$ as a function of time of an ensemble of 100 chains with $N = 7$ flowing along the streamline passing through $x = 0, y = -21.8 \, \mu m$. The chains were allowed to circulate 4 times.

**Convergence in recirculations**

Because diffusion across streamlines is neglected, the chains that are part of the recirculation cycle around continuously. It was found that the computed ensemble-averaged stretch repeated itself after the first complete cycle around in the recirculation. Figure 3.15 shows the ensemble averaged stretch of 100 chains ($N = 7$) over four continuous cycles. The mean fractional extension (along $x$) at $x = 0, y = -21.8 \, \mu m$ after the first four loops were $0.91 \pm 0.01, 0.90 \pm 0.02, 0.91 \pm 0.01$ and $0.91 \pm 0.01$, respectively, i.e, they coincided within their variance. In subsequent simulations, microscopic quantities are reported at the end of one cycle.
Figure 3.16: Extension map of N=7 bead-spring chain in the two-dimensional flow. (a) Streamline through $x = 0, y = -7 \mu m$ in the recirculation region. (b) Ensemble averaged orientation of the chains as a function of position. Zero is along the x-axis or the flow direction. (c) Mean fractional extension along x-direction as a function of position. (d) Mean fractional extension along y-direction as a function of position. A-H are position markers for easier reading. The results are for an ensemble of 100 chains and the error bars are not shown for clarity.
Molecular configuration along streamlines

Before comparing the simulation results with experiments it is important to understand the effect of flow on the molecular stretch as the chains traverse along the streamline in the large recirculation. Figures 3.16c and 3.16d show the ensemble averaged fractional molecular extension along the $x$ and $y$ axis for 7 bead chains along streamline through $x = 0, y = -7 \mu m$ (figure 3.16a). The ensemble started with $\sim 50$% extension along $x$ (extensions after completion of one recirculation are shown) at position A. Since this was above the zero-velocity plane, the chains flowed opposite the roll ($x < 0$) and are aligned with the $x$ axis (flow direction) as shown in figure 3.16b. The chains maintain their extension along $x$ as they approach the free-surface and the contact line at B (figure 3.16b). As they move along the free surface (C), the chains rotate (figure 3.16b) and the extension along $y$ increase. The molecules almost reach full extension along $x$ as they cross D, the stagnation point at the free-surface. The principal axis of extension at D is normal to the free surface (figure 3.17a), and the molecules reach high extensions along $x$ as they pass through D onward to E. This stretched configuration is maintained as the chains cross E, the minimum gap position $x = 0$, because the time taken by the molecule to go from D to E is smaller than the relaxation time. At the stagnation point F on the downstream free surface, the principal axis of compression is normal to the free surface (figure 3.17b), hence the molecules compress along $x$; while the principal axis of extension is along the free-surface and the molecules extend along $y$ (from G to H). The extended molecules rotate to lie along the $x$ axis as they passed near the contact line at H (figure 3.16b). This extension mapping is qualitatively similar on all streamlines in the recirculation region that cross the minimum gap. Figure 3.18 shows snapshots of a single 11 bead chain traversing along a streamline through $x = 0, y = -3.3 \mu m$. Note the highly extended state at F as compared to the configuration at Q. The stretch-tumble dynamics of DNA observed in pure shear flow can be seen from positions M to Q.

Figures 3.19 and 3.20 show mean fractional extension along $x$-direction of 1000
Figure 3.17: Extension dominated flow regions at the free-surface in the roll-knife coating flow at low capillary number. (a) At the upstream position, the principal axis of extension is normal to the free surface (almost along the direction of motion of the roll). (b) At the downstream position, the principal axis of compression is normal to the free surface.
Figure 3.18: A single chain of 11 beads moving along the streamline through $x = 0, y = -3.3 \, \mu m$. Snapshots of the chain at different positions along the streamline are shown. The end beads of the chain are marked in different colors to show the change in the orientation of the molecule as it flows.
chains as a function of time along streamlines corresponding to the two experiments E4 and E8. The $x$-extension (after extrapolating to $N = 250$) at E4 was 0.54 ± 0.03 and that at E8 was 0.93 ± 0.01. The microscopic $x$-extension measured in the experiment (E4; E8) were lower than these predicted values. The prediction at E8 differed from the experiment by 149% (experimental measurement 0.37 ± 0.17), while that at E4 differed by 41% (experimental measurement 0.33 ± 0.13). Figure 3.21d compares the predicted molecular conformation with the experimental observation. Note that blooming and limited resolution result in the larger measurement of the transverse dimensions of DNA (along the vorticity).

The difference in the computed fractional extensions at the two $y$ planes suggests that molecular conformation is position dependent at the minimum gap. This is expected because each plane has a different shear rate and the hydrodynamic drag that the chains experience would thus be different, leading to position dependent extensions. However, in pure homogeneous shear flows, the mean fractional extension is expected to grow gradually and asymptote to 0.5 (Smith et al. 1999, Hur et al. 2000). Although experimental information was obtained at only two planes, simulations were performed at different $y$ planes at $x = 0$. Figure 3.23 shows the mean fractional extension of chains of different beads as a function of position at the minimum gap. An abrupt change in the extension computed by Brownian dynamics was found at the zero-velocity plane. The chains reached about 50% extension in the back-flow region (molecules moving against the roll); below the zero-velocity plane, i.e., in the forward-flow region, the chains approached almost full extension (figure 3.27). The chains arrived at the minimum gap position below the zero velocity plane after experiencing a strong extensional flow at the upstream free surface (with the principal axis of extension nearly aligned along the roll; figure 3.17). The high velocities did not allow the molecules to relax and the molecules remained extended along the roll with near full extension. In none of the DNA experiments at the low capillary number (Chapter 2) the measured extensions were higher than ~50%. The experimental settings—the geometry (gap), viscosity of the liquid and the roll speed—in each experiment were different but the capillary numbers were low and a large recirculation
Figure 3.19: (a) Position $x$ of the chain as a function of time. (b) Mean fractional $x$-extension $\langle L_x \rangle / L$ as a function of time $t$ along the streamline passing through $x = 0, y = -3.3 \mu m$ (E4). The ensemble had 1000 DNA chains. Results for $N = 7, 11, 19$ and 27 are shown.
Figure 3.20: Mean fractional $x$-extension $\langle x \rangle / L$ as a function of time $t$ along the streamline passing through $x = 0, y = -21.8 \mu m$ (E8). The ensemble had 1000 DNA chains. Results for $N = 7, 11$ and 27 are shown.
Figure 3.21: (a) Mean fractional x-extension $\langle L_x \rangle / L$ as a function of number of beads $N$ in E4 ($x = 0$ and $y = -3.3 \text{ \mu m}$). (b) Mean fractional extension $\langle R_t \rangle / L$ as a function of number of beads $N$ at $x = 0$, $y = -3.3 \text{ \mu m}$. (c) Mean orientation $\theta$ as a function of number of beads $N$ at $x = 0$, $y = -3.3 \text{ \mu m}$. (d) Comparison of the molecular conformation at $x = 0$, $y = -3.3 \text{ \mu m}$ between Successive-Fine Graining in the limit $N \rightarrow N_k$ and the experiment. The major and minor axis are normalized with the contour length. The experimental measurement is also plotted.
Figure 3.22: (a) Mean fractional x-extension $\langle L_x \rangle / L$ as a function of number of beads $N$ in E8 ($x = 0$ and $y = -21.8 \, \mu m$). (b) Mean fractional extension $\langle R_e \rangle / L$ as a function of number of beads $N$ at $x = 0, y = -3.3 \, \mu m$. (c) Mean orientation $\theta$ as a function of number of beads $N$ at $x = 0, y = -3.3 \, \mu m$. (d) Comparison of the molecular conformation at $x = 0, y = -21.8 \, \mu m$ between Successive-Fine Graining in the limit $N \rightarrow N_k$ and the experiment. The major and minor axis are normalized with the contour length. The experimental measurement is also plotted.
similar to that in experiments (and simulations) E4 and E8 was observed below the coverslip. It can be argued that a qualitatively similar recirculation region would be computed from a macroscopic flow calculation in each experiment and a similar extension discontinuity at the zero-velocity plane would be computed by Brownian dynamics. Thus, it is essential to understand the disagreement between the measurements and the computations particularly in the forward-flow region (below the zero velocity plane, near the roll).

To ascertain that the extension domination in the flow at the minimum gap position is not due to noise from the macroscopic flow calculation, $\kappa$ was determined on the coarse mesh. Recall that the macroscopic flow was converged in the two meshes; thus results from both the flow solutions should give microscopic predictions within error bars, and the differences in the simulation results would be expected from numerical errors and gradient interpolation. The coarse mesh had half the number of elements at $x = 0$ than the fine mesh; thus the interpolated velocity gradients in the coarse mesh should have lower accuracy. Figure 3.24 shows that the predicted mean fractional extension does not depend on the discretization of the flow field. The microscopic predictions computed on the two meshes do not agree only in the regions near the upstream and downstream free surface, where the curvature of the streamline is very large (stagnation point). However, this small discrepancy had no effect on the microscopic predictions, within error bars, at the minimum gap ($x = 0$; the point of interest).

**Homogeneous mixed flow**

The two-dimensional roll-knife coating flow is a mixed flow, i.e., the flow is a combination of rotation and extension. In homogeneous mixed flows Hur et al. (2002) and Babcock et al. (2003) found that when the strain rate exceeded the vorticity even slightly (extension dominated flows), the polymer molecules deformed to almost fully stretched states and when the vorticity exceeded the strain rate (rotation dominated flows) the molecules deformed in a periodic motion with an average extension lower than that observed in simple shear flows. The flow at $x = 0$ was investigated to
Figure 3.23: Mean fractional extension $\langle x \rangle / L$ as a function of position in the gap $y$ along the streamlines passing through $x = 0$. The ensembles have 1000 chains. Results for $N = 7, 11$ and 19 are shown. The chains were allowed to complete one recirculation. At $y \geq 29 \mu m$ the streamlines were not part of the recirculation.
Figure 3.24: (a) Mean fractional extension \(\langle L_e \rangle/L\) as a function of position \(x\) of an ensemble of 100 chains with \(N = 7\) flowing along the streamline passing through \(x = 0, y = -16 \ \mu m\). The velocity gradient \(\kappa(t)\) was computed in the coarse finite element mesh (○) and in the fine finite element mesh (solid line). For clarity the error bars are not shown. (b) Mean fractional overall stretch \(\langle R_e \rangle/L\). (c) Mean fractional end-to-end distance \(\langle R_o \rangle/L\). (d) Mean orientation \(\theta\) in the \(x - y\) plane.
determine the flow type, whether it is extension dominated or rotation dominated. An analysis of the flow using the Schunk and Scriven (1990) approach (Chapter 2) on the finite-element solution showed that the flow had a slight extension component (figure 3.25). Babcock et al. (2003) classified mixed flows according to the value of a parameter $\lambda$

$$D \equiv \frac{1}{2} (\nabla v + \nabla v^T)$$

$$\Omega \equiv \frac{1}{2} (\nabla v - \nabla v^T)$$

$$\|D\| \equiv \sqrt{\frac{1}{2} \sum_i \sum_j D_{ij}^2}, \|\Omega\| \equiv \sqrt{\frac{1}{2} \sum_i \sum_j \Omega_{ij}^2}$$

$$\lambda = \frac{\|D\| - \|\Omega\|}{\|D\| + \|\Omega\|}$$

where $\lambda = 0$ is pure shear and $\lambda = 1$ is pure extension. This analysis procedure applied to the region of interest ($x = 0$) also showed (figure 3.25) that the flow field had a slight extension below the zero velocity gradient plane ($y < -9 \mu m$), but a slight rotation above the zero velocity gradient plane ($y > -9 \mu m$). It should be noted that the parameter $\lambda$ is not frame invariant, i.e., if the frame of reference is put on the roll, the value of $\lambda$ changes and becomes position dependent because although $D$ remains the same, $\Omega$ changes to $\Omega + \mathbf{W}$ ($\mathbf{W}$ is the vorticity). In steady, homogeneous flows the two classification approaches are equivalent but in steady, inhomogeneous flows the Schunk and Scriven (1990) approach is more appropriate. Note that both flow classification approaches produce an abrupt change from extension to rotation around $y = -9 \mu m$ because the velocity gradient is close to zero; thus, both $\lambda$ and $\|w_{ref}\|/S$ are undefined.

Homogeneous flows with $\kappa$ obtained at different depths at $x = 0$ were applied to the ensemble of chains. It was found (figure 3.26) that the slight extension below the zero velocity gradient plane ($y < -9 \mu m$) was enough to cause the molecules to extend more than that in pure shear (50% molecular extension). The computed extension (along $x$) grows monotonically at $y < -9 \mu m$, unlike the abrupt discontinuity computed in the recirculation simulations (figures 3.23 and 3.27). This reinforces that
Figure 3.25: Analysis of the flow type at x = 0 by Schunk and Scriven (1990) analysis (||\(w_{rel}\)||/S) and by Babcock et. al. (2003) analysis (\(\lambda\)). The former shows that the flow is slightly dominated by extension at all y positions while the latter shows that the flow is rotation dominated at \(y < 9 \mu m\) and extension dominated at \(y > 9 \mu m\). Note that at \(y \sim 9 \mu m\) all the components of the velocity gradient tensor are almost zero, hence the abrupt change from rotation to extension, where both the flow analysis methods are undefined.

the strain history is important in determining the polymer configuration in inhomogeneous flows.

**Effect of wall hydrodynamics**

Jendrejack et al. (2003) and Jendrejack et al. (2004) performed Brownian dynamics simulations and found that in a rectangular micro-channel wall hydrodynamics (interactions of chain with solid impenetrable boundaries) were important in determining the cross-sectional concentration profile of chains. A migration away from the walls and towards the center of the channel was observed. In recent single molecule DNA experiments in a Poiseuille flow through a micro-channel Fang et al. (2005) confirmed that DNA migrated away from the walls and a depletion layer (near the
Figure 3.26: (a) Mean fractional extension $\langle x \rangle / L$ as a function of position $y$ of an ensemble of 100 to 1000 chains with $N = 7$, 11, and 19 under homogeneous flow $\kappa(x = 0, y)$. (b) Weissenberg number, $Wi = \lambda \sqrt{-I_{2D}^{2D}}$ as a function of position in the gap at $x = 0$. $I_{2D}^{2D}$ is the second invariant of $2D$ and $\lambda$ is the relaxation time of DNA.
Figure 3.27: Extrapolated mean fractional $x$-extension $(x)/L$ as a function of position $y$ in the gap for mixed homogeneous flow (red, ■) and for inhomogeneous flow in the recirculation (black, •). The extensions were extrapolated in the limit $N_k \to 250$. 
wall) of about one-third of the contour length of DNA (7 µm) existed. They also
found that in this depletion layer DNA stretch less than in the bulk. Woo et al.
(2004a) also performed Brownian dynamics simulations accounting for confinement
and showed that the effect of the wall on the microscopic properties extended beyond
$20R_g = 14$ µm: confinement (proximity to walls) altered the entropic spring force in a
bead-spring chain because of decrease in the configuration space of the chain, and the
hydrodynamic interaction with the walls increased the viscous drag that the chain
experiences. In the Brownian dynamics simulations in this thesis, wall hydrodynamic
effects and entropic confinement spring force due to interactions between the chains
and the roll surface and the coverslip have been neglected. DNA molecules flowed
through a small gap (about 40 to 50$R_g$) and experienced “confinement”. DNA were
observed 3.3 µm away from the coverslip in experiment E4 and 13.5 µm away from
the roll in experiment E8; thus, they lie within this predicted low extension region.

The low extension measurement in the experiments and the importance of the wall
hydrodynamic effects can be explained in the framework developed by Jendrejjack
et al. (2003), Jendrejjack et al. (2004) and Fang et al. (2005). If contributions from
the wall are neglected, i.e., in bulk, the hydrodynamic interaction from a point force
in space is anisotropic and symmetric (Jendrejjack et al. 2004). The point force on the
liquid can originate because a polymer molecule is stretched away from its equilibrium
coiled configuration. The polymer molecule can be simply modeled as a dumbbell—
two beads connected by a spring. If the spring is stretched by $Q$ from its equilibrium
state and released inside an infinite liquid domain of viscosity $\eta$, then the spring force
acting on the two beads along the connector vector can be described by a simple force
law $F = KQ$. For simplicity, let this be along the x-axis (figure 3.28) and one of the
beads is placed at $(0,0)$. Due to the force in the spring, there is an equal and opposite
force that the bead imparts on the surrounding fluid. The local flow $v$ induced by
this point force $F$ at position $r$ is described by the Oseen-Burgers tensor $\Omega$,

$$v = \Omega \cdot F$$ (3.31)
\[ \Omega \equiv \frac{1}{8\pi \eta r} \left( I + \frac{rr}{r^2} \right). \] (3.32)

As expected (Jendrej et al. 2004) the effect of this point force on the flow field is symmetric (figure 3.28). The effect of the point force decays almost linearly with distance; the effect of the stretch \( Q \) is felt to a distance \( Q \) from the single bead (Fang et al. 2005). This simple analysis can be crudely extended in the presence of an impenetrable wall. Introducing a wall parallel to the dumbbell dampens the induced flow field, for example, if the wall is placed below the dumbbell an upward thrust develops on the beads forcing the dumbbell to move away from the wall (Jendrej et al. 2004). Thus, molecules whose end-to-end distance is larger than the distance from the wall drift away from the wall and the probability of finding such configurations at that particular plane is reduced.

Table 3.2 lists the distances of the imaging planes in the DNA experiments. Almost all the DNA conformations and stretches in the forward flow region were measured at planes with distances \( 13R_g \) to \( 30R_g \) away from the roll surface. The experimental measurements of fractional extensions in the forward flow region were \( \sim 50\% \) and lower than the expected (simulations without wall effects) near-full extensions (\( \sim 20 \mu m \)).

**Chains crossing walls and free surfaces**

Because the chains reach almost full extensions in the micro-scale flow, it is possible that in the simulations a part of the chain may cross the liquid domain boundaries—the solid roll surface, the solid coverslip, and the free surfaces. Single chains were simulated and tracked along the streamline corresponding to \( E4 \). Figure 3.29 shows snapshots of chains of 7 beads near the upstream and downstream free surfaces. Clearly, at a number of locations along the streamline the chains cross the liquid domain. In the simulations it was assumed that the velocity is linear across the dimensions of the chain configuration, i.e., all the beads feel equal velocity gradient. The applied velocity gradient was interpolated at the center of mass of the chain that moved along the streamline. In the minimum gap region, where the velocity changes
Figure 3.28: Effect of a point force induced due to a finite extension $Q$ in the spring of a simple dumbbell representation of a polymer chain. One of the beads is positioned at $x = 0, y = 0$. The force induces velocity in the surrounding fluid. Streamlines and the velocity vectors in the flow are shown.
Table 3.2: Location of the image planes (in depth) at the minimum gap position for the various local Weissenberg numbers and the experimental conditions. R: Roll; C: Coverslip; $R_g = 0.7 \mu m; L = 22 \mu m$.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>$W_i$</th>
<th>$y$</th>
<th>Minimum Gap ($\mu m$)</th>
<th>ZVP</th>
<th>Flow Direction</th>
<th>Distance from Wall ($\mu m$)</th>
<th>Extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>84</td>
<td>-30</td>
<td>-41.7</td>
<td>-27.2</td>
<td>Forward</td>
<td>11.7 (R; 17$R_g$; 0.5$L$)</td>
<td>0.36</td>
</tr>
<tr>
<td>E2</td>
<td>106</td>
<td>-32.9</td>
<td>-42.1</td>
<td>-28.1</td>
<td>Forward</td>
<td>9.2 (R; 13$R_g$; 0.4$L$)</td>
<td>0.35</td>
</tr>
<tr>
<td>E3</td>
<td>147</td>
<td>-31.8</td>
<td>-55.5</td>
<td>-33.8</td>
<td>Back</td>
<td>31.8 (C; 45$R_g$; 1.4$L$)</td>
<td>0.47</td>
</tr>
<tr>
<td>E4</td>
<td>176</td>
<td>-3.3</td>
<td>-35.3</td>
<td>-18.7</td>
<td>Back</td>
<td>3.3 (C; 5$R_g$; 0.15$L$)</td>
<td>0.32</td>
</tr>
<tr>
<td>E5</td>
<td>195</td>
<td>-16.6</td>
<td>-35.3</td>
<td>-16.6</td>
<td>No Flow</td>
<td>16.6 (C; 24$R_g$; 0.75$L$)</td>
<td>0.50</td>
</tr>
<tr>
<td>E6</td>
<td>217</td>
<td>-26.3</td>
<td>-45.8</td>
<td>-20.2</td>
<td>Forward</td>
<td>19.5 (R; 28$R_g$; 0.9$L$)</td>
<td>0.49</td>
</tr>
<tr>
<td>E7</td>
<td>302</td>
<td>-25.4</td>
<td>-45.8</td>
<td>-17.3</td>
<td>Forward</td>
<td>20.4 (R; 29$R_g$; 0.9$L$)</td>
<td>0.53</td>
</tr>
<tr>
<td>E8</td>
<td>361</td>
<td>-21.8</td>
<td>-35.3</td>
<td>-18.7</td>
<td>Forward</td>
<td>13.5 (R; 19$R_g$; 0.6$L$)</td>
<td>0.37</td>
</tr>
</tbody>
</table>
Figure 3.29: Snapshots of a 7 bead chain, traversing along streamline corresponding to E4 ($x = 0$ and $y = -3.3$ μm). (a) Near the upstream free surface. (b) Near the stagnation point at the upstream free surface. (c) Near the stagnation point at the downstream free surface. The liquid domain boundaries—coverslip, roll and the free surfaces—are plotted in red. The black line is the streamline. A few chains that cross the liquid domain are encircled in each plot.
slowly with position and the results of the lubrication approximation agree quite well with the finite element solution, the chains lied inside the fluid domain; thus, the assumption is reasonable. However, near the stagnation points on the upstream and downstream free surfaces, the chains reach near full extension and the velocity field changes abruptly over small (few μm) distances, a part of the chain lies outside the liquid flow domain; thus, this assumption is clearly not valid. At the upstream free surface the velocity of the chains on the streamline is high and the chains reach the minimum gap location (point of observation) very fast; the errors introduced because of the linearity assumption propagate to the minimum gap region below the zero velocity plane, and the computed extension does not agree with the experimental results. On the other hand, at the downstream free surface the velocity of the chain is low as it approaches the contact line onward to the minimum gap region above the zero velocity plane. The chains have enough time to “recover” from errors introduced by the assumption in the linearity of the velocity. The measured extension agreed better with the simulations in this region.

3.4 Conclusions

A macro-micro approach to predict polymer configuration in a complex free surface flow was applied to ultra-dilute solutions of DNA. In ultra-dilute solutions the elasticity from the polymer molecules is negligible and the liquid solutions are Newtonian. The macroscopic Newtonian flow field was solved using Galerkin/Finite Elements method. The complex free surface flow in a scaled down roll-knife coating flow cell was computed in a low capillary number regime; the velocity at the minimum gap position agreed within 3% of experimental measurements. Bead-spring chains modeled DNA molecules were used as tracers in the computed flow field. Rescaling approach called successive fine graining developed by Sunthar and Ravi Prakash (2005) in simple extensional flows was used for the first time in a complex mixed flow. The predictions of the simulations did not agree with the experimental observations because of the assumption that velocity is linear across the dimensions of the chains; in
order to study realistic and complex flows the Brownian dynamics simulations should interpolate the velocity gradient at each bead location. New approaches are already being developed (Jendrejaj et al. 2003, Jendrejaj et al. 2004, Woo et al. 2004a) to include wall hydrodynamics in simulations of microscopic models of dilute polymer solutions. Synthetic polymer molecules that are commonly used in process flows have contour length (and radius of gyration) much smaller than that of DNA molecules used in this study. Thus, in macro-scale flows of synthetic polymers, near-wall hydrodynamics may not be crucial. But, in micro-scale flows such as ink-jet printing, spraying, coating, microfluidic flows and biological flows such as preparation of DNA micro-arrays that operate at microscopic dimensions (about 1 pL volume and spot size about 340 μm (Blossey and Bosio 2002) to as low as 2–5 nL volume and spot size from 15-200 μm (Cooley et al. 2001)), near-wall hydrodynamics are essential to capture the flow-microstructure coupling. The macro-micro approach used here can complement single molecule DNA experiments to understand polymer dynamics in other complex micro-scale flows of ultra-dilute solutions and can be used to test, validate and improve molecular models for dilute polymer solutions. The technique can then be used to study fully coupled problems where the deformation of the microstructure also affects the flow field significantly, i.e., concentrated and entangled polymer solutions.
Chapter 4

Real-time Fluorescence Visualization of Single Walled Carbon Nanotubes

Single walled carbon nanotubes (SWNTs) are allotropes of carbon with high aspect ratio that exhibit complex behavior when dispersed in a liquid. The behavior of SWNTs in liquids is an important subject in the physical, material, and life sciences but, because of lack of viable techniques, little is known on the micro- and nano-scale dynamics of suspensions of SWNTs. Visualization of real-time dynamics of SWNTs in liquids could lead to development of scalable processing techniques like in making fibres, self-assembly and directed assembly into films and nanoscale structures, and key biological studies, chiefly the interaction of SWNTs with cells. In spite of recent progress at direct fluorescence visualization of SWNTs, real time observations of individual pristine SWNTs in suspensions is inconvenient, and the current techniques do not provide sufficiently resolved shapes. In this chapter, a new, simple and convenient technique for fluorescent tagging of individual SWNTs is presented (Section 4.2): this allowed observation of the rotational (section 4.3) and bending dynamics (Section 4.4) of individual SWNTs in water.

4.1 Introduction

Carbon nanotubes like diamond, graphite, and Buckminsterfullerene (C60) are allotropes of carbon. Carbon nanotubes are cylinders of a few nanometers to a few
Figure 4.1: Transmission electron microscopy (TEM) micrograph of single-walled carbon nanotubes showing an individual SWNT and a bundle of SWNTs. Scale bar is 20 nm.

micrometers in length and of diameter below few nanometers, obtained by wrapping up graphite sheets (Iijima 1991, Dai 2001). There are two main types of carbon nanotubes—single walled nanotubes (SWNTs) are cylindrical tubes with a single carbon layer (figure 4.1) and multi walled nanotubes (MWNTs) comprise a concentric array of such single walled nanotubes (Baughman et al. 2002). This work focusses on SWNTs. Depending on the direction about which the graphite sheet is rolled, different classes of nanotubes can be obtained—armchair, zigzag or chiral. This direction also determines the electronic (metallic or semi-conducting) properties of SWNTs (Dai 2001).

There are several methods to produce carbon nanotubes—arc discharge, laser ablation, chemical vapor deposition, and gas phase catalytic growth (Dai 2001). SWNTs used in this study were produced by the HiPco process, where high pressure carbon monoxide is used as the feedstock and catalyst particles are generated from the decomposition of iron pentacarbonyl at reactor temperatures 800-1200°C (Nikolaev et al. 1999, Bronikowski et al. 2001). SWNTs thus produced exist as bundles or aggregates called ropes that are bound tightly by high van der Waals forces (figure 4.1). The size of bundles may vary from about 10 nm (a few nanotubes) to about 100 nm. The individual nanotubes are a mixture of tubes of different types (metallic or semi-conducting), polydisperse in length and diameter. The average diameter of
HiPco tubes is $\sim 1$ nm (Bachilo et al. 2002). Cryo-TEM (Moore et al. 2003) and AFM have shown that HiPco nanotubes that had been suspended in water with the aid of surfactants and sonification ranged from a few nm to over 1 $\mu$m in length.

Carbon nanotubes are stiff and exceptionally strong, i.e., they have a high Young modulus and high tensile strength. The calculated Young modulus of an individual SWNT is about 1 TPa (Gao et al. 1998, Krishnan et al. 1998, Yu et al. 2000). The SWNT tensile strength is calculated to be 30–45 GPa and strain to failure at least 6% (Walters et al. 1999, Yu et al. 2000). In addition to the extraordinary mechanical properties, the nanotubes have an electrical resistivity of about 200 mWcm (Hone et al. 2000) and thermal conductivity better than that for diamond $\sim 3500$W/m K (Hone et al. 1999). Because of these exceptional properties, carbon nanotubes have been the focus of extensive research during the last ten years.

Individual SWNTs with diameters $\sim 1$ nm and lengths less than about 1$\mu$m cannot be visualized using optical microscopy: although bundles of nanotubes have been visualized using phase contrast microscopy (Prakash et al. 2003). High resolution techniques like transmission electron microscopy (TEM) and atomic force microscopy (AFM) allow visualization of individual SWNTs (Moore et al. 2003, Richard et al. 2003), but only static measurements such as length and diameter can be made. Real-time dynamics like diffusion in liquids cannot be observed, and both TEM and AFM cannot be applied to living systems—studying real-time interaction of living cells with SWNTs (Cherukuri et al. 2004).

Real-time visualization of single molecules like DNA (Smith et al. 1996), F-actin (Käs et al. 1994), Mictrotubules (Gittes et al. 1993), and worm-like micelles (Dalhaimer et al. 2003) has been performed by fluorescent tagging. Similarly, individual SWNTs tagged with fluorescent molecules can be used to study the dynamic behavior of SWNTs in liquids. Otobe et al. (2002) covalently attached silicon-based fluorescent polymers to visualize SWNT bundles using fluorescence microscopy. These fluorescent-SWNTs can be used for real-time visualization of the dynamics in liquids, but such covalent attachments are undesirable because they damage the atomic structure of the carbon nanotubes and alter the electronic properties. Prakash et al.
(2003) have labeled MWNTs through non-covalent attachments using several common fluorescent dyes in polar and non-polar solvents. The fluorescent molecules get tagged to the walls of carbon nanotubes by hydrophobic interaction and by π stacking of the aromatic rings at the walls. Recently, Chaudhary et al. (2004) attached surface modified fluorescent quantum-dots (CdSe-ZnS) to SWNT bundles dispersed in a surfactant solution. The quantum-dots arranged at the surface of the SWNTs by electrostatic interactions and allowed fluorescence visualization in solution. The images obtained are poorly resolved. It is also not clear how the surface conjugation alters the atomic structure and properties of the nanotubes. Near-infrared fluorescence microscopy has also been used for visualizing individual SWNTs (Tsvboulski et al. 2005). This technique is quite promising—it does not require any staining or modification of the SWNT; the diameter of the imaged SWNT can be determined from the fluorescence spectra, and the SWNT visualized is definitely individual because only individual SWNTs (HiPco) emit fluorescence. The major drawback is the poor spatial resolution, about 1 μm, which is one-half to one-third of the resolution of visible-light imaging. The time resolution is about 50 ms and to determine the diameter of the nanotube the fluorescent signal has to be integrated over time which requires the nanotube to be fixed at a particular point in space. Another drawback is the need for a special infra-red camera for imaging.

4.2 Visualizing individual SWNTs

O’Connell et al. (2002) and Moore et al. (2003) have dispersed bundles of SWNTs as individuals in aqueous suspensions by using various surfactant. Here sodium dodecyl sulfate (SDS) surfactant was used to disperse of individual nanotubes in water. Raw nanotubes (HiPco, Rice University; HPR 120.3) were dispersed in 200 ml of 1 wt% (34.3 mM) aqueous SDS surfactant solution by 1 hour of high-shear mixing (Poly-science X-520). The resulting suspension was treated in a cup-horn sonicator (Cole Palmer CPX-600) for 10 min at 540 W. Immediately after sonication, samples were centrifuged (Sovall 100S Discovery Ultracentrifuge, Surespin 630 swing bucket rotor)
at 122,000g for 4 hours. The top 75 to 80 % of supernatant was then carefully
decanted, leaving micelle-suspended nanotube solutions at a typical mass concentration
of 20 to 25 mg/l\textsuperscript{1}.

In water, SDS above the critical micelle concentration (8.1 mM) exist as spherical
micelles. The micelles remain spherical up to a concentration of 810 mM (Shirota
et al. 2004). It is believed that sonification splits individual SWNTs at the ends of
the bundles and provides surface for the surfactant to adsorb. As the sonification
progresses, individual SWNTs get unzipped from the bundles and more surfactant
adsorbs on the surface (Strano et al. 2003). Individual SWNTs encased in close-packed
columnar SDS micelles (figure 4.2) are released into the liquid. O’Connell et al. (2002)
computed the diameter of SDS-SWNT complex to be \( \approx 7 \) nm. Surfactant encased
bundles of SWNTs have a higher specific gravity than encased individual SWNTs;
therefore, centrifugation brings bundles and other metallic impurities to the bottom
of the centrifuge tube, leaving a supernatant highly enriched in individual nanotubes
(O’Connell et al. 2002).

The SWNTs were polydisperse in length, and the length distribution was deter-
mined by measuring the specific viscosity as a function of shear rate of the suspension.
A log-normal distribution was fit to the measurements to determine the the first mo-
ment and cube root of the third moment of the length distribution, \( \langle L \rangle \approx 250 \) nm
and \( \langle L^3 \rangle^{1/3} \approx 440 \) nm, respectively (Parra-Vasquez et al. 2005).

Raman scattering is used to investigate and characterize SWNTs. A typical Raman
spectrum from a SWNT sample shows strong scattering signal—the radial breathing
mode (RBM) near 230 cm\textsuperscript{-1} which results from the in-phase vibration of all carbon
atoms in the radial direction, and the tangential carbon stretching mode at 1593 cm\textsuperscript{-1}
which results from the axial stretching of the carbon atoms (Yu and Brus 2001).
O’Connell et al. (2002) performed Raman spectroscopy and showed that individual
SWNTs fluoresce in a dispersion. Addition of surfactant and de-bundling during
the dispersion process was observed as high energy shifts in the Raman and fluorescenc

\textsuperscript{1}The solution was prepared by A. Nicholas G. Parra-Vasquez. He also performed rheology of the
sample.
Figure 4.2: Fluorescent tagging of individual SWNTs. Schematic illustrations (cross-section and side view) of the SWNT-SDS-Dye complex. The surfactant hydrocarbon chains arrange on the surface of the encapsulated SWNT. Spheres represent dye molecules.

spectrum (Strano et al. 2003). These peaks (high energy shifts) were absent from a dispersion with bundles because the electronic excitation was quenched due to non-irradiative energy transfer between the nanotubes in a bundle (O'Connell et al. 2002). Strano et al. (2003) also found that the radial breathing mode indicative of a bundle at about 273 cm$^{-1}$ diminished in intensity as it progressively dispersed into water.

Raman and fluorescence spectroscopy (Kaiser Raman Spectrometer, Ann Arbor, MI) was performed on the aqueous dispersion of SWNTs in 1 wt% SDS using 785 nm excitation. Figure 4.3 shows the Raman and fluorescence spectrum. The presence of high energy (high Raman shifts) peaks (figure 4.3a) and the absence of the RBM of the bundle at $\sim$ 273 cm$^{-1}$ (figure 4.3b) indicated that the SWNTs in the dispersion were individuals\(^2\).

The SDS-SWNT complexes were then labeled with a hydrophobic fluorescent dye (PKH26; Sigma; excitation 551/ emission 567 nm), which was added to the aqueous solution and spontaneously incorporated into the core of the micelles. PKH26 was previously used by Dallaihmer et al. (2003) to tag the hydrophobic core of worm-

\(^2\)See Appendix B
Figure 4.3: Raman fluorescence spectrum of SWNTs dispersed in 1 wt % SDS. The solid line is the spectrum for SDS-SWNT and the broken line is the spectrum for dyed SDS-SWNT. (a) High energy fluorescence (high Raman shift) showing presence of individual SWNTs in solution. (b) Low energy Raman spectrum showing absence of the radial breathing mode of the SWNT bundles at $\sim 273$ cm$^{-1}$. The spectrums are normalized with the intensity of the transverse breathing mode at 1593 cm$^{-1}$.
like micelles formed by diblock copolymers. 1 µl of PKH26 was added to 10 µl of SWNT-SDS solution. The dye concentration inside the SWNT-SDS micelles is about one molecule every 6 nm. To ensure proper mixing, the sample was vortex mixed for about 10 s. Adding the dye did not affect the Raman spectrum of the nanotube suspension (figure 4.3); thus, the SWNTs do not aggregate when the dye is added.

Individual fluorescently tagged SWNTs were visualized by epi-fluorescence microscopy with a Nikon E600 microscope equipped with a filter cube (XF37; excitation 540–550/555 dichroic/ emission 570–600 nm; Omega, Inc., VT) and a 100X oil-immersion objective (NA 1.4). The filter cube is sub-optimal for the dye PKH26, but the fluorescence signal is strong enough to give good images. The depth of focus for the system was about ≈ 0.5 µm. Figure 4.4a shows images of fluorescent individual SWNTs. Diffraction limited the resolution of SWNT dimensions in the radial direction. Tubes longer than about 1 µm were easily identified. The staining technique is simple, and I am convinced that it can be used to fluorescently tag carbon nanotubes (SWNTs and MWNTs) individually suspended in a dispersion using many other surfactant or co-polymers. Images of a fluorescent individual SWNT in an aqueous 2 wt% F68 Pluronic surfactant solution are shown in figure 4.4b.

The aqueous solution of nanotubes had excess surfactant. The pure surfactant micelles (without encased SWNTs) also have a hydrophobic core. Likely, the dye molecules occupied the core of these empty micelles too and stained them. The size of such spherical micelles and micelles with short SWNTs (less than about 500 nm) could not be resolved by optical microscopy because of their small dimensions; but produced a lot of background and reduced the contrast in the images. To get good, sharp and high contrast images of fluorescent SWNTs, the glass slide and the coverslip must be extremely clean (cleaning procedure discussed in Section 4.3.2). The dye and the surfactant adhered to unclean glass and produced an extremely bright background.

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3See Appendix C
Figure 4.4: Fluorescence microscopy images of individual single-walled carbon nanotubes tagged with PKH26 in (a) SDS (b) F68 Pluronic. Scale bar is 10 μm.
4.3 Brownian motion of SWNTs

4.3.1 Introduction

Rod-like polymers are important in biology (e.g., microtubules a filamentous protein that forms the cytoskeleton of cells) and in polymer technology (e.g., poly p-phenylene benzobisoxazole (PBO) is used to prepare very strong fibres). The physical and dynamic properties of rod-like molecules in liquids differ from those of flexible molecules—-at equilibrium rod-like molecules encompass a larger hydrodynamic volume than flexible ones of comparable molecular weight or, more precisely, contour length. Because of this, rod-like molecules start interacting at a much lower number concentration and with increasing concentration spontaneously orient forming lyotropic liquid crystals (Doi and Edwards 1986, Donald and Windle 1992).

When suspended in liquids, small particles such as these rod-like polymers are stabilized from sedimentation by Brownian motion—the random thermal motion of the liquid molecules cause the particles to move randomly, and affect time-dependent properties such as viscoelasticity, diffusion, birefringence and light scattering in liquids. Because of continuous fluctuating momentum exchange with a large number of solvent molecules, the position of the center of mass and the orientation of small rod-like particles fluctuates in time—-i.e., the particles undergo translational and rotational Brownian motion. The rotational behavior of a Brownian rod can be modeled as diffusion of a unit vector \( \mathbf{n} \) on a unit sphere, i.e., the tip of \( \mathbf{n} \) performs a random walk on the surface of a unit sphere (Doi and Edwards 1986). The Debye rotational diffusion equation describes the orientation dynamics of an ensemble of non-interacting rods in polar coordinates (Kirchhoiff et al. 1996)

\[
\frac{\partial \Psi(\mathbf{n}(0), \mathbf{n}(t))}{\partial t} = D^R \left[ \frac{1}{\sin \theta} \frac{\partial}{\partial \theta} \sin \theta \frac{\partial \Psi}{\partial \theta} + \frac{1}{\sin^2 \theta} \frac{\partial^2 \Psi}{\partial \phi^2} \right].
\]

\( \Psi(\mathbf{n}(0), \mathbf{n}(t)) \) is the probability density of rods with orientation \( \mathbf{n}(t) \) at time \( t \) when at \( t = 0 \) the orientation was \( \mathbf{n}(0) \) (at \( t = 0, \Psi(\mathbf{n}) = \delta(\mathbf{n} - \mathbf{n}(0)) \)). \( D^R \) the diffusion coefficient is the rate of reorientation of rods due to Brownian motion. The solution of equation 4.1 can be expressed conveniently in terms of a set of ortho-
nal eigenfunctions (Doi and Edwards 1986)—spherical harmonics, a combination of trigonometric functions and Legendre polynomials $P_k(x)$. The generalized expression called Rodrigues formula and the first three Legendre polynomials are (Kreyszig 1986)

$$P_k(x) = \frac{1}{2^k k!} \frac{d^k}{dx^k} (x^2 - 1)^k$$  \hspace{1cm} (4.2)

$$P_1(x) = x \hspace{1cm} (4.3)$$

$$P_2(x) = \frac{1}{2} (3x^2 - 1) \hspace{1cm} (4.4)$$

$$P_3(x) = \frac{1}{2} (5x^3 - 3x) \hspace{1cm} (4.5)$$

The autocorrelation of the fluctuating orientation $\mathbf{n}(t)$ of rods in the ensemble decays exponentially in time; in two-dimensions the correlation functions are described by (Doi and Edwards 1986, Kirchhoff et al. 1996)

$$\langle P_k(\mathbf{n}(0).\mathbf{n}(t)) \rangle = e^{-D_k^R \frac{\ln p - 0.8}{2} t}$$  \hspace{1cm} (4.6)

where, $D_k^R$ are the diffusion coefficients (rates of decay) corresponding to the different harmonics. A suspension of rods is considered dilute if each rod can rotate freely without being affected by other rods: in this case, all diffusion coefficients are equal ($D_k^R = D^R$) and independent of rod concentration (Kirchhoff et al. 1996).

In dilute solution in a liquid of viscosity $\eta_s$, a thin rod ($L \gg d$; $L$ is the length and $d$ is the hydrodynamic diameter) diffuses twice faster parallel to its axis than it does perpendicular to its axis (Doi and Edwards 1986). The friction coefficients per unit length of a rod-like particle are $\zeta_\perp \simeq 2\zeta_\parallel \simeq 4\pi \eta_s$. Kirkwood and Auer (1951) approximated the hydrodynamic effects on the friction coefficient with a logarithmic dependence on the aspect ratio $p = L/d$

$$\zeta_\parallel = \frac{4\pi \eta_s}{\ln p}. \hspace{1cm} (4.7)$$

Similarly, the friction coefficient for rotational motion is given by (Doi and Edwards 1986, Larson et al. 1999)

$$\zeta_r = \frac{\eta_s L^3}{3(\ln p - 0.8)} \hspace{1cm} (4.8)$$

and the diffusion coefficient for rotational motion ($D_R$) is defined

$$D_R = \frac{k_B T}{\zeta_r}. \hspace{1cm} (4.9)$$
Broersma (1981) performed precise numerical studies to estimate the diffusion coefficients for Brownian rod-like particles in solution, and found that for \( p > 4.6 \) the bulk rotational diffusion coefficient of rods is

\[
P^R = \frac{3k_BT}{\pi \eta_s L^3} (\ln p + \delta) \tag{4.10}
\]

\[
\delta = -0.446 - \frac{0.2}{\ln 2p} - \frac{16}{(\ln 2p)^2} + \frac{63}{(\ln 2p)^3} - \frac{62}{(\ln 2p)^4} \tag{4.11}
\]

The high aspect ratio and rigidity of rod-like molecules impose topological constraints on the motion of the rods as the concentration is increased in a dispersion. A suspension of thin long rods is considered dilute if the rods can rotate freely without being affected by neighboring rods. The transition to the semi-dilute regime, where rod-rod interactions become important, occurs when the number concentration \( \nu \) reaches a value proportional to \( L^{-3} \), where \( L \) is the length of the rods. Theory predicts that in monodisperse samples the transition should occur at \( \nu^* = 1/L^3 \); the volume spanned by a rod of length \( L \) as it rotates in space is proportional to \( L^3 \) (figure 4.5). Bulk measurements on polydisperse samples show that the transition occurs at \( \nu^* \approx 30/(L^3) \) (Larson 1999). The critical volume concentration for the transition from dilute to semi-dilute regime is given by

\[
\phi^* \equiv \frac{\text{Volume of rods}}{\text{Volume of spheres}} = \alpha \frac{d^2 \langle L \rangle}{4 \langle L^3 \rangle} \sim \alpha \frac{0.01}{[\eta_0]} \tag{4.12}
\]

where \([\eta_0] \equiv (\eta_0 - \eta_s) / \eta_s \phi\) is the intrinsic viscosity of the solution (\( \eta_0 \) is the zero-shear viscosity and \( \phi \) is the volume fraction) (Batchelor 1970).

Suspensions of rod-like molecules—Tobacco Mosaic Virus (TMV), bacterial fd-virus and poly-\( \gamma \)-benzyl \( \alpha \)-L-glutamate (PBLG)—have been studied to understand the behavior of anisotropic fluids. Maguire et al. (1980) studied the rotational diffusion of M-13 viruses in solution at semi-dilute concentrations using electric birefringence. Marion et al. (1983) used electric birefringence to measure the rotational relaxation time of DNA molecules \((M_w \approx 5 \text{ Mg/mol})\) and found that it depended strongly on concentration. Bu et al. (1994) used fluorescence photobleaching recovery to observe the diffusion of fluorescently tagged PBLG and found that the transition
Figure 4.5: Transition from a dilute concentration regime to semi-dilute concentration regime for a suspension of rod-like molecules. The transition occurs when there is one rod inside the sphere defined by each rod.

from dilute concentration regime (non-interacting rods) to semi-dilute concentration regime (sterically interacting rods) occurs at concentrations higher than predicted by the Doi-Edwards analytical theory. Cush et al. (1997) used light scattering to measure the rotational and translational diffusion coefficients of TMV in aqueous polymer solutions.

Birefringence and light scattering are bulk techniques that measure response of a large ensemble of molecules that are typically polydisperse in length. Single molecule observations reveal individual molecular behavior that can be significantly different from the bulk average behavior, and one requires fewer assumptions, frequently.

### 4.3.2 Experimental section

Solutions of dyed SWNTs were prepared at two concentrations from a stock solution of undyed SDS-SWNT. The SDS-SWNT solution had intrinsic viscosity $[\eta] = 7520 \pm 250^4$. The two dyed samples had number concentration $\nu_2 = 0.3 \nu^*$ and $\nu_2 = 2 \nu^*$ (mass concentrations 10.6 and 1.6 mg/l, respectively).

A 2 µl drop of the suspension labeled SWNTs was placed on hydrophilic glass and then covered with a hydrophilic glass coverslip. The glass slides and coverslips were previously cleaned by soaking for 30 minutes in a mixture of 70% sulphuric acid and

\footnote{Solution rheology was performed by A. N. G. Parra-Vasquez.}
30% hydrogen peroxide. After rinsing with water, the glass slides were wiped clean with methanol and were ready for use. The sample was sealed with vacuum grease to prevent convective flow due to evaporation. The average gap $H$ between the glass slide and the coverslip was measured at $2.48 \pm 0.3 \mu m$. Marks were made at the top and bottom of the two glass surfaces forming the experimental cell. The gap was measured by focussing at the two marks and measuring the translation of the motorized microscope stage. Three different cells were prepared for the gap calibration. The diffusing nanotubes were imaged 10 minutes after sealing the experimental cell. This was done to ensure the cessation of any convective flow induced by pipetting the sample and clamping of the coverslip.

The Brownian motion of fluorescent SWNTs was recorded at 30 fps using an EBCCD camera (Hamamatsu, Japan), controlled by MetaMorph software (Universal Imaging Co., USA). A 100X oil immersion objective (NA = 1.4, depth of focus $\approx 0.5 \mu m$) was used to image the tubes. The motion of a rod $L > H$ was restricted in one direction (figure 4.6) and rods longer than the gap were observed to stay in the focal plane. The movement of individual tubes was tracked, and their positions and orientations measured in 500 to 1000 consecutive frames using MetaMorph. Diffusivities were computed from each single particle trajectory by evaluating the orientational correlation functions.

4.3.3 Results and discussion

Brownian motion of 27 fluorescent SWNTs was observed at the two solution concentrations. The measured length $L_m$ of the SWNTs varied from 2 $\mu m$ to 5 $\mu m$. The SWNTs were continuously visualized for 33 s during which the nanotubes remained in focus. Although the SWNTs were confined, the measured length fluctuated over time as the SWNTs oriented in the gap. This was because the depth of focus of the imaging system was smaller than the gap which was comparable to the length of the nanotubes. The images of the SWNTs were 2-D projections of a rod (figure 4.6). It can be shown that in bulk (no confinement), if all orientations were equally probable
Figure 4.6: A thin rod of length $L$ between two parallel plates separated by a gap $H$. The center of mass of the rod is at $y$ and the rod has an orientation $\phi$. Imaging is done from the top and the measured length $L_m$ is the 2-D projection of $L$, $L_m = L \sin \phi$.

then the average measured length $L_m$ is

$$\langle L_m^2 \rangle = L^2 \int_0^{2\pi} \int_0^{\pi/2} \sin^2 \phi \sin \phi d\phi d\theta$$

$$= \int_0^{2\pi} \int_0^{\pi/2} \frac{1}{4} \sin^2 \phi \sin \phi d\phi d\theta$$

$$\langle L_m^2 \rangle = \frac{2}{3} L^2.$$  \hspace{1cm} (4.13)

While in a confined space (figure 4.6) (rods of length $L$ in a gap $H$ ($L \geq H$)), if all orientations $0 < \phi < \phi_0$ are equally probable, the average measured length is

$$\langle L_m^2 \rangle = L^2 \left[ \frac{3}{4} + \frac{1}{12} \frac{\cos 3(\pi - \phi_0) - \cos 3\phi_0}{\cos(\pi - \phi_0) - \cos \phi_0} \right]$$

$$\phi_0 = \arccos \left( \frac{H}{L} \right).$$  \hspace{1cm} (4.15)

The measured lengths were corrected using equation 4.15. Figures 4.7 shows time lapse images of fluorescent SWNTs diffusing. The orientation $\theta$ of the SWNTs were tracked over time (figure 4.8) and correlation functions (equation 4.6) were computed. The diffusion coefficient of each SWNT was determined from the slope of the linear fits to logarithm of the correlation functions. Figures 4.9a and 4.9b show that the orientational correlation functions decay exponentially at both concentrations. At
Figure 4.7: Time lapse images of diffusing fluorescent SWNTs. Scale bar is 10 μm. Note that the two SWNTs (bottom) are interacting and avoiding each other as they rotate and translate.

$\nu_1 = 0.3 \nu^*$ (figure 4.9a), the first three harmonics of a $3.3 \pm 0.2$ μm SWNT decay at same rate, indicating that the SWNT is in a dilute suspension. At the higher concentration $\nu_2 = 2 \nu^*$ (figure 4.9b), the decay rates of the first three harmonics of a $3 \pm 0.3$ μm SWNT are different, i.e., this SWNT is interacting with others. Figure 4.10 shows the ratio of the second and third diffusion coefficients with respect to the first, measured on 27 SWNTs at the two concentrations. At the lower concentration $\nu_1$, $\langle D^R_2/D^R_1 \rangle = 0.95 \pm 0.04$, $\langle D^R_3/D^R_1 \rangle = 0.90 \pm 0.08$, i.e., the diffusion coefficients $D^R_k$ are equal and the suspension is dilute. At the higher concentration $\nu_2$, $\langle D^R_2/D^R_1 \rangle = 0.91 \pm 0.14$, $\langle D^R_3/D^R_1 \rangle = 0.87 \pm 0.15$ and the suspension is transitioning to semi-dilute.
Figure 4.8: Orientation $\theta$ of a SWNT over time $t$.

Figure 4.11a shows the measured two-dimensional rotational diffusivity as a function of rod length of 11 nanotubes at concentration $n_1$. The measurements of 16 nanotubes at concentration $n_2$ are plotted in figure 4.11b. The measurements were lower than that theoretically predicted for non-interacting rigid rods in bulk (solid line in the figures, equation 4.10). In the experiments the nanotubes were confined between two glass plates separated by a small gap; thus, wall effects which increase the hydrodynamic drag that the nanotubes experience slow down the diffusion, are important. Li and Tang (2004) have modeled the two-dimensional diffusion of a rod constrained to move in a gap between two parallel plates by superposing the friction drag that the rod experiences due to its proximity to either of the two walls. The drag coefficient per unit length on an infinitely long cylinder close to a wall is (Hunt et al. 1994)

$$\zeta = \frac{4\pi \eta_s}{\text{arccosh}(2h/d)}$$  \hspace{1cm} (4.17)

here $h$ is the distance of the cylinder axis from the wall. The rotational drag on a rod of finite length at position $y$ and orientation $\phi$ in the gap (figure 4.6) can thus be written as (Li and Tang 2004)
Figure 4.9: Exponential decay of the first three harmonic orientation correlation functions at concentration (a) \( \nu_1 \) and (b) \( \nu_2 \). Linear fits \( W_k = D_k^R t \) over \( t < 0.15 \text{ s} \) to obtain the diffusion coefficients. (a) \( D_1^R = 0.1691 \pm 0.009 \text{ s}^{-1}, D_2^R = 0.1666 \pm 0.009 \text{ s}^{-1} \) and \( D_3^R = 0.163 \pm 0.009 \text{ s}^{-1} \). The length of the SWNT was \( L = 3.25 \pm 0.18 \mu\text{m} \). (b) \( D_1^R = 0.4689 \pm 0.017 \text{ s}^{-1}, D_2^R = 0.4432 \pm 0.02 \text{ s}^{-1} \) and \( D_3^R = 0.4083 \pm 0.025 \text{ s}^{-1} \). The length of the SWNT was \( L = 3.03 \pm 0.31 \mu\text{m} \). 

\[ W_k = -\frac{2}{k(k+1)} \ln \langle P_k(\mathbf{n}(t), \mathbf{n}(0)) \rangle \]
Figure 4.10: Dilute to semi-dilute transition of SWNTs in solution. The second $D^{R}_2$ (open symbols) and third $D^{R}_3$ (filled symbols) two dimensional rotational diffusion coefficients with respect to the first $D^{R}_1$, at concentrations $\nu_1$ (red ○) and $\nu_2$ (black Δ). The lines are the averages: $D^{R}_2/D^{R}_1$ solid line and $D^{R}_3/D^{R}_1$ broken line.
\[ \xi_r(y, \phi) = \int_{-L/2}^{L/2} \left( \frac{4\pi \eta x^2 \cos^2 \phi}{\text{arccosh} \frac{y + r \sin \phi}{r}} + \frac{4\pi \eta x^2 \cos^2 \phi}{\text{arccosh} \frac{H - y - r \sin \phi}{r}} \right) dx \]  

(4.18)

\[ D^R(y, \phi) = \frac{k_B T}{\xi_r(y, \phi)} . \]  

(4.19)

The overall rotational diffusion coefficient is an average over all possible orientations and positions that a freely diffusing rod can occupy

\[
D^R = \frac{\int_0^{H/2} dy \int_{\arcsin(2y/L)}^{\arcsin(2y/L)} D^R(y, \phi) d\phi}{\int_0^{H/2} dy \int_{-\arcsin(2y/L)}^{\arcsin(2y/L)} d\phi}
\]  

(4.20)

where, \( y \) is the position of the center of mass of the rod in the gap and \( \phi \) is the orientation of the rod.

Confined rotational diffusivity were calculated for rods of different length in the gap \( H = 2.48 \mu m \) using \( \text{equation 4.20} \) (broken lines in figures 4.11a and 4.11b), and there is a reasonable agreement between the experimentally determined diffusivity of the SWNTs and those predicted by the confinement model.

### 4.4 Rigidity of individual SWNTs

#### 4.4.1 Introduction

The stiffness of an inextensible elastic thread-like object is characterized by its bending stiffness or rigidity \( \kappa \) (Landau and Lifshitz 1980). When a microscopic thread is placed in a liquid, the balance between Brownian forces, which tend to bend the thread, and elastic forces, which oppose curvature, is characterized by the persistence length \( L_p \equiv \kappa/k_B T \)—the distance along the thread over which the tangent to the thread contour remains correlated, in the absence of flow and other forces (Doi and Edwards 1986). The Brownian motion of a thread in a liquid is described by the Langevin equation (Shankar et al. 2002)

\[
\zeta L \frac{\partial \mathbf{r}}{\partial t} = -\kappa \frac{\partial^4 \mathbf{r}}{\partial s^4} + \frac{\partial}{\partial s} \mathbf{u} + f^B
\]  

(4.21)
Figure 4.11: Two dimensional rotational diffusion coefficients of SWNTs of different lengths at solution concentration (a) $\nu_1 = 0.3\nu^*$ and (b) $\nu_2 = 2\nu^*$. The symbols are the experimental measurements. The solid line is prediction in bulk (equation 4.10). The broken line is the numerically computed diffusion coefficient averaged over all possible configurations in a two-dimensional confined geometry (equation 4.20). The two parallel plates are separated by $2.48 \pm 0.3 \,\mu m$ and the solvent viscosity is $0.95 \, mPa.s$. The error bars are standard deviation of the length and the errors in the linear fits. Times less than 0.15 s were used to get the linear fit.
where, \( \mathbf{r}(s) \) is the shape of the thread, \( \mathbf{u}(s) = \partial \mathbf{r} / \partial s \) is the local unit tangent vector, \( \tau \) is fluctuating tension that introduces a constraint force to make the thread locally inextensible \( |\partial \mathbf{r}(s)/\partial s| = 1 \), and \( f^B \) is a random Brownian force. The persistence length for the thread is defined as

$$
(\mathbf{u}(s) \cdot \mathbf{u}(s + \Delta s)) = \exp \left( \frac{-\Delta s}{L_p} \right).
$$

(4.22)

The literature is divided on whether, in the absence of flow, individual SWNTs in liquids should be considered rigid \( (L_p \gg L) \) or semi-flexible \( (L_p \sim L) \). Based on the in-plane bending stiffness \( (C = 345 \text{ N/m}) \) calculated using density functional theory (Kudin et al. 2001), at room temperature the persistence length of a single SWNT of typical diameter \( d \) between 0.6 and 1.3 nm (Bachilo et al. 2002), was estimated (Yakobson and Couchman 2004) to be in the range 7 to 74 \( \mu \text{m} \). The generalized expression for the persistence length \( L_p \) is

$$
L_p = \frac{\pi C d^3}{8 k_B T}.
$$

(4.23)

Salvetat et al. (1999) measured the bending stiffness of ropes of SWNTs by using Atomic Force Microscopy (AFM). The AFM tip was used to apply a load to a SWNT rope bridging a pore of the membrane substrate and the resulting deflection was measured to estimate stiffness. Based on this measurement the persistence length should be in tens of microns. Sano et al. (2001) chemically reacted SWNTs to form closed rings with an average diameter of 540 nm. Based on this size, they estimated a persistence length of 800 nm. Recent neutron scattering data (Zhou et al. 2004) shows that SWNTs behave as rigid rods on length scales up to \( \sim 150 \text{ nm} \) (no conclusions could be drawn on the behavior at longer length scales because of tube-tube interactions). Contrary to this finding, X-ray scattering (Schaefer et al. 2003) found that the SWNTs do not display rod-like behavior. A direct measurement of the rigidity of the SWNTs in solvent is thus important in settling this controversy about whether SWNTs in liquids should be considered rigid or semi-flexible, and thus furthering understanding of their solution phase behavior.

The bending stiffness of a macromolecule can be measured directly by observing its response (deflection) and relaxation to an imposed force. The externally applied
force could be from a homogeneous flow field that introduces a drag force that bends
the molecule (Riveline et al. 1990); optical tweezers that use focused lasers to in-
duce deformations in a molecule (Felgner et al. 1996); cantilever tips on atomic force
microscopes (Radmacher 2002). Bundles of SWNTs have been optically trapped; but, individual SWNTs have dimensions (≈ 1 nm) that may be too small to im-
mobilize in a focused laser beam. Thus optical tweezing may not be achievable for
controlled deformation of individual SWNTs. Atomic force microscopes provides local
measures of the stiffness of sub-micron molecules; thus, it can be used on individual
SWNTs. However, in such measurements it is difficult to eliminate deformations aris-
ing from substrate-molecule and tip-molecule interactions, and solvent evaporation
can induce uncalibrated deformations if performed on a dried sample. In contrast
to the techniques mentioned above, where an external force is applied, stiffness can
be measured from changes in the local curvature of the molecules caused by thermal
motion. Such deformations are explained by the laws of statistical physics. Intrinsic
thermal vibrations in vacuum also induce undulations and have been used to measure
the mechanical stiffness of SWNTs using Transmission Electron Microscopy (Treacy
et al. 1996).

Measuring deflections caused by an applied flow field or thermal motion requires
visualizing the molecules in solution. Gittes et al. (1993), Ott et al. (1993), Käs et al.
(1994) and Janson and Dogterom (2004) used fluorescence microscopy to observe ther-
mal deflections in biological macromolecules—microtubules and actin filaments—and
measured their persistence length. Ott et al. (1993) measured the persistence length
of actin filaments $L \approx L_p \approx 17 \mu m$ with the correlation of the unit tangent vectors
along the contour of the molecule. Sequential images of fluorescent actin filaments
were processed to obtain single pixel backbones. Two-dimensional correlation func-
tion along the molecule contour $s$ was used to compute the persistence length

$$
(u(s) \cdot u(s + \Delta s)) = \exp \left( \frac{-\Delta s}{2L_p} \right). \quad (4.24)
$$

Gittes et al. (1993) analyzed thermally driven fluctuations in shape to measure the
rigidity of microtubules (long cylindrical protein filaments) and found that $L \ll L_p$
\( L = 25-65 \ \mu \text{m and } L_p = 5200 \ \mu \text{m} \). The shape of the filaments was characterized by a sum of cosine waves of increasing frequency. The wave amplitudes at each frequency of a single filament over time were used to measure the rigidity. Janson and Dogterom (2004) have used a similar technique to measure the rigidity of growing microtubules.

Another correlation that has been used to measure the rigidity of actin filaments is the mean end-to-end distance (in 2-D) is (Käs et al. 1994)

\[
\langle R^2 \rangle = L^2 \left[ \frac{L_p}{4L} - 8 \frac{L_p^2}{L^2} \left( 1 - \exp \left( -\frac{L}{2L_p} \right) \right) \right].
\]  

Equation 4.25 is a two point measurement on an ensemble of time lapse images. Gittes et al. (1993) state that persistence length measurements from equation 4.25 are not reliable for microtubules because in a very stiff filament the signal to noise (deflection to pixelation error) is low. Bending mode analysis that uses measurements of deflections at a number of points along the contour is more accurate than end-to-end analysis that uses only the two end points.

Real-time fluorescence visualization of SWNTs (Section 4.2) allowed observation of the bending dynamics of SWNTs in water in order to directly measure the persistence length of SWNTs.

### 4.4.2 Thermal fluctuations in a rigid-rod

**Method 1: Shankar et. al. (2002)**

In dilute solutions, the conformation of a single inextensible Brownian thread of contour length \( L \) and diameter \( d \) is parameterized in space by \( \mathbf{r}(s) \), where \( s \) is the arc length measured along the thread contour. The thread is locally inextensible \( |\partial \mathbf{r}(s)/\partial s| = 1 \). With reference to a straight rod parallel to a unit vector \( \mathbf{n} \), the thread can be written as

\[
\mathbf{r}(s) = [s + f(s)]\mathbf{n} + \mathbf{h}(s)
\]  

where \( f(s) \) and \( \mathbf{h}(s) \) are the longitudinal and transverse displacements, respectively. The transverse displacement \( \mathbf{h}(s) \) is orthogonal to \( \mathbf{n} \), and can be expanded as

\[
\mathbf{h}(s) = \sum_{a=1,2} h_a(s) \mathbf{e}_a
\]
where \( \mathbf{e}_1 \) and \( \mathbf{e}_2 \) are two unit vectors orthogonal to \( \mathbf{n}(t) \) and to each other. The bending energy of a thread with a bending rigidity \( \kappa \) is given by

\[
U_{\text{bend}} = \frac{1}{2} \kappa \int_0^L ds \left| \frac{\partial \mathbf{u}(s)}{\partial s} \right|^2
\]

(4.28)

where \( \mathbf{u}(s) = \partial \mathbf{r}(s)/\partial s \) is the local tangent vector. The balance between the elastic restoring force and the hydrodynamic drag for a thread can be written as

\[
\zeta \frac{\partial \mathbf{r}}{\partial t} = -\kappa \frac{\partial^2 \mathbf{r}}{\partial s^2}
\]

(4.29)

Here, \( \zeta \) is a local friction coefficients for motion perpendicular to the local tangent \( \mathbf{u}(s) \). Solutions of Eq. 4.29 must satisfy boundary conditions requiring that \( \frac{\partial^2 \mathbf{r}}{\partial s^2} = \frac{\partial^3 \mathbf{r}}{\partial s^3} = 0 \) at both the chain ends \( s = 0, L \).

The transverse displacement \( h_\alpha(\hat{s}) \) along the contour \( \hat{s} = s/L \) can be expanded as

\[
h_\alpha(\hat{s}) = \sum_j \hat{h}_{\alpha,j} W_j(\hat{s}),
\]

(4.30)

where \( W_j \) is the \( j \)th normalized eigenfunction of the fourth order eigenvalue problem (Aragón and Pecora 1985, Wiggins et al. 1998)

\[
\frac{\partial^4 W_j(\hat{s})}{\partial \hat{s}^4} = \alpha_j^4 W_j(\hat{s})
\]

(4.31)

subject to the homogeneous boundary conditions \( \frac{\partial^2 W_j(\hat{s})}{\partial \hat{s}^2} = \frac{\partial^3 W_j(\hat{s})}{\partial \hat{s}^3} = 0 \) at \( \hat{s} = 0 \) and \( \hat{s} = 1 \). The eigenfunctions (shape functions) \( W_j \) are orthogonal and are taken to be orthonormal, so that \( \int_0^1 d\hat{s} W_j W_k = \delta_{jk} \). The nontrivial eigenfunctions, corresponding to bending modes, are (Shankar et al. 2002)

\[
W_j(\hat{s}) = A_j \left\{ [\sinh(\alpha_j) + \sin(\alpha_j)][\sin(\alpha_j \hat{s}) - \sin(\alpha_j \hat{s})] 
+ [\cosh(\alpha_j) + \cos(\alpha_j)][\cosh(\alpha_j \hat{s}) - \cos(\alpha_j \hat{s})] \right\}
\]

(4.32)

where \( A_j = \left[ \frac{1}{2} + \frac{1}{2} \cosh(2\alpha_j) + \alpha_j^{-1} \cosh(\alpha_j \sin(\alpha_j)) \right]^{-1/2} \). The eigenvalues \( \alpha_j \) satisfy \( \cos(\alpha_j) \cosh(\alpha_j) = 1 \). The first three eigenvalues are \( \alpha_1 = 4.73 = 3\pi/2 + 0.0176 \), \( \alpha_2 = 5\pi/2 + 0.0007816 \), and \( \alpha_3 = 7\pi/2 + 0.000033 \). The higher eigenvalues are \( \alpha_j = (2j+1)\pi/2 \). The first three shape functions are shown in figure 4.12. Note that the trivial eigenvalue \( \alpha_0 = 0 \) corresponds to the orientation of the thread.
Figure 4.12: Shape functions $W(\dot{s} = s/L)$ of the first three bending modes.

The bending energy stored in the thread can be expanded in terms of mode amplitudes as

$$U_{\text{bend}} = \frac{1}{2} \frac{\kappa}{L^3} \sum_j \alpha_j^4 (\tilde{h}_{\alpha,j})^2 \ .$$  \hspace{1cm} (4.33)

The equipartition theorem states that each mode contributes on an average $\frac{1}{2} k_B T$ to the total bending energy; therefore, the variance of each mode amplitude equals

$$\langle \tilde{h}_{\alpha,j}^2 \rangle = \frac{L^3}{L_B \alpha_j^4} \ .$$  \hspace{1cm} (4.34)

To measure the persistence length using the mode amplitudes, the ensemble should be un-correlated—the relaxation time of the bending modes is given as (Shankar et al. 2002)

$$\tau_j^b = \frac{\zeta_{\perp} L^4}{\kappa \alpha_j^4} \ .$$  \hspace{1cm} (4.35)
Method 2: Gittes et. al. (1993)

The shape of a thread is expressed as a superposition of a number of Fourier modes of increasing frequency,

$$\theta(s) = \left(\frac{2}{L}\right)^{1/2} \sum_{n=0}^{\infty} a_n \cos\left(\frac{n\pi s}{L}\right).$$  \hspace{1cm} (4.36)

where, $\theta(s)$ is the tangent at every point $s$ along the arc of a rod of length $L$. The bending energy is the quadratic sum of the mode amplitudes $a_n$

$$U_{\text{bend}} = \frac{1}{2} \kappa \sum_{n=1}^{\infty} \left(\frac{n\pi}{L}\right)^2 a_n^2. \hspace{1cm} (4.37)$$

The zero-order mode ($n = 0$) corresponds to the orientation of the rod and does not contribute to the bending energy. From equipartition theorem, the variance in each mode amplitude equals

$$\langle a_n^2 \rangle = \frac{1}{L_p} \left(\frac{L}{n\pi}\right)^2. \hspace{1cm} (4.38)$$

The mode amplitudes can be computed for each rod by taking the fourier inverse transform of equation 4.36 for $n > 0$

$$a_n = \left(\frac{2}{L}\right)^{1/2} \int_0^L ds \theta(s) \cos\left(\frac{n\pi s}{L}\right). \hspace{1cm} (4.39)$$

4.4.3 Shape parametrization

The relaxation times of the first bending mode in our experimental system was estimated to be below 10 ms in the worst case ($L = 4 \mu m$ and $L_p \sim 20 \mu m$). The higher order bending modes have shorter relaxation times. If images in the ensemble are taken at $t > t^b$, they are un-correlated. About 100 SWNT images at 333 ms time interval were used in this analysis. The images were processed by a custom software written in Matlab (Chapter 2). The average and standard deviations of the intensity of each image were computed. Linear combinations of the average and the standard deviation were used to threshold the SWNT from the background. These “objects” were skeletonized to a single pixel backbone using standard image processing technique Skeletonization in Matlab which recursively eroded the thick object to
get to the backbone. Figure 4.13 shows the backbones of three images of a nanotube obtained form the corresponding gray-scale images. All the backbones were analyzed in custom softwares written in Matlab for the two analysis methods. The outline of the procedures are presented next.

**Method 1**

The shape functions (equation 4.32) were fit to the backbones to obtain the mode amplitudes.

- The code read $N+1$ discrete points $(x_i', y_i', s_i)$ along the backbone and computed the slope of the best linear fit to the backbone. The backbone was rotated so that it lied along the x-axis.

- The transformed $y$ positions of the discrete points $(x_i, y_i, s_i)$ were corrected so that the area they enveloped around the x-axis was zero.

$$h_i(s) = y_i(s) - \int_0^L y(s)ds$$  \hspace{1cm} (4.40)

The integral was approximated using trapezoidal rule

$$h_i(s) = y_i(s) - \sum_{i=1}^N \frac{s_{i+1} - s_i}{2} (y_{i+1} + y_i) .$$  \hspace{1cm} (4.41)

- The amplitudes of the first 10 modes $(k)$ were computed using

$$\tilde{h}_k = \int_0^L h(s)W_k(s/L)ds$$  \hspace{1cm} (4.42)

by trapezoid rule, i.e,

$$\tilde{h}_k = \sum_{i=1}^N \frac{s_{i+1} - s_i}{2} (W_{k,i+1}h_{i+1} + W_{k,i}h_i) .$$  \hspace{1cm} (4.43)

- The variance $\langle \tilde{h}_k^2 \rangle$ of the mode amplitudes was used to compute the persistence length $L_p$ using equation 4.34.
Figure 4.13: Fluorescent SWNTs as imaged (left column) and after skeletonization (right column). A single pixel backbone is recovered for each SWNT.
Method 2

Waves of increasing frequency (equation 4.36) were fit to the backbones to obtain the mode amplitudes. The outline of the procedure is as follows:

- The code read $N + 1$ discrete points $(x_i, y_i, s_i)$ along the backbone. The length of $N$ segments $\Delta s_i = [(x_{i+1} - x_i)^2 + (y_{i+1} - y_i)^2]^{1/2}$ and the tangent angles $\theta_i = \arctan[(y_{i+1} - y_i)/(x_{i+1} - x_i)]$ were computed.

- The inverse Fourier transform (equation 4.39) was computed using the approximation (for $n = 1$ to 10)

$$ a_n = \left( \frac{2}{L} \right)^{1/2} \sum_{i=1}^{N} \theta_i \Delta s_i \cos \left( \frac{n\pi}{L} s_i^{\text{mid}} \right) $$

where

$$ L = \sum_{i=1}^{N} \Delta s_i $$

and

$$ s_i^{\text{mid}} = \Delta s_1 + \Delta s_2 + \ldots + \Delta s_{i-1} + \frac{1}{2} \Delta s_i $$

- The variance of the mode amplitudes was computed

$$ \langle a_n^2 \rangle = \frac{1}{L_p} \left( \frac{L}{n\pi} \right)^2 $$

The estimates of the mode amplitudes from equation 4.44 included error due to the limited resolution of the imaging system. Gittes et al. (1993) estimated this experimental error $\langle a_n^2 \rangle^{\text{noise}}$ for each filament and adjusted the measured mode amplitudes to get a more accurate measurement of the bending rigidity (for detailed analysis see original reference). The noise was estimated as

$$ \langle a_n^2 \rangle^{\text{noise}} = \frac{4}{L} \langle \epsilon_i^2 \rangle \left[ 1 + (N - 1) \sin^2 \left( \frac{n\pi}{2N} \right) \right] $$

The experimentally measured mode amplitudes are thus the sum of equations 4.47 and 4.48 with two unknowns $L_p$ and $\langle \epsilon_i^2 \rangle$

$$ \langle a_n^2 \rangle^{\text{measured}} = \frac{1}{L_p} \left( \frac{L}{n\pi} \right)^2 + \frac{4}{L} \langle \epsilon_i^2 \rangle \left[ 1 + (N - 1) \sin^2 \left( \frac{n\pi}{2N} \right) \right] $$
• $\langle \epsilon_n^2 \rangle$ was obtained for each SWNT from a least-square fit of the measured variance of the mode amplitude as a function of $n$.

• The persistence length was calculated as

$$\frac{1}{L_p} = \left( \frac{n \pi}{L} \right)^2 \left[ \langle a_n^2 \rangle_{\text{measured}} - \langle a_n^2 \rangle_{\text{noise}} \right].$$  \hspace{1cm} (4.50)

• The uncertainty in the persistence length was estimated by assuming the uncertainty in the noise to be equal to the noise itself (Gittes et al. 1993)

$$\ln (L_p) \equiv \ln (L_p^{\text{true}}) \pm \frac{2}{M-1} \left[ \frac{\langle a_n^2 \rangle_{\text{measured}}}{\langle a_n^2 \rangle_{\text{noise}}} \right]^{\frac{1}{2}} \frac{\langle a_n^2 \rangle_{\text{measured}}^2 + \langle a_n^2 \rangle_{\text{noise}}^2}{\langle a_n^2 \rangle_{\text{measured}} - \langle a_n^2 \rangle_{\text{noise}}}. \hspace{1cm} (4.51)$$

Here, $M$ is the number of sample values.

### 4.4.4 Procedure validation

The two analysis procedures were tested on an ensemble of filaments of known length and persistence length. In two-dimensional space, filaments with length $L$ between 2 and 4 $\mu$m and persistence length 15 $\mu$m to 90 $\mu$m, whose shape amplitude (equation 4.34) sampled the Gaussian distribution

$$P(a_n) = \frac{1}{\sigma_n \sqrt{2\pi}} \exp \left[ -\frac{a_n^2}{\sigma_n^2} \right] \hspace{1cm} (4.52)$$

($\sigma_n^2$ is the variance) were generated. The filaments were constructed using the shape functions of the first 10 modes (equation 4.30). These filaments were randomly oriented between 0 and $\pi/4$ and positioned on a 2-D square lattice with spacing equal to the pixel size in our optical system (0.14 $\mu$m). If a segment of the simulated filament passed through a lattice-pixel it was chosen as part of the “imaged” filament backbone. One such chain is shown in figure 4.14. The single pixel backbone thus obtained was used to recover the mode amplitudes by following both the image analysis procedures outlined in the previous section. Ensembles of 100, 400 and 1000 simulated chains were used in the analysis. Digitization of the chain lead to about 9% error on the computed contour length (figures 4.15a and 4.16).
Figure 4.14: Randomly generated filament with contour length $L = 3 \mu m$ and persistence length $L_p = 30 \mu m$ on a square lattice $0.14 \times 0.14 \mu m^2$. The red line is the chain and the blue circle mark the vertices of the pixels that would be "lighted" in a binary image.
Figure 4.15: (a) Error in the calculated contour length (method 1) of random samples of 400 chains with contour length $L = 3 \, \mu m$ and persistence length $15 \leq L_p \leq 120 \, \mu m$. (b) Errors in the persistence length of these random samples using method 1. The open symbols show the error in the initial ensemble and closed symbols show error in the calculated persistence length from the initial persistence length. Errors for the first three modes are reported.
Figure 4.16: Error in the calculated contour length of random samples of 100, 400 and 1000 chains with contour length $L = 2, 3$ and $4 \, \mu m$ and persistence length $20 \leq L_p \leq 90 \, \mu m$ using method 2.

The error in the calculated persistence length from the first three modes computed using method 1 is plotted as a function of the persistence length in figure 4.15b. The generated random numbers gave a 10 % error in the persistence length of the initial simulated ensembles. The errors in the computed $L_p$ (figure 4.15b) were measured with respect to the values of the initial starting configuration. As the chains became stiffer (higher $L_p$) the calculated measurements from the first mode amplitudes agree well (< 10 % error) with the initial persistence lengths. The measurements from the second and third modes predict higher flexibility because of the errors introduced by the resolution (pixelation) of the imaging system. Thus, only the measurements of the first bending mode will give the correct $L_p$ of SWNTs. Similarly, figures 4.17a and 4.17b show the error in the calculated persistence length from the first two bending modes computed using method 2. Here also the first mode gave more accurate
measure of the persistence length. Using a finer lattice (0.07 μm pixel size) did not alter the results significantly.

The mean square end-to-end distance was also computed for the ensemble of simulated chains. Figure 4.18a plots the measured mean square end-to-end distance $\langle R^2 \rangle$ as a function of the persistence length. The error in the measurement of the contour length lead to high errors in the computed $L_p$. Figure 4.18b shows equation 4.25 as a function of $L_p$. The left side of equation 4.25 is very sensitive to $L_p$ when $L_p$ is greater than 20 μm; thus, small errors in the end-to-end distance measurements or the contour length (due to noise in the images) lead to incorrect estimation of $L_p$.

4.4.5 Results and discussion

Thermal fluctuation in the shape of individual single-walled carbon nanotubes were observed using fluorescence microscopy. The fluctuation in the curvature of the SWNTs was small and could not be identified by naked eye in real-time. Though some curvature could be identified by the naked eye when looking at still individual frames. Backbones of SWNTs recovered from the gray-scale (intensity) images of the SWNTs showed the shape fluctuations (figure 4.13). Using the two procedures described in Section 4.4.3, the mode amplitudes of the fluctuations were computed and persistence lengths of 13 fluorescently tagged individual SWNTs were measured. Shape functions (method 1) fit on one such SWNT are shown in figure 4.19. Note that as the number of modes is increased, all the points start fitting perfectly onto the shape curve.

Tables 4.4.5 and 4.4.5 report the measured persistence lengths $L_p$ of 13 SWNTs analyzed using method 1 and method 2, respectively. There is a disagreement between the measurements from the different modes. This is attributed to noise dominating thermal undulations of the higher order modes. The noise in the experiment is due to the resolution of the imaging system that introduced digitization errors and blooming (scattering of light into adjacent pixels). Figure 4.20 shows the variance of the mode amplitude computed using method 2 for a SWNT. The experimentally measured mode amplitudes were dominated by noise (broken curve) at modes $n > 1$. Thus
Figure 4.17: Errors in the persistence length of random samples of 100, 400 and 1000 chains with contour length $L = 2$, 3 and 4 $\mu$m and persistence length $20 \leq L_p \leq 90$ $\mu$m calculated using method 2. Error for the first mode (a) and the second mode (b) are reported.
Figure 4.18: (a) Mean square end-to-end distance for simulated chains of contour length \( L = 3 \mu m \). Top: Open symbols are the mean square end-to-end distance \( \langle R^2 \rangle \), for an ensemble of 400 chains. Closed symbols are the square of the mean contour length \( \langle L^2 \rangle \). The broken line is \( \langle R^2 \rangle = \frac{9}{4} \mu m^2 \). Bottom: The ratio of the mean square end-to-end distance \( \langle R^2 \rangle \) and the mean contour length \( \langle L^2 \rangle \) as a function of persistence length \( L_p \). Note that there is no noticeable difference in the mean square end-to-end distance even as the persistence length changes by a factor of 3. (b) Equation 4.25 as a function of persistence length \( L_p \) for chains of contour length \( L = 3 \mu m \).
Figure 4.19: Shape fitting to backbone of a SWNT. The points are the backbone pixels. The black solid lines is the shape fit with only the first mode. The red line is the shape fit with the first two modes.
Figure 4.20: The variance of the mode amplitudes for a SWNT of length $L = 3.63 \pm 0.24 \mu$m. ● are the experimentally measured mode amplitudes using method 2. The solid curve is the least-square fit (equation 4.49). The estimated variance from experimental noise is estimated by the broken curve (equation 4.48).

the measured $L_p$ for SWNTs are the the first mode measurements that had a higher signal to noise ratio.

In this study, the $L_p$ measurements of the SWNTs using the two analysis procedures differ by a factor of $\sim 2.6$. $L_p$ calculated using method 2 (Gittes et al. 1993) is more accurate because the systematic error due to the limited resolution of the microscopy system is subtracted from each measured variance of the mode amplitude. No such correction was performed in method 1. Method 1 has been used by Janson and Dogterom (2004) to measure $L_p$ of growing microtubules that had one end of the filament fixed. Method 2 has been used by Gittes et al. (1993) to measure the persistence length of microtubules which are high rigidity filaments. The results from both the methods agreed with within a factor of 2 with previously reported $L_p$ measurements of microtubules using various other analysis methods.
Table 4.1: Persistence length $L_p$ of fluorescently tagged SWNTs computed using method 1 for 13 SWNTs of contour length $L$. The results from the first three modes are presented.

<table>
<thead>
<tr>
<th>$L$ (µm)</th>
<th>$L_p$ (µm)</th>
<th>$L_p$ (µm)</th>
<th>$L_p$ (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n = 1$</td>
<td>$n = 2$</td>
<td>$n = 3$</td>
</tr>
<tr>
<td>3.49 ± 0.28</td>
<td>164.5</td>
<td>24.1</td>
<td>9.8</td>
</tr>
<tr>
<td>3.63 ± 0.24</td>
<td>120.9</td>
<td>35</td>
<td>14.9</td>
</tr>
<tr>
<td>3.93 ± 0.27</td>
<td>440.3</td>
<td>87.9</td>
<td>16.1</td>
</tr>
<tr>
<td>4.49 ± 0.23</td>
<td>543.8</td>
<td>81.7</td>
<td>21.4</td>
</tr>
<tr>
<td>3.17 ± 0.27</td>
<td>130.2</td>
<td>18.9</td>
<td>7.5</td>
</tr>
<tr>
<td>4.1 ± 0.26</td>
<td>295</td>
<td>60.4</td>
<td>17.1</td>
</tr>
<tr>
<td>4.64 ± 0.33</td>
<td>255.1</td>
<td>45.1</td>
<td>17.2</td>
</tr>
<tr>
<td>3.31 ± 0.24</td>
<td>162.3</td>
<td>25.2</td>
<td>7.8</td>
</tr>
<tr>
<td>3.92 ± 0.33</td>
<td>123</td>
<td>23.6</td>
<td>10.6</td>
</tr>
<tr>
<td>3.76 ± 0.22</td>
<td>226.9</td>
<td>41.9</td>
<td>14.4</td>
</tr>
<tr>
<td>3.17 ± 0.24</td>
<td>71.8</td>
<td>15.2</td>
<td>38.7</td>
</tr>
<tr>
<td>3.52 ± 0.28</td>
<td>282.7</td>
<td>33.2</td>
<td>12.3</td>
</tr>
<tr>
<td>4.11 ± 0.22</td>
<td>404.8</td>
<td>55.3</td>
<td>13.6</td>
</tr>
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</table>
Table 4.2: Persistence length $L_p$ of fluorescently tagged SWNTs computed using method 2 for 13 SWNTs of contour length $L$. The results from the first three modes are presented.

<table>
<thead>
<tr>
<th>$L$ (µm)</th>
<th>$L_p$ (µm)</th>
<th>$L_p$ (µm)</th>
<th>$L_p$ (µm)</th>
</tr>
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<td></td>
<td>$n = 1$</td>
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<td>$n = 3$</td>
</tr>
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<td>3.49 ± 0.28</td>
<td>67.1</td>
<td>14.4</td>
<td>10</td>
</tr>
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<td>3.63 ± 0.24</td>
<td>68.6</td>
<td>20.7</td>
<td>13.8</td>
</tr>
<tr>
<td>3.93 ± 0.27</td>
<td>167.8</td>
<td>60.5</td>
<td>19</td>
</tr>
<tr>
<td>4.49 ± 0.23</td>
<td>174</td>
<td>47.8</td>
<td>22.3</td>
</tr>
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<td>3.17 ± 0.27</td>
<td>56.9</td>
<td>9.7</td>
<td>7.6</td>
</tr>
<tr>
<td>4.1 ± 0.26</td>
<td>120.2</td>
<td>34.6</td>
<td>17.8</td>
</tr>
<tr>
<td>4.64 ± 0.33</td>
<td>135.3</td>
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<td>23</td>
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<td>3.31 ± 0.24</td>
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<td>9.1</td>
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<td>3.92 ± 0.33</td>
<td>52</td>
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<td>11.7</td>
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<td>2.7</td>
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<td>3.52 ± 0.28</td>
<td>154.6</td>
<td>17.3</td>
<td>14.5</td>
</tr>
<tr>
<td>4.11 ± 0.22</td>
<td>73.9</td>
<td>25.4</td>
<td>7.3</td>
</tr>
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</table>
Figure 4.21: Measured persistence length $L_p$ of SWNTs in water from the first bending mode by the method of Gittes et al. (1993).

Figure 4.21 shows the persistence length of 13 nanotubes measured from the first bending mode by the method of Gittes et al. (1993). The measurements range from 32 $\mu$m to 174 $\mu$m and are consistent with the theoretical estimates of Yakobson and Couchman (2004)---$L_p$ in the range 7 $\mu$m to 74 $\mu$m. SWNTs in a liquid sample are polydisperse in diameter and $L_p$ is directly related to the cube of the diameter. The diameters of individual SWNTs ($\sim 1$ nm) could not be measured due to diffraction limitations; thus, the wide range of measured persistence lengths. The bending rigidity the SWNTs can be calculated from the persistence length and is in the range $1.3 \times 10^{-25}$ to $7.1 \times 10^{-25}$ Nm$^2$.

4.5 Conclusions

A simple and convenient fluorescent tagging procedure for visualizing in real-time individual surfactant-stabilized SWNTs in water by standard visible-light microscopy
was presented. By using this method, the first data on SWNT rotational diffusion coefficient was measured and it was shown that the measurements agree with predictions based on theory of confined diffusion of slender rods. It was also found that the SWNTs in water start transitioning from dilute, non-interacting suspensions to semi-dilute at a number concentration equal to about \( \langle L^3 \rangle^{-1} \).

The first direct measurement of the persistence length was performed; \( L_p \) ranged from 32 \( \mu \)m to 174 \( \mu \)m. Most SWNTs samples comprise SWNTs shorter than 1 \( \mu \)m; thus, clearly \( L_p \gg L \) and single walled carbon nanotubes in liquids can be considered as rigid in the absence of imposed external fields (e.g., flow). This conclusion agrees with inferences from neutron scattering by Zhou et al. (2004) and estimates from the rigidity results measured by Salvetat et al. (1999), and disagrees with the X-ray scattering study by Schaefer et al. (2003) and also results by Sano et al. (2001). The direct measurement of \( L_p \) in this study ends this controversy about whether to treat nanotubes in liquids as rigid or as semi-flexible macromolecules.

Understanding of SWNTs in liquids can be furthered by fluorescence visualization using video-microscopy\(^5\). Moreover, this new visualization method will be invaluable in material sciences, for example, liquid-phase directed assembly or self-assembly in scalable processing like in sorting of nanotubes by flow-fractionation, assembly into films and coatings, SWNT fiber production and electrophoresis, and in biological and life sciences, for example, studying the interaction of SWNTs with living organisms (e.g., cells) and biological molecules like DNA, making SWNT bio-sensors, targeted gene and drug-delivery using SWNTs.

\(^5\)See Appendix D
Chapter 5

Drop Drying of Surfactant Stabilized Aqueous Dispersion of Single Walled Carbon Nanotubes

Self-assembly is largely a natural process, e.g., biological processes to build molecules, viruses, cells, etc., where ordered macroscopic structures result from assembly of micro and nano sized particles. In recent times, engineers and scientists have been able to initiate self-assembly processes to produce functional materials such as photonic crystals (Vlasov et al. 2001) and DNA structures (Bensimon et al. 1995, Jing et al. 1998). Drying of drops on substrates is ubiquitous—coffee stains on counter tops, ring-deposits on drying utensils and window panes. Evaporation of the solvent induces flow inside the drop which can be used as a driving force for the self-assembly of suspended particles onto substrates. Single-walled carbon nanotubes are currently the focus of extensive interdisciplinary studies because of their unique physical and chemical properties and potential electronic applications, for example, in making sensors and field-emission devices (Baughman et al. 2002). Self-assembled SWNTs on substrates can be used to produce novel materials with excellent electronic properties. Investigation of drying of a sessile drop of individually suspended SWNTs in an aqueous solution of F68 Pluronic on glass is presented in this chapter.
5.1 Introduction

Single walled carbon nanotubes have been used to produce novel materials that utilize the extraordinary mechanical and electrical properties (Section 4.1). Recent efforts include preparing fibers and ribbons of SWNTs (Vigolo et al. 2000). Nanotubes dispersed in an aqueous surfactant solution were aligned and assembled into indefinitely long ribbons and fibres by flowing polymer solution. Thin, transparent and electrically conducting films of pure SWNTs have been prepared by filtration of a surfactant stabilized aqueous dispersion of SWNTs through a membrane and subsequent removal of the surfactant by washing (Wu et al. 2004). Filtration was also used by Zhang et al. (2004) to produce thicker films called bucky paper. The films were prepared from aqueous dispersions containing nitric acid. The mechanical properties of the films were a function of the acid concentration. Another technique that was used to form films of nanotubes was in-situ growth of SWNTs on a substrate Murakami et al. (2004). SWNTs were grown by catalytic chemical vapor deposition on quartz substrates. About 1 μm thick films with vertical aligned SWNTs were prepared. Continuous self-assembled films of SWNT bundles on glass near a receding contact line by solvent evaporation have been prepared by Shimoda et al. (2002). Homogeneous films of nanotube-polymer composites have also been formed; due to the high aspect ratio of the nanotubes, only a small concentration of SWNTs in the composite was required to significantly improve the electrical conductivity of the polymer matrix (Ramasubramaniam et al. 2003, Kim et al. 2003).

Drying of liquid drops on solid substrate has been the subject of several studies (Birdi et al. 1989, Rowan et al. 1995, Deegan et al. 1997). The focus has been understanding of the drying mechanism and utilization of the drying process to prepare ordered structures. Drops of a solution on a substrate follow one of two drying mechanisms: either the drop maintains a constant contact angle by de-pinning the contact line (e.g., water on non-wetting substrates (Birdi and Vu 1993)), or the contact line gets pinned and the drop maintains a fixed contact area (e.g., colloidal dispersions (Deegan et al. 1997)). Birdi et al. (1989) have studied the evaporation of small water
droplets placed on smooth solid surfaces like glass; they found that the evaporation rate depends on the radius of the liquid-solid interface. This radius remains constant for contact angles less than 90° (wetting surface) (Rowan et al. 1995) and the decrease in the volume yields a diminishing in the contact angle. When the contact angle exceeds 90° (non-wetting surface), the evaporation rate is nonlinear and the radius of the interface decreases while the contact angle remains constant (Rowan et al. 1995). Rowan et al. reported direct measurements of the changing contact angle of a water drop on poly-(methyl methacrylate) for angles less than 90°. They concluded that the evaporation rate depends on the height of the drop rather than the radius of the interface.

Deegan and co-workers (Deegan et al. 1997, Deegan 2000, Deegan et al. 2000) have studied drying of drops of colloidal dispersions and found that the particles deposit in a ring at the periphery of the drop (e.g., coffee stains on counters) due to capillary flow in which the pinned contact line causes the solvent to flow towards the edge. Microscopic evidence for the growth in the ring mass was provided for colloidal suspensions of microspheres. Abramchuk et al. (2001) have directly observed DNA molecules in a convection flow of a drying drop of poly-(ethylene glycol) under ambient conditions. The DNA molecules were observed to stretch in the vicinity of the droplet contact line due to velocity gradients in the viscous drop. Ordered array of particles were created spontaneously on substrates of patterned wettabiliity by evaporation of drops of colloidal suspensions (Fan and Stebe 2004). Drying of salt solutions on substrates resulted in microscopic and macroscopic fractal patterns (Okubo et al. 2004). Figure 5.1a shows fractal patterns formed on glass from a salt buffer solution. Such fractal patterns were also formed by protein solutions (Annarelli et al. 1999). Self-assembled morphologies that depend on the size of the particle and its concentration appeared on drying of colloidal dispersion of nano-particles (Rabani et al. 2003) (figure 5.1b). Macroscopic regular radial stripe-crack patterns formed on drying aqueous solutions of surface modified polymer nano-spheres at high concentrations (Chen et al. 2004) (figure 5.1c). Similar to these self-assemblys, the moving contact line of a drying drop could be used to form aligned patterns of SWNTs
on substrates for making films or for nano-fabrication.

Recent investigations have also shown the formation of a skin or crust at the free surface of drops of polymers and colloidal suspensions (Pauchard and Allain 2003b, Pauchard and Allain 2003a). Pauchard and Allain (2003a) found that the crust may collapse and evolve into different shapes as the surface area remains constant while the drop volume decreases due to solvent evaporation. "Crusting" on the surface of spin cast films (Bornside et al. 1989, Cimapi and McDonald 2003) is a well known phenomenon. Because the glass transition or the gelation temperature of a pure polymer/colloid is higher than that in solution (Pauchard and Allain 2003a), at any temperature below the glass transition there is a critical particle concentration at which the system transitions from fluid to glassy or gel-like. Evaporation of solvent from the free surface leads to a local increase in concentration of the polymer/suspension at the free surface, and a very thin glassy or gelled crust is formed at the free surface. de Gennes (2002) suggested a transport model for crust formation in spin cast films from glassy polymers (figure 5.2). Assuming that diffusion is rate limiting and a steady state inside the crust, the mass balance can be written as

\[ J = D(\psi) \frac{d\psi}{dx} \]  

(5.1)

where \( \psi \) is the solvent volume fraction and \( D(\psi) \) is the diffusion coefficient of the solvent in the mixture. \( J \) is the solvent evaporation current to the atmosphere (de Gennes 2002)

\[ J = \frac{v_{th} a}{l} \frac{p_v}{p_a} \psi_a . \]  

(5.2)

Here \( v_{th} = (k_B T/m)^{1/2} \) is the thermal velocity of solvent molecules of mass \( m \) and size \( a \), \( l \) is the diffusion layer in air, \( p_a \) is the atmospheric pressure and \( p_v \) is the vapor pressure of pure solvent. The thickness of the crust at the free-surface is estimated by (the concentration at the surface \( \psi_a \) reaches a critical value \( \psi^* \))

\[ h = \frac{l p_a}{p_v v_{th} a} \frac{D(\psi^*)}{\psi_a} . \]  

(5.3)

de Gennes (2002) estimated the crust to be extremely thin \( \approx 70 \) nm. The crust would experience increasing tension as its volume decreases due to solvent loss and crack.
Figure 5.1: (a) Fractal assembly of salt in a dried drop of DNA buffer (TBE buffer, 1 mM NaCl) on glass observed by dark-field microscopy. Scale bar is 100 μm. (b) Self-assembled morphologies on glass in a dried drop of aqueous colloidal suspension of 500 nm diameter carboxyl modified polystyrene beads. Scale bar is 50 μm. (c) Radial-crack patterns formed in a dried drop of 2 wt % colloidal suspension of 20 nm dia. carboxyl modified polystyrene beads. Scale bar is 50 μm.
Figure 5.2: Schematic of de Gennes model describing crusting in a polymer film on a substrate. $\psi$ is the solvent volume fraction and $\psi^*$ is the critical concentration for glass transition. The crust is an entangled mesh of the polymer and has a thickness $h$. $l$ is the diffusion layer in air from solvent evaporation. Adapted from de Gennes (2002).

5.2 Experimental

5.2.1 Materials

SWNTs were produced by the HiPco process at Rice University (HPR 120.3). F68 (Sigma-Aldrich, St. Louis, MO) is a high molecular weight ($M_w = 8400$ g/mol) Pluronic surfactant with two poly-ethylene oxide chains connected together by a poly-propylene oxide chain, $\text{PEO}_{78}(\text{PPO}_{30})\text{PEO}_{78}$. Glass slides were cleaned in a solution of 70% sulphuric acid and 30% hydrogen peroxide for 30 minutes, rinsed in water and wiped clean with methanol. Freshly cleaved mica was used for atomic force microscopy. Clean aluminium posts were used for scanning electron microscopy. Bare copper grids (without film) were purchased from SPI Supplies (West Chester, PA) for transmission electron microscopy.

5.2.2 Solution preparation and characterization

Dispersions of the SWNTs were prepared in a 2 wt% or 2.4 mM aqueous solution of F68 Pluronic. F68 has a critical micelle concentration at 1.4 mM and forms ly-
otropic cubic phases at high concentrations and/or low temperatures (Lopes and Loh 1998, Wanka et al. 1994). SWNTs were dispersed in the surfactant solution through a technique developed by O’Connell et al. (2001) involving homogenization, ultra-sonication, and ultra-centrifugation to get suspensions with final concentration between 20 to 30 mg/l (Section 4.2). The hydrophobic segment of the surfactant (poly-propylene oxide) wraps around individual carbon nanotubes, whereas the two hydrophilic poly-ethylene oxide segments expand in water. The polymer provides a steric barrier to bundling induced by van der Waals forces between nanotubes; such pluronic-stabilized suspensions of individual SWNTs are stable for months. HiPco SWNTs have a diameter \( \sim 1 \) nm (O’Connell et al. 2002) and F68 coated individual SWNT in solution have an estimated diameter \( \sim 13 \) nm (using de Gennes polymer brush theory (Sedev et al. 2000): \( L_o = a^{5/3}N^{1/3}A^{-1/3} \), where \( L_o \) is the brush length in the solvent, \( N = 78 \) is the number of ethylene oxide monomers, \( a = 0.24 \) nm is the size of the ethylene oxide monomer, \( A = 1.8 \) nm\(^2\) is the area occupied by each molecule of F68 at the air/water interface). The SWNTs in the suspensions were dispersed as individuals (Section 4.2): Raman fluorescence spectroscopy showed high energy fluorescence peaks, and the Raman peak at \( \sim 273 \) cm\(^{-1}\) (indicative of bundles (Strano et al. 2003, Moore et al. 2003)) was suppressed (figure 5.3).

### 5.2.3 Microscopy

1 \( \mu \)l drops of the dispersion were deposited on the substrates and allowed to dry under ambient conditions (23°C and 38% relative humidity). Dried drop samples were investigated using a Zeiss Axioplan-2 optical microscope, a tapping mode Digital Instrument Nanoscope IIIA atomic force microscope (AFM), a JEM 2010 transmission electron microscope (TEM) and a JEOL 5300 scanning electron microscope (SEM). The crust was floated on water and transferred to appropriate substrates—on mica for AFM, on clean SEM posts, and on copper grids for TEM. The crusts could float on water for over 48 hours without re-suspending.

The contact angle of the dispersion was measured by video microscopy on Contact Angle Goniometer (Rame Hart Inc, Mountain Lakes, NJ). A Retiga 1300 video camera
Figure 5.3: Raman fluorescence spectrum of SWNTs dispersed in 2 wt% F68 Pluronic. High energy fluorescence (high Raman shift) show presence of individual SWNTs in solution. Inset: Low energy Raman spectrum showing absence of the radial breathing mode of the SWNT bundles at $\sim 273 \text{ cm}^{-1}$. 
(QImaging, Canada) was used to monitor the radius of the drops as they dried on a glass substrate. Images were captured at 30 s time interval for about 10 min. Measurements of the drop radius were performed using QCapture Pro (QImaging, Canada). The diameter of each drop was measured at four different angles and then averaged.

5.2.4 Weight measurement

16 drops, each of 1 µl volume, were deposited on a clean glass substrate placed on an analytical balance (Denver Instrument Company, Arvada, CO) to monitor the weight loss due to solvent evaporation. The resolution of the balance was 0.1 mg. Weight measurements were recorded every 30 s after all the drops were deposited.

5.2.5 Rheology

Bulk rheological measurements of the surfactant solutions were done using an ARES 1000FRT Rheometer (TA Instruments, New Castle, DE). The Couette (bob diameter = 32 mm, bob length = 34 mm) testing fixture was used to perform measurements on solutions up to 40 wt% surfactant concentration. The 50 wt% solution was measured in the parallel plate geometry (plate diameter = 50 mm). The temperature of the fixtures was controlled using a Neslab RTE-130M refrigerated bath/circulator and tests were performed at 25°C.

Solutions were prepared in water (18.2 MΩ, Barnstead NANOpure Diamond water purification system) by stirring overnight at 5°C. The set-up consisted of an ice bath maintained at about 5°C on a magnetic stirring plate. Both the hydrophilic (PEO) and the hydrophobic (PPO) segments of the chain are soluble in water at 5°C (Eiser et al. 2000). Micelles get formed when the temperature is increased as the PPO segment of the molecules become increasingly hydrophobic. To ensure proper mixing, wide bottom bottles should be used and the surfactant should be added very slowly while maintaining a vortex at the free-surface of the solution. Solutions with polymer concentration greater than 40 wt% were difficult to prepare.
5.2.6 Differential scanning calorimetry

Differential scanning calorimetry was done using DSC Q10 (TA Instruments, New Castle, DE) on pure F68 surfactant and F68-SWNT mixture. The F68-SWNT mixture was obtained by drying overnight the SWNT solution on clean glass substrates and then carefully scraping the dry deposits.

5.3 Results and discussion

Video microscopy showed that the initial drying progressed by de-pinning of the contact line, i.e., the radius of the base decreased with time (figure 5.4). Figure 5.5a shows the drop radius (normalized by the initial radius) as a function of time. After about 360 s the drop attained a fixed base radius and a foot started appearing. Drops of pure water on the same substrate dried by maintaining a fixed base radius, in agreement to the findings by Birdi et al. (1989).

Assuming quasi-stationary conditions, if diffusion of water in air is rate controlling, then for a sessile drop receding with a constant contact angle the square of the base radius is linear with time (Parisse and Allain 1997)

\[ R^2(t) = 1 - B(\theta_o)D\left(\frac{n_{ws} - n_{w\infty}}{n_1}\right)t \]  

(5.4)

where, \( R \) is drop radius normalized with radius at \( t = 0 \), \( B \) is a geometrical factor that depends on the contact angle \( \theta_o \), \( D \) is the diffusion coefficient of water in air, \( n_1 \) is the molar water concentration for pure liquid, \( n_{ws} \) is the molar water concentration at the air-water interface and \( n_{w\infty} \) is the molar water concentration far from the
Figure 5.5: (a) Normalized Drop Radius $R$ versus time $t$ for SWNT-FO8 drops (1 μl) deposited on glass. After $\approx 360$ s the drops attain a fixed base radius. Results for five drops are plotted here. The error bars are the standard deviations. The drop radius of water (•) remained constant as the drying progresses. Images after 270 s did not have enough contrast for accurate measurements. (b) $1 - R^2$ vs. time (equation 5.4). The good linear fits ($t < 240$ s) show that the drying process is diffusion limited. Results for four drops are plotted here.
Figure 5.6: Contact angle of two drops of SWNT-F68 drying on a glass substrate. Contact Angle Goniometer was used to measure the contact angle over time. Note that the contact angle changed by a small amount as the drying progressed.

drop. While the drop radius decreased, the contact angle between the drop and the glass substrate was about 10 – 15° (figure 5.6). The resolution of the measuring system was about 10 – 15°. Mc Hale et al. (1998) have argued that a small change in the contact angle was expected in experiments as the base contact radius decreased and the constant contact angle expression equation 5.4 for diffusion controlled drying was still valid. Upto \( t \sim 210 \text{ s} \), we find that the assumption that diffusion is rate controlling is fairly accurate (figure 5.5b).

The weight of the drops was monitored over time as they dried (figure 5.7) and the initial evaporation rate (\( t \leq 360 \text{ s} \)) was \( J_o \approx 2 \times 10^{-6} \text{ cm}^3/\text{s} \). After 360 s the drying rate decreased. In contrast, drops of water on the same substrate dried at a constant evaporation rate (figure 5.7). As the drying progressed further, the drop attained a constant base radius at \( t \approx 360 \text{ s} \), and a surface undulation appeared at the top of
the drop (figure 5.9(1)). A thin crust appeared at the free surface, with a convective flow toward the foot, underneath. The formation of the crust slowed down the solvent loss from the initial evaporation rate \( J_0 \) (figure 5.7) by reducing the diffusion of water from the core of the drop to the free surface. Loss of the solvent decreased the volume enclosed by the thin crust, and the crust thus inverted, forming an undulation similar to a collapsing dome. The drying process is summarized in a schematic in figure 5.8.

As soon as the drop was deposited solvent loss began. The drop attained a fixed base radius; a foot formed as the suspension deposited at the contact line and progressively extended toward the middle of the drop, and the spherical-cap shape got distorted. The increased concentration of the polymer at the free surface lead to the formation of a solid-like crust. The crust enveloped the fluid drop and slowed the loss of water. Once the solvent loss was complete the crust settled on the deposits that formed the foot.

The inhomogeneities (due to SWNTs) in the thin crust served as nucleation points for fractures. “Volcanic landscapes” appeared when the crust ruptured at isolated points (figures 5.9(2) and 5.10a). Aligned liquid crystalline domains (observed under polarization, figures 5.9(3-6) and 5.10b) were formed as the evaporation front moved across the drop from the rupture sites. The increased volume fraction due to solvent loss lead to formation of a gel-like solid that got packed due to the moving evaporation front. Defect planes appeared over time when two evaporation fronts met (figure 5.9(4)), akin to the formation of grain boundaries and defects in crystals. Further water loss occurred with the horizontal dimensions remaining fixed and the extremely thin crust comes under increasing tension (as it tried to shrink) and then fractured forming cracks (figure 5.9(6)). The fan-like arrangement (figure 5.10b) observed is typical of hexagonal liquid crystalline arrangement where the solute is cylindrical in shape. Drops of only F68 also show birefringence patterns in the foot of the drop (figure 5.11a). F68 form only cubic liquid crystalline phase and thus have no birefringence. The convective flow in the drop aligned the micelles into columns, and yield the characteristic hexagonal arrangement. Drying drops of only F68 did not develop a surface undulation, but a gelled foot appeared at the contact line, and
Figure 5.7: Variation of drop weight versus time. • SWNT-F68 and □ Water. Water drops dry with a constant drying rate (broken line). SWNT-F68 drops dry at a constant drying rate for $t < 360 \text{ s}$ (solid line). For $t > 360 \text{ s}$ the evaporation rate slows down. The averages are for 5 sets of 16 drops (SWNT-F68) and 3 sets of 16 drops (Water). The error bars are the standard deviations.
Figure 5.8: Schematic of the drying process. (1) & (2) The drop maintains a fixed contact angle as the base radius shrinks. (3) The contact line gets pinned and a gelled foot appears. A crust gets formed at the free surface that reduces the evaporation rate. (4) The volume that the crust envelopes decreases as evaporation proceeds, and a surface undulation appears. (5) The crust sits on the surfactant-SWNT deposit on the substrate, when the drying reaches completion.
Figure 5.9: Drying drops of SWNT-F68 on glass. (a) Progression of drying under crossed polarizers. (1) Contact line gets pinned and the drop forms a gelled foot. Note the undulation of the free-surface in the middle. (2) Crust ruptures at a point and birefringent patterns start appearing. (3) Crust ruptures at another point and the drying fronts move radially. (4) The drying fronts meet to form a grain boundary. (5) The drying fronts move across the whole drop. (6) A circular crack appears around the rupture site (at the top).

A crust was formed. Structures similar to those on glass were formed on mica; figure 5.12a shows an AFM image of aligned SWNT-F68 micelles near the edge of the drop. The striations (also seen in figure 5.10a, the spokes originating from the rupture sites (A)) are the assembled surfactant-nanotube micelles. The presence of SWNTs on the surface of the dried drop was found be imaging at the cracks by scanning electron microscopy. The long rod-like structures are the SWNT-micelles and not to be confused with the flow induced columnar pluronic micelles (figure 5.12b). Such structures are particularly prominent in cracks which they bridge. The sample was coated with gold to prevent widening of the cracks due to the high energy electron beam, which covers the striations.

When aqueous drops of a SWNT-SDS suspension were dried under similar conditions, a crust did not form, and the SWNTs deposited in the center of the drop (figure 5.11b). Both SDS and F68 are surface adsorbing molecules which arrange
Figure 5.10: Dried drop of SWNT-F68 on glass. (a) Brightfield image showing the foot of the drop, volcanic landscape, and cracks. A: Rupture sites B: Cracks C: Grain boundaries D: Foot. Scale bar is 100 μm. (b) Under crossed polarizers. The birefringent fan-like arrangements of the micelles, characteristic of hexagonal liquid crystalline domains. Scale bar is 100 μm.
Figure 5.11: (a) Under crossed polarizers, the birefringent patterns formed by F68 micelles in the foot of a dried drop of aqueous solution of F68. (b) Brightfield image of a dried drop of aqueous solution of SDS-SWNT on glass. Note that the SWNTs deposited in the center of the drop. Scale bar is 100 μm.
Figure 5.12: (a) AFM amplitude scan (10 µm × 10 µm) at the surface of a dried drop of SWNT-F68 on mica. Scans were done near the edge of the drop. (b) SEM image showing SWNT-F68 bundles bridging a crack.
at the free-surface with the hydrophobic part at the air-water interface and the hydrophilic part suspended in water. The hydrophobic PPO chain in pluronic surfactant arranges itself at the air-water interface and the hydrophilic PEO chains extend as brushes in the water (Muñoz et al. 1999a). SDS—a small molecule—cannot form a mesh to support the SWNTs, while F68—a long-chain polymer—can form an entangled network entrapping the SWNTs. Both, evaporative loss of the solvent and also the preferential adsorption of surfactant at the air-solution interface leads to a local increase in the concentration of the SWNT-F68 complex at the free surface, leading to the formation of a crust.

In order to investigate the structure and arrangement of the SWNT-F68 micelles in the crust we first suspended it in water, and floated on aluminium SEM posts, and coated with gold to obtain scanning electron microscopy images. Mats of entangled strands were imaged. Some monolayer thin regions of the mat (figure 5.13b) where the mesh was under tension (figure 5.13a) were observed. The size of the strands in the mesh was $\sim 10$ nm. The thickness of the crust was between 10 and 100 nm. Figure 5.14a shows an AFM scan of the mesh-like structure of the crust. Accurate film thicknesses could not be measured by AFM due to folding of the crust during transfer onto mica. The crust was monolayer thick ($\sim 10$ nm) at the edges; toward the middle the surface roughness was 50–80 nm. The presence of SWNTs in the thin crust is apparent from the dark color (figure 5.15 inset). Raman spectrum of the crust shows the characteristic transverse breathing mode of SWNTs at 1592 cm$^{-1}$ and the radial breathing mode at 234 cm$^{-1}$ (figure 5.15). To confirm the presence of SWNTs in the strands of the mat, transmission electron microscopy was also performed on the crust fished onto non-coated copper grids. Images of the mat were obtained and SWNTs were imaged in the entangled strands (figure 5.14b). The SWNTs appeared bundled but smaller in size as compared to conventional Bucky papers. The low bundling is attributed to surfactant-SWNT dispersion that had most of the SWNTs as individuals and the spontaneous self-assembly of individual SWNT-F68 micelles at the free surface. The thickness ($\sim 100$ nm) and the low bundling can be potentially used to prepare thin optically transparent conductive films and coatings.
Figure 5.13: SEM image of SWNT-F68 crust formed on glass showing entangled mesh-like morphology of the crust. Note that the crust is under tension in the crack. Scale bar is 200 nm.
Figure 5.14: (a) AFM scan (5 μm × 5 μm) of the crust on mica showing entangled mesh-like morphology. The height scale is 80 nm. (b) Transmission Electron Micrograph of the crust, showing the presence of SWNTs in the strands. Scale bar is 40 nm.
Figure 5.15: Raman spectrum of the crust displaced from a drying drop of SWNT-F68 on glass (780 nm diode laser). The peaks at $\sim 1592$ cm$^{-1}$ and $\sim 234$ cm$^{-1}$ correspond to the axial and radial breathing modes of SWNTs, respectively. Inset: A displaced crust. Scale bar is 200 μm.
The simple deGennes theory (de Gennes 2002) is based on two assumptions: the polymer has a glass transition and the polymer is non-surface adsorbing. Differential Scanning Calorimetry (DSC) was performed to find the glass transition temperature of F68 Pluronic. F68 is a semi-crystalline polymer and its melting point was measured at 57.2°C (figure 5.16 dashed line). Also, F68 did not exhibit glass transition above the room temperature. It is well known that the glass transition behavior of polymers is altered by addition of even small amounts of nanotubes. A dried sample of the aqueous dispersion of SWNTs in the surfactant was tested by DSC and it was found that the presence of SWNTs did not alter the melting point of the mixture (figure 5.16 solid line). Moreover, F68 is a surface adsorbing polymer molecule; thus, deGennes theory cannot be used to explain the SWNT-F68 system. However qualitatively, the phenomenon is similar, i.e., an entangled mesh is formed at the free surface because the mixture transitions from liquid-like to solid-like at the free surface due to loss of solvent, and also due to the preferential adsorption of the surfactant at the surface.

To find the critical concentration of the polymer required at the free surface to form a crust, the linear viscoelastic response of the surfactant/water system was measured by solution rheology. Figure 5.17a shows the elastic ($G'$) and viscous ($G''$) moduli of aqueous F68 solutions at different concentrations. The solutions are liquid-like below 40 wt%. The 50 wt% solution showed solid-like behavior: the elastic modulus was independent of frequency and became greater than the viscous modulus. Figure 5.17b shows $\tan \delta = G''/G'$ as a function of concentration. The solution transitioned from liquid-like ($\tan \delta > 1$) to solid-like ($\tan \delta < 1$) between 40 and 50 wt% polymer concentration and the critical gel concentration thus was between 40 and 50 wt%.

Recall that the drop radius measurements (figure 5.5b) showed that the drying process is diffusion limited for $t < 210$ s. In this regime, from the conservation of material flux at the free-surface, the time required for the concentration to reach the critical value at which crust formation occurs can be estimated by $t_c = D(\phi_c)(\phi_e - \phi_o)^2 S^2 / J_c^2$, where $\phi_o$ is the initial volume fraction of the polymer, $\phi_e$ is the polymer volume fraction at the free surface, $D$ is the diffusion coefficient and $S$ is the surface area (Pauchard and Allain 2003b). The diffusion coefficient of F68 in aqueous solution at the critical
Figure 5.16: Differential Scanning Calorimetry (DSC) of F68 (dashed line) and F68-SWNT mixture (solid line). F68 has a melting point at 57.2°C and does not have a glass transition temperature above room temperature. Note that the addition of SWNTs did not alter the melting properties of pure F68.

concentration (50 wt % ≈ 59 mM) is $2 \times 10^{-6}$cm$^2$/s (Muñoz et al. 1999b). Using these values and the surface area of the drop when it reaches a fixed base radius gives $t_c = 102$ s. The drying time corresponding to a fixed evaporation rate $J_o$ is $t_d = 540$ s. Similar to the findings of Pauchard and Allain (2003a), because $t_c < t_d$, the crust gets formed before the drying of the drop is complete; evaporation proceeds at a slower rate through a porous mesh-like crust, and loss of volume enclosed leads to the surface undulation.

5.4 Conclusions

Evaporation of dilute aqueous solution of surfactant stabilized SWNTs exhibited complex dynamics. The drops of the solution dried with the drop radius initially decreasing and then attaining a fixed base radius. The initial drying was diffusion controlled.
Figure 5.17: (a) Frequency dependent elastic $G'$ and viscous $G''$ moduli of F68 aqueous solutions. Below 40 wt% the solutions are liquid-like. At 50 wt%, both $G'$ and $G''$ become frequency-independent and the solution is solid-like. (b) $\tan \delta = G''/G'$ as a function of F68 concentration at different frequencies. Gelation occurs between 40 and 50 wt% when $\tan \delta$ crosses 1.
Solvent loss due to evaporation resulted in the surface concentration of the surfactant reaching a critical concentration above which it formed a gel. The crust slowed the evaporation of the solvent, and a free surface inversion was observed. Crust formation can be explained qualitatively by de Gennes theory but to get quantitative agreement for the thickness of the crust, polymer adsorption at the free-surface needs to be considered.

The suspended nanotubes instead of assembling on the substrate self-assembled into a thin (~ 100 nm) entangled mesh-like crust at the free surface. The phenomena of crusting at the free surface due to solvent mass transfer across an interface, demonstrated in a drying drop in this study, can be used to fabricate thin crusts and coatings of SWNTs on substrates.
Appendix A

Image analysis

Image processing—both acquisition and subsequent analysis—has an important relevance to a microscopist. The objective is to extract the information from the image for which the image was acquired. The images of flowing stained λ-DNA have to be analyzed to determine the molecular size and orientation.

A.1 Thresholding

This technique isolates objects of interest from the background. Each pixel is classified as either belonging to an object (pixel value 1) or to the background (pixel value 255). Pixels below a certain threshold gray scale are set to 0 and those above it to 255. An automatic thresholding technique was used, where a threshold was calculated from a linear combination of the mean gray level $\mu$ and the standard deviation $\sigma$ of the gray levels of the source image, $\tau = k_1 \mu + k_2 \sigma$ (Ritter and Wilson 1996). For low resolution images $k_1 = k_2 = 1$ and for high resolution images $k_1 = 1$ or $k_1 = 1.5$ and $k_2 = 2$ (Ritter and Wilson 1996). Figure A.1 displays the binary images obtained by processing the raw DNA image. Before the thresholding, median filtering was performed on the raw images. Median filtering is a non-linear image processing operation that reduces noise but preserves edges (Ritter and Wilson 1996). Each processed pixel is assigned the median gray-value from a 3-by-3 neighborhood gray values of the raw image. In the software written in Matlab, raw data was automatically read and median filtered, and then thresholded either automatically
Figure A.1: Thresholding of image of a stretched DNA molecule. The image was acquired using a 100X Oil immersion objective. (0): original image; (1) Threshold gray value 0.45 times the maximum gray value, $\tau = 98$; (2) $\tau = k_1 \mu + k_2 \sigma = 193$, $k_1 = 1.5, k_2 = 2.0$; (3) $\tau = 149$, $k_1 = 1, k_2 = 2$; (4) $\tau = 118$, $k_1 = 1, k_2 = 1$.

using prescribed $k_1$ and $k_2$ (determined by inspection before starting analysis of a set of images) or by user defined $k_1$ and $k_2$ for each image.

### A.2 Molecular properties

The centroid ($\bar{x}$, $\bar{y}$) of binary image of each molecule can be calculated using the following expression

\[
\bar{x} \equiv \frac{\sum_{i=1}^{n} x_i}{n}
\]

\[
\bar{y} \equiv \frac{\sum_{i=1}^{n} y_i}{n}
\]

(A.1)

where $(x_i, y_i)$ is the pixel belonging to the object and $n$ is the total number of pixels belonging to the object (Russ 1995). The moment axis of the molecule is the axis around which the moment of the 2-D object is minimum (Russ 1995) and the axis orientation is calculated using the the object pixels, from the following equations (Sunada and Blanch 1998, Petera and Muthukumar 1999)
\[
\begin{aligned}
M_{xx} &= \frac{1}{n} \sum_{i=1}^{n} (x_i - \bar{x})^2 \\
M_{yy} &= \frac{1}{n} \sum_{i=1}^{n} (y_i - \bar{y})^2 \\
M_{xy} &= \frac{1}{n} \sum_{i=1}^{n} (x_i - \bar{x})(y_i - \bar{y}) \\
2\theta &= \arctan \left( \frac{2M_{xy}}{M_{xx} - M_{yy}} \right)
\end{aligned}
\] (A.2)

Note that \( M \) is the conformation of the molecule and is equivalent to the gyration-tensor \( R_g \). The eigen-values of the \( M \) are the major and minor radii of equivalent ellipses, and the eigen-vectors give the orientation of the ellipse (molecule). The maximum stretch of the molecule is the longest distance between any two pixels that are part of the molecule. All these molecular properties were evaluated using standard were evaluated for each DNA molecule using standard image analyzing functions in Matlab.
Appendix B

Atomic force microscopy of fluorescent SWNTs

To determine whether the SWNTs in the suspension were individuals, atomic force microscopy was performed. The diameter of a single SWNT (HiPco) is between 0.6 and 1.3 nm (Bachilo et al. 2002). An silicon-aminated wafer was dipped in the fluorescently labeled SWNT sample (Parra-Vasquez et al. 2005). The chip was washed immediately with water and iso-propyl alcohol and dried under clean air. Scans on the chip were performed on a tapping mode Digital Instrument Nanoscope IIIA atomic force microscope (AFM). Figure B.1 shows the height and the amplitude images of 4 nanotubes (longer than 1 µm). The height of the four nanotubes is about 1-2 nm, thus the nanotubes are individuals. It should be mentioned here that AFM imaging of SWNTs dispersed with SDS is extremely difficult because the SWNTs do not stick to the substrate surface and also because the residual surfactant forms patches on the substrate surface, making it difficult to find SWNTs.

Based on the length distribution in the sample (Parra-Vasquez et al. 2005), about 0.12% of the SWNTs are longer than 2 µm, which yields $\sim 10^7$ above 2 µm SWNTs in 2 µl of suspension.
Figure B.1: AFM images of SWNTs on silicon-aminated wafer. The height and amplitude images are shown along with the height analysis of the SWNTs. Note that the height of the four SWNTs is about 1–2 nm, and thus these nanotubes are individuals.
Appendix C

Fluorescence visualization of SWNTs

A green fluorescent hydrophobic dye (PKH67; Sigma; excitation 490/ emission 502 nm) was also used for labeling the SDS-SWNT complexes. This dye can offer better resolution than the red dye (PKH26) because the emission wavelength of this dye is lower than the emission wavelength of the red dye. The staining procedure for PKH67 was the same as that for PKH26; the dye was added to the aqueous solution and spontaneously incorporated into the core of the micelles. 1 µl of dye was added to 10 µl of SWNT-SDS solution and to ensure proper mixing, the sample was vortex mixed for about 10 s.

Individual fluorescently tagged SWNTs were visualized by epi-fluorescence microscopy with a Nikon E600 microscope equipped with a filter cube (XF100-2; excitation 450-500/500 dichroic/ emission 520-560 nm: Omega, Inc., VT) and a 100X oil-immersion objective (NA 1.4). The filter cube is sub-optimal for the dye PKH67, and better images can be obtained with an appropriate filter cube. Figure C.1 shows images of fluorescent individual SWNTs.
Figure C.1: Fluorescence microscopy images of individual single-walled carbon nanotubes tagged with PKH67 in SDS. Scale bar is 10 μm.
Appendix D

SWNTs in Water

D.1 Exfoliation of SWNT bundles using light

Long slender objects (sometimes longer than 10 μm) were found in fluorescently tagged SWNT samples that had spent several days on the shelf. These objects were found to be bundles of SWNTs that broke quickly (within tens of seconds) into smaller parts when they were continuously exposed to light under the microscope (figure D.1 shows such an exceedingly long bundle). This suggests the possibility of using light for exfoliating individual SWNTs from bundles in liquids\(^1\). These long breakable objects were never observed in fresh samples of fluorescent SWNTs; this shows that all the nanotubes in the diffusion and bending measurements were individuals.

D.2 SWNT at a free surface

SWNTs are amphiphobic, i.e., they do not like either water or organic solvents. Wang and Hobbie (2003) used SWNTs similar to surfactant, and formed stable emulsions. The arrangement of SWNTs at the interface is not yet understood. Real time fluorescence visualization can be used to understand the behavior of SWNTs at interfaces such as an air-water free surface. An air bubble was introduced inside a drop of fluorescently tagged SWNTs in water, on a glass slide. SWNTs were observed at the air-water interface, and they moved parallel to the free surface (figure D.2). Possibly

\(^1\)From a 100W Hg lamp (light source for epi-fluorescence microscopy set-up), the amount of excitation light incident on the field of view (100X; 90 μm x 70 μm) is about 0.07W (Herman 1998).
Figure D.1: Snapshots of a fluorescently tagged (PKH26) nanotube bundle breaking on exposure to light (540–550 nm).
Figure D.2: Images of a SWNT at an air-water interface. Note that the SWNT is oriented parallel to the free surface.

the curvature of the free surface determines the preferential orientation that a SWNT takes at the interface.
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