Mathematical Biology of the Cell: Cytoskeleton and Motility (11w5050)

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1 Overview of the Field

1.1 Introduction and Rationale

Cell biology and mathematics have traditionally been distinct areas of science, each with its own culture, techniques, and approaches. Until recent times, the only points of contact were a few key biophysical concepts, with relevant mathematical formulations, that were applied in a cell biology context. However, this situation has drastically changed. First, cell biology has become increasingly quantitative and new experimental techniques allow researchers to collect extremely detailed information for both *in vitro* as well as *in vivo* systems. Concomitant with this, there has been explosive growth of computational biology with essential aspects of mathematics, physics, and computational methods at its core. These new techniques and areas of focus have been used to gain a deeper understanding of eukaryotic cells, how these cells orient and move, and what roles are played by the underlying biopolymers and regulators.

Cell biology, and the cytoskeleton in particular, constitute a particularly promising area of contact between mathematics and the life sciences due to the abundance and complexity of the available data. This means that biologists are more inclined to actively seek collaboration with mathematicians, physicists, and computer scientists, and there is an obvious need for mathematical analysis of complex pathways to decipher data sets that cannot be understood by traditional methods. This workshop was in essence a follow-up to a BIRS meeting held in the summer of 2005 (Mathematical Modeling of the Cell: Cytoskeleton and Motility 05w5004). That first meeting planted many ideas that have now come to fruition, and many participants from the 2005 meeting were able to return and participate in this workshop.

1.2 The Cytoskeleton and the Cell

In order to put the developments and presentations from the workshop in the proper context, it is useful to first present some basic background on the relevant biology. All eukaryotic cells, or cells with a nucleus, contain an array of polymers collectively referred to as the cytoskeleton. There are three primary filament types: actin filaments, microtubules and intermediate filaments. The cytoskeleton is involved in a wide range of cellular functions including cell division and cell migration. Further, the cytoskeleton provides a structural framework within the cell, allowing it to both exert and respond to extracellular stimuli.

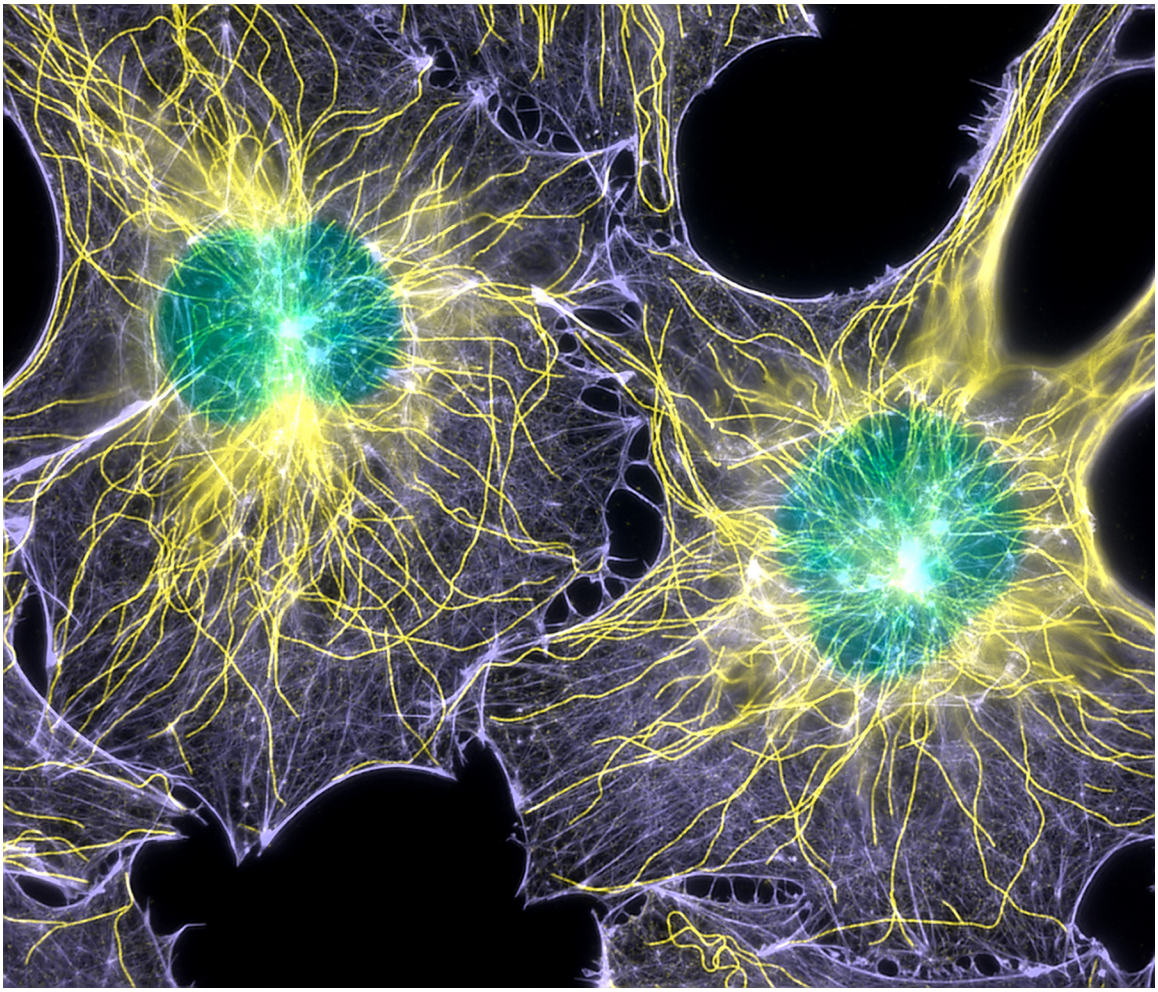

Figure 1: Fluorescent micrograph of a cell highlighting the microtubules (yellow), actin filaments (blue) and cell nucleus (green). Image courtesy of Torsten Wittmann (UCSF).

As shown in Figure 1, actin filaments and microtubules often assume semi-regular arrays within cells. Microtubules are the most rigid structures in the cell, typically emanating from the microtubule organizing center (MTOC) next to the nucleus. Actin filaments can assume a variety of different conformations including bundles, where the filaments are arranged in a parallel or antiparallel fashion, or branched networks. These branched networks typically found at the edge of migrating cells, form a broad thin structure called a lamellipod. The cytoskeleton is involved in determining cell shape and movement.

It should not be surprising that actin filaments and microtubules do not act alone. Rather, their function is regulated by a multitude of associated proteins. Some of these associated proteins help to polymerize or depolymerize the filaments, crosslink filaments into networks or bundles. Motor proteins exert forces between filaments, organelles, cell membrane and extracellular matrix. Biochemistry and cell biology have given us a wealth of data about the components and parts of this system, but many details are still missing. More than that, how the system is organized to work coherently in space and time is still an elusive question. Given the inherent complexity of this system, mathematical and physical models are playing an increasingly important role in elucidating the underlying behaviour of the cytoskeleton and its role in many cellular functions.

2 Recent Developments and Open Problems

2.1 Experimental Advances

Cell biology has been significantly impacted by the introduction of many new experimental techniques that allow more accurate and quantitative measurements to be made. There are several burgeoning areas of technology development, such as super-resolution microscopy, that hold promise to further revolutionize the field, but in this section we highlight a few recent advances that have had major impact on our understanding of cell motility and the cytoskeleton.

In 2008, the Nobel Prize in Chemistry was awarded to Shimomura, Chalfie and Tsien for their discovery and development of green fluorescent protein (GFP). The ability to express GFP tagged proteins in cells and observe their localization, movement and interaction has led to a completely new understanding of the cell. This technology was further exploited with Total Internal Reflection Fluorescence (TIRF) microscopy. This technology uses the evanescent wave resulting from total internal reflection to illuminate only a narrow portion of the viewing field, eliminating the background that would result in standard fluorescent microscopy. This technology allows researchers to view processes at or near the cell membrane, in the case of live cells, but is also very useful in purely *in vitro* systems where the dynamics and interactions of actin filaments or microtubules can be observed in detail. These advances in protein labelling and microscopy now allow researchers to make detailed measurements on cell processes to the point of determining the order of events and assigning kinetic rate constants to individual interactions. One of the workshop participants, Tom Pollard (Yale), has long advocated that to truly understand a process knowledge of rate constants is essential. This viewpoint meshes well with mathematical modellers, whose description of these cellular phenomena using coupled differential equations relies on estimates for such rate constants.

One clever extension of fluorescent labeling is known as fluorescent speckle microscopy. In this scenario the concentration of the fluorescent proteins is low enough that individual proteins appear as dots or speckles. For example, polymers appear as a series of puncta that move and flow as filaments polymerize or are translocated by motor proteins. Multiple particles can be simultaneously followed using different fluorescent labels. Gaudenz Danuser (Harvard Medical School) has developed elaborate methods to track these particles and quantify their movements. These methods have been widely adopted, giving new insight into many complex processes including cell migration, dynamics within lamellipodia, and cell division.

One final advance that must be mentioned is the development of new (biological) model systems and cell environments. For many years, researchers studying cell motility and migration used two-dimensional systems of cell(s) crawling on glass. This has been ideal for microscopy and, in some cases, for determining biochemical details underlying motility. However it has become apparent that some essential details are missing. First, the mechanics of the substrate strongly affect the cell, and many researchers, including workshop participant Paul Janmey (University of Pennsylvania), have now shown that hard surfaces, like glass, affect cells differently than softer, more biologically appropriate substrates such as collagen or fibrin. Second, although it is easier to study cells in a 2D environment, cells within the body interact in a 3-dimensional network of cells and matrix. Denis Wirtz (Johns Hopkins), has shown how motility in 3D differs in many aspects from previous 2D studies. This factor will undoubtedly become more fully explored in future research.

2.2 Theory and Modeling Advances

Recent years have seen an increasingly close two-way interaction between theory and experiment, in which theory is beginning to guide experimentalists to particular systems and parameter ranges for performing experiments that discriminate between competing hypotheses. This trend has been manifested in several methodologies and approaches to the theory of complex systems which have expanded over the past decade.

Multiscale modeling, which originated in materials science but is now increasingly practiced in biology, involves grafting approaches which treat a complex system at differing length scales. For example, it could constitute a "wedding" of an atomistically based approach with one based on continuum elasticity. Such modeling has the advantage that it can, in principle, combine the accuracy of atomistic methods with the ability of more coarse-grained methods to treat spatial scales of micrometers and time scales of minutes. One could never treat all of the molecules in a cell in a single computer simulation, but with the use of continuum models based on molecular-level calculations one can incorporate molecular-level information

into a treatment of a whole cell. Examples of such work at the meeting included simulations of whole-cell behavior from the lab of Alex Mogilner (UC Davis), and work by David Sept (University of Michigan) and Fred MacKintosh (Vrije Universiteit) developing elastic models of microtubules based on information from intensive molecular-level simulations of small microtubule fragments.

Stochastic simulation has increasingly become a standard component of a theorist's portfolio. This is important for cell biology because a number of phenomena, such as transcription regulation by small-copy-number proteins or the growth of a microtubule, involve a small number of constituents so that fluctuations are important. This arm of theory is further driven by the ability to watch the growth of biopolymers such as microtubules at sufficiently high resolution to see the stochastic nature of the growth. Stochastic simulation, in its simplest form, involves assigning a random number to each possible event which could occur during a time interval, and in more complex realizations uses algorithms in which the time step is variable, and determined by the rates. Provided that distinct events are not too strongly correlated, such methods describe the fluctuations accurately. Applications of stochastic-simulation methods at the workshop included simulations by Pollard and Dimitrios Vavylonis (Lehigh University) of the self-assembly of the contractile ring in yeast, simulations by Anders Carlsson (Washington University) of F-actin waves moving on the substrate-attached surface of cells, simulations by the labs of Holly Goodson (University of Notre Dame) and Melissa Gardner (University of Minnesota) of the growth of microtubules, and simulations of chemotaxis and sensing from Herbert Levine (UC San Diego).

An increasing influx of nonlinear-dynamics methods into the mathematical biology of cells has been a recent trend. Simple mathematical models based on reaction-diffusion equations and related nonlinear interaction models provide key concepts for understanding complex cell phenomena, such as bifurcations leading to spontaneous symmetry breaking or limit cycles, or even to chaos. Such nonlinear-dynamics models are being applied in an increasingly quantitative way to cells, based on our growing understanding of the underlying protein-protein interactions. Such quantitative applications were seen in work from the group of Leah Edelstein-Keshet (University of British Columbia) treating cell polarization and migration using a model based on several modules, including membrane lipids, Rho GTPAses, and actin. Basic nonlinear-dynamics concepts were also utilized in work presented by the Levine lab on cell chemotaxis and sensing, work from the lab of Adriana Dawes (Ohio State University) treating Par protein segregation, work from the Mogilner, Vavylonis, and Carlsson labs of F-actin waves, and very recent work from the Vavylonis lab on Cdc42 oscillations.

3 Presentation Highlights

3.1 Cell Migration

The meeting included several outstanding talks about recent progress in tracking and modeling cell migration. Alex Mogilner (UC Davis) talked about a recent models under development joint with experimentalists (Kinneret Keren (Technion, Israel), Julie Theriot and others) for the migration of cells derived from fish scales (keratocytes). The models are based on the idea that actomyosin behaves like a viscoelastic material, graded from front to back in these cells, and that membrane tension forms an important dynamically changing regulator of the motion. His talk was followed by Kinneret Keren's beautiful lecture on the experimental results probing the role of membrane tension in assembly, contraction of actomyosin, and formation of adhesions. Keren's lab is able to experimentally manipulate each of these variables, and measure the response of the others using innovative experiments. For example, she fuses giant vesicles to motile cells to rapidly change their membrane area. Surprisingly, the cells keep moving with little change. She summed up the results by the statement "tension is generated by growing actin filaments at the leading edge pushing against the inextensible membrane, and is relieved due to centripetal actin flow generated by myosin-powered contraction and mediated by the adhesion strength".

Alexander (Sasha) Verkhovsky (EPFL Lausanne) described similar keratocyte experiments. He used osmotic manipulations to investigate the role of tension, and concluded that cell speed increases with membrane tension. In his hands, inhibiting myosin resulted in irregular cell shapes, lower tension, and slower speed. Some of the differences in results between the Verkhovsky experiments and those of Keren are not fully resolved.

Several talks focused on the importance of understanding multiscale processes in cell motility for both basic science and clinical applications. John Condeelis (Albert Einstein College of Medicine) presented an overview of his research on the motility and invasive properties of mammary carcinoma cells and their response to a growth factor (EGF). Using bioinformatic analysis of the invasive tumor cells, he has deciphered the changes in their actin-related regulatory networks that are a "signature" of invasion/metastasis. Among these factors is a well-know protein, cofilin, that breaks actin filaments. Work in recent years identifies cofilin as an actin-nucleating agent (creating new filament ends that can grow and lead to membrane protrusion). Condeelis described the spatial distribution of cofilin and its regulators (Rho, LIM kinase) in structures called invadopodia and lamellipodia, and pointed to recent modeling work (with UBC postdoc Nessy Tania) that has helped to decipher the dynamics of the intermediates.

Inke Näthke (University of Dundee) gave a visually stunning lecture about how changes in tissue structure (crypts) are linked to colorectal cancer. Hers was a second talk about tissue organization. Jennifer Zallen (Sloan-Kettering Institute) showed the polarity reorganization in fly embryos that results in elongation along the head-tail axis. By labeling proteins responsible for adhesion and contraction, Zallen showed that they become asymmetrically distributed and generate cell polarization. She returned to the dominant theme of tension, noting that myosin generates tension and tension then leads to recruitment of more myosin to the cortex of the cells.

A number of lectures specifically addressed the issue of single-cell polarization, both in the context of chemotaxis and in other cases. Adriana Dawes described her joint experiment-modeling work on the polarity of (PAR) proteins in the worm embryo, *C. elegans*. Herb Levine summarized recent work with experimental and theoretical colleagues on the chemotaxis of the social amoeba (*Dictyostelium discoideum*). He described a 2D motility simulation that can account for the unique motion of this kind of cell by successive random formation of extensions (pseudopods). The model is based on a reaction-diffusion system operating around the cell periphery, and giving rise to hot spots that become protrusions. Good comparison with experimental observation was evident.

Orion Weiner (UC San Francisco) gave an intriguing lecture about probing the polarity of white blood cells (neutrophils). Returning to the theme of tension, his group showed that manipulating membrane tension in these cells (e.g. by micropipette aspiration) can lead to dramatic changes in cell polarization from the level of signaling proteins (such as Rac) up to the level of actin and protrusive activity. He also described unusual experiments with cells that form two parts, connected by long thin membranous "tubes". So long as the tube remains intact, only one of the parts acts as "cell front". Once the tube is severed, the second part also develops a "front". Such experiments were used to infer that tension plays a role in polarity signaling, and that diffusible inhibitors cannot be the only mechanism for preventing multiple fronts.

Talks in this workshop had a spectrum of levels of detail. Les Loew (University of Connecticut) presented an argument in favor of including details in models of actin regulation and turnover. Herb Levine noted that, as few details are known, models with known dynamic properties are useful. Edelstein-Keshet's talk motivated a range of approaches, including detailed as well as simplified (analytically tractable) "toy models". She pointed out that some details (e.g. differences in diffusion of proteins on the membrane versus in the fluid interior) are important. Other details (such as how feedback is wired, enhancing rates of activation or damping rates of inactivation) may be less important for the dynamic behavior of a given signaling circuits.

3.2 Cell Mechanics

The role of cell mechanics in cell migration and morphology, and potential mutual regulation of mechanical and biochemical processes, was a common thread through many presentations at the workshop. About the role of forces and tension, Michael (Misha) Kozlov (Tel Aviv University) summarized the status in his introduction to a lecture on focal adhesions. He said that ".. in past years, membrane tension was hardly mentioned as an exotic factor. Now, everyone talks about membrane tension, and we who know how to model it physically have become very popular.".

Sean Sun (Johns Hopkins University) addressed the topic of mechanosensation in migrating cells. Cells form stress fibers, bundles of polymerized actin filaments, to adhere to a substrate or neighboring cells. The formation of these stress fibers can be induced by applying an external force, indicating that cells can modulate internal structures in response to external forces. His results suggest that in this case, mechanics may be the origin of the biochemical response.

During migration, some cell types exhibit bipedal motion where the front extends at a constant speed, but there is an alternating body contraction that moves the sides of the cell forward in a rhythmic pattern that is reminiscent of walking. Using a highly abstracted mechanical model of a migrating cell consisting of cross-linked springs, Jay Tang (Brown University) was able to reproduce the bipedal motion. He was able to demonstrate configurations that give the same qualitative agreement with experimental observations and suggestions for experiments to distinguish between the potential configurations.

One talk focused on understanding mechanical properties of actin networks with a novel use of atomic force microscopy (AFM). Actin polymers can organize into many different types of structures that are branched or bundled and can extend over long or short space scales. Dan Fletcher (UC Berkeley) demonstrated that imposing stiffness on an actin network can alter its behavior. Using a broad flat tip during AFM, he applied either a constant force or constant height to a sample of actin polymers and measured the equilibration time. The results varied depending on the imposed conditions suggesting that actin networks dynamically remodel in response to mechanical regulation.

A series of talks looked at the assembly, stability and regulation of contractile actomyosin networks. Karen Oegema (Ludwig Institute for Cancer Research) uses the nematode worm *C. elegans* to explore mechanisms of cell division and regulation of the contractile ring. Her experiments have shown that during constriction the contractile ring maintains a constant concentration and thickness, although the constriction rate decreases, potentially by contacting the spindle midzone. One of the highlights of the conference was an outstanding lecture by Ewa Paluch (MPI for Molecular Cell Biology and Genetics), who specializes in the phenomenon of cell blebbing. Paluch studies blebbing both in the context of motility and cell-division. Large cytoplasmic oscillations during cytokinesis lead to failure of division, while small cytoplasmic oscillations accompanied by bleb formation at the pole allow the cell to divide successfully. She proposes that the blebs are essential to release membrane tension at the poles and allow cytokinesis to proceed. Tatyana Svitkina (University of Pennsylvania) discussed assembly of sarcomere-like structures of actin and myosin in non muscle cells. The exquisite and compelling images showed a striated organization before and after recovery from blebbistatin treatment, and demonstrated that unpolymerized non-muscle myosin II may play a role in forming focal adhesions.

3.3 Microtubule and Actin Dynamics

There was a provocative series of talks relating to microtubule dynamics and mechanics. Jennifer Ross (University of Massachusetts Amherst) started the session by discussing microtubule length regulation and severing and two newly identified microtubule severing proteins, katanin and fidgetin. Using TIRF microscopy, she has been able to characterize and localize the function of these proteins *in vitro*. Melissa Gardner also spoke on microtubule length regulation, but via different mechanisms. Microtubules exhibit an interesting property called *dynamic instability* where they undergo stochastic periods of growth and shrinkage. Gardner showed how the distribution of catastrophes, the transition from growth to shrinkage, follows a gamma distribution with $k = 3$, suggesting there is a series of 3 transitions that precede a catastrophe. She next demonstrated how microtubule associated proteins can alter this behavior by either affecting the rate of these individual steps or by changing the number of steps. David Odde (University of Minnesota) spoke on microtubule assembly kinetics and showed how our understanding of this seemingly simple process is incorrect. Polymerization processes have typically been modeled using mass action chemical kinetics where the growth rate is a second-order process that depends on the free monomer concentration and the off rate is simply a first order rate constant. New data from high-resolution experiments that can track polymerization at the nanoscale have shown that this model is too simplistic since the structure of the microtubule tip changes as the growth rate increases. This gives rise to a very different kinetic model that fundamentally changes the way we understand microtubule polymerization.

Holly Goodson spoke on computational modeling of microtubule dynamics and how observed dynamics can be related to the biochemistry, mechanics, and structure of the microtubule. As with Odde's talk, her results suggest that we may need to fundamentally rethink the details of microtubule polymerization. Finally, David Sept spoke on efforts to connect all-atom molecular dynamics simulations of microtubules with more coarse-grained simulations and ultimately continuum mechanics. In his talk he showed how a bootstrap approach allows one to connect atomically detailed descriptions to polymer level models and thereby explain the effects of drugs or protein mutations on the mechanics that are observed.

New results for actin dynamics focused on spontaneous patterning and wave formation. In cell types including neutrophils and the slime mold Dictyostelium, polymerized actin often forms spontaneous patterns including clumps of actomyosin crucial for cytokinesis, traveling waves moving around the edge of the cell pushing the membrane out, waves traveling along the substrate-attached surface of the cell, or patches which can either remain stationary or move. Work out of the Pollard and Vavylonis laboratories demonstrated that actomyosin clumping and subsequent ring formation results from a "search, capture, pull, and release" mechanism in which actin filaments growing from preexisting foci find other foci, pull on them, and then release due to the action of actin-binding proteins which disassemble actin filaments. They showed that such a mechanism explains contractile ring formation for parameters consistent with experiment. Furthermore, they showed that under other conditions, the clumps remain isolated - without forming rings; this prediction is consistent with experiments.

Complementary to this work, calculations out of the MacKintosh laboratory showed that the active motion of myosin in actin networks can give rise to diffusive-like behavior of the networks. Several talks and posters, out of the laboratories of Vavylonis, Mogilner, and Carlsson, treated these waves using a combination of positive and negative feedback acting between two chemical constituents. Such feedback effects, when coupled to diffusion of at least one of the constituents, are known from the mathematics of nonlinear dynamics to have the ability to produce spontaneous traveling waves and patches. The models generally focused on the combination of F-actin and the nucleation-promoting factors which act upstream of it, with either of the constituents assumed to feed back on itself positively. The negative feedback terms were based on a mechanism in which F-actin "bites the hand that feeds it" - by inactivating the nucleation-promoting factors. Arpita Upadhyaya (University of Maryland) found experimental evidence for actin based traveling waves in spreading kinetics of T cells. Work out of Martin Falcke's laboratory (Max Delbrück Center Berlin) focused on the dynamics of filament attachment and detachment from the membrane. This work showed that the velocity response of a cell to a sudden applied force is often has a delayed component coming from dynamic rearrangements of the actin network.

John Cooper (Washington University) investigated actin assembly during cytokinesis in yeast cells. In particular, he focussed on the mechanism of Arp2/3 action and regulation. His lab has found that N-WASP associates with Arp2/3 at the membrane where it nucleates an actin filament branch point, but N-WASP is replaced by cortactin as Arp2/3 is pushed away from the membrane by actin polymerization. Mutants of Arp2/3 regulators exhibit longer actin filaments and fewer branches, consistent with reduced Arp2/3 activity. Margaret Gardel (University of Chicago) gave a comprehensive overview of recent understanding of the lamellar actin cytoskeleton, while Cécile Sykes (Institut Curie) discussed biomimicking systems of cell shape changes. When actin gels form around rigid beads, the ability of the bead to break symmetry and initiate movement depends on the ratio of Arp2/3 to capping protein: too much Arp2/3 and the actin gel is overly branched while too much capping protein produces an actin shell that is too small. To further clarify the role of the actin cortex, she presented recent work with liposomes (oil droplets with no actin) and membrane blebs (with an intact actin cytoskeleton). She demonstrated that the actin cytoskeleton slows spreading compared to liposomes, but when force is applied using a tube pulling assay, force exerted by the liposomes depends on the tube length. Taken together, this suggests that differences in liposomes and cells d not lie entirely in the cytoskeleton, but also in the membrane proteins, and the membrane bilayer in cells can help regulate mechanical properties in the cell.

4 Scientific Progress Made

The cytoskeleton and motility community has a diverse constituency, made up of cell biologists, biophysicists, mathematicians and engineers. There are few opportunities and/or venues that brings this group together. This alone makes the BIRS workshop unique. Although these researchers continually read and follow each other's work, there is often not an efficient transfer of information since a biologist can face challenges in reading an article in a *SIAM* journal, and likewise an engineer may not glean all the details and nuances from a *Nature Cell Biology* paper.

The small size of the workshop, the ample time for discussion, and the informal interactions during meals and other social times, gave valuable opportunities for in-depth and informative scientific exchange. Experimentalists were exposed to new theoretical ideas and modeling platforms. Likewise, theorists and

modelers saw novel experimental measurements and data that provides contact with reality and motivation for new approaches. This type of interaction goes far beyond what is possible at a typical mathematics (or cell biology) meeting. The workshop created many new opportunities that will bear fruit in the coming months and years.

5 Outcome of the Meeting

Experimental biology has come a long way in allowing precise measurements of forces between cells, cells and their environment, and even between individual molecules. Similarly, our visualization techniques have dramatically improved, with fluorescent labeling techniques and ever improving microscopy. Consequently, we have more data at every level, and a greater need for synthesis and understanding of that data. Modeling is playing a prominent role in this process and has become an integral part of quantitative cell biology, helping to focus experimental directions and distinguish between competing hypotheses.

Although this report is being written in the weeks immediately following our workshop, we can already document at least 11 new collaborations that have been established, 3 grant proposals that are being written or planned, and 2 manuscripts that will acknowledge the contribution of BIRS. The previous workshop held in 2005 had far-reaching effects that impacted all sectors of this interdisciplinary field. We anticipate nothing less to arise from this latest workshop and look forward to the next opportunity to bring together such an interdisciplinary group at BIRS.

6 Remaining Open Questions

When comparing the 2005 meeting to the one in 2011, there has been a significant move from modeling at the level of individual proteins and polymers to large systems of proteins, cells and even tissues. This trend will undoubtedly continue in the future, but significant challenges remain. Experimental complexity greatly increases when dealing with full-scale signaling cascades in cells, cell-cell interactions, and communication between cells in a tissue, let alone a whole organism. Modeling methods and techniques for the analysis of models, as well as simulation methods are still emerging to treat large-scale dynamics that depend on both time and space.

Although we have a plethora of data, some very basic questions remain, such as "*Where is force produced in the cell?*", "*What is the relative contribution of polymerization forces and motor proteins?*", "*What are the key feedback elements leading to cell polarization?*", and "*What are the specific roles of the various actin nucleators and their activators?*". At this point, each of these questions has been addressed by a substantial body of theory and experiment, and investigations are crystallizing around a few competing hypotheses. Over the next five years, we would expect several of these questions be definitively answered. But we are only beginning to understand the larger question of the logic of the complex mechano-chemical protein networks that control cell behavior. Systems biology efforts are well-poised to begin addressing these issues and we hope that at least rudimentary answers will be given over the next few years.