Organization of kinase signaling at the cell center for cytokinesis

Radhika Subramanian

The Aurora B kinase-containing Chromosomal Passenger Complex (CPC) is an essential cytokinesis regulator. During anaphase, CPC concentrates at the spindle midzone, overlapping antiparallel microtubules at the cell center. How CPC is selectively enriched at the midzone within the dense, heterogeneous and dynamic spindle microtubule network is unknown. Here, we define a minimal CPC midzone enrichment module. We purified full length recombinant human CPC and characterized its architecture and dynamics using Atomic Force Microscopy. Using a bottom-up reconstitution approach, we found that the maximal enrichment of CPC at antiparallel overlaps requires PRC1-crosslinked microtubules and two kinesins, KIF4A and KIF20A. The motors exhibit a division of labor: KIF4A retains CPC at overlaps, while KIF20A delivers it from non-overlapping regions of the PRC1-crosslinked microtubules. Single-molecule imaging of CPC transport by the two motors show that this spatial segregation of motor activity is governed by CPC (i.e., cargo) density. At high density, as in overlaps, KIF20A is inhibited and KIF4A dominates; at low density, as in the non-overlapping regions of microtubules, the roles reverse. Conditional-depletion experiments in mitotic cells establish KIF4A as the CPC anchor at the midzone. Our findings uncover a new organizational principle: cargo density governs motor selection. Such an adaptation to cargo density may represent a general strategy for positioning signaling proteins within complex cytoskeletal networks.

Principles of Bipolar Spindle Formation Learned from an Energetic Framework

Jing Chen

In animal cells, each pole of a normal mitotic spindle is organized by a centrosome. Centrosome defects can disrupt this organization, producing abnormal monopolar or multipolar spindles. Remarkably, cells can often recover bipolarity by separating centrosomes in monopolar spindles or clustering them in multipolar spindles. To investigate the physical basis of this adaptability, we developed a parsimonious biophysical model, grounded in experimental data, that represents key mechanical forces during spindle assembly through effective potential energies. The model predicts general biophysical factors essential for robust bipolarization of spindles that start monopolar or multipolar. These include balanced attractive and repulsive forces between centrosomes, appropriate force fluctuations, exclusion of centrosomes from

the cell center, suitable cell size and geometry, and a limited centrosome number. Consistent with these predictions, our experiments show that bipolar clustering of centrosomes is favored as mitotic cell aspect ratio and volume decrease in tetraploid cancer cells with supernumerary centrosomes. By unifying diverse experimental phenomena within a single energetic framework, our model distills fundamental principles underlying bipolar spindle formation. These principles not only provide mechanistic insight, but also provide guidance for future development of more complex and realistic models for mitotic spindle assembly, particularly when integrated with data-driven and machine learning approaches

Investigating models and mechanisms of cell migration to better understand and target cancer metastasis

Madeleine Oudin

Metastasis, the dissemination of primary tumor cells to secondary organs in the body, is the leading cause of death in cancer patients. Metastasis requires the local invasion of tumor cells into the surrounding environment, followed by entry into the lymphatics or vasculature and subsequent colonization of distant organs to form metastases. All steps of the metastatic cascade require tumor cell migration: tumor growth, local dissemination and intravasation, and colonization. While mutations and epigenetic changes play a critical role in driving cancer progression, no single mutation renders cells metastatic or distinguishes a local from a metastatic tumor. Instead, metastasis is driven by multiple signaling pathways inside the cell in response to many different cues from the local tumor environment. Eradicating metastatic disease and improving outcomes in cancers requires an in-depth understanding of the mechanisms that drive tumor cell migration. We will review the different assays used to study tumor cell migration and invasion in vitro and how they can be used to understand how cells respond to different cures in their microenvironments. We will also discuss efforts to develop computational models to identify the best assays to predict effects on invasion in vitro and metastasis in vivo, how to identify invading cells in tumors, and understand how different migration cues cooperate to drive invasion, with a focus on breast cancer. Increasing our understanding our mechanisms of metastasis will help us better identify which patients are at risk of developing metastatic disease and develop better treatments for patients with metastasis.

Modeling mechanisms of microtubule dynamics and polarity in living neurons

Anna Nelson

In neurons, sustained intracellular transport of cargo depends on a stable and correctly oriented microtubule cytoskeleton, which is important to ensuring the long-term survival of these cells. However, microtubules also need to be dynamic and reorganize in response to injury events. How this balance is achieved remains an open question. Here, I will give examples of mechanistic modeling of microtubule growth dynamics in Drosophila neurons that utilize in vivo experimental data and a variety of mathematical frameworks. These models can provide testable predictions and lead to new biological insight to better understand mechanisms underlying microtubule filament behavior in neurons.

Fantastic forces and where to find them

Julien Berro

Mechanical forces are central to countless biological processes in health and disease. However, despite their ubiquity and importance in cellular processes, our understanding of biomechanical forces lags far behind our understanding of the underlying biochemistry. Studying forces within cells is difficult because tools and approaches to directly probe forces at the molecular level are scarce, difficult to use or have limited applications. In this seminar, I will present approaches based on quantitative microscopy, mathematical modeling and molecular force sensor engineering that my lab has developed to readily measure biophysical quantities so far impossible or difficult to measure in vivo. Using clathrin-mediated endocytosis as a model system, I will show how these methods have uncovered new molecular mechanisms of force production, force transmission and force sensing by the actin cytoskeleton.

Mechanical Information Processing in Adherent Cells

Margaret Gardel

My lab studies how the movement and shape of living cells is controlled by living materials constructed by protein assemblies within the cell interior. In this talk, I will describe our recent efforts to understand the design principles of the active, soft materials that drive morphogenesis of multicellular tissue. In particular, I will discuss design principles by which the cellular cytoskeleton senses, generates, and adapts to mechanical forces and couples to biochemical and transcriptional pathways. Such mechanical information processing controls diverse processes including cell proliferation, barrier function and cell fate determination.

Learning the dynamics and interaction of migrating cells

Chase Broedersz

Single and collective cell migration are fundamental processes critical for physiological phenomena ranging from embryonic development and immune response to wound healing and cancer metastasis. Yet, the underlying dynamics of how cells move and interact with each other, and their environment is still unclear. We employ a data-driven approach to infer the dynamics of cell movement, morphology and interactions of cells confined in artificial environments. By inferring a stochastic equation of motion directly from experimental data, we show that cells exhibit intricate non-linear deterministic dynamics that adapt to the geometry of confinement. We extend this approach to interacting systems, by tracking the repeated collisions of confined pairs of cells. After identifying distinct interactions modes for diverse cell types, we show how these interactions can be captured by a unifying mechanistic model. Finally, I will discuss how our approach can be extended to the collective dynamics multicellular systems.

Ongoing efforts to connect molecular and cellular scale behaviors in microtubule self-organization

Daniel Needleman

How microtubules self-organize to form different structures in different contexts, such as the spindle during cell division and bundles in neuronal processes, remains poorly understood. My group is studying this issue by integrating different experimental modalities that provide quantitative information at different time-scales and length-scales, and interpreting our results with biophysical theories. A key difficulty in developing multiscale theories of microtubule self-organization is our lack of understanding of the relevant physics and behaviors at the scale of tens of nanometers, which is the bridge between the molecular and cellular scales. Measurements at such mesoscales have been challenging. I will describe our ongoing efforts to leverage recent advances in cryo-electron microscopy to study mesoscale behaviors of the microtubule cytoskeleton. Our work so far has focused on developing new cryo-electron microscopy data analysis procedures, including using approaches pioneered by the machine learning community.

Data-centered approach and mechanics of the mitotic spindle

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The mitotic spindle, a self-organising, force-generating apparatus, ensures accurate chromosome segregation. Its microtubules, extending from two poles to kinetochores, undergo dynamic instability, while pulling/pushing forces, exerted from the cell periphery to the poles, position the spindle. Concurrently, tension on the kinetochores must be tightly regulated to ensure the correct chromosome attachment.
The spindle pole-to-pole length was established as a functional proxy. We investigated it in the Caenorhabditis elegans zygote across ~100 experimental conditions and 1,600 individuals. Principal component analysis revealed that the first three eigenvectors captured 95 % of the inter-individual variability. The third component (≈ 6 %) represented a transient shortening during late metaphase in every condition and resembled a kinetochore defect phenotype, suggesting a core contractile mechanism acting against cortical pulling forces. Importantly, the eigenvector profiles spanned both metaphase and anaphase; machine-learning regression demonstrated that late metaphase elongation alone can predict anaphase length. Beyond highlighting that solely three independent mechanisms govern spindle length, this study also provides a predictive tool for identifying candidate proteins involved in these mechanisms.
Metaphasic half-spindles are commonly viewed as a gelified microtubule network undergoing poleward flux, i.e., microtubules polymerise at chromosome-proximal plus ends, slide to the poles, and possibly depolymerise at the poles. In contrast, sliding-window Fourier analysis of length fluctuations, modelled as a Kelvin-Voigt system with inertia, revealed a minimal spring contribution versus viscous and inertial elements. Fluorescence recovery after photobleaching the half-spindle revealed only kinetochore microtubule fluxes, sliding along pole-anchored ones. Such a mechanism may buffer cortical forces, ensuring regulated tension on the kinetochores.

Beyond motility and migration: Cell shape and cytoskeleton as regulators of signals

Gaudenz Danuser

(Tuesday 11/4 if should participate in a Roche event until 10 AM. If possible, please do not schedule my talk before 10:30 AM or so.)

‘Form follows function’ explains why giraffes have long necks, airplane wings are airfoil-shaped, and syringes are pointed. Darwin made the case for genetic form selection based on the beaks of finches. At the level of cells, neurons have evolved to

extend axons to connect to other neurons, immune cells form protrusions that look like suction cups to engulf pathogens and other cells, and blood cells are biconcave disks to optimize the surface to volume ratio for gas exchange. Cells alter their shapes on the time scale of seconds to adapt to different functional tasks. Since microscopes existed, pathologists have exploited cell shape for diagnosis and stratification of disease. Cell morphotype and function have been associated even more tightly in a growing number of machine learning applications that predict cell behavior as well as genetic and molecular states from shape features. Yet, in most of these works morphotype is downstream of regulation. Our recent work indicates, however, that cell shape and cytoskeleton architecture are at least part of, if not even at the top of regulation. Hence, 'function follows form'. We are particularly interested in the reversal of the 'form follows function' paradigm in the context of cancer. We find that cancer cells control through shape how they survive, proliferate and metabolize in hostile environments. These discoveries, enabled by twenty years of innovation in live cell microscopy and computer vision, may inspire expansion of the scope of the machine learning discussed at the workshop.

A dynamical systems framework applied to live cell imaging suggests a first-order phase transition between morphology-based cell states

Susanne Rafelski

Efforts to quantify a Waddington-like landscape from the perspective of dynamical systems have largely focused on describing cell states from the molecular perspective such as via gene regulatory networks. Here we, instead, use morphological data to quantify stable cell states. We take advantage of the ability of human induced pluripotent stem cell-derived endothelial-like cells (hiPSC-ECs) to undergo an inducible cell state transition as they change their alignment behavior (cell organization and migration) in relation to the direction of flow under low and high fluid shear stress (FSS), respectively. We developed a segmentation-free unsupervised machine learning (ML) approach to extract features from the transmitted light images from timelapse videos of hiPSC-ECs expressing GFP-tagged VE-Cadherin under different FSS. We applied a dynamical systems framework to these features and constructed vector field representations of the cell state dynamics, from which we identified stable fixed points at varying levels of FSS. We found that the way these attractors change with FSS suggests a first-order phase transition. We validated biological interpretation of these results by using the ML model to reconstruct representative images of the VE-Cadherin channel at these fixed points. By further comparison with more standard cell-centric approaches including segmentation-based individual cell trajectories and their metrics

(eg., cell density and alignment), we found that cells take varied paths towards the fixed points. Our work acts as a proof-of-concept framework for quantifying the non-equilibrium driving forces of cell state switching and response to perturbation from microscopy image-based data.
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High resolution imaging of Spindle Structure in mitosis: From Plastic to Cryo

Stefanie Redemann

Understanding the architecture of mitotic spindles is fundamental to cell division, yet traditional microscopy techniques have long limited our ability to resolve the fine-scale organization of microtubules. In the classical model, microtubules nucleate at spindle poles and extend toward chromosomes, with minus ends anchored at the poles. However, this view has been challenged by recent advances in high-resolution imaging. Leveraging cutting-edge technologies, specifically large-scale 3D electron tomography, cryo-focused ion beam scanning electron microscopy (cryo-FIB SEM), and cryo-electron tomography (cryo-ET), we have achieved unprecedented structural resolution of mitotic spindles in RPE-1 cells. These tools have enabled us to visualize microtubule polarity and organization at the molecular level, revealing that approximately 50% of spindle microtubules are not connected to poles or chromosomes, but instead form a complex, interdigitated network.
Our cryo-ET data show that while kinetochore fibers are composed of parallel microtubules, the surrounding spindle microtubules are arranged in antiparallel bundles. Remarkably, these antiparallel structures form even in monopolar spindles, indicating that bipolarity is not a prerequisite. Instead, their formation depends on the activity of kinesin-5 (Eg5), highlighting a critical role for this motor protein in establishing local microtubule polarity.
These findings underscore the transformative power of advanced cryo-imaging techniques in resolving previously inaccessible features of spindle architecture. By

integrating large-scale tomography with molecular-level cryo-ET, our study provides the first detailed insights into the spatial arrangement and polarity of spindle microtubules, insights that are essential for understanding spindle mechanics, stability, and the fidelity of chromosome segregation.

Unravelling Cellular Morphodynamic Heterogeneity with Live-Cell Imaging and Machine Learning

Kwonmoo Lee

Live-cell imaging provides critical insights into dynamic cellular behaviors, insights that static imaging cannot capture. Cellular morphodynamics are essential for understanding not only cancer progression but also the complex and heterogeneous processes of stem cell differentiation. In both contexts, cells exhibit diverse trajectories and phenotypes, even under identical conditions, posing significant analytical challenges. Tumors and stem cell populations alike contain highly heterogeneous cells, contributing to variability in disease progression, therapeutic response, and developmental outcomes. Yet, conventional analytical methods often focus on predefined phenotypes and rely on feature selection strategies that may inadvertently compress this phenotypic complexity and obscure subtle but biologically meaningful variation.

To address these challenges, our lab has developed machine learning tools designed to preserve and analyze the full spectrum of morphodynamic heterogeneity. In this talk, I will present a suite of these tools, including deep learning–based cell segmentation, heterogeneity-preserving feature selection, and trajectory embedding. Using these approaches, we applied unsupervised learning to uncover morphodynamic phenotypes in both cancer cells and megakaryocytes. This enabled highly accurate discrimination between MDA-MB-231 and MCF10A cells, and supported high-resolution phenotyping of megakaryocyte morphodynamics, capturing subtle yet functionally important transitions during development. Together, these methods offer a powerful framework for decoding cell behavior and enabling precision therapeutic strategies based on dynamic phenotypic features.

A kinetochore tracking perspective - the complex mechanics of human cell division.

Nigel Burroughs, Catriona Conway, Abdullahi Daniyan, Constandina Koki, Andrew McAinsh.
Warwick University

Cell division is a beautifully choreographed mechanical process in which a microscopic microtubule-based mitotic spindle self assembles. The spindle facilitates physical segregation of the duplicated chromosomes into 2 daughter cells. Chromosomes connect to
the mitotic spindle via kinetochores, large multi-protein complexes that form an attachment
site for microtubules.
By using lattice light sheet microscopy and near-complete kinetochore tracking we are able
to visualise kinetochore 3D dynamics across mitosis at 3-4s resolution over 10-20mins. In
previous work we used Bayesian inference methods to fit mechanical models to metaphase
oscillations, demonstrating that there is substantial kinetochore heterogeneity, both spatially
across the metaphase plate and temporally with biophysical parameters maturing towards
anaphase. Extending this analysis to prometaphase presents substantial challenges, since it
is highly complex and stochastic, with multiple kinetochore-microtubule attachment states.
We address these challenges with two analysis approaches. Firstly, we analyse the collective of kinetochores in each cell, showing that prometaphase progresses through distinct mechanical phases. Secondly, we analyse individual kinetochores, using switching
models to dynamically partition the trajectory into phases where the external forces are stable and distinct. From these data we are beginning to understand the sequence of dynamic phases that kinetochores progress through during congression.

Opportunities and challenges in tracking mitotic subcellular structures.

Viji Draviam

Accurate 3D tracking of fast-moving subcellular structures remains limited by temporal and spatial (axial) discontinuities imposed by photo-toxicity constraints[1]. This trade-off is particularly acute when combining high-throughput and high-resolution imaging of mitotic spindles and their dynamic kinetochores or microtubule plus-ends. To overcome this, we developed SpinX[2] and its extension MultiSpinX[3]**,** computational frameworks that integrate AI-guided spindle segmentation with predictive mathematical modelling of

spindle pole dynamics and kinetochore tracking to reconstruct continuous 3D trajectories from discontinuous datasets. I will present our ongoing efforts to integrate these with liquid lens–based adaptive optics[4] to achieve rapid axial refocusing and significantly minimize sampling gaps in volumetric imaging — a step that could enable true high-throughput, high-resolution imaging of mitotic microtubule-end dynamics required for drug discovery screens[4]. Lastly, I will introduce optogenetic perturbation tools[5] being implemented for localised spindle microtubule-end ablation and force modulation, providing a platform to dissect cortical pulling and microtubule-driven pushing mechanisms we reported in human cells[6].

1. Chai et al., TiCB 2024, PMID: 38030542
2. Dang et al., JCB 2023, PMID: 36880744
3. Chai et al., Comput Biol Med 2025, PMID: 39847944
4. Efstathiou and Draviam JCS 2021, PMID: 34409445
5. Efstathiou et al., Biorxiv 2025
6. Zulkipli et al., JCB 2018. PMID: 29941476

Studying Multicellular Living Systems with Graph Neural Networks.

Ming Guo

Multicellular tissues are sculpted by the spatial and temporal coordination of cells and their interactions. Yet, the organizational principles that govern these events, and their disruption in disease, remain poorly understood. In this talk, I will first discuss our recent experimental work investigating multicellular dynamic organization in several physiologically relevant systems, including human breast cancer model and mouse embryos. Next, I will present our recent efforts in using Graph based deep learning to predictively model multicellular developmental processes, including collective cell migration and Drosophila embryogenesis.

Extending Molecular Dynamics Timescales for Biological Macromolecules through Machine Learning Approaches

Tamara C Bidone

Molecular dynamics (MD) simulations provide atomistic insights into the structural dynamics of biological macromolecules but are inherently limited by accessible timescales. To overcome these limitations, we apply machine learning (ML) approaches to accelerate all-atom simulations and extend their temporal reach. In the first case, unsupervised ML is employed to derive a coarse-grained (CG) model of microtubules

directly from atomistic trajectories. Clustering reduces the number of degrees of freedom, and effective interaction potentials are extracted to preserve essential structural and dynamic features while enabling simulations over extended timescales. In the second case, graph neural networks (GNNs) are combined with reinforcement-learning–inspired reward functions to learn and propagate protein fluctuations. The GNN encodes the connectivity of the protein structure, while the reward mechanism guides the model toward physically consistent long-timescale motions. Together, these approaches represent our initial efforts to show that ML can extract critical biophysical information from atomistic simulations and translate it into predictive, efficient models. By implementing unsupervised identification of coarse-grained representations and data-driven learning of dynamical rules, we aim to establish a framework for extending MD timescales and uncovering emergent behavior in complex biomolecular systems.

Biophysics of mitotic spindle-nuclear envelope coupling in closed mitosis

Meredith Betterton

During closed mitosis the mitotic spindle elongates within an intact nuclear envelope. This architecture requires coordination of chromosome segregation with nuclear division and coupled shape and force changes between the spindle and envelope. Previous work has demonstrated the coupling of the spindle and nuclear envelope, but it is not yet fully clear how and to what extent the nuclear envelope and mitotic spindle shape each other. We combined quantitative imaging, mechanical perturbation, and biophysical modeling of closed mitosis in the fission yeast S. pombe. Increased envelope tension bends the spindle and slows elongation, and relaxing tension can reverse this. Severing either the spindle or envelope by laser ablation relaxes force on the other. Our computational model of spindle elongation in closed mitosis predicts how nuclear shape change is driven by the force distribution at spindle poles. These results illustrate how force balance between the cytoskeleton and lipid membranes can drive remodeling.

Decoding behavioral algorithms in single cells

Ben Larson

Abstract: Single cells can display remarkably sophisticated behaviors, managing the flow of information and orchestrating intricate processes to carry out essential functions. Proper execution of cellular behavior is essential not only for survival of microbes in the diverse, fluctuating environments they inhabit, but also for proper development and

function of multicellular organisms. In animals, behavioral control is generally achieved through neural activity. In cells, however, behaviors emerge directly from the joint action of chemical reactions, cellular architecture, and physical mechanisms and constraints within the cell and in its environment. How do cells coordinate the ordered sequences of actions that constitute behaviors in the face of constraints on the precision of sensing and actuation? Despite increasingly sophisticated knowledge of the components of cells, it remains challenging to synthesize this knowledge into understanding of how cells work. To meet this challenge, we are developing tools and approaches for decoding complex cellular behaviors. Applying these methods to diverse eukaryotic cells, we obtain low dimensional phenotypic spaces in which behaviors can be decomposed into stochastic yet stereotyped sequences of sub-behaviors. This perspective on cell behavior allows us to predict and infer functional physiological states of cells and also to interpret effects of perturbative experiments, yielding mechanistic insights into behavioral control. Altogether, our work sheds new light on how geometry and mechanics encodes cell function. In addition to delivering fundamental insights into the physical and molecular mechanisms and principles of behavioral control in eukaryotic cells, our work stands to enable new ways to predict, guide, and engineer cell function and generate new bio-inspired designs for autonomous control systems.

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From Fading Stains to Foundation Models: Adapting Digital Pathology to Real-World Complexity
Binghao Chai

Sayandeepa Raha
Quantitative Characterization of Nonequilibrium Steady States for Dynamical Systems of Microtubules

Engineering a Virtual Cell to Probe Spindle Assembly and Beyond
Saptarshi Chatterjee

Wenzheng Shi
Reconstructing Actin Dynamics of the Cell Leading Edge from Observational Data

Stanley Nicholson
Collective Nuclear Motion Driven by Mitotic Waves in the Drosophila Blastoder