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as of 24-Feb-2022

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INVESTIGATOR(S):

Name: Denis Tsygankov
Email: denis.tsygankov@bme.gatech.edu
Phone Number: 4043854747
Principal: Y

Organization: **Georgia Tech Research Corporation**

Address: 505 Tenth Street NW, Atlanta, GA 303320420

Country: USA

DUNS Number: 097394084

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Final Report for Period Beginning 15-Sep-2017 and Ending 14-Apr-2021

Title: An integrative methodology for the multi-scale study of collective behavior emerging in a heterogeneous cell population

Begin Performance Period: 15-Sep-2017

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Submitted By: Denis Tsygankov

Email: denis.tsygankov@bme.gatech.edu

Phone: (404) 385-4747

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STEM Degrees: 10

STEM Participants: 18

Major Goals: The major goal of this project was to develop an integrative methodology to couple experimental approaches with multi-scale modeling, simulation, and image analysis in a synergetic manner and to provide a deeper, systems-level understanding of the collective cell behavior during endothelial patterning. Specifically, the project aimed to establish a comprehensive biomechanical representation of multicellular behavior ranging from molecular to cellular to tissue levels by:

1. developing a predictive model of vascular endothelial cells, which is parametrized based on experimental observations at the cellular level. This model is used in simulations of collective cell behavior during vascular tube formation.
2. developing an image-analytics tool for the quantification and direct comparison of numerically simulated and experimentally observed multicellular patterns. This tool provides a computational platform for the systematic study of the relationships between mechanical properties of individual cells, their physical interactions with the extracellular matrix (ECM) and with each other, and the development of various patterns of multicellular organization.

These integrative approaches allowed us to tackle the project's central hypothesis that the interaction-induced segregation of endothelial cells into functionally distinct subpopulations ("linkers" and "anchors") is achieved through the dynamic regulation of cell-cell and cell-ECM junctions and plays the key role in maintaining the integrity and function of the vascular system.

The specific aims were stated as:

Aim 1. Develop a comprehensive multi-scale model of collective cell behavior in three-dimensional microenvironments.

Indeed, we developed a comprehensive cell model that included: 1) stochastic dynamics of protrusions, by means of which cells interact with each other and the extracellular matrix; 2) an elastic cell body that moves and changes its shape in response to the mechanical forces resulting from interactions with its environment; 3) ECM-mediated sensing of cell proximity for protrusion guidance. This model allowed us to simulate the emergence of collective cell behavior with sufficient level of detail to capture the complex interplay of the molecular regulation of the cell stiffness, the mechanics of cell interactions and shape changes, and the self-organization of cells into multicellular structures.

Aim 2. Investigate the role of mechanosensitive regulation of cell-cell and cell-matrix interactions in self-organization and stability of heterogeneous cell formations.

Using our multi-scale model for numerical simulations of vasculature formation, we aimed to determine the role of functional heterogeneity in the stability of resulting cellular patterns. We initiated a long-term objective to collect live

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and fixed cell fluorescence images of multiple proteins associated with adherens junctions and focal adhesions and compare their spatial distributions among different patterns of tube formation under various genetic and environmental perturbations. Through the iterative cycling between the model and experiments, we explore alternative hypotheses about the regulation of cell-cell and cell-ECM junctions, such as titration of signaling proteins that are shared between cell-cell and cell-ECM adhesions.

Aim 3. Develop an image analysis platform for automated characterization of multicellular formations with complex geometries.

We developed a computational platform for the quantification/classification of multicellular patterns and learning /extracting characteristic features in cell networks with subtle structural differences. We tested the performance of our image analysis algorithms with an extensive imaging data on the formation of capillary-like patterns under different experimental conditions and apply the quantification approach to the comparative analysis of tube formations from experiments and simulations of Aim 1 and 2. We also converted these algorithms into a user-friendly graphical interface (MATLAB-based GUI) to ensure easy accessibility of our methods to a broader scientific community.

Accomplishments: We are happy to report that the key objectives of this project have been accomplished.

Aim 1. Develop a comprehensive multi-scale model of collective cell behavior in three-dimensional microenvironments.

At this time Aim1 in its originally proposed form is completed. We designed a model to explicitly account for protrusive activity, cytoskeletal stiffness, and forces developed through cell interactions. In this model, cells and ECM interact through the extension of cell protrusions and establishment of elastic contacts within the 3D substrate. The ellipsoidal cell body allows the cell to stretch and spread on the substrate due to lateral cell-cell interactions. In the model, we also account for long-distance (ECM-mediated) sensing between plated cells guiding their directed protrusion towards each other. Together, these features allowed us to achieve exceptionally accurate quantitative correspondence between the simulated and the experimentally observed dynamics both at the single-cell and multi-cellular levels. We used the model to dissect biomechanical contributions to the collective behavior of endothelial cell under different biochemical and genetic perturbations. The results of this work are now published in *iScience* (Cell Press). To build upon this completed Aim, we are working on coupling the morphodynamic cell model with a model of molecular signaling coordinating the balance of cell-cell and cell-ECM interactions, which is necessary for the formation of the robust multicellular structures.

Aim 2. Investigate the role of mechanosensitive regulation of cell-cell and cell-matrix interactions in self-organization and stability of heterogeneous cell formations.

Our main goal was to apply our integrative methodology to answer the fundamental questions regarding the development of the biomechanical deficiencies caused by mutations in one of the three Cerebral Cavemous Malformation proteins and the rescue of the despise phenotypes by pharmacological treatments.

First, we discovered that despite significantly elevated phosphorylation of cofilin and MLC in all three disease phenotypes, only CCM1 and CCM2 have significantly increased stiffness as compared to the control cells. This result is important from the pharmacological treatment perspective because targeting the components of the RhoA signaling pathway may rescue the phosphorylation levels of cofilin and MLC, but has limited effect on the collective behavior of CCM3 cells since the overall stiffness of these cells appears not to be the major factor defining their deficient biomechanics.

Second, our simulations with the model developed in Aim 1 led us to the conclusion that central to the loss of multicellular integrity in CCM1 and CCM3 cells is the decrease in cell-ECM and cell-cell contact stability, respectively. We confirmed this model prediction with the gene ontology analysis of our RNA-seq data and, more directly, with the non-adherent cell washout assay.

Third, quantitative comparison of our experimental data with a systematic scan of model parameters in our simulations allowed us to show that the rescue of cell interactions, taking place upon the treatment of cells with a Rho kinase inhibitor, leads to additional (predictable!) perturbations of cell biomechanics unrelated to the deficiency of CCM proteins. These perturbations are associated with the modulation of cell stretching and spreading, contractility of protrusions, and the efficiency of long-range cell-cell sensing.

Collectively these findings illustrated the predictive power of our model and its ability to dissect intertwined processes driving the complex behavior of endothelial cells in health and disease.

Further analysis of gene expression has revealed that some of the genes with a more than 2-fold change in expression as compared to wild type (e.g. DAG1, PALS1, and PYGO1) are related to the WNT/ β -catenin pathway. Since this pathway is known to be involved in the regulation of cell-cell adhesions, reorganization of the

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cytoskeleton, and assembly of ECM, our results bring WNT/ β -catenin into the spot as an important player in the regulation of functional heterogeneity of endothelial cells or its perturbations due to CCM mutations. Thus, our results in this project motivated a new line of research aiming to establish cellular localization of β -catenin, DAG1, PALS1, and PYGO1 in different parts of the multicellular formations to correlate their spatial distributions with the shift in the balance of cell-cell and cell-ECM adhesions. These data will guide our simulations and enable a thorough analysis of the role of WNT/ β -catenin pathway in the homeostasis and failure of vascular tubule formation, with a long-term goal to achieve a high level of CCM rescue through a combination of rationally chosen inhibitors.

Aim 3. Develop an image analysis platform for automated characterization of multicellular formations with complex geometries.

We developed a novel algorithm for morphometric analysis of imaging data using the shape-to-graph mapping that allows precise and efficient identification of multiple structural features. With this algorithm, we can now extract a comprehensive set of geometric measures from imaging data with arbitrarily complex cell shapes and multicomponent structures. We have integrated this information-rich shape representation with a novel machine-learning algorithm and developed a pipeline for automated classification of complex cellular patterns. We have demonstrated an exceptional accuracy and efficiency of our approach using annotated multicellular patterns with subtle structural variations. The method was also validated on experimental data. We showed that our methodology not only allows to extract subtle image-level differences that are very difficult or even impossible to determine by eye, but also relate these differences to specific structural characteristics, thus providing an insight into the effects of various genetic or pharmacological perturbations of the cell behavior. Finally, to show that our methodology is not limited to mesh-like cell formations characteristic to specific cell types, we applied it to completely different type of data. Specifically, we analyzed confluent cultures of U2OS cells subjected to an extensive set of small molecule treatments. The global (image-scale) nature of our graph structure, which captures both the shapes of all individual cells and their relative spatial positioning in the field of view, allowed us to outperform the conventional shape metrics in terms of precision and sensitivity of the phenotypic classification. Big paper reporting this three-year effort within this project is published in PLOS Computational Biology. Developing this platform also led us to a new idea for modeling highly realistic shapes of cells within a tight cluster, which would nicely complement the Aim 1 model for confluent plated cells. Results on this topic are now being prepared for another publication.

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Training Opportunities: During the first year of the award for following individuals participated in the project, completed their training, and moved on to the next stages of their careers: Dr. Olga Chernaya (Postdoctoral research associate); Kian Pourak, Justin Cha, Dae Eun Park, Sidi Zhao, Brooke Trogdon, John Miles, Rajan Jayasankar (undergraduate students).

During the second year of the award, the following undergraduate students participated in the project, completed the training, and moved on to the next stages of their careers: Arjun Patel (Research topic: "Inverse Voronoi mapping for analysis of cell shapes in tight clusters." A paper on this topic is now in preparation for submission); Ethan Offenbacher (Research topic: "Spatiotemporal dynamics of actin waves within the three-dimensional shape of a moving cell".); Gilad Stolarski (Research topic: "Modeling biomechanics of tight cell clusters.").

During the last period of the award, the following undergraduate students participated in the lab research: Asher Altman (Research topic: "Establishing the role DAG1 and PALS1 in distinct CCM1 and CCM3 phenotypes of endothelial cells"); Samantha Smith (Research topic: "Regulation of static and dynamic patterns of Rho-GTPase activity at the cell-level scale"); Farhan Rahman (Research topic: "The role of noise-induced excitable dynamics of Rho family GTPases in the regulation of actin cytoskeleton").

The following Ph.D. and Master's level students have been trained through the activities supported by this project:

1. Graduate research assistant, Ms. Anastasia Zhurikhina: Ms. Zhurikhina has been responsible for the development of the computational multi-scale models and for performing numerical simulations of multicellular pattern formation. She analyzes simulation results to predict outcome and systematically test hypotheses of the project. She works closely with the other team members to ensure that the model design fits the format of the experimental data and the image-analytics components of the project. She is a co-author on 5 published papers resulted from this project.
2. Graduate research assistant, Mr. William Pilcher: Mr. Pilcher has been responsible for the development of a computational platform for the morphometric analysis of multicellular patterns, including automated feature extraction for quantification and classification of complex cell networks with subtle structural differences. He closely interacts with the other team members to carry out the comparative analysis of tube formations from both experimental imaging and numerical simulations. He is a co-author on 2 published and 1 in-preparation papers resulted from this project.
3. Master's student, Yinghan Xu: Mr. Xu has been responsible for converting image analysis routines into a user-friendly graphical interface to be easily accessible to researchers from different disciplines and backgrounds. He is a co-author on a paper recently published in PLOS Computational Biology.

All students in the lab report their progress at our weekly lab meetings. William Pilcher also presented his findings as a poster at the 2017 and 2018 Annual Meetings of the Biomedical Engineering Society: "Automated graph-based quantification of complex multicellular formations in imaging data." Anastasia Zhurikhina presented a poster and a flash talk on her findings at the 2019 Annual Meeting of the Biophysical Society: "Biophysical Model Reveals the Role of CCM Proteins in Collective Behavior of Endothelial Cells." Asher Altman presented his work as a poster at the National Collegiate Research Conference by the Harvard College Undergraduate Research Association: "Establishing the role of DAG1 and PALS1 in the development of different types of Cerebral Cavernous Malformation" and as a poster at the Engineering in Healthcare: Industry and Research Symposium: "The Biomechanical Implications of DAG1 and PALS1 in the development of Cerebral Cavernous Malformation Phenotypes".

Beyond the lab work, the students involved in the project are encouraged to be engaged in the broader impact activities. Previously the lab participated in the Emory University Science-Art-Wonder initiative that facilitates the integration of science and visual arts. Anastasia Zhurikhina served as an instructor in SigmaCamp (sigmacamp.org), a week-long math and science sleepaway camp aiming to ignite a spark of interest and curiosity in 12-16 years old students. Anastasia Zhurikhina also served as a Module Teaching Assistant at Bioignite Biomedical STEM Summer Camp (bioignite.org). The camp creates a unique environment, where the complex research topics are being broken down into engaging activities and intertwined with school material in a way that helps students connect their basic scientific knowledge and interests with the cutting-edge advances in the field of Biomedical Engineering. Most recently, with the additional funding provided by the NSF Career Award, the lab initiated broader impact activities in partnership with Spelman College and the Georgia Tech GIFT program to facilitate engagement, preparedness, and well-rounded training of students from minority groups in biomedical research.

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Results Dissemination: Our goal is to disseminate software tools, models, and analysis methods to the broadest audience. During the award period, our findings have been disseminated through the following publications and presentations, where the ARO grant was formally acknowledged:

1. H. Lei, J.-G. Chiou, A. Zhurikhina, T.R. Zyla, D. Tsygankov, D.J. Lew. "Temporal regulation of morphogenetic events in *Saccharomyces cerevisiae*." *Molecular Biology of the Cell* 29(17): 2069 (2018).
2. A. Zhurikhina, T. Qi, K.M. Hahn, T.C. Elston, D. Tsygankov. "EdgeProps: A computational platform for correlative analysis of cell dynamics and near-edge protein activity." *Methods in Molecular Biology* 1821: 47 (2018).
3. W. Pilcher* and D. Tsygankov. "Automatic characterization of complex cell shapes and patterns in biological data." Biomedical Engineering Society, 2017 BMES Annual Meeting, Phoenix, AZ, October 11, 2017 (poster presentation)
4. M. Shaaya, V. Huyot, A. Zhurikhina, D. Tsygankov, V. Natarajan, A. Karginov. "Allosteric regulation of protein kinases using optogenetics." *FASEB Journal*, 32 (1), 99650. Annual Meeting of American Society for Investigative Pathology, San Diego, CA, April 21, 2018 (oral presentation)
5. D. Tsygankov. "Mechanobiology of collective cell behavior in the contexts of vascular malformation and metastasis." Winship Center's Cell Biology Program Seminar, Emory University, September 6, 2018. (oral presentation)
6. Chernaya, O., A. Zhurikhina, S. Hladyshau, W. Pilcher, K.M. Young, J. Ortner, V. Andra, T.A. Sulchek, D. Tsygankov. "Biomechanics of endothelial tubule formation differentially modulated by Cerebral Cavernous Malformation proteins." *iScience* 9: 347 (2018).
7. K.D. Moran, H. Kang, A.V. Araujo, T.R. Zyla, K. Saito, D. Tsygankov, D.J. Lew. "Cell-cycle control of cell polarity in yeast." *Journal of Cell Biology* 218(1): 171 (2019).
8. A. Zhurikhina, O. Chernaya, S. Hladyshau, W. Pilcher, K.M. Young, J. Ortner, V. Andra, T.A. Sulchek, D. Tsygankov. "Biophysical model reveals the role of CCM proteins in collective behavior of endothelial cells." *Biophysical Journal*, 116(3), p.416a. (2019).
9. D. Tsygankov. "A Methodology for automated characterization of complex shapes, patterns, and compound structures." STAMI Industrial Partners Day and Exposition, Georgia Tech, September 27-28 (oral presentation).
10. W. Pilcher*, A. Zhurikhina, O. Chernaya, Y. Xingyu, P. Qiu, D. Tsygankov. "Automated graph-based quantification of complex multi-cellular formations in imaging data." 2018 BMES Annual Meeting, Atlanta, GA, October 17-20, 2018 (poster presentation).
11. W. Pilcher*, P. Qui, D. Tsygankov. "A novel image -analytic tool for automated characterization of complex cell shapes and patterns in 3D tissue models." MC3M Symposium, Georgia Tech, December 7, 2018 (oral presentation).
12. D. Tsygankov. "Image-guided simulation models of cellular biomechanics." CISMM Advisory Board Meeting, UNC at Chapel Hill, NC, February 11, 2019 (oral presentation).
13. E.C. O'Shaughnessy, O.J. Stone, P.K. LaFosse, M.L. Azoitei, D. Tsygankov, J.M. Heddleston, W.R. Legant, E. S. Wittchen, K. Burrige, T.C. Elston, E. Betzig, T.-L. Chew, D. Adalsteinsson, K.M. Hahn. "Software for lattice light-sheet imaging of FRET biosensors, illustrated with a new Rap1 biosensor." *Journal of Cell Biology* 218(9): 3153, 2019.
14. W. Pilcher, X. Yang, A. Zhurikhina, O. Chernaya, Y. Xu, P. Qiu, D. Tsygankov. "Shape-to-graph mapping method for efficient characterization and classification of complex geometries in biological images." *PLoS Computational Biology* 16(9): e1007758, 2020.
15. D. Tsygankov. "Application of machine learning to quantification of complex cellular patterns in imaging data using a set of metrics from the shape-to-graph mapping." Symposium on Machine Learning in Science and

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Engineering, Georgia Tech, June 10, 2019 (oral presentation).

16. D. Tsygankov. "Multiscale modeling of coordinated endothelial cell behavior during vascular development." 2nd Annual Symposium on Multiscale Cell Fate Research, University of California Irvine, CA, October 29, 2019 (oral presentation).

17. S. Hladyshau, S. Cohen, S. Nie, D. Tsygankov. "An integrative computational model of cellular morphodynamics." The Career, Research, and Innovation Development Conference, Georgia Tech, February 13, 2019 (poster presentation).

18. S. Hladyshau, S. Cohen, S. Nie, D. Tsygankov. "An integrative computational model of cell migration." 63rd Annual Meeting of the Biophysical Society, Baltimore, MD, March 2-6, 2019 (poster presentation).

19. S. Hladyshau and D. Tsygankov. "A computational platform for understanding complex regulatory processes driving cell migration." Winship Cancer Immunology Retreat, Emory University, Atlanta, GA, January 10, 2020 (poster presentation).

20. D. Tsygankov. "Integrating shape-to-graph mapping with machine learning approaches for automated characterization of complex geometries and patterns in imaging data." Meeting of the Marcus Center for Therapeutic Cell Characterization and Manufacturing with Applied Materials, Inc., Georgia Tech, April 24, 2019 (oral presentation).

21. D. Tsygankov. "Integrative systems biology approach to understanding biomechanics of cellular formation." Cutting Edge Technologies showcase for Denning Technology and Management Program, Georgia Tech, February 6, 2020 (oral presentation).

22. Shaaya M, Fauser J, Zhurikhina A, Conage-Pough JE, Huyot V, Brennan M, Flower CT, Matsche J, Khan S, Natarajan V, Rehman J, Kota P, White FM, Tsygankov D, Karginov AV. Light-regulated allosteric switch enables temporal and subcellular control of enzyme activity. *eLife* 9: e60647 (2020).

23. Hladyshau S, Kho M, Nie S, Tsygankov D. "Spatiotemporal development of coexisting wave domains of Rho activity in the cell cortex". *Scientific Reports* 11: 19512 (2021).

24. Marston DJ, Slattery S, Hahn KM, Tsygankov D. "Correcting artifacts in ratiometric biosensor imaging; an improved approach for dividing noisy signals". *Frontiers in Cell and Developmental Biology* 9: 685825 (2021).

25. Errede B, Hladyshau S, Nivedita N, Tsygankov D, Elston TC. "Bistability in the polarity circuit of yeast". *Molecular Biology of the Cell* doi: 10.1091/mbc.E20-07-0445 (Accepted on April 27, 2021).

26. Tsygankov D. "Unveiling the development of coexisting wave domains of Rho activity in the cell cortex with a mass-conserved activator-substrate model". PDE & Applied Math Seminars in the Department of Mathematics, University of California, Riverside, CA, April 21, 2021 (oral presentation).

27. Tsygankov D. "Pushing the limits of ratiometric precision at the cell edge with the NCF method". Klaus M. Hahn's Lab Seminar, UNC at Chapel Hill, NC, April 26, 2021 (oral presentation).

28. Tsygankov D. "Integrative systems biology approach to understanding biomechanics of cell motion and their collective behavior". The Georgia Tech Chapter of the Biomedical Engineering Society, October 21, 2021 (oral presentation).

29. Tsygankov D. "Multi-cellular biomechanics of endothelial cells in cerebral cavernous malformation". Presentation, Multi-cellular Engineering of Living Systems Workshop, Georgia Tech, December 3, 2021 (oral presentation).

30. Altman A, Zhurikhina A, Tsygankov D. "Establishing the role of DAG1 and PALS1 in the development of different types of Cerebral Cavernous Malformation". National Collegiate Research Conference by the Harvard College Undergraduate Research Association, Cambridge, MA, January 21-23, 2022 (poster presentation).

31. Altman A, Zhurikhina A, Tsygankov D. "The biomechanical implications of DAG1 and PALS1 in the

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development of Cerebral Cavemous Malformation phenotypes". Engineering in Healthcare: Industry and Research Symposium, Ohio State University, Columbus, OH, February 18-19, 2022 (poster presentation).

Papers in preparation:

32. A. Patel, W. Pilcher, M. Kemp, D. Tsygankov. "Efficient high-precision representation of cell shapes with an inverse Voronoi mapping."

Honors and Awards: 1. Denis Tsygankov (PI): Faculty Early Career Development (CAREER) Award, National Science Foundation (NSF), Division of Civil, Mechanical, and Manufacturing Innovation (CMMI), 2020.

2. Asher Altman (undergraduate researcher): Summer 2021 President's Undergraduate Research Award.

3. Asher Altman (undergraduate researcher): Alfred H. Gibeling Family Research Fund Award, 2021.

4. Asher Altman (undergraduate researcher): PURA Travel Award to present at the 2022 National College Research Conference by the Harvard College Undergraduate Research Association.

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PARTICIPANTS:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)

Participant: Olga Chernaya

Person Months Worked: 12.00

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National Academy Member: N

Participant Type: Graduate Student (research assistant)

Participant: Anastasia Zhurikhina

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National Academy Member: N

Participant Type: Graduate Student (research assistant)

Participant: William Pilcher

Person Months Worked: 6.00

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Participant Type: Other Professional

Participant: Yinghan Xu

Person Months Worked: 12.00

Funding Support:

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Participant Type: Undergraduate Student

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Participant: Kian Pourak

Person Months Worked: 6.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Undergraduate Student

Participant: Justin Cha

Person Months Worked: 6.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Undergraduate Student

Participant: Brooke Trogdon

Person Months Worked: 6.00

Project Contribution:

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Participant Type: Undergraduate Student

Participant: Sidi Zhao

Person Months Worked: 6.00

Project Contribution:

National Academy Member: N

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Participant Type: Undergraduate Student

Participant: Dae Eun Park

Person Months Worked: 6.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Undergraduate Student

Participant: Rajan Jayasankar

Person Months Worked: 6.00

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Participant Type: Undergraduate Student

Participant: John Miles

Person Months Worked: 6.00

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Participant: Gilad Stolarski

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Participant: Ethan Offenbacher

Person Months Worked: 6.00

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Participant: Farhan Rahman

Person Months Worked: 6.00

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Participant: Samantha Smith

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Participant: Asher Altman

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Abstract: Tip growth in fungi involves highly polarized secretion and modification of the cell wall at the growing tip. The genetic requirements for initiating polarized growth are perhaps best understood for the model budding yeast *Saccharomyces cerevisiae*. Once the cell is committed to enter the cell cycle by activation of G1 cyclin/cyclin-dependent kinase (CDK) complexes, the polarity regulator Cdc42 becomes concentrated at the presumptive bud site, actin cables are oriented toward that site, and septin filaments assemble into a ring around the polarity site. Several minutes later, the bud emerges. Here, we investigated the mechanisms that regulate the timing of these events at the single-cell level. Septin recruitment was delayed relative to polarity establishment, and our findings suggest that a CDK-dependent septin "priming" facilitates septin recruitment by Cdc42. Bud emergence was delayed relative to the initiation of polarized secretion, and our findings suggest that the delay reflects

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Authors: Anastasia Zhurikhina, Timothy Qi, Klaus M. Hahn, Timothy C. Elston, Denis Tsygankov

Keywords: GTPase activity, Image analysis, Morphodynamics, Protrusive activity, Correlation

Abstract: Developing molecular tools to visualize and control Rho GTPase signaling in living cells has been instrumental in elucidating the mechanisms of cytoskeletal reorganization and causal relationships between activation events in cell function. An indispensable part of such studies is the quantitative characterization of the spatiotemporal GTPase activity. Here we present a computational pipeline, EdgeProps, designed for comparative/correlative analysis of cell dynamics (edge velocity) and near-edge protein activity (intensity of a fluorescent signal). The tool offers a user-friendly interface with three functional modules for processing, visualization, and statistical characterization of single-cell imaging data.

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Journal: PLOS Computational Biology

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Publication Identifier: <https://doi.org/10.1371/journal.pcbi.1007758>

Volume: 16

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Date Submitted: 2/22/22 12:00AM

Date Published: 9/3/20 4:00AM

Publication Location:

Article Title: Shape-to-graph Mapping Method for Efficient Characterization and Classification of Complex Geometries in Biological Images

Authors: William Pilcher, Xingyu Yang, Anastasia Zhurikhina, Olga Chernaya, Yinghan Xu, Peng Qiu, Denis Tsyg

Keywords: morphometric analysis, cell formations, machine learning

Abstract: With the ever-increasing quality and quantity of imaging data in biomedical research comes the demand for computational methodologies that enable efficient and reliable automated extraction of the quantitative information contained within these images. One of the challenges in providing such methodology is the need for tailoring algorithms to the specifics of the data, limiting their areas of application. Here we present a broadly applicable approach to quantification and classification of complex shapes and patterns in biological or other multi-component formations. This approach integrates the mapping of all shape boundaries within an image onto a global information-rich graph and machine learning on the multidimensional measures of the graph. We demonstrated the power of this method by (1) extracting subtle structural differences from visually indistinguishable images in our phenotype rescue experiments using the endothelial tube formations assay, (2) training the algorithm to identify

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Publication Location:

Article Title: Software for lattice light-sheet imaging of FRET biosensors, illustrated with a new Rap1 biosensor

Authors: Ellen C. O'Shaughnessy, Orrin J. Stone, Paul K. LaFosse, Mihai L. Azoitei, Denis Tsygankov, Jo

Keywords: image analysis, 3D, molecular engineering

Abstract: Lattice light-sheet microscopy (LLSM) is valuable for its combination of reduced photobleaching and outstanding spatiotemporal resolution in 3D. Using LLSM to image biosensors in living cells could provide unprecedented visualization of rapid, localized changes in protein conformation or posttranslational modification. However, computational manipulations required for biosensor imaging with LLSM are challenging for many software packages. The calculations require processing large amounts of data even for simple changes such as reorientation of cell renderings or testing the effects of user-selectable settings, and lattice imaging poses unique challenges in thresholding and ratio imaging. We describe here a new software package, named ImageTank, that is specifically designed for practical imaging of biosensors using LLSM. To demonstrate its capabilities, we use a new biosensor to study the rapid 3D dynamics of the small GTPase Rap1 in vesicles and cell protrusions.

Distribution Statement: 2-Distribution Limited to U.S. Government agencies only; report contains proprietary info

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Publication Location:

Article Title: Spatiotemporal development of coexisting wave domains of Rho activity in the cell cortex

Authors: Siarhei Hladyshau, Mary Kho, Shuyi Nie, Denis Tsygankov

Keywords: GTPases, actin dynamics, simulation model

Abstract: The Rho family GTPases are molecular switches that regulate cytoskeletal dynamics and cell movement through a complex spatiotemporal organization of their activity. In *Patiria miniata* (starfish) oocytes under in vitro experimental conditions (with overexpressed Ect2, induced expression of ?90 cyclin B, and roscovitine treatment), such activity generates multiple co-existing regions of coherent propagation of actin waves. Here we use computational modeling to investigate the development and properties of such wave domains. The model reveals that the formation of wave domains requires a balance between the activation and inhibition in the Rho signaling motif. Intriguingly, the development of the wave domains is preceded by a stage of low-activity quasi-static patterns, which may not be readily observed in experiments. Spatiotemporal patterns of this stage and the different paths of their destabilization define the behavior of the system in the later high-activity (observable) stage. Acco

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Publication Identifier: <https://doi.org/10.7554/eLife.60647>

Volume: 9

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Publication Location:

Article Title: Light-regulated allosteric switch enables temporal and subcellular control of enzyme activity

Authors: Mark Shaaya, Jordan Fauser, Anastasia Zhurikhina, Jason E Conage-Pough, Vincent Huyot, Martin Bre

Keywords: biosensors, Src kinase, spatiotemporal activity

Abstract: Engineered allosteric regulation of protein activity provides significant advantages for the development of robust and broadly applicable tools. However, the application of allosteric switches in optogenetics has been scarce and suffers from critical limitations. Here, we report an optogenetic approach that utilizes an engineered Light-Regulated (LightR) allosteric switch module to achieve tight spatiotemporal control of enzymatic activity. Using the tyrosine kinase Src as a model, we demonstrate efficient regulation of the kinase and identify temporally distinct signaling responses ranging from seconds to minutes. LightR-Src off-kinetics can be tuned by modulating the LightR photoconversion cycle. A fast cycling variant enables the stimulation of transient pulses and local regulation of activity in a selected region of a cell. The design of the LightR module ensures broad applicability of the tool, as we demonstrate by achieving light-mediated regulation of Abl and bRaf kinases as well

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Journal: Frontiers in Cell and Developmental Biology
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Volume: 9 **Issue:** **First Page #:** 685825
Date Submitted: 2/22/22 12:00AM **Date Published:** 8/20/21 4:37AM
Publication Location:

Article Title: Correcting Artifacts in Ratiometric Biosensor Imaging; an Improved Approach for Dividing Noisy Signals

Authors: Daniel J. Marston, Scott D. Slattery, Klaus M. Hahn, Denis Tsygankov

Keywords: ratiometric analysis, FRET biosensors, cell morphodynamics, image processing, Rho (Rho GTPase)

Abstract: The accuracy of biosensor ratio imaging is limited by signal/noise. Signals can be weak when biosensor concentrations must be limited to avoid cell perturbation. This can be especially problematic in imaging of low volume regions, e.g., along the cell edge. The cell edge is an important imaging target in studies of cell motility. We show how the division of fluorescence intensities with low signal-to-noise at the cell edge creates specific artifacts due to background subtraction and division by small numbers, and that simply improving the accuracy of background subtraction cannot address these issues. We propose a new approach where, rather than simply subtracting background from the numerator and denominator, we subtract a noise correction factor (NCF) from the numerator only. This NCF can be derived from the analysis of noise distribution in the background near the cell edge or from ratio measurements in the cell regions where signal-to-noise is high. We test the performance of the m

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Volume: **Issue:** **First Page #:**
Date Submitted: 2/22/22 12:00AM **Date Published:** 5/6/21 4:42AM
Publication Location:

Article Title: Bistability in the polarity circuit of yeast

Authors: Beverly Errede, Siarhei Hladyshau, Nivedita Nivedita, Denis Tsygankov, Timothy C. Elston

Keywords: polarization, computational model

Abstract: Cells polarize their growth or movement in many different physiological contexts. A key driver of polarity is the Rho GTPase Cdc42, which when activated becomes clustered or concentrated at polar sites. Multiple models for polarity establishment have been proposed. All of them rely on positive feedback to reinforce regions of high Cdc42 activity. Positive feedback can lead to bistability, a scenario in which cells can exist in either a polarized or unpolarized state under identical external conditions. Determining if the signaling circuit that drives Cdc42 polarity is bistable would provide important information about the mechanism that underlies polarity establishment and insights into the design features required for proper cellular function. We studied polarity establishment during the mating response of yeast. Using microfluidics to precisely control the temporal profile of mating pheromone and live-cell imaging to monitor the polarity process in single living cells, we found that

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CONFERENCE PAPERS:

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Conference Name: 2017 IEEE Global Conference on Signal and Information Processing (GlobalSIP)
Date Received: 31-Aug-2020 **Conference Date:** 14-Nov-2017 **Date Published:** 08-Mar-2018
Conference Location: Montreal, QC
Paper Title: Graph-based extraction of shape features for leaf classification
Authors: Andrew Zhao, Denis Tsygankov, Peng Qiu
Acknowledged Federal Support: Y

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WEBSITES:

URL: <https://github.com/tsygankov-lab/NoiseCorrectionFactor>

Date Received: 22-Feb-2022

Title: Methodology for identifying and correcting ratio-imaging artifacts

Description: Scripts and documentation for the methods presented in <https://pubmed.ncbi.nlm.nih.gov/34490242/>

URL: https://github.com/tsygankov-lab/yeast_polarization_bistability

Date Received: 22-Feb-2022

Title: Simulation model of bistability in yeast polarity

Description: Script and documentation for the cell polarization model presented in <https://pubmed.ncbi.nlm.nih.gov/33956497/>

URL: <https://github.com/tsygankov-lab/WaveDomains>

Date Received: 22-Feb-2022

Title: Methodology for analyzing and modeling wave domains

Description: Script and documentation for the methods presented in <https://pubmed.ncbi.nlm.nih.gov/34593939/>

Partners

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I certify that the information in the report is complete and accurate:

Signature: Denis Tsygankov

Signature Date: 2/22/22 12:47AM

CREATIVE PRODUCTS (software)

Methodology for analyzing and modeling wave domains

<https://github.com/tsygankov-lab/WaveDomains>

Script and documentation for the methods presented in <https://pubmed.ncbi.nlm.nih.gov/34593939/>

Methodology for identifying and correcting ratio-imaging artifacts

<https://github.com/tsygankov-lab/NoiseCorrectionFactor>

Scripts and documentation for the methods presented in <https://pubmed.ncbi.nlm.nih.gov/34490242/>

Simulation model of bistability in yeast polarity

https://github.com/tsygankov-lab/yeast_polarization_bistability

Script and documentation for the cell polarization model presented in

<https://pubmed.ncbi.nlm.nih.gov/33956497/>