BIOGRAPHICAL SKETCH

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NAME: Legant, Wesley Ryan

eRA COMMONS USER NAME (credential, e.g., agency login): NA

POSITION TITLE: Research Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Washington University, St. Louis, MO	B.S.	12/05	Biomedical Engineering
University of Pennsylvania, Philadelphia, PA	Ph.D.	05/12	Bioengineering
HHMI Janelia Research Campus, Ashburn, VA	Postdoctoral	--	Optical Physics

A. Personal Statement

The long term goal of my research is to combine advanced optical microscopy techniques, quantitative computational algorithms, and microfabricated cell culture systems to study the biochemical and biophysical mechanisms that drive cell migration and tissue formation. My undergraduate and graduate work in Bioengineering has given me the background and skills necessary to contribute both to instrument design, algorithm development and novel studies of basic cell biology. In my graduate work at the University of Pennsylvania I used microfabrication to develop highly controlled cell culture systems that permitted both measurement and manipulation of the cellular microenvironment. During my final year as a graduate student, I worked on a collaborative project at ETH in Zurich to use these systems to study extracellular matrix deposition and conformation. At HHMI Janelia Research Campus, I am developed novel fluorescence microscopy techniques and computational algorithms with a focus on live-cell and super-resolution modalities. In addition to instrument design, I have also worked directly with many biological collaborators to apply these methods in a range of settings. Over the past three years, these collaborations have produced 7 peer reviewed publications with several more currently in preparation. In addition to my scientific work, both in graduate school and as a post doc, I have pursued an interest in teaching and outreach. In graduate school, I took additional coursework in teaching methods for undergraduates, was a graduate teaching assistant, and mentored undergraduate students in the lab. As a post doc, I have organized workshops to assist other research groups in replicating our imaging technologies in their own labs. I feel that my experience and contributions at the interface between engineering and biology uniquely positions me to build a research program that is fluent in both fields.

1. Chen, B.C.*, **Legant, W.R.***, Wang, K.*, Shao L., Milkie, D.E., Davidson, M.W., Janetopoulos, C., Wu, X. S., Hammer, J. A., Liu, Z., English, B. P., Mimori-Kiyosue, Y., Romero, D. P., Ritter, A. T., Lippincott-Schwartz, J., Fritz-Laylin, L., Mullins, R. D., Mitchell, D. M., Bembenek, J. N., Reymann, A. C., Bohme, R., Grill, S. W., Wang, J. T., Seydoux, G., Tulu, U. S., Kiehart, D. P., Betzig, E., Lattice light-sheet microscopy: imaging molecules to embryos at high spatiotemporal resolution. Science 346, 1257998 (Oct 24, 2014). ***equal contribution**

2. **W.R. Legant**, L. Shao, J.B. Grimm, T. Brown, D.E. Milkie, B Avants, L.D. Lavis, E. Betzig, High density three-dimensional localization microscopy across large volumes. Nature Methods. 13(4): 359-365 (April 2016)

3. **Legant, W. R.,** Miller, J. S., Blakely, B. L., Cohen, D. M., Genin, G. M., Chen, C. S., Measurement of mechanical tractions exerted by cells in three-dimensional matrices. Nature Methods 7, 969 (Dec, 2010).

4. **Legant, W. R.***, Choi, C. K.*, Miller, J. S., Shao, L., Gao, L., Betzig, E., Chen, C. S., Multidimensional traction force microscopy reveals out-of-plane rotational moments about focal adhesions. Proceedings of the National Academy of Sciences of the United States of America 110, 881 (Jan 15, 2013). ***equal contribution**

5. **Legant, W. R.**, Pathak, A., Yang, M. T., Deshpande, V. S., McMeeking, R. M., Chen, C. S., Microfabricated tissue gauges to measure and manipulate forces from 3D microtissues. Proceedings of the National Academy of Sciences of the United States of America 106, 10097 (Jun 23, 2009).

B. Positions and Honors

Positions and Employment

2005-2006 Research Scientist, InVivo Sciences LLC, St. Louis, MO
2006-2012 Graduate Student, Dept. of Bioengineering, University of Pennsylvania, Philadelphia, PA
2011-2012 Whitaker International Fellow, Dept. of Biologically Oriented Materials, ETH, Zurich, Switzerland
2012- Postdoctoral Associate, HHMI Janelia Research Campus, Ashburn, VA

Other Experience and Professional Membership

2005 Teaching Assistant for Quantitative Physiology course, Washington University, St. Louis
2009 Teaching Assistant for Cells to Tissues Bioengineering Course, University of Pennsylvania
2010 Co-chair, Gordan-Kenan Research Seminar, Signal Transduction by Engineered ECMs
2016 Organizer, Lattice light sheet microscopy workshop, Janelia Research Campus

Honors

2002-2005 Calvin M. Woodward Fellow (1/2 tuition, 4 years at Washington University in St. Louis)
2006 Valedictorian and Graduation Speaker (Washington University in St. Louis)
2015 Newcomb Cleveland Prize for most outstanding research article in the journal Science

Complete List of Published Work in My Bibliography, NCBI:
http://www.ncbi.nlm.nih.gov/sites/myncbi/1B_plcl_WnV5q/bibliography/42378457/public/?sort=date&direction=ascending

C. Contribution to Science

1. In my undergraduate and early graduate work, I described the generation and use of microfabricated cell culture platforms to measure and manipulate the forces that arise during tissue remodeling. Although larger, centimeter-scale, engineered tissue constructs had been used for many years, miniaturization via microfabrication permitted simultaneous control and microscopic measurement of cellular forces and extracellular events such as matrix remodeling. This further enabled high throughput measurements of hundreds of constructs in parallel with potential applications in drug screening. We used this system to investigate the effects of matrix and boundary rigidity on the contractile force and protein expression in model cardiac and skin tissues. During my final year of graduate school, I combined this system with fluorescent biosensors to investigate how mechanical forces drive changes in extracellular matrix protein conformation and signaling.

a. Marquez, J.P.*, **Legant, W. R.***, Lam, V., Cayemberg, A., Elson, E., Wakatsuki, T., High-throughput measurements of hydrogel tissue construct mechanics. Tissue engineering. Part C, Methods 15, 181 (Jun, 2009). PMCID: PMC2819830, ***equal contribution**

b. **Legant, W. R.**, Pathak, A., Yang, M. T., Deshpande, V. S., McMeeking, R. M., Chen, C. S., Microfabricated tissue gauges to measure and manipulate forces from 3D microtissues. Proceedings of the National Academy of Sciences of the United States of America 106, 10097 (Jun 23, 2009)., PMCID: PMC2700905

c. **Legant, W. R.**, Chen, C. S., Vogel, V., Force-induced fibronectin assembly and matrix remodeling in a 3D microtissue model of tissue morphogenesis. Integrative biology: quantitative biosciences from nano to macro 4, 1164 (Oct, 2012). PMCID: PMC3586566

d. Boudou, T., **Legant, W. R.**, Mu, A., Borochin, M. A., Thavandiran, N., Radisic, M., Zandstra, P. W., Epstein, J. A., Margulies, K. B., Chen, C. S., A microfabricated platform to measure and manipulate the mechanics of engineered cardiac microtissues. Tissue engineering. Part A 18, 910 (May, 2012). PMCID: PMC3338105

2. In my later graduate work, I combined novel biomaterials, 3D imaging, and mathematical modeling to perform the first quantitative sub-cellular measurements of the forces that cells use to drive their migration through a 3D matrix. Such "traction force microscopy" had been developed and applied previously to cells grown on flat planar substrates and has been a key technique to aid in models of cell migration. However, extending these 2D measurements quantitatively into 3D matrices required the generation of a synthetic linearly elastic hydrogel material that would support both cell adhesion and invasion as well as advances in finite element modeling and linear inverse source solutions. We used these materials and methods to describe the role of matrix rigidity and adhesiveness on angiogenic sprouting, to describe the forces cells exert as they spread and invade into a 3D matrix, and to reveal the presence of rotational torques about cell-matrix contacts. Future applications of these methods will be critical to advancing our understanding of mechanotransduction (how mechanical forces are converted to biochemical signals) and migration in 3D matrices and in vivo.

a. Miller, J. S., Shen, C. J., **Legant, W. R.**, Baranski, J. D., Blakely, B. L., Chen, C. S., Bioactive hydrogels made from step-growth derived PEG-peptide macromers. Biomaterials 31, 3736 (May, 2010). PMCID: PMC2837100

b. **Legant, W. R.,** Miller, J. S., Blakely, B. L., Cohen, D. M., Genin, G. M., Chen, C. S., Measurement of mechanical tractions exerted by cells in three-dimensional matrices. Nature Methods 7, 969 (Dec, 2010). PMCID: PMC3056435

c. **Legant, W. R.***, Choi, C. K.*, Miller, J. S., Shao, L., Gao, L., Betzig, E., Chen, C. S., Multidimensional traction force microscopy reveals out-of-plane rotational moments about focal adhesions. Proceedings of the National Academy of Sciences of the United States of America 110, 881 (Jan 15, 2013). PMCID: PMC3549134, ***equal contribution**

3. In my early postdoctoral work we developed a new type of light sheet microscope ("Lattice light sheet microscopy") that dramatically increases speed and reduces phototoxicity compared to confocal or widefield microscopes. We used this instrument both in diffraction limited and super-resolution imaging modalities to investigate specimens ranging in size from single molecules to small embryos. After our initial demonstration of the technique, I have also worked together with biological collaborators to develop additional computation analysis and to apply this instrument to address fundamental problems in cell biology. My on-going postdoctoral work focuses on combining light sheet microscopy, super-resolution and adaptive optical imaging modalities to perform high resolution, low phototoxicity in vivo imaging.

a. Chen, B.C.*, **Legant, W.R.***, Wang, K.*, Shao L., Milkie, D.E., Davidson, M.W., Janetopoulos, C., Wu, X. S., Hammer, J. A., Liu, Z., English, B. P., Mimori-Kiyosue, Y., Romero, D. P., Ritter, A. T., Lippincott-Schwartz, J., Fritz-Laylin, L., Mullins, R. D., Mitchell, D. M., Bembenek, J. N., Reymann, A. C., Bohme, R., Grill, S. W., Wang, J. T., Seydoux, G., Tulu, U. S., Kiehart, D. P., Betzig, E., Lattice light-sheet microscopy: imaging molecules to embryos at high spatiotemporal resolution. Science 346, 1257998 (Oct 24, 2014). PMCID: PMC4336192, ***equal contribution,** *awarded the Newcomb Cleveland prize for most outstanding article published that year in the journal Science.*

b. **W.R. Legant**, L. Shao, J.B. Grimm, T. Brown, D.E. Milkie, B Avants, L.D. Lavis, E. Betzig, High density three-dimensional localization microscopy across large volumes. Nature Methods. 13(4): 359-365 (April 2016)

c. Liu, Z., **W. R. Legant**, Chen, B. C., Li, L., Grimm, J. B., Lavis, L. D., Betzig, E., Tjian, R., 3D imaging of Sox2 enhancer clusters in embryonic stem cells. eLife 3, e04236 (2014). PMCID: PMC4381973

d. Ritter, A. T., Asano, Y., Stinchcombe, J. C., Dieckmann, N. M., Chen, B. C., Gawden-Bone, C., van Engelenburg S., **Legant, W. R.**, Gao, L., Davidson, M. W., Betzig, E., Lippincott-Schwartz, J., Griffiths, G. M., Actin depletion initiates events leading to granule secretion at the immunological synapse. Immunity 42(5) (May 19, 2015). PMCID: PMC4448150

e. Yamashita, N., Morita, M., **Legant, W. R.**, Chen, B. C., Betzig, E., Yokota, H., Mimori-Kiyosue, Y., Three-dimensional tracking of plus-tips by lattice light-sheet microscopy permits the quantification of microtubule growth trajectories within the mitotic apparatus. Journal of Biomedical Optics, 20(10) (Nov 03, 2015). PMID: 26527322

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

Howard Hughes Medical Institute, 2012-present
As a postdoctoral researcher in Eric Betzig's group, I am developing novel light microscopy technologies and applying them to fundamental applications in cell biology.

Completed Research Support

Graduate Assistance in Areas of National Need Fellowship, 2006
Provided tuition and funding for the first year of graduate school at the University of Pennsylvania. Also included additional courses focusing on methods to teach engineering to undergraduate students.

National Science Foundation Graduate Research Fellowship, 2007-2010
Provided funding for three years of graduate school at the University of Pennsylvania. During this time, I used microfabrication techniques and mathematical modeling to study the physical and biological mechanisms that drive cellular migration, extracellular matrix assembly and tissue formation.

Whitaker International Fellowship, 2011-2012
Provided one year of funding to work on a collaborative project at ETH Zurich in Switzerland. Here, I investigated the relationship between mechanical stress, tissue organization and fibronectin conformation in microfabricated 3D model tissues.