BIOGRAPHICAL SKETCH

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NAME: Legant, Wesley R.

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

POSITION TITLE: Assistant Professor of Biomedical Engineering and Pharmacology

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.	12/2005	Biomedical Engineering
University of Pennsylvania, Philadelphia, PA	Ph.D.	05/2012	Bioengineering
HHMI Janelia Research Campus, Ashburn, VA	Postdoctoral	12/2017	Optical Physics

A. Personal Statement

Research: The overarching goal of my work is to develop and apply novel imaging technologies to answer challenging problems in cell biology. These directions are complimentary. New technologies enable new biological studies and the results/challenges of these studies feedback to inform new tools. Specifically, my group is focusing on three primary directions. Microscope Development: We harness fundamental physics and engineering principles to conceive, design, and test new fluorescence microscopes to study cells. Specifically, we are developing Lattice Light Sheet Microscopy to allow rapid 3D imaging across scales from single molecules to developmental organisms. We are combining these with advances in computer vision, machine learning, and image analysis to overcome the spatial and temporal resolution limits of existing microscopes. Chromatin Biology: We are applying our new instruments to better understand the relationship between chromatin organization, protein diffusion, and diverse functions within the cell nucleus. We use both live-cell and multiplexed super-resolution imaging to quantify the organization of local nuclear environments with different biological functions and to visualize single proteins as they search for and bind to target genes in these different settings. By combining these measurements with polymer physics models and pharmacological and genetic perturbations, we are studying how cells orchestrate diverse and often conflicting biochemical reactions within the same cell nucleus. Collaborative applications in cell and developmental biology: We also work collaboratively with groups at UNC and at other institutions to apply our technologies to study biological areas including cytoskeletal and organelle dynamics, mechanotransduction, cell division, endothelial cell biology, and drosophila development.

Together, work has led to over 43 peer reviewed manuscripts, two book chapters, and three US patents that have collectively been cited more than 11,000 times (via Google Scholar). Two of the patents have been licensed for further development and the lattice light sheet microscope is now sold commercially by Carl Zeiss Microscopy as the “Lattice Light Sheet 7”.

Mentorship, Teaching, and Outreach: To accomplish the above research goals, I recruit students from both biomedical engineering and pharmacology/biophysics programs, integrate them within a cohesive research program, and develop strong collaborations with experts in both mathematics and biology communities. As an assistant professor at UNC, I am committed to training graduate students and postdoctoral researchers in a timely manner within a supportive and inclusive research environment. This includes mentoring in both experimental design, research methodology, and critical interpretation of experimental data. As part of my joint appointment in the Biomedical Engineering and Pharmacology departments, I have completed the Faculty

Mentor Training Series through the Office of Graduate Education at UNC. I also teach a semester-long class, "Super Resolution – Imaging Beyond Limits (BME 490/890)" that discusses ways to increase resolution in various biomedical imaging modalities as well as give various lectures in the Pharmacology and Cell Biology departments. Beyond normal mentoring and committee duties, I am also developing new interactive lesson plans that utilize our 4D microscopy datasets to teach undergraduate biology at UNC (BIOL 205) and starting in 2025, I will became the new course director for the Optical Microscopy and Imaging in Biomedical Sciences (OMIBS) at the Marine Biology Laboratory in Woods Hole, MA. In summary, I provide a unique training environment that bridges both engineering and biology disciplines and ensures that students are well positioned to transition to careers in today's interdisciplinary biomedical workforce. Recent alumni from my group have gone on to graduate, post-doctoral, and assistant professor as well as industry positions.

Important research publications from my work:

1) Shi Y, Tabet JS, Milkie DE, Daugird TA, Yang CQ, Ritter AT, Giovannucci A, **Legant WR**. Smart lattice light-sheet microscopy for imaging rare and complex cellular events. Nat Methods. 2024 Jan 2;. doi: 10.1038/s41592-023-02126-0. [Epub ahead of print] PubMed PMID: 38167656. *Featured on the cover.*

2) Rahman F, Augoustides V, Tyler E, Daugird TA, Arthur C, **Legant WR**. Mapping the nuclear landscape with multiplexed super-resolution fluorescence microscopy. bioRxiv [Preprint]. 2024 Jul 29:2024.07.27.605159. doi: 10.1101/2024.07.27.605159. PMID: 39211261; PMCID: PMC11360932. *In Revision at Nature Communications.*

3) Daugird TA, Shi Y, Holland KL, Rostamian H, Liu Z, Lavis LD, Rodriguez J, Strahl BD, **Legant WR.** Correlative single molecule lattice light sheet imaging reveals the dynamic relationship between nucleosomes and the local chromatin environment. Nat Commun. 2024 May 16;15(1):4178. doi: 10.1038/s41467-024-48562-0. PMID: 38755200; PMCID: PMC11099156.

4) Chen BC*, **Legant WR*,** Wang K*, Shao L, Milkie DE, Davidson MW, Janetopoulos C, Wu XS, Hammer JA 3rd, Liu Z, English BP, Mimori-Kiyosue Y, Romero DP, Ritter AT, Lippincott-Schwartz J, Fritz-Laylin L, Mullins RD, Mitchell DM, Bembenek JN, Reymann AC, Böhme R, Grill SW, Wang JT, Seydoux G, Tulu US, Kiehart DP, Betzig E. Lattice light-sheet microscopy: imaging molecules to embryos at high spatiotemporal resolution. Science. 2014 Oct 24;346(6208):1257998. doi: 10.1126/science.1257998. Epub 2014 Oct 23. PMID: 25342811; PMCID: PMC4336192., *equal contribution. *Featured on the cover.*

Ongoing and recently completed projects that I would like to highlight include:
DP2-GM136653
Legant (PI)
09/30/2019 – 05/31/2024
Connecting the Dots Between Single Molecule Dynamics and Cell Differentiation

Beckman Young Investigator Award
Legant (PI)
09/01/2019 – 08/31/2024
Intelligent Microscopes to Observe and Interact with Dynamic Biological Specimens

Searle Scholars Program
Legant (PI)
07/01/2019 – 06/30/2023
Single molecule dynamics of differentiation

Packard Fellowship in Science and Engineering
Legant (PI)
10/15/2019 – 10/14/2024
AI-enhanced microscopy

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2018-	Assistant Professor, Departments of Biomedical Engineering and Pharmacology, University of North Carolina, Chapel Hill
2015-2017	Research Scientist, HHMI Janelia Research Campus, Ashburn, VA
2012-2015	Postdoctoral Associate, HHMI Janelia Research Campus, Ashburn, VA
2011-2012	Whitaker International Fellow, Dept. of Biologically Oriented Materials, ETH, Zurich, Switzerland
2006-2012	Graduate Student, Dept. of Bioengineering, University of Pennsylvania, Philadelphia, PA
2005-2006	Research Scientist, InVivo Sciences LLC, St. Louis, MO

Other Experience and Professional Memberships

2019 -	Instructor for "Optical Microscopy and Imaging in the Biomedical Sciences" at the Marine Biology Laboratory, Woods Hole, MA
2017	Teaching Assistant for Physiology Course at the Marine Biology Laboratory, Woods Hole, MA
2016	Organizer, Lattice light sheet microscopy workshop, Janelia Research Campus
2010	Co-chair, Gordan-Kenan Research Seminar, Signal Transduction by Engineered ECMs

Honors

2021	Joint Dept of BME at UNC/NCSU - Teaching and Mentorship award
2019	David and Lucile Packard Foundation – Packard Fellow
2019	National Institute of Health – New Innovator Award
2019	Searle Scholars Program - Searle Scholar Award
2019	Arnold and Mabel Beckman Foundation - Beckman Young Investigator Award
2015	Newcomb Cleveland Prize for most outstanding research article in the journal Science
2011-2012	Whitaker International Fellowship
2006-2007	Graduate Assistance in Areas of National Need Fellowship
2007-2010	National Science Foundation Graduate Research Fellowship
2006	Valedictorian and Graduation Speaker (Washington University in St. Louis)

C. Contributions to Science

1. **Lattice light sheet and super-resolution microscopy:** I have pioneered new techniques in super-resolution and light sheet microscopy. With a small team of researchers at Janelia Research Campus, we developed the lattice light sheet microscope which increases speed and reduces phototoxicity nearly 10-fold 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. In my group at UNC, we have developed "smart lattice light sheet microscopy" (a). This is a multifunctional instrument that incorporates widefield and multiphoton imaging together with AI-based instrument control algorithms to autonomously search for and image complex samples. Additionally, we developed and demonstrated light sheet compatible microfluidics (b), and rigorously characterized the performance of different types of light sheets to image different biological specimens (c). I have also worked on collaborative projects to develop computational analysis for FRET microscopy and to apply this instrument to address fundamental problems in cell biology and gene transcription. This work has been awarded several US patents (on which I am a co-inventor), has been commercially licensed, and has been rapidly adopted by other groups throughout the world. To support these efforts, I have organized build workshops, videos of which are freely available online, and I manage an online user forum (with over 100 members) where people share troubleshooting and build tips. As part of this proposal, I will continue to build these resources by freely sharing developments for our new microscopes and image analysis tools. This work is significant because it describes the theory, development and implementation of a new microscope that greatly expands our ability to study cells in 3D settings.

a. Shi Y, Tabet JS, Milkie DE, Daugird TA, Yang CQ, Ritter AT, Giovannucci A, **Legant WR**. Smart lattice light-sheet microscopy for imaging rare and complex cellular events. Nat Methods. 2024 Jan 2;. doi: 10.1038/s41592-023-02126-0. [Epub ahead of print] PubMed PMID: 38167656. Featured on the cover.

b. Shi Y, Daugird TA, **Legant WR**. A quantitative analysis of various patterns applied in lattice light sheet microscopy. Nat Commun. 2022 Aug 8;13(1):4607. doi: 10.1038/s41467-022-32341-w. PMID: 35941165; PMCID: PMC9360440.

c. Moore RP, O'Shaughnessy EC, Shi Y, Nogueira AT, Heath KM, Hahn KM, **Legant WR**. A multi-functional microfluidic device compatible with widefield and light sheet microscopy. Lab Chip. 2021 Dec 21;22(1):136-147. doi: 10.1039/d1lc00600b. PMID: 34859808; PMCID: PMC9022779.

d. O'Shaughnessy EC, Stone OJ, LaFosse PK, Azoitei ML, Tsygankov D, Heddleston JM, **Legant WR**, Wittchen ES, Burridge K, Elston TC, Betzig E, Chew TL, Adalsteinsson D, Hahn KM. Software for lattice light-sheet imaging of FRET biosensors, illustrated with a new Rap1 biosensor. J Cell Biol. 2019 Sep 2;218(9):3153-3160. doi: 10.1083/jcb.201903019. Epub 2019 Aug 23. PMID: 31444239; PMCID: PMC6719445.

2. **Imaging of transcription and chromatin biology:** Single molecule imaging has revealed that the majority of protein-protein or protein-DNA interactions in the nucleus are highly transient, occurring for only a few seconds. This starkly contrasts with the earlier picture of the stable and ordered assembly of molecular machines like the pre-initiation complex. Working together with Bob Tjian's group at Berkeley and Zhe Liu's group at Janelia Research Campus, we applied lattice light sheet microscopy to demonstrate a hierarchical binding mechanism for enhancer-binding pluripotency regulators Sox2 and Oct4, namely that Sox2 is kinetically favored to bind first and then recruit Oct4 to target sites on the genome. This work also revealed that non-sequence-specific chromatin binding is a major regulator for how a transcription factor finds its target (a). However, due to technical constraints, most single molecule imaging approaches lack correlative information about the local microenvironments in cells in which the molecules move. To overcome these constraints, we have independently developed correlative single-molecule lattice light sheet microscopy (b). We used this approach to measure how nucleosomes and different nuclear proteins move relative to variations in chromatin density. This work solidified an emerging hypothesis that transcription actually stabilizes nucleosome motion and added to it by demonstrating that this effect only happens in low-density chromatin regions in the nucleus. We envision that this will be a broadly useful tool to better understand how the local microenvironment and different epigenetic modifications regulate nuclear protein search and function. Additionally, we collaborated with the Liu lab to develop a new technique (ATAC-PALM) (c) to spatially map transposase-accessible regions in the nucleus and with Greg Wang's lab at UNC to demonstrate that oncogenic chimeric fusion proteins drive malignant transformation by forming phase separated condensates at target genomic loci (d).

a. Chen J, Zhang Z, Li L, Chen BC, Revyakin A, Hajj B, **Legant W**, Dahan M, Lionnet T, Betzig E, Tjian R, Liu Z. Single-molecule dynamics of enhanceosome assembly in embryonic stem cells. Cell. 2014 Mar 13;156(6):1274-1285. doi: 10.1016/j.cell.2014.01.062. PMID: 24630727; PMCID: PMC4040518.

b. Daugird TA, Shi Y, Holland KL, Rostamian H, Liu Z, Lavis LD, Rodriguez J, Strahl BD, **Legant WR.** Correlative single molecule lattice light sheet imaging reveals the dynamic relationship between nucleosomes and the local chromatin environment. Nat Commun. 2024 May 16;15(1):4178. doi: 10.1038/s41467-024-48562-0. PMID: 38755200; PMCID: PMC11099156.

c. Xie L, Dong P, Chen X, Hsieh TS, Banala S, De Marzio M, English BP, Qi Y, Jung SK, Kieffer-Kwon KR, **Legant WR**, Hansen AS, Schulmann A, Casellas R, Zhang B, Betzig E, Lavis LD, Chang HY, Tjian R, Liu Z. 3D ATAC-PALM: super-resolution imaging of the accessible genome. Nat Methods. 2020 Apr;17(4):430-436. doi: 10.1038/s41592-020-0775-2. Epub 2020 Mar 16. PMID: 32203384; PMCID: PMC7207063.

d. Ahn JH, Davis ES, Daugird TA, Zhao S, Quiroga IY, Uryu H, Li J, Storey AJ, Tsai YH, Keeley DP, Mackintosh SG, Edmondson RD, Byrum SD, Cai L, Tackett AJ, Zheng D, **Legant WR**, Phanstiel DH, Wang GG. Phase separation drives aberrant chromatin looping and cancer development. Nature. 2021 Jul;595(7868):591-595. doi: 10.1038/s41586-021-03662-5. Epub 2021 Jun 23. PMID: 34163069; PMCID: PMC8647409.

3. **Precision measurements of 3D cellular forces.** I have combined novel biomaterials, 3D imaging tools, and mathematical modeling to perform first-of-their-kind measurements of the forces that cells use to drive their migration through a 3D matrix (a,b). 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 into 3D matrices was a long standing challenge in the field that allowed for the measurement of forces in more physiologically relevant and complex settings. In my group at UNC, we are developing new applications for traction force microscopy on mechanically

heterogeneous surfaces and in micropatterned matrices that better mimic native tissue architecture (c). We are utilizing these approaches to understand the role of mechanical forces and cytoskeletal proteins in directional cell migration (d).

a. **Legant WR**, Miller JS, Blakely BL, Cohen DM, Genin GM, Chen CS. Measurement of mechanical tractions exerted by cells in three-dimensional matrices. Nat Methods. 2010 Dec;7(12):969-71. doi: 10.1038/nmeth.1531. Epub 2010 Nov 14. PMID: 21076420; PMCID: PMC3056435.

b. **Legant WR***, Choi CK*, Miller JS, Shao L, Gao L, Betzig E, Chen CS. Multidimensional traction force microscopy reveals out-of-plane rotational moments about focal adhesions. Proc Natl Acad Sci U S A. 2013 Jan 15;110(3):881-6. doi: 10.1073/pnas.1207997110. Epub 2012 Dec 31. PMID: 23277584; PMCID: PMC3549134. *equal contribution

c. Hockenberry MA, Ulmer AJ, Rapp JL, Leibfarth FA, Bear JE, **Legant WR**. Measurement of cellular traction forces during confined migration. bioRxiv 2024.09.27.615466; doi: https://doi.org/10.1101/2024.09.27.615466

d. Hakeem RM, Subramanian BC, Hockenberry MA, King ZT, Butler MT, **Legant WR,** Bear JE. A Photopolymerized Hydrogel System with Dual Stiffness Gradients Reveals Distinct Actomyosin-Based Mechano-Responses in Fibroblast Durotaxis. ACS Nano. 2023 Jan 10;17(1):197-211. doi: 10.1021/acsnano.2c05941. Epub 2022 Dec 7. PMID: 36475639; PMCID: PMC9839609.

4. Microfabricated cell cultures. In my undergraduate and early graduate work, I generated 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 work further enabled high throughput measurements of hundreds of constructs in parallel. 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. With funding from the Whitaker International Fellowship, I worked with Viola Vogel's group at ETH Zurich to combine this system with fluorescent biosensors and determined how mechanical forces lead to changes in extracellular matrix protein conformation and signaling. In total, this contribution was significant because it was one of the first microfabricated "organ on chip" systems. This platform was awarded a US patent for drug screening using microfabricated cardiac tissue constructs, was the basis for the formation of a startup company, was rapidly adopted by other group members and outside collaborators, and has enabled research and publications in diverse areas of biology and engineering.

a. Marquez JP*, **Legant W*,** Lam V, Cayemberg A, Elson E, Wakatsuki T. High-throughput measurements of hydrogel tissue construct mechanics. Tissue Eng Part C Methods. 2009 Jun;15(2):181-90. doi: 10.1089/ten.tec.2008.0347. PubMed PMID: 19196123; PubMed Central PMCID: PMC2819830. ***equal contribution**

b. **Legant WR,** Pathak A, Yang MT, Deshpande VS, McMeeking RM, Chen CS. Microfabricated tissue gauges to measure and manipulate forces from 3D microtissues. Proc Natl Acad Sci U S A. 2009 Jun 23;106(25):10097-102. doi: 10.1073/pnas.0900174106. Epub 2009 Jun 16. PMID: 19541627; PMCID: PMC2700905.

c. **Legant WR,** Chen CS, Vogel V. Force-induced fibronectin assembly and matrix remodeling in a 3D microtissue model of tissue morphogenesis. Integr Biol (Camb). 2012 Oct;4(10):1164-74. doi: 10.1039/c2ib20059g. PMID: 22961409; PMCID: PMC3586566.

d. Chen CS, Margulies K, Boudou T, **Legant WR,** Yang MT, "In vitro microphysiological system for high throughput 3D tissue organization and biological function." U.S. Patent 9,512,396, issued December 6, 2016.

Complete List of Published Work in My Bibliography, NCBI:
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