BIOGRAPHICAL SKETCH

NAME: L. Stirling Churchman, PhD

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

POSITION TITLE: Associate Professor of Genetics, Harvard Medical School, Boston, MA

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Cornell University, Ithaca, NY	B.A.	05/1999	Physics
Stanford University, Stanford, CA	Ph.D.	01/2008	Physics
University of California, San Francisco, San Francisco, CA	Postdoctoral Fellow	07/2011	Cellular and Molecular Pharmacology

A. Personal Statement

My role in the proposed project is that of PI. Over the past six years in my first independent position at Harvard Medical School, I have lead my group through projects that seek to quantitatively visualize gene expression processes at higher levels of resolution (e.g. Mayer, di Iulio *et al. Cell,* 2015, Couvillion *et al. Nature,* 2016 and Yin, Eser *et al. Cell,* 2017).

My interest in quantitative, high resolution measurements stems from my time as a physics graduate student in the laboratory of Dr. James Spudich (Stanford University). There I developed super-resolution single-molecule fluorescence microscopy techniques to elucidate the mechanism by which myosin V and myosin VI achieve directional and processive movement along actin filaments (Okten, Churchman *et al. NSMB*, 2004 and Churchman *et al. PNAS* 2005, Mortensen*, Churchman* *et al. Nature Methods,* 2010, *equal authorship). *My quantitative background in physics provides me with the ability to lead my group in the development of novel bioinformatics approaches proposed in this grant application.*

As a postdoctoral fellow with Dr. Jonathan Weissman (UCSF/HHMI), I developed native elongating transcript sequencing (NET-seq) in budding yeast, *Saccharomyces cerevisiae* (Churchman and Weissman, *Nature,* 2011). Yeast NET-seq data revealed frequent pausing during transcription and a general nucleosomal barrier for elongating RNA polymerase. In my independent career, I lead my group in re-engineering NET-seq to human cells (Mayer, di Iulio *et al*., *Cell,* 2015). We have also investigated the coordination of mitochondrial and nuclear gene expression processes that required adapting ribosome profiling to interrogate yeast mitochondrial ribosomes (Couvillion *et al. Nature*, 2016). In order to perform an unbiased analysis of these data and to integrate them with other datasets, we have developed integrative deep learning models to extract key parameters, such as transcription start sites, from NET-seq data (Eser and Churchman, *bioRxiv*, 2016) *My extensive expertise in developing, re-engineering and optimization of functional genomic approaches strongly positions my group to perform the proposed research.*

Publications most relevant to proposed research:

1. **Churchman, L. S.**, and Weissman, J. S. (2011). Nascent transcript sequencing visualizes transcription at nucleotide resolution. *Nature,* **461**, 186-192. PMCID: PMC3880149

2. Mayer, A.*, di Iulio, J.*, Maleri, S., Eser U., Vierstra, J., Reynolds, A., Sandstrom R., Stamatoyannopoulos J.A., **Churchman, L.S.** (2015). Native elongating transcript sequencing reveals human transcriptional activity at nucleotide resolution. *Cell,* **161**, 541-554. PMCID: PMC4528962

3. Eser, U and Churchman, L.S. (2016). FIDDLE: An integrative deep learning framework for functional genomic data inference. *bioRxiv*, doi: 10.1101/081380

4. Jin, Y.*, Eser, U.*, Struhl, K.#, **Churchman, L.S.**#. (2017). The ground state and evolution of promoter region directionality. *Cell,* **170***,* 889-898.

* denotes co-first authorship;
# denotes co-corresponding authorship

B. Positions and Honors

Positions and Employment

1997-1999	Undergraduate researcher in the lab of Watt W. Webb, Department of Applied Physics, Cornell University; Imaging intracellular oxidative stress using confocal and two-photon microscopy.
2000-2002	Graduate student in the labs of Steven Chu and Daniel Herschlag, Departments of Physics and Biochemistry, Stanford University; Single-molecule FRET studies of the folding of the *Tetrahymena* ribozyme.
2002-2008	Graduate student in the lab of James Spudich, Department of Biochemistry, Stanford University; Mechanisms of myosin processivity observed by super-resolution single molecule fluorescence techniques.
2008-2011	Postdoctoral Fellow in the lab of Jonathan Weissman, Department of Cellular and Molecular Pharmacology, University of California, San Francisco; Global analysis of transcription at nucleotide resolution.
2011-2017	Assistant Professor of Genetics, Harvard Medical School, Boston, MA
2017-present	Associate Professor of Genetics, Harvard Medical School, Boston, MA
2011-present	Associate Member of the Broad Institute of Harvard and MIT

Honors and Awards

2002	Kirkpatrick Teaching Award - Stanford Department of Physics
2008-2011	Merck Postdoctoral Fellow of the Damon Runyon Cancer Research Foundation
2011	Dale F. Frey Award for Breakthrough Scientists from the Damon Runyon Cancer Research Foundation
2011	Career Award at the Scientific Interface from the Burroughs Wellcome Fund
2013	New Scholar in Aging Award from the Ellison Medical Foundation
2015	Scialog Fellow of the Research Corporation and the Gordon and Betty Moore Foundation
2016	Glenn Award for Research in Biological Mechanisms of Aging

C. Contributions to Science:

Complete list of published work:

https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/45025847/?sort=date&direction=descending
* denotes co-first authorship
# denotes co-corresponding authorship

1) Regulatory control of transcription elongation and co-transcriptional processes

To deeply investigate the regulation of transcription elongation and coupled co-transcriptional processes, we need high-resolution approaches to localize RNA polymerase genome-wide. As a postdoctoral fellow, I pioneered the development of native elongating transcript sequencing (NET-seq) that directly monitors Pol II at single nucleotide resolution across the *S. cerevisiae* genome (ref. 1). In my independent position, my group re-engineered NET-seq to enable its straightforward application to higher organisms, resulting in a transformative

research tool that is empowering new research directions into gene expression regulation and genome biology (ref. 2). By resulting in a non-perturbative measure of transcription initiation, elongation and termination, NET-seq allows for the in-depth investigation of transcriptional complexities and provides insight into the *in vivo* dynamics of Pol II that are directly comparable to *in vitro* biophysical studies. We have discovered that transcription is interrupted by pausing events at nucleosomes, sites of transcription factor occupancy and the boundaries of exons to be retained in the mature mRNA (ref. 1&2). The NET-seq approach has been applied by many researchers to shed light on the roles of transcription, chromatin and termination factors. Most recently we applied NET-seq and quantitative proteomics to identify RNA polymerase II interactomes that revealed unappreciated connections between RNA polymerase II and splicing regulation (ref. 3). In order to *extract the most information from the rich NET-seq datasets*, we have also developed innovative bioinformatics analyses using *deep neural networks to seamlessly integrate NET-seq* with other high resolution genomic data (ref. 4). Together, NET-seq exposes the topography and regulatory complexity of human gene expression.

1. **Churchman, L. S.**, and Weissman, J. S. (2011). Nascent transcript sequencing visualizes transcription at nucleotide resolution. *Nature,* **461**, 186-192. PMCID: PMC3880149

- *Research Highlight*: Schuldt A. (2011) Teasing out transcription. *Nature Reviews Genetics*. 12, 152.

2. Mayer, A.*, di Iulio, J.*, Maleri, S., Eser U., Vierstra, J., Reynolds, A., Sandstrom R., Stamatoyannopoulos J.A., **Churchman, L.S.** (2015). Native elongating transcript sequencing reveals human transcriptional activity at nucleotide resolution. *Cell,* **161**, 541-554. PMCID: PMC4528962

- *Research Highlight*: Nawy T. (2015) Catching Pol II in the act. *Nature Methods*, 12, 597.

3. Jin, Y.*, Eser, U.*, Struhl, K.#, **Churchman, L.S.#** (2017). The ground state and evolution of promoter region directionality. *Cell*, **170***,* 889-898.

4. Winter G.E.*, Mayer A.*, Buckley D.L.*, Erb M.A.*, Roderick J.E., Vittori S., Reyes J., di Iulio J., Souza A., Ott C.J., Roberts J.M., Zeid R., Scott T.G., Paulk J., Lachance K., Olson C.M., Dastjerdi S., Bauer S., Lin C.Y., Gray N.S., Kelliher M.A., **Churchman L.S.#**, Bradner J.E.# (2017) BET bromodomain proteins function as master transcription elongation factors independent of CDK9 recruitment. *Mol. Cell.*, 67, 5-18.

2) More than a sum of parts: integration of genome-wide data using deep learning

Over the past decade, many functional genomics studies to understand gene regulatory networks have analyzed the genome-wide annotation of numerous molecular events for many biological systems. However, due to expense and time constraints, many systems have not been fully analyzed by all available genomics approaches. Even large-scale consortium efforts that have mapped dozens of molecular events across dozens of cell types cannot realistically characterize all molecular events across the genomes of all cells.

To address this limitation, we are developing new computational algorithms to circumvent the need to determine each molecular event separately, and instead use data on hand to infer the status of unmapped events. We are developing deep neural networks (DNN) to predict unavailable datasets. DNN, or deep learning, is a type of machine learning that requires no *a priori* knowledge of the data being analyzed and can be applied to accommodate any type of input data. Our approach, FIDDLE (Flexible Integration of Data with Deep LEarning), builds a model in a completely unsupervised manner using available genome-wide datasets (e.g.,NET-seq, ChIP-seq, RNA-seq, etc.)[23]. The model is capable of predicting multiple unavailable genome-wide datasets, with accuracy similar to that of biological replicate data. For example, using genomic datasets from *S. cerevisiae*, we demonstrated that FIDDLE can infer Transcription Start Site sequencing (TSS-seq)[24] data from NET-seq, MNase-seq, TFIIB-ChIP-seq, RNA-seq, and DNA sequence (ref 1). We took advantage of this approach to reduce the number of TSS-seq experiments for our recent study of promoter region directionality (ref 2). Thus, FIDDLE can fill out large experimental matrices (experiment type versus condition) using a handful of existing datasets.

1. Eser, U and Churchman, L.S. (2016). FIDDLE: An integrative deep learning framework for functional genomic data inference. *bioRxiv*, doi: 10.1101/081380

2. Jin, Y.*, Eser, U.*, Struhl, K.#, **Churchman, L.S.#** (2017). The ground state and evolution of promoter region directionality. *Cell*, **170***,* 889-898*.*

3) Co-regulation of mitochondrial and nuclear gene expression programs

Oxidative phosphorylation (OXPHOS) complexes pose a unique challenge for cells because their subunits are encoded on both the nuclear and the mitochondrial genome. Genomic approaches designed to study nuclear/cytosolic and bacterial gene expression have not been broadly applied to mitochondria, so the co-regulation of OXPHOS genes remains largely unexplored. *We have developed approaches to monitor mitochondrial gene expression with the same accuracy and resolution afforded by nuclear genomic methodologies.* We used these approaches to monitor mitochondrial and nuclear gene expression in *Saccharomyces cerevisiae* during mitochondrial biogenesis, when OXPHOS complexes are synthesized. We observed that nuclear- and mitochondrial-encoded OXPHOS transcript levels do not increase concordantly. Instead, mitochondrial and cytosolic translation are rapidly, dynamically and synchronously regulated. Furthermore, cytosolic translation processes control mitochondrial translation unidirectionally. Thus, the nuclear genome coordinates mitochondrial and cytosolic translation to orchestrate the timely synthesis of OXPHOS complexes, representing an unappreciated regulatory layer shaping the mitochondrial proteome. Our whole-cell genomic profiling approach establishes a foundation for studies of global gene regulation in mitochondria.

1. Couvillion, M.T, Soto, I.C., Shipkovenska, G. and **Churchman, L.S.** (2016). Synchronized mitochondrial and cytosolic translation programs, *Nature,* **533**, 499-503. PMID: 27225121

- *News and Views*: Ott M. (2016) Choreography of protein synthesis. *Nature*. 533, 472-473.
- *Preview*: St-Pierre J and Topisirovic I. (2016) Nucleus to Mitochondria: Lost in Transcription, Found in Translation. *Dev Cell*. 37,490-492.
- *Principles of Systems Biology – No. 6*: Couvillion MT and Churchman LS. (2016) Synchronized in Translation. *Cell Systems*. 2, 356-359.

4) Molecular mechanism of myosin motor processivity

Myosin-based motility is responsible for trafficking molecular cargo across the actin cytoskeleton at the cell periphery. When I began my graduate work in the laboratory of Dr. James Spudich, the mechanism of processive myosin motors was still under debate. To directly observe myosin motility, *I developed a novel super-resolution microscopy method* that revealed that the two catalytic heads of the myosin V homodimer move alternatively along actin filaments using a 'hand-over-hand' mechanism, 36-nm at a time (ref 2). We observed a similar mechanism for myosin VI motility (ref 1). The mechanisms that power processive myosin motion have been used as models in subsequent work to determine how other cytoskeletal motors transport their cargo across the cell. Furthermore, *this mechanism has critically influenced our thinking on how multiple molecular motors function and the role of accessory factors in living cells*.

1. Okten, Z., **Churchman, L. S.**, Rock, R. S., and Spudich, J. A. (2004). Myosin VI walks hand-over-hand along actin. *Nat Struct Mol Biol* **11**, 884-887. PMID: 15286724

2. **Churchman, L. S.**, Okten, Z., Rock, R. S., Dawson, J. F., and Spudich, J. A. (2005). Single molecule high-resolution colocalization of Cy3 and Cy5 attached to macromolecules measures intramolecular distances through time. *PNAS* **102**, 1419-1423. PMCID: PMC545495

5) Super-resolution light microscopy technology development and analysis

The resolution of conventional microscopy is limited to ~250 nm by light diffraction, mathematically described by the point-spread function (PSF). In contrast, the resolution of super-resolution localization microscopy is limited by the number of collected photons, the signal-to-noise ratio and the computational analysis method. We developed two-color super-resolution microscopy, single molecule high resolution colocalization (SHREC), to co-localize two fluorescent probes to within 10 nm (ref 1; also see description of our biological application in section 2 above). In another study, we derived and verified an accurate physical model for light microscopy images of point sources, *enabling the highest precision possible by information statistics for super-resolution localization microscopy*, such as PALM and STORM (ref 2). Using wave optics applied to a dipole emitter, we derived the PSF of the light microscope and showed that it precisely describes images of isolated fluorophores. We found that Maximum Likelihood estimation using the true PSF improved the localization precision by a

factor of two, yielding the highest precision possible by information statistics (Cramér–Rao lower bound). Finally, we theoretically derived and confirmed experimentally the accurate *localization precision formula that is now the standard in the field* (ref 2). In other work, we developed ways to increase signal-to-noise using zero-mode waveguides (ref 3) and by deriving optimal models for determining distances from noisy measurements (ref 4).

1. **Churchman, L. S.**, Okten, Z., Rock, R. S., Dawson, J. F., and Spudich, J. A. (2005). Single molecule high-resolution colocalization of Cy3 and Cy5 attached to macromolecules measures intramolecular distances through time. *PNAS* **102**, 1419-1423. PMCID: PMC545495

2. Mortensen, K. I.*, **Churchman, L. S.***, Spudich, J. A., and Flyvbjerg, H. (2010). Optimized localization analysis for single-molecule tracking and super-resolution microscopy. *Nat Methods* **7**, 377-381. PMCID: PMC3127582

- *News and Views:* Larson DR. (2010) The economy of photons. *Nature Methods.* 7, 357-359.

3. Elting, M.W., Leslie, S.R., **Churchman, L.S.**, Korlach, J., McFaul, C.M., Leith, J.S., Levene, M.J., Cohen, A.E., Spudich, J.A. (2013). Single-molecule fluorescence imaging of processive myosin with enhanced background suppression using linear zero-mode waveguides (ZMWs) and convex lens induced confinement (CLIC). *Opt Express* **21(**1):1189-1202. PMCID: PMC3632498

4. **Churchman, L. S.**, Flyvbjerg, H., and Spudich, J. A. (2006). A non-Gaussian distribution quantifies distances measured with fluorescence localization techniques. *Biophys J* **90**, 668-671. PMCID: PMC1367071

D. Research Support

Ongoing Research Support

R01 HG007173 Churchman 4/1/13 – 3/31/18
NIH / NHGRI
Mechanisms of transcriptional control revealed by nascent transcript sequencing
The major goals of this project are to adapt native elongating transcript sequencing to human cells and to determine mechanisms of transcriptional elongation regulation.

R01 GM117333 Churchman 9/01/15 - 8/31/20
NIH / NIGMS
High resolution analysis of transcription-splicing coupling
The goals of this grant are to identify determinants of transcription-splicing coupling through using proteomics, sequencing and genetic screens.

R21 HG009264 Churchman 9/28/16 - 7/31/18
NIH / NHGRI
Global measurement of splicing kinetics
The goal of this grant is to develop a genome-wide tool to measure splicing kinetics at high resolution.

AG-NS-1068-13 Churchman 12/1/13 – 11/30/18
Ellison Medical Foundation – New Scholar in Aging Award
Mitochondrial gene expression landscape of S. cerevisiae during aging and life span extension
The major goal of this grant is to determine how mitochondrial gene expression is altered during yeast aging.

R01 GM123002 Churchman 7/1/17 – 6/30/21
NIH/ NIGMS
Nuclear-mitochondrial co-regulation during mitochondrial biogenesis
The goal of this grant is to determine how mitochondrial and cytosolic translation programs are synchronized.

Research Support Completed During the Last Three Years

Charles E.W. Grinnell Medical Research Award Churchman 3/3/15 – 2/28/16
Charles E.W. Grinnell Trust
High-resolution Analysis of Estrogen-Induced Transcription in Rare Human Mammary Cells using Native Elongating Transcript Sequencing
In this project we will adapt our NET-seq approach to require fewer cells in order to analyze rare cell populations.

Junior Faculty Grant Churchman 3/18/13 – 6/30/16
Giovanni Armenise-Harvard Foundation
Visualizing the regulation of mitochondrial gene expression with nucleotide resolution
The major goal of this grant is to develop methodologies to follow yeast mitochondrial gene expression with nucleotide resolution.

1010244.01 Churchman 9/1/11 – 6/30/16
Burroughs Wellcome Fund- Career Award at the Scientific Interface
Regulation of the RNA polymerase motor mechanism in vivo
The goal of this grant is to determine the biophysical properties of RNA polymerase II that allow it to be dynamically regulated.