# 2024 Symposium on Physical Genomics Poster Abstracts

1. **Repurposing Drug Combinations for the Treatment of Brain Cancers**

*Jorin Graham, Thomas P. Wytock, Arthur N. Montanari, Zachary G. Nicolaou, Erisa J. Apantaku, Sara F. Dunne, Craig M. Horbinski, John Marko, Adilson E. Motter*

Department of Physics and Astronomy, Northwestern University, Evanston, IL, USA

**Abstract:** Due to state-of-the-art techniques for interrogating the physical structure of the genome like Hi-C, it is supposed that chromatin conformation directs gene expression by partitioning the genome into active and inactive compartments. If the relationship between chromatin conformation and gene expression can be further elucidated, it could facilitate the repurposing drug combinations by providing a scalable, data-driven framework to interpret their nonadditive impacts on gene expression. The development of such a framework requires a large corpus of training data, a reduction in the dimensionality of the feature space, and a sensible method to compare data across experiments. We develop and experimentally validate such a framework for transcriptional data, applying it to predict anti-cancer drug combinations for brain cancers. The predictions are validated by screening 180 candidate drug combinations that can steer cells to a less cancerous state. The identification of nonadditive interactions at the transcriptional level that materially influence phenotype facilitates the search for corresponding signatures in the physical genome.

2. **Histone H3.1 is a Chromatin-Embedded Redox Sensor Triggered by Tumor Cells Developing Adaptive Phenotypic Plasticity and Multidrug Resistance**

*Flavio R. Palma[1], Diego R. Coelho[1], Kirthi Pulakanti[2,3], Marcelo J. Sakiyama[1], Yunping Huang[1], Fernando T. Ogata[1], Jeanne M. Danes[1], Alison Meyer[2,3], Cristina M. Furdui[4], Douglas R. Spitz[5], Ana P. Gomes[6], Benjamin N. Gantner[3], Sridhar Rao[2,3], Vadim Backman[7], Marcelo G. Bonini[1]*, [1]Northwestern University, Feinberg School of Medicine, Chicago, IL, USA, [2]Blood Center of Wisconsin, Milwaukee, WI, USA; [3]Department of Medicine, Medical College of Wisconsin, Milwaukee, Wi, USA; [4]Wake Forest University School of Medicine, Winston-Salem, NC, USA; [5]Carver College of Medicine, University of Iowa, Iowa City, IA, USA; [6]Moffitt Cancer Center, Tampa, FL, USA and Coimbra University, Coimbra, Portugal; [7]Biomedical Engineering Department, Northwestern University, Evanston, IL, USA

**Abstract:** Chromatin structure is regulated through posttranslational modifications of histone variants that modulate transcription. Although highly homologous, histone variants display unique amino acid sequences associated with specific functions. Abnormal incorporation of histone variants contributes to cancer initiation, therapy resistance, and metastasis. This study reports that, among its biologic functions, histone H3.1 serves as a chromatin redox sensor that is engaged by mitochondrial $H_2O_2$. In breast cancer cells, the oxidation of H3.1Cys96 promotes its eviction and replacement by H3.3 in specific promoters. We also report that this process facilitates the opening of silenced chromatin domains and transcriptional activation of epithelial-to-mesenchymal genes associated with cell plasticity. Scavenging nuclear $H_2O_2$ or amino acid substitution of H3.1(C96S) suppresses plasticity, restores sensitivity to chemotherapy, and induces remission of metastatic lesions. Hence, it appears that increased levels of $H_2O_2$ produced by mitochondria of breast cancer cells directly promote redox-regulated H3.1-dependent chromatin remodeling involved in chemoresistance and metastasis.

3. **Disruption of the lncRNA CHASERR Regulates Chromatin Dynamics by Altering Gene Expression within its TAD and Upregulating Expression of Chromatin Remodeler CHD2**

*Esther Yoon, Vijay Ganesh, Anne O-Donnell, Gemma M. Carvill*
Department of Neurology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

**Abstract:** *CHD2* is a chromatin remodeler critical for chromatin organization and developmentally regulated gene expression. Pathogenic loss of function *CHD2* variants cause treatment-resistant epilepsy. Upstream of the *CHD2* gene is a conserved lncRNA *CHASERR*. We recently identified *de novo CHASERR* deletions in individuals with epilepsy, who overexpress *CHD2* and have a more severe clinical presentation. When both *Chd2* and *Chaserr* are disrupted on the same allele in mice, the phenotypic consequence of *Chaserr* deletion is rescued, demonstrating *Chaserr*'s direct role in increased *Chd2* levels. To investigate the mechanism of *CHASERR* disruption on *CHD2* regulation and global chromatin dynamics, we used patient-derived *CHASERR*$^{+/-}$ induced pluripotent stem cells (iPSCs) and genome-edited diploid HAP1 *CHASERR* deletion and inversion lines as our model. Like the patients' deletions, the genome-edited HAP1 deletion lines removed the 5' UTR region and the first three exons of *CHASERR*. The same region was deleted for one allele and inverted in another for the inversion line, resulting in the expression of the first three *CHASERR* exons, but not the last two.

*CHASERR* deletions increased CHD2 expression at the RNA and protein level in both the HAP1 and patient iPSC lines. Similar to the observations in mice, *CHASERR* acts as a negative regulator of *CHD2* in *cis*. Furthermore, *CHASERR* inversion not only increased *CHD2* expression, but genes upstream within the topologically associated domain (TAD), suggesting that different parts of *CHASERR* have different regulatory roles. Specifically, given that *CHASERR* is concentrated in the transcriptional locus and acts as a repressive regulator of *CHD2* expression, our data further suggests that the last two exons of *CHASERR* may be important in recruiting repressive chromatin remodeling complexes to its TAD. We will conduct RAP-MS to determine which proteins *CHASERR* interacts with to regulate *CHD2* and its TAD.

To determine the downstream effects of such disruption on transcription, we performed differential gene expression analysis. Pathways related to nucleosome assembly, nucleosome organization, and structural constituent of chromatin were differentially upregulated with *CHASERR* deletion compared to wildtype, which may be the result of increased CHD2 chromatin remodeler activity. Additionally, gene ontology analysis of differentially expressed genes that overlapped between the inversion and deletion lines were performed. GO terms such as nucleus, cell migration, adhesion, and regulation of actin cytoskeleton organization were generated with the overlapping downregulated genes (n=28). Overlapping upregulated genes (n=169) were involved in pathways such as positive regulation of gene expression and negative regulation of cell proliferation. Future studies will involve epigenomic approaches (CUT&RUN) to elucidate which changes in global gene expression are directly due to increased CHD2 protein abundance via *CHASERR* deletion.

4. **Exploring the Role of Formamide-Free Gene Labeling Methods in Cancer Research**

*Tiffany Kuo[1], Anne R. Shim[2], Jane Frederick[1], Emily M. Pujadas Liwag[1], I Chae Ye[1], Joshua A. Pritchard[1], Cody L. Dunton[1], Paola C. Gonzalez[1], Nicolas Acosta[1], Surbhi Jain[3], Nicholas M. Anthony[1], Luay M. Almassalha[4], Igal Szleifer[1], Vadim Backman[1]*

[1]Biomedical Engineering Department, Northwestern University, Evanston, IL, USA; [2]Paul Scherer Institut, Villigen PSI, Switzerland; [3]Sana Biotechnology Inc., San Francisco, CA, USA; [4]Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

**Abstract:** Changes in the three-dimensional organization of chromatin play a major role in cancer and other disease states. Therefore, it is crucial to utilize techniques that label gene loci to study higher-order chromatin structure and how it relates to global transcriptional processes. Three-dimensional fluorescence *in situ* hybridization (3D FISH) is the most common method of labeling gene loci. However, the protocol requires formamide to denature DNA, which allows a fluorescent probe to bind to the single-stranded DNA. Our work has shown that using formamide in the 3D FISH protocol causes significant changes to nanoscale chromatin structure. The primary goal is to label genes without changing the overall chromatin architecture in the nucleus. Therefore, we explored other formamide-free DNA labeling protocols to determine if they impacted chromatin organization. We selected resolution after single-strand exonuclease resection (RASER)-FISH as it uses exonuclease digestion to form single-stranded DNA that allows probes to bind. We also chose clustered regularly interspaced short palindromic repeats (CRISPR)-Sirius as it allows for visualization of gene loci cells. We found that these two formamide-free labeling methods had minimal impact on nanoscale chromatin organization compared to 3D FISH. This suggests that we should consider moving towards alternative DNA labeling techniques that minimize or remove formamide when studying the functional consequences of chromatin domains in the context of cancer.

5. **Increasing the Acclimatization Potential of Reef-Building Corals to Warmer Oceans by Remodeling Gene Transcription - A Framework**

*Kayla Shen, Andy Curran, Shanna Davidson, Maeve Brady, George Warfel, Wing Shun Li, Karla I. Medina, Cody L. Dunton, Emily M. Pujadas, Reiner Bleher, Kate Virsik, Joel K. Kim, Joshua A. Pritchard, Natalie G. Schlepp, Tiffany Kuo, Jianning Yang, Anna Simons, Vadim Backman, Luisa A. Marcelino*

Department of Civil and Environmental Engineering, Northwestern University, Evanston, IL, USA

**Abstract:** Corals are symbiotic associations of animals (coral) and photosynthetic unicellular algae (Symbiodiniaceae), which provide the host with most of their nutrition. However, heat stress impairs the photosynthetic centers of the algae, leading instead to the production of toxic radicals of oxygen and the breakdown of the partnership (coral bleaching). Increasing climate change-induced ocean warming has caused coral bleaching and mortality worldwide, resulting in the collapse of many reef ecosystems, loss of ecosystem services (coastal protection), and loss of livelihoods for people who depend on them. While many corals are dying, some others resist bleaching or quickly recover. Thermotolerance is a combination of genetic ("thermotolerant genes") and epigenetic (changes in gene expression that are unrelated to changes in DNA sequence) effects. Transcriptomic plasticity allows corals to survive heat stress by quickly achieving optimal physiological states and is regulated by changes in chromatin structure and chromatin marks. Here, we combine modeling, technology development, and experimental testing to determine changes in chromatin structure that foster optimal gene expression and relate those changes with transcriptome profiles that result in coral survival to stress. The ability to regulate transcriptional plasticity via chromatin regulation in corals may allow us to choose epigenetically resilient corals that quickly acclimate to more frequent heat stress episodes. This strategy could be combined with "assisted evolution" to propagate the most genetic and epigenetic robust colonies and

build resilient coral ecosystems. Ultimately, we are "buying time" for species to adapt to a warming ocean until an effective climate mitigation plan is implemented.

6. **Tandem Peak Analysis of Chromatin Domains as a Predictor for Gene Expression**

*Delan Hao, Benjamin Brenner, Elizabeth Bartom, Daniela Matei, Hao Zhang*
Biomedical Engineering Department, Northwestern University, Evanston, IL, USA

**Abstract:** Many technologies, such as RNA-seq and Chip-seq, exist today and allow for the collection of genome-wide data in cells. Furthermore, with the importance of epigenomic events on transcriptomic expression having been widely explored, the need for the integration of CHIP-seq and RNA-seq data is more important than ever. Grouping CHIP-seq peaks of chromatin modification into clusters defined by domain regions can tell us the relative concentration of histone modifications in a genomic region. Using this clustering analysis on h3k27ac, a modification associated with transcriptionally active euchromatin regions, and comparing it to clusters of h3k27me3, a modification associated with transcriptionally silent heterochromatin regions, we can predict the resultant changes in gene expression due to entinostat treatment in OVCAR5 cancer cells, which we then verified using RNA-seq. This analysis provides a novel method for predicting gene expression using CHIP-seq data and will be vital to elucidating the behaviors of cancer treatments, such as entinostat and GSK inhibitors, on gene expression.

7. **Multiplex Profiling Gene Functions Through Barcoded, Inducible Degron Technology**

*Ozlem Neyisci, De Xing, Tao Bai, Seyedeh Zahra Paylakhi, Alexandra Griffiths, Hengqiang Zhao, Praeploy Pongpamorn, Paul Burridge, Feng Yue, Mazhar Adli*
Robert H Lurie Comprehensive Cancer Center, and the Department of ObGyn, Northwestern University, Feinberg School of Medicine, Chicago, IL, USA

**Abstract:** Reversible Auxin inducible degron (AID) technology can achieve rapid deletion of the target protein and generate conditional null alleles of target essential human genes. Our primary goal is creating null phenotypes of 250 human genes including 200 essential genes and 50 non-essential genes in iPSC. Characterization of these null alleles in iPSC and three differentiation lineages using bulk RNA-seq and ATAC a seq is labor-intensive, time-consuming, and cost-ineffective. Although the advent of single-cell multi-omics technology has made it possible to obtain a snapshot of the transcriptome and chromatin accessibility of thousands of single cells, it is still challenging to track all different null alleles phonotypes over time and space with high throughout way. Therefore, we performed gene-specific "barcoded" degrons knock-in, allowing hundreds and thousands of null-allele cell lines to be pooled, co-cultured and differentiated. Such a resource will be a powerful screening strategy to study various cellular phenotypes not limited to only survival fitness but also other phenotypes such as cell shape, motility, and expression patterns of multiple gene markers. Additionally, such barcoded cell libraries can be combined with various single-cell genomics strategies to track the fate and contribution of null alleles to various cellular states.

8. **Deep Learning Modeling of RNA Translation Initiation in Human Cells and Diseases**

*John Carinato, Emily K Stroup, Vadim Backman, Zhe Ji*
Biomedical Engineering Department, Northwestern University, Evanston, IL, USA

**Abstract:** The dysregulation of gene expression is the cause of many human diseases including cancers. Specifically, RNA translation initiation plays a large role in determining the translational efficiency of a gene. Mutations in the 5' untranslated regions of gene transcripts have been shown to affect translation initiation and drive specific disease states. However, the genomics field is still missing a systematic method to analyze translation initiation. Modeling molecular biological processes from a genetic

standpoint has been difficult due to the wide range of impactful motifs in the human genome. Neural networks have recently been able to overcome this problem in genomics research by leveraging genetic feature extraction. The flexibility of deep learning models gives this method the capability to identify all cis-regulatory elements involved in controlling the process of translation initiation. Here, we built a deep learning model with advanced architectural units that has produced highly accurate predictions of translation initiation sites across the genome. Deep learning has afforded us the ability to quantify the impact an AUG codon, the canonical start codon, has on translation initiation compared to one of its single nucleotide variants. Determining where translation initiation occurs in the genome will allow for a comprehensive translation initiation landscape, and thus gene expression profile, to be understood in human diseases such as cancers. This approach synthesizes research being done in computational genomics, molecular biology, and genetics as we try to better understand fundamental RNA biology, and the effects of its dysregulation on human disease.

9. **Resolving Variants of Uncertain Significance in CHD2 at Scale with High-Throughput Epigenetic and Transcriptomic Profiling of Edited Cell Lines**

*Nico Bodkin, Jeffrey Calhoun, Gemma M Carvill*
Department of Neurology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

**Abstract:** Childhood epilepsies often have a genetic basis, with over 100 genes having been implicated. However, determining the pathogenicity of variants within these genes remains a challenge. Classifying variants as either disease-causing (pathogenic) or not disease-causing (benign) improves rates of diagnosis and often patient outcomes. Variants in some epilepsy-related genes cause global epigenetic changes that can be measured through DNA methylation with modern genomic technologies. Because these changes have been well-characterized, “epi-signatures” have been designed to distinguish between pathogenic and benign variants in the clinical setting. However, this method is limited by the number of clinical samples with inconclusive genetic testing that can be obtained. To overcome this limitation, epi-signatures can be leveraged to classify thousands of variants *in vitro* with recent advances in single-cell sequencing methods. Variant resolution at this scale would also provide the ability to better understand protein function.

Methods: We will leverage known epigenetic differences between pathogenic and benign *CHD2* variants for *in vitro* screening. We will first use prime editing to create isogenic HAP1 cell lines with known pathogenic and benign variants. We will utilize Illumina EPIC methylation arrays with these positive/negative control variants to establish a HAP1-specific epi-signature for *CHD2* pathogenicity. We will identify 200 differentially methylated loci that we will use to develop a high-throughput assay for *CHD2* variant resolution.

Next, we will construct a comprehensive HAP1 variant library harboring all known possible *CHD2* variants. Each pegRNA will be integrated into the genome with a unique barcode that will allow for multi-modal profiling of cells. Using the Mission Bio Tapestri platform, each cell will be targeted for relevant epigenetic loci and the *CHD2* gene, and digested with the methylation-sensitive enzyme HhaI. Targeted DNA will be amplified and sequenced at the single-cell level with a standard short-read protocol. A similar workflow will be applied using the 10x Chromium instrument to identify transcriptomic changes in

individual cells. Following demultiplexing and variant calling, we will analyze the methylation and transcriptomic profile of each cell and create an associated pathogenicity score for its introduced variant. This score will be based on a signature that is trained with data from the known pathogenic and benign variants in the experiment.

Preliminary Data: In preliminary experiments, we have observed global transcriptomic and epigenetic changes in HAP1 *CHD2* knockout and *CHD2* patient-specific cells. Additionally, proteomic data revealed significant changes in Nestin protein abundance based on variant pathogenicity. With significant changes in the transcriptomic, epigenome, and proteome, we expect to successfully develop and implement a multimodal machine learning signature for variant classification.

Conclusion: This project proposes a novel method for *CHD2* variant classification using epigenetic and transcriptomic profiling of engineered HAP1 cells. This approach holds promise to improve genetic diagnoses and enable future targeted therapies in childhood epilepsies.

### 10. The Impact of Charge Regulation and Ionic Intranuclear Environment on Chromatin Charge and Structure

*Rikkert J. Nap[1], Paola Carrillo Gonzalez[1], Aria Coraor[2], Ranya K. A. Virk[3], Marcelo A. Carignano[1], Juan J. de Pablo[2], Vadim Backman[1], Igal Szleifer[1,4]*

[1]Biomedical Engineering Department, Chemistry of Life Processes Institute Northwestern University, Evanston, IL, USA, [2]Pritzker School of Engineering University of Chicago, Chicago, IL, USA, [3]Department of Pharmaceutical Chemistry, University of California, San Francisco, CA, USA, [4]Department of Chemistry, Northwestern University, Evanston, IL, 60208, USA

**Abstract:** Chromatin consists of DNA polyacid and histone proteins. DNA carries a negative charge due to the phosphate in its sugar-phosphate backbone, while the chargeable amino acids of the histone proteins imbue the core protein with a net positive charge. However, the amino acid residues of histones only partially neutralize the highly negative charge of DNA, producing an overall net negative charge on chromatin. The structural organization of chromatin within the nucleus while managing electrostatic repulsions is not fully understood. Intranuclear environment factors, including ions, pH, and crowding density, including that of chromatin and macromolecules, are expected to regulate overall structure and charge. Experiments have demonstrated that the electrolyte environment influences chromatin at differing length scales of organization, producing differing transcriptional and phenotypic outcomes and even cell death. However, experiments are unable to concomitantly measure all relevant properties of the system to identify the principal mechanisms of charging and packing. Computational approaches, ranging from all-atomistic to coarse-grained simulations, have helped to mechanistically uncover the effects of ions on chromatin, but assume invariably fixed charges and overlook chemical equilibrium. Here, we employ a Molecular Theory (MT) approach to investigate how the intranuclear environment influences the structure and charge by explicitly considering the size, shape, conformations, charges, and chemical states of all molecular species, linking the structural state with the chemical or charged state of the system. Using MT, we investigate how the packing and charge of small nucleosome arrays change in response to changes in monovalent and divalent magnesium concentrations.

### 11. Nuclear Multi-Color Single Molecule Localization Microscopy Reveals Coupling of Constitutive Heterochromatin, Euchromatin and Active RNA

*Nicolas Acosta[1], Ruyi Gong[1], Yuanzhe Su[1], Jane Frederick[1], Luay Almassalha[2], Vadim Backman[1]*

[1]Biomedical Engineering and [2]Applied Physics Departments, Northwestern University, Evanston, IL, USA; [2]Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

**Abstract:** Super-resolution microscopy has transformed our ability to visualize structures beyond the diffraction limit of traditional optical microscopy. By circumventing diffraction limitations, techniques such as STORM, STED, and SIM enable the exploration of biological samples at unprecedented spatial resolutions, revealing intricate details with sub-200 nm precision. This enhanced capability is particularly useful for investigating complex biological targets like chromatin, which exhibits a hierarchical organization with structural compartments and domains at various length scales. Single molecule localization microscopy (SMLM) methods, such as STORM, are particularly valuable for studying chromatin at the supra-nucleosomal level due to their ability to target molecular labels marking epigenetic features that define chromatin organization. Multi-label imaging of chromatin is essential to unravel its structural complexity. However, such efforts are challenging due to the dense nuclear environment, which affects the binding affinities, diffusibility, and non-specific interactions of antibodies. Achieving accurate multi-label imaging requires careful optimization of buffer conditions, fluorophore stability, and antibody specificity to ensure proper antibody conjugation. This meticulous optimization is crucial for successfully visualizing the intricate details of chromatin structure and its regulatory mechanisms.

### 12. Artificial Intelligence-driven Automatic Nuclei Segmentation of Label-free Partial Wave Spectroscopic Microscopy Images for Cancer Research

*Md. Shahin Alom[1], Nicolas Acosta[2], Joshua A Pritchard[2], Natalie G Schlepp[2], Ali Daneshkhah[2], Vadim Backman[2], Sunil K Gaire[1]*,

[1]North Carolina AT&T State University, NC, USA; [2] Biomedical Engineering Department, Northwestern University, Evanston, IL, USA

**Abstract:** Accurate nucleus segmentation in Partial Wave Spectroscopic (PWS) microscopy images is crucial for chromatin analysis, but manual method is often biased and inefficient, leading to inaccurate nuclear outlines and segmentation errors. In this study, we introduce an AI-driven method employing an attention-based U-Net model to accurately segment nuclei in label-free PWS microscopy images of live HCT116 cell lines. Our approach significantly improves accuracy with a median IoU of 0.79 and an F-1 score of 0.88, greatly benefiting nuclei analysis for cancer research and diagnosis.

### 13. Investigating Chromatin Dynamics and Transcriptional Regulation in Cancer

*Paola Carrillo-Gonzalez*, *Rikkert Nap, Igal Szleifer, Vadim Backman*

*B*iomedical Engineering Department, Northwestern University, Evanston, IL, USA

**Abstract:** Cancer is characterized by aberrant nuclear morphology, indicating disruptions in chromatin structure, a crucial component of the nucleus comprising DNA and associated proteins. The spatial arrangement of chromatin significantly impacts gene expression, thus alterations in its organization likely contribute to carcinogenesis. To elucidate the connection between chromatin structure and cancer-related gene expression, we investigate the dynamics of chromatin organization to comprehend cancer

pathogenesis. We specifically focus on the mechanisms governing the formation of ~80 nm packing domains that exhibit power law scaling and have been shown to govern transcriptional activity. We propose a computational model to explore the underlying processes driving packing domain formation. Our model assumes that long-range interactions or loops play a central role in mediating domain assembly, influenced by entropic forces and enzymatic processes. Entropic forces, encompassing loop formation and degradation as well as core crosslinking, stabilize the structure. Enzymatic processes involve RNA Polymerase-mediated supercoiling and cohesion loop extrusion, crucial for transcriptional loop formation. Our simulations highlight the indispensability of transcription in packing domain formation, as it generates the majority of loops essential for domain stabilization. Absence of transcription impedes domain formation and stabilization. Integrating these findings, our study illuminates the intricate interplay between chromatin organization, transcriptional regulation, and cancer pathogenesis, providing insights into potential therapeutic avenues targeting chromatin dynamics in cancer treatment.

### 14. Chromatinomics: Early Screening of Cancer Using Multi-Aspect Chromatin

*Andrew Chang, Sravya Prabhala, Ali Daneshkhah, Vadim Backman*
Biomedical Engineering Department, Northwestern University, Evanston, IL, USA

**Abstract:** Colonoscopy is accurate but inefficient for colorectal cancer (CRC) prevention due to the low (7%) prevalence of target lesions, advanced adenomas. We leveraged rectal biopsy samples to identify patients who harbor CRC field carcinogenesis by evaluating chromatin 3D architecture. Supranucleosomal disordered chromatin chains (~5-20 nm, ~1 kbp) fold into chromatin packing domains (~100-200 nm, ~100–1,000 kbp). In turn, the fractal-like conformation of DNA within chromatin domains and the folding of the genome into packing domains has been shown to influence multiple facets of gene transcription, including the transcriptional plasticity of cancer cells. We evaluate the packing density scaling D of the chromatin chain conformation within packing domains using csPWS and its synergistic effect with biomolecular markers from matching samples. mRNA and miRNA expression levels from RNA sequencing have been evaluated as potential biomarkers, specifically global expression patterns such as sensitivity curve showed divergence in RNA expression. The diagnostic performance using chromatin D alone was AUC = 0.84, and when combined with the biomarker extracted from divergence pattern in miRNA (AUC=0.68) increased to 0.90. Further addition of mRNA marker increased the AUC to 0.95.

### 15. Multiplexed Single-Molecule Fluorescence Measurements Enabled by Multi-Parameter Spectroscopic Detection of Nanostructured FRET Labels

*Jiachong Chu, Ayesha Ejaz, Kyle M. Lin, Madeline R. Joseph, Aria E. Coraor, D. Allan Drummond, Allison H. Squires,*
Pritzker School of Molecular Engineering, University of Chicago, Chicago, IL, USA

**Abstract:** Traditionally, single-molecule fluorescence measurements of multiple molecular species are limited to just 3-4 colors due to detector and signal-to-noise considerations. Barcoding approaches to labeling achieve excellent multiplexing, but require multiple rounds of measurement for each molecule. To capture molecular identity in one measurement, additional spectroscopic information such as fluorescence lifetime, brightness, or anisotropy enables discrimination among chemically different fluorophores with overlapping emission spectra. Here, we demonstrate that the distinct spectroscopic signatures of a series of DNA-based FRET constructs, called FRETFluors, offer a new avenue for real-time multiplexing at the single-molecule level. Through strategic positioning of donor-acceptor pairs on a DNA

scaffold, we have created many structural variations that generate distinct spectroscopic signatures from just three chemical building blocks: DNA, Cy3, and Cy5. In addition to tuning fluorescence output with FRET, we utilize the sensitivity of fluorophore photophysics to local sequence and attachment chemistry to access an even wider range of characteristic emission properties. A custom Anti-Brownian ELectrokinetic (ABEL) trap provides high-precision multi-parameter identification of FRETfluors, allowing mixtures of tens of FRETfluor labels to be identified in complex sample mixtures down to ~10 fM concentration. The shared chemical properties of FRETfluors ensure consistent performance across changing pH, salt, and different nanoscale environments. We present applications for FRETfluors targeted to various biomolecular species, including mRNAs and proteins, using standard sequence- and site-specific attachment chemistries. FRETfluors hold the potential to facilitate the multiplexing of tens or even hundreds of unique molecular targets at the single-molecule level, representing a significant advance in the capabilities of single-molecule sensing platforms for complex biological analyses.

16. **Enhancing Cancer Diagnosis Relevance: Statistical Modeling of Biomarkers from Partial Wave Spectroscopy Digital Images**

*Ali Daneshkhah[1], Emily M Pujadas Liwag[1], Andrew S Chang[1], Sravya Prabhala[1], Hariharan Subramanian[2], Hemant K Roy[3], Vadim Backman*

[1]Biomedical Engineering Department, Northwestern University,Evanston, IL, USA; [2]NanoCytomics LLC, Skokie, IL, USA; [3]Baylor College of Medicine, Houston, TX, USA

**Abstract:** Field carcinogenesis allows assessment of easily accessible sites like buccal mucosa for lung cancer and rectal mucosa for colorectal cancer, independent of lesion size or stage. Nanoscale alterations in chromatin domains (20-300 nm; sub-Mbp genomic scales) are crucial in promoting the transcriptional plasticity of (pre)cancerous cells. Chromatin-sensitive partial wave spectroscopic (csPWS) microscopy detects these alterations by analyzing scattered light interfaces caused by spatial refractive index variations, illustrating packing scaling D Image. Using an HCT116/Oxaliplatin model in temporal analysis, we examined the correlation between initial packing scaling D and cell fate. Nucleus images were segmented based on D image distribution and spatial location to evaluate the predictive value of averaging and standard deviation markers for cell fates. The cell fate was successfully detected using univariate markers in the initial D image, demonstrated by an effect size of 0.7. We developed an advanced image processing algorithm that identifies features from D images, surpassing the predictive capability of average D alone. This proposed biomarker was further evaluated in a human study, showing enhanced classification with an effect size of 1.13.

17. **Assessing the Impact of Buccal Brushing on packing Scaling D: Protocols and Methodology Using Partial Wave Microscopy**

*Ali Daneshkhah, Natalie G Schlepp, David VanDerway*

Biomedical Engineering Department, Northwestern University, Evanston, IL, USA

**Abstract:** Researchers have adopted a novel approach utilizing field carcinogenesis to evaluate buccal cells within the injury field for lung cancer assessment. Buccal brushing, a simple and accessible technique, collects cells from lung cancer patients, but its impact on cells deposited on glass slides is uncertain. This study designed protocols to assess the impact of buccal brushing on packing scaling, using HCT116/oxaliplatin cells and csPWS microscopy. Findings indicated an increase in D and nucleus

area among chemotherapy-surviving cells, with consistent drug treatment effect sizes between protocols with and without brushing (1.34 vs 2.03). These results suggest minimal influence of the buccal brushing protocol. Future experiments are recommended to validate these findings across diverse cell lines.

### 18. Cationic Regulation of Chromatin Conformation: A Novel Approach for Chromatin Engineering

*Cody L. Dunton[1], Paola C. Gonzalez[1], Wing Shun Li[2], Ruyi Gong[1], Nico Acosta[1], Rachel Ye[1], Luay Almassalha[3], Jane Frederick[1], Zois Syrgiannis[4], Matias Alvarez-Saavedra[4], Chris Hogg[4], Gareth Williams[4], Samuel Stupp[4], Igal Szleifer[1], Vadim Backman[1]*

[1] Biomedical Engineering Department and [2]Department of Applied Physics, Northwestern University, Evanston, IL, USA; [3]Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; [4]Department of Chemistry, Northwestern University, Evanston, IL, USA

**Abstract:** The intricate organization of chromatin within the nucleus is essential for regulating gene expression and cellular function. Chromatin, composed of DNA and histone proteins, exists in dynamic states that determine genetic accessibility, influencing processes such as transcriptional regulation and DNA repair. Despite recognizing the importance of chromatin dynamics, the precise mechanisms governing these processes, especially in living cells, remain elusive. This study investigates the role of nuclear ions, particularly divalent cations like Mg2+, in modulating chromatin structure through electrostatic interactions with DNA. Previous research suggests that ions are crucial for chromatin compaction, yet the exact mechanisms by which Mg2+ drives hierarchical chromatin structures from DNA self-assembly to mitotic chromosomes are not fully understood. Utilizing a multidisciplinary approach, combining advanced imaging, biochemical assays, and computational modeling, we aim to explore the role of nuclear ions in shaping chromatin conformation in living human cancer cells. This study investigates how nuclear ion concentrations affect chromatin structure and cellular function, particularly under stress. We used various methods to modulate nuclear ions within live cells, including chelators like BAPTA-AM and APDAP-AM, and ion-doped peptide amphiphiles (IDPAs), and found that chelation of divalent cations decreases chromatin packing density, whereas $Mg^{2+}$ doping increases it. Chelation effects are rapid, reducing nuclear $Mg^{2+}$ within 5 minutes, while $Mg^{2+}$ doping increases concentrations within 10 minutes and maintains elevated levels for hours. ChromSTEM imaging showed that chelation decreases chromatin domain size and compaction, whereas excess $Mg^{2+}$ can disrupt mature domains but retains some organization. STORM imaging indicated that divalent cation chelation reduces H3K9me3 staining, particularly at the nuclear periphery, while $Mg^{2+}$ doping increases it, suggesting alterations in heterochromatin structure and transcriptional activity. Functional consequences include significant downregulation in transcription upon chelation of divalent cations. Our findings provide insights into the complex interplay between nuclear ions and chromatin dynamics, with potential implications for understanding cancer biology and developing novel therapeutic strategies. This research represents a significant step towards unraveling the mechanisms of ion-mediated chromatin regulation and its effects on cellular physiology and disease pathogenesis.

### 19. Low Frequency Electromagnetic Stimulation Induced Chromatin Reprogramming for Chromatin Engineering Applications

*Cody Dunton, Cody L. Dunton[1], Nancy Rivera-Bolanos[1], Paola C. Gonzalez[1], Ruyi Gong[1], Rikkert Nap[1], Pei-An Lo[2], Ben Yi Tew[2], Dimitrios Pollalis[3], Pranav Bajaj[1], Alejandra Gonzalez[2], Gianluca Lazzi[3], Bodour Salhia[2], Igal Szleifer[1], Mark Humayun[2], Guillermo Ameer[1], Vadim Backman*

[1] Biomedical Engineering Department, Northwestern University, Evanston, IL, USA; [2]Keck School of Medicine of the University of Southern California, Los Angeles, CA, USA; [3]Roski Eye Institute, University of Southern California, Los Angeles, CA, USA

**Abstract:** Retinal degenerative diseases such as age-related macular degeneration (AMD), retinitis pigmentosa (RP), and diabetic retinopathy (DR) result from genetic and environmental factors. With modern technological and industrial advancements, the incidence of these diseases is rising due to increased exposure to stressors like blue light, digital eye strain, air pollutants, and poor diet. These stressors activate oxidative pathways, leading to retinal pigmented epithelial (RPE) cell layer disruption and increased reactive oxygen species (ROS) production, culminating in photoreceptor and RPE apoptosis, tissue necrosis, and loss of visual acuity. Current treatments, including pharmacological interventions, gene therapy, and surgical procedures, have limitations such as off-target effects, moderate success rates, and invasiveness. A promising new therapeutic approach is low-frequency electrical stimulation (LFES), which has the potential to reprogram retinal cells early in degeneration, restoring functionality and preventing further progression. The precise mechanisms by which LFES exerts its effects are not fully understood, but recent evidence suggests that it may involve alterations in chromatin conformation, impacting cellular plasticity and function. This study investigates the impact of LFES on hierarchical chromatin structures within retinal cells and examines the subsequent effects on cellular function. Using a multidisciplinary approach that integrates advanced imaging techniques, biochemical assays, and computational modeling, we treated retinal cells with varying LFES parameters and monitored changes in chromatin structure in real time. Our findings reveal that LFES can induce significant changes in chromatin organization, which in turn affects cellular processes such as ion flux, mitochondrial activity, and apoptosis. Furthermore, we explored the translational potential of LFES by evaluating its effects on retinal cells in vivo using a rat model of retinal degeneration. LFES treatment resulted in increased chromatin packing density and improved cellular organization, correlating with enhanced visual acuity. Overall, our research provides novel insights into the mechanisms of LFES-mediated chromatin remodeling and its implications for therapeutic interventions in retinal degenerative diseases. Through rigorous experimentation and interdisciplinary approaches, we aim to elucidate the role of chromatin dynamics in retinal cell plasticity and function, paving the way for new treatments to combat retinal degeneration.

### 20. Preventing Non-Genetic Adaptation to Chemotherapy Through Modulation of Chromatin Structure

*Jane Frederick[1], Ranya Virk[2], Luay Almassalha[3], Greta Wodarcyk[4], David VanDerway[1], I Chae Ye[1], Rikkert Nap[1], Vasundhara Agrawal[4], Anne Shim[5], John Carinato[1], Wing Shun Li[6], Emily Pujadas Liwag[1], Surbhi Jain[7], Shohreh Shahabi[8], Guillermo Ameer[1], Igal Szleifer[1], Vadim Backman[1]*

[1] Biomedical Engineering Department, Northwestern University, Evanston, IL, USA; [2]Pharmaceutical Chemistry Department, School of Pharmacy, UCSF, San Francisco, CA, USA; [3]Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; [4]CatalystBio, Chicago, IL, USA;

[4]Spatial Biology Image Analysis, Leica Microsystems, Boston, MA, USA; [5]Paul Scherer Institut, Villigen PSI, Switzerland; [6]Applied Physics Department, Northwestern University, Evanston, IL, USA; [7]Sana Biotechnology Inc., San Francisco, CA, USA; [8]Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

**Abstract:** Advances in cancer treatment, including precision oncology, are significantly hindered by cancer cell plasticity. The ability to adapt to various external stressors is due to two attributes of highly plastic cells: transcriptional malleability, which is the ability of a cell to upregulate survival pathways, and transcriptional heterogeneity, or the variance in the pathways utilized by cells to evade cell death. Both aspects of phenotypic plasticity have been directly linked to nuclear chromatin organization, which facilitates changes in gene expression. To study the role of chromatin in plasticity-mediated survival, we performed time-course imaging of chemotherapy-treated cancer cells using label-free Partial Wave Spectroscopic (PWS) microscopy. Our results demonstrated that the population of cells that survive chemotherapy have altered chromatin organization. We developed a computational model of cell fate based on changes in transcription due to chromatin, which could recapitulate experimental results. We predicted that pharmacologically targeting chromatin would reduce plasticity and improve chemotherapeutic efficacy. Specifically, we screened candidate transcriptional plasticity repressors (TPR) that facilitate chromatin-nucleoplasm interactions through histone tail modifications or changes in the nuclear ionic environment. Adding a TPR to chemotherapy treatment increased the probability of cell death compared to chemotherapy alone, which matched model predictions. We next tested these treatments *in vivo* in a PDX model where our model predicted that although tumors would still be able to adapt to chemotherapy treatment, the combination treatment would exhibit a higher cell growth inhibition rate and stunt tumor adaptation. Our results demonstrated that targeting chromatin structure improves outcomes even in a conservative treatment with low-dose chemotherapy.

### 21. Deep Learning-Driven Reconstruction Algorithm for Spectroscopic Single Molecule Localization Microscopy

*Sunil Gaire[1], Ruy Gong[2], Jane Frederick[2], Ethan Flowerday[3], Ali Daneshkhah[2], Vadim Backman[2],*
[1]North Carolina AT&T State University, NC, USA; [2]Biomedical Engineering Department, Northwestern University, Evanston, IL, USA; [3]Cybersecurity department, University of Tulsa, OK, USA

**Abstract:** We introduce DsSMLM, a novel deep-learning algorithm for the reconstruction of label-free and fluorescence-labeled spectroscopic single-molecule localization microscopy (sSMLM) data. Deep learning is leveraged for the precise localization of single-molecule emitters and accurate estimation of their emitter spectra. DsSMLM achieved a resolution of 6.22 nm on label-free single-stranded DNA fiber data and offered simultaneous multicolor imaging of multi-labeled nanoruler sample, distinguishing two dyes labeled in three emitting points with a separation distance of 40 nm, showing promising potential for super-resolution imaging of both label-free and labeled nanostructures.

### 22. Molecular Specificity of Nucleic Acids Via Intrinsic Stochastic Fluorescence Under Visible Light Illumination

*Ruyi Gong[1], Luay Almassalha[2], Patrick Su[1], Vadim Backman[1]*
[1]Biomedical Engineering Department, Northwestern University, Evanston, IL, USA; [2]Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

**Abstract:** We measured the emission spectra of 20-base-pair nucleotides (adenine, guanine, cytosine and thymine) and nucleotide pairs (GC, AT), as well as their photophysical parameters (blinking frequency and on-off cycles) under visible light illumination (405 nm, 488 nm, 532 nm). Using a machine learning classification algorithm can easily distinguish AT and GC double stranded DNA molecules to an accuracy of more than 90%. The spectral information and the photophysical parameters of these DNA molecules may serve as end**ogenous labels in probing DNA density, chromatin conformation and structure in the future.**

### 23. Limited-Angle ChromSTEM CT Reconstruction Using Diffusion Model

*Jiaqui Guo[1], Santiago Lopez-Tapia[1], Wing Shun Li[2], Yunnan Wu[1], Vinayak P. Dravid[2], Aggelos K. Katsaggelos[1],*
[1]Electrical and Computer Engineering, and [2]Applied Physics Departments, Northwestern University, Evanston, IL, USA

**Abstract:** Chromatin electron scanning transmission electron microscopy (ChromSTEM) is a CT-based imaging technique that provides high-resolution chromatin conformation in fixed cells. Due to experimental setting limitations, a complete range of scanning angles cannot be obtained, leading to unwanted degradation in the 3D chromatin structure reconstructed using classical CT methods. In this work, we propose Residual Null-space Diffusion Stochastic Differential Equations (RN-SDE) to refine those low-fidelity CT reconstructions. Our experiments show that RN-SDE outperforms other state-of-the-art deep-learning-based methods in ChromSTEM CT reconstruction tasks under different missing-angle scenarios.

### 24. Investigating Topologically Associating Domains Targeted by a Synthetic Reader-Actuator in H3K27me3-Enriched Chromatin

*Seong Hu Kim[1], Ranjit Pelia[1], Isioma Enwerem-Lackland[1], Natecia L., Williams[1], Christopher Plaisier[2], Karmella A. Haynes[1],*
[1]Wallace H. Coulter Department of Biomedical Engineering, Emory University and Georgia Tech, Atlanta, GA, USA; [2]School of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ, USA

**Abstract:** Epigenetic plasticity is the idea that once set, gene expression states can be altered to drive long-term changes in cell state. Tools to probe and manipulate epigenetic plasticity are primarily small molecules that bind chromatin-modifying enzymes: histone methyltransferases, deacetylases, and DNA methyltransferases. Additional tools include fusion proteins, epigenome editors, to recruit chromatin modifying enzymes directly to target genes. Neither approach directly targets transcriptional regulatory machinery that regulates the ultimate step in transcription, RNA PolII activity. To couple chromatin modifications with gene expression outputs, we developed a platform called “epigenome actuation” where customizable synthetic reader-actuators (SRAs) bind chromatin marks and regulate transcription. Our previous work has shown that one version of SRA named SRA-polycomb transcription factor (SRA-PcTF) binds histone H3K27me3 and activates some, but not all genes with promoters near H3K27me3. To fully elucidate the activation mechanism of SRA-PcTF we have developed stable MCF7 cell lines that inducibly express full-length SRA-PcTF or a truncated protein that binds H3K27me3 but lacks the activation domain (control). Time-dependent RNA-seq identified different groups of genes that responded specifically to SRA-PcTF at different time points (10, 24 and 48 hours). Early-response genes

including HOXB9 and HOXC9 were activated in a dose-dependent manner at 15 hours (RT-qPCR assay). These genes are known to have H3K27me3-enriched enhancers. To investigate regulation at a larger scale that is relevant to enhancer activity, we looked for topologically associated domains (TADs) that were enriched for SRA-activated genes using a public MCF7 Hi-C dataset. Genes such as HOXC8, HOXC9 and HOXC10 reside within a single TAD on chromosome 12. We expect our ongoing TAD mapping and ChIP-seq analyses to confirm that SRA-PcTF binds at H3K27me3 at enhancers, and to produce a model to predict activatable genes in any chromatin and TAD landscape.

### 25. ChromSTEM: Capturing Changes of Three-Dimensional Chromatin Packing Domain Structure using Electron Tomography

*Wing Shun Li[1], Emily Pujadas Liwag[2], Lucas Carter[2], Cody Dunton[2], Luay Almassalha[3], Vinayak P. Dravid[1], Vadim Backman[2]*

[1]Applied Physics and [2]Biomedical Engineering Department, Northwestern University, Evanston, IL, USA; [3]Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

**Abstract:** Chromatin structural organization determines fundamental cellular activities such as transcriptional regulations. Highly dense heterochromatin plays a role in gene silencing while the open structure of euchromatin allows a higher transcriptional rate. However, the study of 3D chromatin structure at a nanoscale level is limited by resolution of light microscopy techniques. In this work, we applied electron tomography together with ChromEM staining method to resolve the 3D chromatin structure with ultra-high resolution. Perturbations including RAD21 inhibition and calcium ion depletion were applied and changes in chromatin structures were analyzed. Our work shows that chromatin forms packing domains with sizes ~200nm with dense core and domain properties are largely affected by both short-range and long-range interaction of chromatin.

### 26. The Chromatin Gradient of Cellular Senescence

*Ioana Olan, Aled Parry, Stefan Schoenfelder, Tetsuya Handa, Masami Ando-Kuri, Masako Narita, Masashi Narita*,

Cancer Research UK Cambridge Institute, Cambridge, UK

**Abstract**: Cellular senescence exhibits striking re-organization of 3D chromatin structure as revealed by microscopy and chromatin conformation studies. This occurs at different scales: increased compartmentalization, the formation of heterochromatic foci (SAHF), and rewiring of cohesin loops and enhancer-promoter interactions. Current constructs used to characterize chromatin interactions derived from Hi-C experiments, such as A/B compartments and TADs, are insufficient for capturing the complexity of the epigenetic environments within the cell nucleus. We propose an integrative model called the 'chromatin gradient' which supports a more detailed characterization of chromatin states and their relative positioning within the chromatin network. We show that this is a useful tool for inferring crucial insights regarding chromatin rewiring during senescence and for building a universal model of chromatin organisation and its modulation during development and cancer.

### 27. Evaluating Deep Learning Features of Chromatin-Sensitive Partial Wave Spectroscopic Microscopy for Early Lung Cancer Diagnosis

*Sravya Prabhala[1], Andrew S. Chang[1], Ali Daneshkhah[1], Ankit Bharat[2], Hariharan Subramanian[1], Hemant K. Roy[3], Vadim Backman[1]*,

[1]Biomedical Engineering Department, Northwestern University, Evanston, IL, USA; [2]Canning Thoracic Institute, Department of Surgery, Northwestern University, Feinberg School of Medicine, Chicago, IL, USA

[3]Baylor College of Medicine, Houston, TX, USA

**Abstract:** This study investigates the application of Chromatin-Sensitive Partial Wave Spectroscopic Microscopy (csPWS) for early lung cancer detection using buccal mucosa cells. Utilizing deep learning, we extract intricate features from csPWS data to understand chromatin structure alterations. Our goal is to unveil the biological pathways of lung cancer and identify distinct diagnostic markers in clinical data.

### 28. The Roles of B-type Lamins & Heterochromatin in Chromatin Dynamics, Gene Regulation, & Nuclear Blebbing

*Emily M. Pujadas Liwag[1], Xiaolong Wei[2], Nicolas Acosta[1], Lucas Carter[1], Jiekun Yang[3], Luay Almassalha[1], Ali Daneshkhah[1], Suhas S.P. Rao[1], Vasundhara Agrawal[1], Fidan Seker[1], Erez Lieberman Aiden[3,4], Masato T. Kanemaki[5], Andrew Stephens[6], Mazhar Adli[1], and Vadim Backman[1]*

[1]Department of Biomedical Engineering, Northwestern University, Evanston, Illinois, 60208, USA, [2]Department of Biochemistry and Molecular Genetics, University of Virginia School of Medicine, Charlottesville, Virginia, 22903, USA, [3]Broad Institute of MIT and Harvard, Cambridge, Massachusetts, 02142, USA, [4]The Center for Genome Architecture, Baylor College of Medicine, Houston, Texas, 77030, USA, [5]Department of Genetics, The Graduate University for Advanced Studies, Mishima, Shizuoka, 411-8540, Japan, [6]Biology Department, University of Massachusetts Amherst, Amherst, MA 01003, USA

**Abstract:** B-type lamins are critical nuclear envelope proteins that interact with the three-dimensional genomic architecture. However, identifying the direct roles of B-lamins on dynamic genome organization has been challenging as their joint depletion severely impacts cell viability. To overcome this, we engineered mammalian cells to rapidly and completely degrade endogenous B-type lamins using Auxin-inducible degron technology. Using live-cell Dual Partial Wave Spectroscopic (Dual-PWS) microscopy, Stochastic Optical Reconstruction Microscopy (STORM), in situ Hi-C, and fluorescence in situ hybridization (FISH), we demonstrate that lamin B1 and lamin B2 are critical structural components of the nuclear periphery that create a repressive compartment for peripheral-associated genes. Lamin B1 and lamin B2 depletion minimally alters higher-order chromatin folding but disrupts cell morphology, significantly increases chromatin mobility, redistributes both constitutive and facultative heterochromatin, and induces differential gene expression both within and near lamin-associated domain (LAD) boundaries. Critically, we demonstrate that chromatin territories expand as upregulated genes within LADs radially shift inwards. Our results indicate that the mechanism of action of B-type lamins comes from their role in constraining chromatin motion and spatial positioning of gene-specific loci, heterochromatin, and chromatin domains. Our findings suggest that, while B-type lamin degradation does not significantly change genome topology, it has major implications for three-dimensional chromatin conformation at the single-cell level both at the lamina-associated periphery and the non-LAD-associated nuclear interior with concomitant genome-wide transcriptional changes. This raises intriguing questions about the individual and overlapping roles of lamin B1 and lamin B2 in cellular function and disease. Further, the nuclear blebs that arise from either B-type lamin or heterochromatin

disruption are linked to various pathologies, including cancer and premature aging disorders. We therefore also investigate alterations in higher-order chromatin structure within blebs, revealing fragmentation of nanoscopic heterochromatin domains.

### 29. ISOCT: 4D Spectral OCT for Tissue Imaging

*Yuanzhe (Patrick) Su[1], Aya Eid[2], Rongrong Liu[3], James Winkelmann[2], Graham Spicer[4], Luisa Marcelino[5], Vadim Backman[1]*

[1]Biomedical Engineering Department, Northwestern University, Evanston, IL, USA; [2]Presage Technologies, Oakland, CA, USA; [3]Apple, San Francisco, CA, USA; [3]Applied Physics Laboratory, The Johns Hopkins University, Laurel, MD, USA; [5]Department of Civil and Environmental Engineering, Northwestern University, Evanston, IL, USA

**Abstract:** Optical Coherence Tomography (OCT) is a label-free non-invasive 3D optical imaging technique that excels in acquiring cross-sectional images from scattering samples. This poster features an extension version of OCT named Inverse Spectroscopic OCT (ISOCT) that acquires spectral information alongside tomography. It is achieved by solving the inverse problem that associates tissue optical properties with OCT Aline signal and introducing short-time Fourier Transform to get spectral domain 4D OCT signals. The authors also describe the proof of theory via Finite Difference Time Domain (FDTD) simulation methods and an automated layer segmentation algorithm for improved image analysis workflow. Finally, the poster demonstrates the potential applications of ISOCT: angiography based purely on spectral contrast, field carcinogenesis marker by measuring tissue ultrastructure and imaging of biological tissues like corals.

### 30. Abel-Pie: Pulsed Interleaved Excitation for High- Pricision Measurements of Single Molecule Protein- Protein interactions

*Ayesha Ejaz, Kavya Vaidya, Kyle Lin, Diangen (Dana) Lin, Jiachong Chu, David A. Drummond, Allison Squires,*

Pritzker School of Molecular Engineering, University of Chicago, Chicago, IL, USA

**Abstract:** The Anti-Brownian Electrokinetic (ABEL) trap is a powerful platform for manipulating and studying single molecules in free solution, simultaneously allowing us to obtain high-precision measurements of multiple spectroscopic properties as well as transport parameters. We have now introduced pulsed-interleaved excitation (PIE) to the ABEL trap system so that trapped molecules can be excited in multiple color channels simultaneously. ABEL-PIE brings new capabilities to the ABEL trap, including the ability to dynamically control excitation patterns, and providing a built-in reference for enhanced Förster resonance energy transfer (FRET) measurements using DNA-FRET constructs, allowing us to study completely new dynamical systems. Here, we demonstrate the expanded capabilities of ABEL-PIE to record dynamic interactions of molecular chaperones with protein condensates: Pab1 is an RNA binding protein that aggregates into condensates under cellular stress conditions such as fever. As cells return to normal functionality, disaggregation of these condensates occurs via molecular chaperones including heat shock protein 70 (hsp70). Using ABEL-PIE we can study the dynamics of this system at a molecular level under varying conditions, shedding light on stability, structure and disaggregation of the condensates and chaperones while freely diffusing in solution. By understanding the molecular dynamics and interplay of condensates and chaperones in cellular stress response, we

aim to contribute to broader knowledge of phase-separated condensates and their significance in cellular physiology.

### 31. Targeting Epigenetic Plasticity in Cancer Stem Cells to Overcome Chemotherapeutic Resistance

*I Chae Ye[1], Jane Frederick[1], Yinu Wang[2], Elizabeth Bartom[2], Luay Almassalha[2], Yaqi Zhang[2], Greta Wodarcyk[3], Hao Huang[2], Paola Carrillo Gonzalez[1], Karla Medina[1], Josh Pritchard[1], John Carinato[1], Alex Duval, Zhe Ji[1], Mazhar Adli, Vadim Backman[1], Daniela Matei[2],*

[1]Biomedical Engineering Department, Northwestern University, Evanston, IL, USA; Northwestern University, Feinberg School of Medicine, Chicago, IL, USA; [3]CatalystBio, Chicago, IL, USA

**Abstract:** Non-genetic mechanisms are pivotal in the rapid development of therapeutic resistance in cancer cells, operating independently of genetic mutations. Specifically, alterations in chromatin organization and epigenetic reprogramming within cancer cells induce shifts in gene accessibility, thereby influencing transcriptional profiles associated with chemoresistance. Cancer stem cells (CSCs) exhibit enhanced adaptability to therapeutic stress, transitioning between stem-like and differentiated states through tight regulation of epigenetic processes and chromatin dynamics.

Our investigation reveals that CSCs inherently possess a chromatin conformation conducive to plasticity compared to non-CSCs. Furthermore, CSCs exhibit a distinct transcriptional signature characterized by an enrichment of stemness and stress-response genes at baseline, along with a modified gene set promoting survival post-chemotherapy. Exposure of CSCs to chemotherapy induces heightened transcriptional plasticity, manifested by increased upregulation of survival pathways and enhanced transcriptional heterogeneity compared to non-CSCs. We investigated different epigenetic modifiers and found that inhibition of DOT1L, a methyltransferase targeting H3K79me3, has the most impact in reducing the plasticity-fostering chromatin conformation. Epigenetic manipulation of CSCs using DOT1Li prevents activation of survival pathways and facilitates the transition of CSCs to a non-CSC state. Treatment with DOT1Li results in a reduction in the plasticity-fostering chromatin conformation of CSCs, accompanied by diminished transcriptional malleability, particularly within DNA repair pathways. In summary, our findings underscore the critical role of epigenetic mechanisms, specifically chromatin organization and transcriptional regulation, in mediating the adaptive responses of CSCs to therapeutic stress in cancer. Targeting these non-genetic determinants presents a promising avenue for overcoming chemoresistance and improving treatment outcomes in cancer patients.

### 32. Exploring Chromatin Structure Across Scales: Integrating Advanced Microscopy Techniques

*Geng Wang[1], Wing Shun Li[2], Yuanzhe Su[1], Ruyi Gong[1], Nicolas Acosta[1], Yue Li[1], Adam Eshei[3], Ranya Virk[4], Vasundhara Agrawal[5], Luay Almassalha[6], Vinayak P. Dravid[2], Vadim Backman[1],*

[1]Biomedical Engineering and [2]Applied Physics Departments, Northwestern University, Evanston, IL, USA; [3]Dexcom, San Diego, CA, USA; [4]Pharmaceutical Chemistry Department, School of Pharmacy, UCSF, San Francisco, CA, USA; [5]Spatial Biology Image Analysis, Leica Microsystems, Boston, MA, USA; [6]Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

**Abstract:** The three-dimensional organization of the human genome (chromatin) is critically involved in the regulation of gene expression and is highly complex. To investigate chromatin conformation, four imaging technologies were integrated to develop a new nanoscale imaging platform with molecular

resolution and cross-scale capabilities (from molecular level to the whole chromatin in hundreds of cells), including 3D chromatin scanning transmission electron microscopy (ChromSTEM), multi-label spectroscopic single-molecule localization microscopy (sSMLM), spectroscopic intrinsic-contrast photon-localization optical nanoscopy (SICLON), and partial wave spectroscopic microscopy (PWS). Label-free PWS provides the chromatin packing context, multi-label sSMLM/SICLON shows the precise location of individual molecules without the danger of artifacts from heavy labeling/ without labeling, and electron microscopy tomography allows zooming in on the finest details of DNA organization.

### 33. Effects of Reader Protein Crosstalk on Chromatin Organization Revealed by Physical Simulation

*Joseph Wakim[1], Andrew Spakowitz[1,2,3,4]*

Stanford University [1]Department of Chemical Engineering, [2]Department of Materials Science and Engineering, [3]Biophysics Program, [4]Department of Applied Physics

**Abstract:** Epigenetic factors and the resulting spatial organization of chromatin contribute to the physical regulation of gene expression, enabling diverse cellular phenotypes to be encoded by a shared genome. While dysregulation of chromatin architecture has been linked to age-related disorders, such as Alzheimer's disease and various cancers, the physical mechanisms governing chromatin folding remain underspecified. We leverage mechanistic simulation and deep learning to develop a model of chromatin organization based on the cooperative binding of epigenetic reader proteins. Using our model, we evaluate how conditions in the nuclear environment and crosstalk between epigenetic marks affect chromatin compartmentalization. We demonstrate that direct interactions between reader proteins are not required to facilitate epigenetic crosstalk. Rather, due to the shared scaffold to which reader proteins bind, chromatin condensation by one reader protein may indirectly support the binding of another. Our model suggests that the binding and unbinding of reader proteins facilitate transitions between architectural programs adopted by the chromatin fiber. By characterizing modes of epigenetic crosstalk, we demonstrate the interdependent roles of multiple marks on the spatial organization of DNA.

### 34. Relating Tenocyte Chromatin States to Native Tendon Physiology using Expansion Microscopy

Marcus Woodworth, Melike Lakadamyali

University of Pennsylvania Medical School, Philadelphia, PA, USA

**Abstract:** Tendinopathies are a widely occurring and costly clinical problem, with few strategies for tendon repair and limited knowledge on what mechanisms lead to proper tissue regeneration. One highly under-studied aspect of tendinopathies is how chromatin, the native form of genomic DNA, is organized and epigenetically marked for proper gene regulation within tenocytes, the resident tendon cells that secrete and build up the extracellular matrix (ECM). Current methods are unable visualize, at an appropriate resolution, chromatin architecture in the context of native tissue ECM. To better understand tendon physiology and pathology, it is imperative to develop new tools capable of co-visualizing ECM and tenocyte chromatin organization within healthy and diseased tissue. I will thus leverage recent advancements in tissue labeling and imaging that allow the visualization of tenocyte nanoscale chromatin organization and epigenetic states, at the whole nucleus or single gene level, within native tendon ECM.