Presenters please upload your slides here and add a link to them in the agenda.
Meeting attendance spreadsheet.
Schedule for talks (2/12 - 4/23)
Schedule for Scientific Talks (10/22 -)
Collaborative projects
CCF - Common Coordinate Reference Framework agenda/minutes

Zoom link:
https://4dnucleome-org.zoom.us/j/92967666626?pwd=L3BVeml3amtQZjBWUC8wTG1WSURrQT09

June 27, 2025

Agenda:
Zoom recording

June 13, 2025

Agenda:
Zoom recording

May 23, 2025

Agenda:
Zoom recording

May 9, 2025

Agenda:
Zoom recording

April 25, 2025 - CANCELED

April 11, 2025

Agenda:
Zoom recording

March 28, 2025 - CANCELED

Agenda:

March 14, 2025

Agenda:
Zoom recording

February 28, 2025 - CANCELED

Agenda:

February 14, 2025

Agenda: CCF updates
Zoom recording

January 24, 2025 - CANCELED

January 10, 2025 - CANCELED

November 22, 2024

Agenda: CCF meeting - next steps after data acquisition
(Zoom recording)

November 8, 2024

Agenda: CCF meeting - next steps after data acquisition
(Zoom recording)

October 25, 2024

Agenda: Updates on CCF data acquisition efforts by Quan Zhu and Bogdan Bintu
(Zoom recording)

October 11, 2024 - CANCELED

September 27, 2024

Agenda: Susanne Rafelski presenting nuclear PILRs in a CCF WG
(Zoom recording)

September 13, 2024

Agenda: summer updates for the CCF (Common Coordinate Framework) inter-WG group
(Zoom recording)

June 28, 2024 - August 23 Canceled

June 14, 2024

Zoom recording

Presentation by Katy Borner @Indiana University about the HuBMAP Human Reference Atlas CCF and how it could be extended to connect with our nascent Nuclear CCF

K_Borner_2024-4DN-HRA.pdf

Zoom recording link
Agenda: CCF (Common Coordinate Framework) inter-WG group meeting
See this link for updates
https://docs.google.com/document/d/1RyQKGlrNiEAIpdXFHDl3npZoVpl-hapS4XEqZSpeq3c/edit

May 24, 2024

Zoom recording link
Agenda: CCF (Common Coordinate Framework) inter-WG group meeting

See this link for updates
https://docs.google.com/document/d/1RyQKGlrNiEAIpdXFHDl3npZoVpl-hapS4XEqZSpeq3c/edit

May 10, 2024

Zoom recording link
Agenda: CCF (Common Coordinate Framework) inter-WG group meeting
See this link for updates
https://docs.google.com/document/d/1RyQKGlrNiEAIpdXFHDl3npZoVpl-hapS4XEqZSpeq3c/edit

April 26, 2024

Zoom recording link
Agenda: CCF (Common Coordinate Framework) inter-WG group meeting
See this link for updates
https://docs.google.com/document/d/1RyQKGlrNiEAIpdXFHDl3npZoVpl-hapS4XEqZSpeq3c/edit

April 12, 2024 - CANCELLED

Zoom recording link
Agenda:

March 22, 2024

Zoom recording link
Agenda: CCF (Common Coordinate Framework) inter-WG group meeting to discuss next steps
See this link for updates
https://docs.google.com/document/d/1RyQKGlrNiEAIpdXFHDl3npZoVpl-hapS4XEqZSpeq3c/edit

March 8, 2024

Zoom recording link

Agenda: CCF (Common Coordinate Framework) inter-WG group meeting to discuss next steps
See this link for updates
https://docs.google.com/document/d/1RyQKGlrNiEAIpdXFHDl3npZoVpl-hapS4XEqZSpeq3c/edit

February 23, 2024

Zoom recording link
Agenda: CCF (Common Coordinate Framework) inter-WG group meeting to finish up the survey
See this link for updates
https://docs.google.com/document/d/1RyQKGlrNiEAIpdXFHDl3npZoVpl-hapS4XEqZSpeq3c/edit

February 9, 2024

(Zoom recording link)
Agenda: CCF (Common Coordinate Framework) inter-WG group meeting
See this link for updates
https://docs.google.com/document/d/1RyQKGlrNiEAIpdXFHDl3npZoVpl-hapS4XEqZSpeq3c/edit

January 26, 2024

(Zoom recording link)
Agenda: CCF (Common Coordinate Framework) inter-WG group meeting

January 12, 2024

(Zoom recording link)
Agenda: Continuation from October 27: Ran Zhang (Noble lab) and Susanne Rafelski (AICS) presentation and discussion on “what we have learned trying to match images and omics in WTC11”

December 22, 2023 CANCELLED (Holidays)

December 8, 2023 CANCELLED (Annual Meeting)

November 24, 2023 CANCELLED (Thanksgiving Friday)

November 10, 2023 CANCELLED (Veterans Day)

October 27, 2023

(Zoom recording link) (Meeting saved chat)
Agenda: Ran Zhang (Noble lab) and Susanne Rafelski (AICS) presentation and discussion on “what we have learned trying to match images and omics in WTC11”

October 13, 2023

(Zoom recording link)
Agenda: CCF (Common Coordinate Framework) inter-WG group meeting
See this link for updates
https://docs.google.com/document/d/1RyQKGlrNiEAIpdXFHDl3npZoVpl-hapS4XEqZSpeq3c/edit

September 22, 2023

Agenda: (Zoom recording link)
CCF (Common Coordinate Framework) inter-WG group meeting
See this link for updates
https://docs.google.com/document/d/1RyQKGlrNiEAIpdXFHDl3npZoVpl-hapS4XEqZSpeq3c/edit

September 8, 2023 - CANCELLED

August 25, 2023

Agenda: (Zoom recording link)

- CCF (Common Coordinate Framework) inter-WG group meeting

See this link for updates
https://docs.google.com/document/d/1RyQKGlrNiEAIpdXFHDl3npZoVpl-hapS4XEqZSpeq3c/edit

August 11, 2023 - CANCELLED

July Meetings - CANCELLED

June 23, 2023

Agenda: (Zoom recording link)
June CCF (Common Coordinate Framework) inter-WG group meeting

June 9, 2023 CANCELLED

May 26, 2023 CANCELLED

May 12, 2023

Agenda: (Zoom recording link)
- May CCF (Common Coordinate Framework) inter-WG group meeting

April 28, 2023

Agenda: (Zoom recording link)
- April CCF (Common Coordinate Framework) inter-WG group meeting

April 14, 2023 - CANCELLED

March 24, 2023

Agenda:(Zoom recording link) (meeting chat)

- Follow up meeting between WG/IG co-chairs to discuss coordinating and streamlining WG's and IG's
    - **Google doc (Talk Series discussion)**

March 10, 2023

Agenda:(Zoom recording link)

- CCF (Common Coordinate Framework) inter-WG group meeting

Current status
https://docs.google.com/document/d/1RyQKGlrNiEAIpdXFHDl3npZoVpl-hapS4XEqZSpeq3c/edit#

February 24, 2023

Agenda: (Zoom recording link)
Talk by Lorenzo Boninsegna (Alber lab)

Minutes:

- Chromatin organization is functional and fluid
- The data-driven modeling protocol: A computational microscope
- Integrative genome modeling
    - Population of S diploid genome structures
    - Input data Dk from K different sources
    - Ensemble hi-c contacts are deconvolved into a population of single cell structures.
    - [good explanation of question about how multiple data sources can cross check one another to compensate for errors in each data type. If there is too much error then the algorithm will not converge. ~13 minutes in]
    - Uncertain data doesnt mess up the model, but false data does.
    -
- Model predictivitiy: GM12878
    - Using just hi-C data, the models can predict other data types
    - And single chromosome conformations predicted by model even match patterns observed in chromosome dtracing experiments
- The approach is multimodal
- Benefits of multi-modal data integration.
- Why we should care about genome structures? The nuclear microenvironment
- Describe the microenvironment of genes
- Mapping the nuclear microenvironment of genes by structural features
    - Characterizing hi-C subcomparments with structural features
- Changes in gene expression vs changes in nuclear microenvironment
- Nuclear speckles as scaffolding in genome structure organization
    - “Our models allow to estimate the number/location of nuclear speckles”
    - Low variability in cell-cell variability of radial position for loci that have super low speckle distance and those that have high speckle distance. (lamina and speckles are scaffolding)
- Nuclear speckles are hubs of interchromosomal interaction and transcription
    - Interchromosmal interactions are higher when distance to speckles is lower

- Identify conformational chromatin states
    - Clusters of conformational states identified by lookin at latent vector of deep learning encoder/decoder
- Conformational morphologies affect the nuclear microenvironment fo genes
- Towards omics-imaging integrative modeling?
- Towards and omics and imaging single cell integration
    - Ensemble hi-C + single cell live-cell imaging (lamina & nucleoli)
    - Population of whole genome WTC11 structures
    - Example of DNA merfish/DNA seqFISH
        - Uncertainty in how to trace the data is overcome by multimodal integration.

February 10, 2023 - CANCELLED

January 27, 2023 - SPECIAL MEETING BETWEEN IOWG AND WG/IC CHAIRS

Agenda:(meeting saved chat) (Zoom recording link)

1. Meeting to discuss coordinating and streamlining WG's and IG's. (Regular IOWG meeting will resume in February.)
2. Members representing the following WG/IG were presented:
    a. WGs
    - 4DNsc4ALL
    - Cells
    - Joint Analysis
    - Imaging
    - Inreach/Outreach/Training/Webinar
    - Integrating Imaging and Omics
    - Predictive Modeling/Models and Mechanisms

    b. IG
    - Phase Separation
    - Real-time Chromatin Dynamics and Function
    - Neuro
    - Inflammation/Stress

January 13, 2023 - CANCELLED

Nov 25, Dec 9 & Dec 23, 2022 Meetings - CANCELLED

Nov11, 2022 - CANCELED (HOLIDAY)

October 28, 2022

Agenda: CCF (Common Coordinate Framework) inter-WG group meeting to draft a rationale for the importance of a CCF in the 4DN and beyond
Minutes (Zoom recording link)

October 14, 2022

Agenda: Mattia Conte - application of polymer physics models to investigate the mechanisms of chromatin folding at the single-cell level

September 23, 2022

Agenda:
CCF (Common Coordinate Framework) inter-WG group meeting
Follow up on the presented "use cases" and identify action items and tasks that this set of participants will work on together, meet regularly (~once per 1-2 months depending on bandwidth) to discuss, and report back to the WG's and 4DN (a wider email will be sent out shortly)

Minutes (zoom recording link):
https://docs.google.com/spreadsheets/d/1PO-eHjtf74ywpd4bcrxcz1nIuMPM8289x89vQNdHS_Q/edit#gid=0

Caterina: It would be great if this group would be more connected with the 4DNsc4all

Lacra: One important place to pitch and pilot the CCF ideas (written as one page, and/or in a presentation) is the 4DNsc4all, because if we don't do it there, what chance is others in the community will adopt it?
Bill: I am not sure what the CCF should be, but it is clear to me that a CCF would be a game changer for anyone trying to use 4DN data to build predictive models. Anchoring the results and building models around it
Frank: nuclear shapes are vastly different. Why? How does this influence biology? If we do not capture this information we can notr ask these questions.
Lorenzo: We are already doing work to place events (spots etc.) in the nuclear space. We realized that without a CCF comparing data across different labs is really difficult. We need a CCF and a standardized way of representing it

TO DO ITEMS/NEXT STEPS

1) Draft rationale for what CCF is important
2) Minimal recommendations for nuclear boundary, centroid and radial distance
    a) DAPI better alternatives thereof (NucBlue and NucViolet)
    b) Transmitted light
3) How to best get this in a standardized way
4) Beyond nuclear boundaries/radial

September 9, 2022

Agenda:

- Yun Li UNC - methods to call loops using DNA MERFISH and seqFISH data.
- Discuss opportunities of presenting works at future IOWG meetings.

Minutes (zoom recording link):

Yun Li presenting *SnapFISH: identify chromatin loops from super resolution chromatin imaging data*

Outline

- Background
- Super-resolution single cell DNA imaging technologies
- Our snap fish method
- Conclusions and discussion

Background

- GWAS:
    - most variants are intronic. How do we determine their function?
- Example of variants in FTO gene.

    - Variants in FTO locus, but the effect seems to be mediated by loops to nearby genesis.
- Chromatin loops identified from bulk Hi-C and single cell hi-C.

Super resolution imaging technologies

- Powerful methods for genome tracing. A few landmark papers and many labs doing incredible work and generating data.
- PROBLEM: no rigorous 3D loop caller for SR imaging data
    - Existing methods: use some distance threshold (200-300 nm)
    - Do not consider information from neighborhood loci pais.

SnapFISH method

- Takes 3D coordinates of each locus in each cell (1 thru kK)
- Euclidean distance between all loci matrix in cells (1 thru K)
- Then two sample T-test for each locus pair of interest (comparing with loci in local neighborhood)
- Post-processing: concert P-value into FDR. Define a locus pair as a loop candidate if: (T<-4, fD#<10%, local neighborhood-based filtering). Group nearby loop candidates into cluster, identify loop summits.
- Example application: mESC chromatin tracing data at Sox2 locus
    - Huang et al 2021 NAt Genet. 1416 single cells. Allele specific data in these mouse cells. (alleles are “129” and “CAST”)
    - Detects known loops called with Hiccup for example from hi-C
- Example: DNAseqFISH+ data from meSC (takei et al data)
    - Finds known loops.
- More examples from Mateo et al and Sexton et al.

Conclusions:

- SnapFISH is the first model-based method to identify chromatin loops from super-resoilution imaging data
- SnapFISH has been applied to chromatin tracing, DNA seqFISH+ and ORCA data showing high accuracy
- Future directions
    - Impute missing data
    - Differential loops
    - Identify multi-way chromatin contacts
    - Integrative analysis with data from other modalities (transcriptome, etc)

Questions:

[Lacra]: This was great. I was wondering if you could also report in your loops the euclidean distance. It could be that some are closer than their neighbors, but they may be 200 nm or 600 nm apart and those distances would have different biological meanings .

[Yun Li] That is a great idea. We could definitely report on that. I do think relative closeness is informative, and absolute distance provides important complimentary data.

[Frank] If you identify the loops, you could go back and ask how often is it present in single cells and try to understand the dynamics of the loops.
[Chris] how long does this take to run?
[Lindsay] it is very fast. On the order of minutes … so it could be run on the whole genome.

AUGUST - NO MEETINGS

July 22, 2022

Agenda:
Common Coordinate Framework (CCF)

- David Grunwald CONFIRMED (rescheduled from May 27)
- Discussion of CCF summary table/next steps

Minutes (Zoom recording link)
Nuclear transport of single molecules: dwell times at the nuclear pore complex

- Example of how they identify the NE via NPC labeling (2D fitting to identify NE)

Next example is 3D using a multifocal microscope.
Probability-based particle detection that enables threshold-free and robust in vivo single molecule tracking

- Difficult math involved.
- Tells you probability it is a spot AND the reliability of the prediction.

Estimating nuclear boundaries from smFISH signals
MetaMAX update
NPCs about 3/um2 in diff'ed cells …good for getting nuclear shape

In many cases where cells start to polarize,
You can use DNA dye that emits in a wide spectra to have one dye that is excited with one color that can emit in all channels to do channel alignment.

July 8, 2022

Agenda:
Common Coordinate Framework (CCF) -

- Susanne Rafelski

Minutes (Zoom recording link)
Susanne Rafelski presentation

- Describing 3D imaging workflow of endogenously tagged fluorescent proteins and cell membrane and nucleus.
- Describing workflow for integrating omics with segmented images
    - Label-free predicted nucleoli are generated for each structure.

[Lacra] do you see laminar invaginations always going toward the nucleolus?
[Susanne] invagintions seem to be present early on (soon after reformation of lamin shell) and then seems to go away. But we are have not characterized this to see if is always related to nucleolar proximity.

- Two coordinate systems for cell organization
    - Shape space (parametrization of nuclear and cell shape)
    - PILR (parametrized intracellular localization representation)
        - Location of structure within nucleus and cell
        - Roughly radial organization
- Alignment considerations
    - Currently always align to the longest axis of cell because shape variation is main focus (and Z kept vertical)
        - But other alignments could be chosen. The best alignment will depend on the biological question/system.
            - Example: for edge cells in colony, the team aligned based on part of cell touching edge
- Shape space
    - Choose how to align cell/nucleus and align
    - Decompose cell and nuclear shapes using spherical harmonics.
        - Spherical harmonics is 3d equivalent of fourier expansion
        - Now every cell and nuclear shape is described by a single combined vector for each cell.
    - Run PCA on spherical harmonic vectors
        - 578 spher. Harmonic coefficients → 8 principal components (explains 69% of variance of all cell nuclear shapes)
    - Now you can identify cell/nuclear shapes along the different principal component axes.
        - Find mean cell and find how shape changes along each individual principal component axes.
        - [nucleus only shape space was also computed]
- Parametrizing intracellular locations
    - Use spherical harmonic coordinates to get concentric shells

        - Compute interpolation from spherical harmonics at key points (nucleus centroid to nucleus boundary to cell boundary)
        - Then you get concentric shells from centroid to nuclear boundary along which you can sample the fluorescence intensity or segmentation value.
        - After sampling the 3D image at the surface of each shell you get a matrix representation of cellular organization of that structure. This is called the parametrized intracellular localization representation (PILR)
    - Now you can compare organization from cells of different shapes by using these matrices ("PILRs"). Or put take that matrix and put it into a cell of any shape (morphed cell)
    - You can also average these PILRs and look at average location of these structues
- See bioRxiv paper for full analysis!
- https://www.biorxiv.org/content/10.1101/2020.12.08.415562v1
- Now lets look at average locations in nucleus
    - 10 structures (nucleoli, speckles, telomeres, replication foci)
    - Radial profile plots show interesting localizations of histones, RNA pol II and and telomeres
    - Replication foci change avg organization as size increases as expected
- Future work:
    - Testing new coordinate systems for alignment.
        - Nucleolus? Chromosomes?
-

June 24, 2022

Agenda:
Xiaokun Shu, UCSF - Role of phase separation of transcription factors on chromatin structure and gene transcription

Minutes (Zoom recording link)

June 10, 2022

Agenda:

Alistair Boettiger - Discussion around "Challenges and opportunities in computational image-based omics" + CCF example

Minutes (Zoom recording link)
Computational opportunities in FISHomics

- FISHomic data commonly includes chromatin trace,
    - mature RNA
    - nascent RNA
    - And maybe more
- Computational challenges
    - Improve detection sensitivity
    - Identify artifacts
        - Such as uncorrected drift
    - Increase throughput

[Andy] do we know how much thermal drift remains after common fixation methods
[Alistair] we do see some thermal drift even after fixation. Doing an extra gluteraldehyde fix helps reduce this but its definitely something we see.

Long discussion about how we can determine confidence of spot calling, how much to focus on probe choice vs post-processing of data to filter out bad spots, and the importance of reporting how the data was processed, spots called, etc.

Discussion of “A spatial genome aligner for multiplexed DNA-FISH” as an example of how working with the same raw data in a slightly different way can derive potentially improved spot calling and chromosome tracing.

Discussion about confidence of spot calling.

[susanne] What I can do now that we have had this chat, if there are things that can transfer well to this space, we can share them. We are doing a lot of playing around with generating confidence maps when we are doing image segmentations and machine learning.

Common coordinate framework
Example of embryonic axes in drosophila–anterior and posterior.
Universal sub nuclear coordinate systems?

- Stage coordinates
- Genome sequence
- Radial position
- Dataset and question dependent?

May 27, 2022 - CANCELLED

Agenda:

Common Coordinate Framework (CCF)

- David Grunwald CANCELLED - will reschedule to later in summer
- Discussion of CCF summary table/next steps - postponed till next meeting

Minutes (Zoom recording link)

May 13, 2022

Agenda:

Visual Cell Sorting - Hyeon-Jin Kim, Gang Li, University of Washington

Minutes (Zoom recording link)

Towards understanding nuclear heterogeneity in mouse embryonic development
Presented by Hyeon-Jin Kim and Gang Li (UW genome sciences)

- Want to be able to understand heterogeneity in nuclear morphology (E16.5 Fetal liver cells as example)
- Pathogenic lamin mutations alter nuclear morphology. Lamin B1 and nucleolus
- Nuclear proteins undergo morphological changes during stem cell differentiation.
    - And even within the same cell type there is heterogeneity.
- Can we characterize these morphological differences with different molecular assays?
    - E.g. how much does nuclear morphology explain transcriptional heterogeneity.
- Samples prep
    - Diff'ing cells or embryo
    - Isolate nuclei
    - Measure morphology with visual cell sorting
    - Sequence sorted cells (gene expression, chromatin accessibility , chromatin interactions
- Visual cell sorting
    - First image samples, then image analysis to classify phenotypes, then photoconvert Dendra2 or use antibody with PAJF549 to photoconvert in fixed samples
    - Photoactivate cells of interest and FACS to sort.
    - Example: LMNA variants (wt vs punctate)

[Susanne] – how does time-scale work for this experimental workflow? Some shapes and morphologies change rapidly…how do you take that fact into account?
[Hyeon-Jin Kim] the phenotypes we've currently looked at don't change rapidly. But we also do fixed cells

- Visual cell sorting
    - Can recover four visually distinct populations by tuning photoactivation dose
    - 2-3 million cells per day
    - Compatible with live and fixed cells/nuclei
    - Sorted samples are compatible with single-cell/nuclei genomic assays
    - [Micro-mirror stage is the enabling technology. Also automation.]
- Features
    - Lamin B1, nucleolin, hp1a, dapi,
    - 40x objective, Zstacks, max-z projections
- Nuclear shape changes during ES cell differentiation.
    - +RA (Diff'ing cells) become more circular. (previously observed in publications)
    - They asked how does shape change over diff using visual cell sorting
        - Shape factor = Area divided by perimeter squared
        - They see shape factor increases along diff, as expected
    - Ellipitcal Fourier coefficient ratio
        - Parameterize the shape (see Tamashunas et al MBoC 2020).
        - This ratio seems more sensitive to irregularities in shape…
    - They can bin cells into based on EFC ratio along the different time points (3 bins)
- Nucleoli morphology changes are also clear along diff. That's the next focus
    - Trained a deep neural net for nucleoli segmentation.
    - Nucleolar size decreases during ESC diff.
- Future directions: collect cell nuclei from embryos

Gang Li

- VCS outputs cell morphologies and single cell measurements
    - A matrix of single cell sequencing measurements
    - Segmented single cell images
    - Challenge: no 1=1 correspondence between cells from sequencing and the segmented images.
    - Goal is to match the two using manifold alignment
- scRNA-seq (n1xp1) vs microscopy image (n2xp2) (n=cells, p = features or pixels)
    - Assume that data share manifold structure.
- Maximum mean discrepancy (MMD) between distributions
- MMD manifold alignment (MMD-MA) minimizes the distance between two or more distributions
- How to evaluate integration performance
    - When 1-1 cell mapping exists
        - Fraction of samples closer than true match (FOSCTTM)
        - Neighborhood overlap
    - When 1-1 cell mapping does not exist

- Dataset as an example for integration–Paclitaxol or DMSO (lobulated morphology or not) then RNA-seq
- Compute kernel similarity matrix for scRNA-seq data.
    - Correct batch effects between normal and lobulated datasets.
- Compute kernel (similarity) matrix for images
    - UMAP ResNet18 embeddings.
- Current best MMD-MA results on taxol data
    - Co-embedding space shows good overlap of gene expression and microscopy image manifolds.
    - Clear cell cycle gradient across the UMAP
        - Seems like their method is aligning cell cycle with pixel maximum of images
        - Need an image based control to estimate cell cycle from images to validate this result
- Potential imrpovements
    - The image embeddings are driven by the texture of the cells rather than the morphology.
- Can we get cell cycle information from microscopy images? Try PCNA?

[Jian] have you tried other methods to visualize the embeddings besides UMAP? Also do you have some matched data already where you can identify some marker genes
[Gang] we have only done UMAP or PCA so far for visualization. But we do want to try that…[?]

[Andy] I'm a bit confused by the underlying assumptions… could you use markers for cell types that you could integrate and then ask how shape correlates with those? [i may have poorly paraphrased this question…apologies]

[Gang Li] there is a paper called Phenosequencing that did something like this

[Chris] have you built toy models?

Previous papers:
Here is the LSMMD-MA implementation:
https://www.biorxiv.org/content/10.1101/2022.03.23.485536v1
Original MMD-MA paper: https://drops.dagstuhl.de/opus/volltexte/2019/11040/

April 22, 2022

Agenda:

Common Coordinate Framework (CCF)
- Yodai Takei CONFIRMED
- Frank Alber CONFIRMED

Minutes (Zoom recording link)

Yodai Takei
Title: Overview of nuclear coordinate systems and analysis used in seqFISH+ projects
- Integrated spatial genomics (mouse ES cells; n =446 cells; nature 2021)(mouse brain cortex tissues; Science 2021 and Nature 2021)
- Overview of current DNA seqFISH+ data format that will be uploaded to 4DN data portal
- Overview of segmentation approach for identifying subnuclear structures
    - Binary algorithm approach// heterochromatin from DAPI example
        - Challenge is the sensitivity to background subtraction radius and threshold value
            - Have to manually check.
    - Z-score based approach
- Comparison of different criteria for imaging-based chromatin profiling (nuclear lamina)
    - Different z-score methods give similar profiles
    - But profiles appear somewhat different than lamin B1 DAM ID profile

Available information
- Center + periphery of nucleus
- Centroid for each genomic locus imaged
- Segmentation of each IF target/nuclear structure
- Features of segmented objects

Separate question is how to report all of this information

[Caterina] FISH omics format has proposed methods for reporting some of these details, including the surface of a structure

Frank Alber
Title: Reference coordinate systems
Integration of genomics data with imaging data. *See paper Boninsegna et al 2021 bioRxiv*
Prediction of nuclear speckles. *See Yildrim et al 2021 bioRxiv*
Mapping nuclear microenvironment of genes by structural features.
- For each region of the genome look at how close it is to each subnuclear compartment
    - E.g. make a vector quantifying distance (or other feature) for each locus relative to nuclear bodies.

Spatial features

- Nuclear positions with respect to nuclear landmarks
    - Radial position in nucleus
    - Distance to nuclear bodies
- Signal accumulation using an exponential decay function
    - Simialr to TSA-Seq profile
- Local features of chromatin
    - E.g. where is locus relative to its own chromosome and other local regions.
    - Surface or interior of chromosome
- Chromatin-nuclear body association
- Cell-to-cell variability of all these different features features

Feature calculation in realistic images

- Calculating normal distances to nucleolus

Example of yeast

- Spindle pole body to nucleolus axis is a reference axis.
- So you can just calculate location along axis, radial position and alpha.

[discussion] each specific cell type has its own natural coordinate system. Neuron vs yeast cell vs cell with only one nucleolus vs cell with multiple nucleoli, vs polarized cell.
The scientific question then is how meaningful are these axes we choose? Do we find they help us understand organization?

[Chris] Is there a way we could get the same conclusions from the yeast analysis without using the previous yeast coordinate system? How can we evaluate how useful this coordinate system is relative to others? And are there others that might generalize better to other non-yeast systems.

April 8, 2022 - Canceled

March 25, 2022 - Canceled

March 11, 2022

Agenda:
Chris Frick/Susanne Rafelski updates on mitotic synchronization of WTC11 for better omics-imaging data matching
Slides = 20220311_IOWG_g2m_synchronization_work_in_progress_forsharing.pptx

Minutes (Zoom recording link)

February 25, 2022

Agenda:
David Gilbert updates about ROCCS cell cycle interest group

Minutes (Zoom recording link)

February 11, 2022

Agenda:

Meeting saved chat

Minutes (Zoom recording link)
Caterina shared Slides to give a brief recap of the idea of standardized coordinate system (aka common coordinate framework).

- Agree that it is worth it – 13 people

Benoit – worried that positions of things in the nucleus are not stereotyped enough that assigning them as references for positions won't have as much meaning as one likes.

Susanne: I agree that becomes a scientific question. I think the question is what are our core landmarks (edge of nucleus…maybe also nucleolus and speckles)

Sarah Aufmkolk: in 3d coordinates… one big hub of functional organization…say u decide on 4-5 functional hallmarks to include so you can those references present [might have missed some of this]. I propose functional hallmarks as a coordinate system

Jian: The relative positions I think are more informative than the absolute positions. A microenvironment focused aproach would be useful

Frank: The location of the genes in the nucleus are incredibly important. The first coordinate system that people did was radial position and that had a predictive relevance and use. OF course the radial position itself is an indirect measure of other things that happen. In our modeling we are calculating the nuclear microenvironment byu trying to determine how close each region is to the nearest x,y,z landmark/nuclear body. And I think for some regions these details are very important. And it would be great to see this in images

Alistair: One challenge is cells don't exactly know x,y,z but the microscope does. And we know our lenses cause distortions which can disrupt our measurements affect our interpretations of the data. We have numerous discussions in our lab about how difficult it is to compare data collected from two different micrsocpes if corrections for microscope aberrations are not made.

Caterina: I wanted to push back on two things: 1) I think cells do care about xyz coordinates. They are relevant. I also want to know what absolute, vs relative mean. Everything we measure is relative.

Susanne: Our flagship paper has looks at average locations of structures in the nucleus in a consistent average coordinate system. I think the use of something like this is that it enables us to compare all of our experiments across our systems and cell types. [I missed a lot that was said here too]

Sarah: I want to also add to what Alistair said. E.g. if I want to look at how some region is compacted in the nucleus…and I don't give a proper error estimation we could learn that things closer to the lamina are closer together just because our microscope has worse resolution at that location because it is farther from the coverslip of the microscope. The biases and aberrations of the data are very important and I think we should have a separate discussion on this.

Susanne: I think an uncertainty map would be great.

Yodai: I think the distance to nuclear bodies depends heavily on how you threshold the nuclear bodies. So comparing datasets requires us to care deeply about segmentations/thresholding too

Mike Pazin: [how many details do we have in our coordinate systems .. e.g. distance from innervation in muscle or distance from cell to hair follicle.]

Susanne: I think these kind of coordinate systems should be system specific. [example of hiPSC colony and how to think about aligning that]

… missed some conversation…

Sarah: I would love to have a wishlist…and something that can tell you what kind of details you should measure when your experiment is looking at X feature at Y resolution that will makre you measure interpretable. [Decision tree approach that allows an experiment to set their experiment in a coordinate system that is appropriate or interesting for their resolution / cell type / target gene.]

… missed some conversation…

Sarah: It would be good to also define how we define borders…e.g. How do we define lamina for our segmentation? Can we have a shared code base / target for those segmentations.

Susanne: if you want to be in the first conversations, put your name in the chat
17 hands up for people who agree.

January 28, 2022

Agenda:

1. **Discussion session**
   Discussion led by Caterina Strambio on “Toward a standardized nuclear coordinate system for 4DN - part 1”
   a. Discussion outline
   b. Slides

Minutes (Zoom recording link)

- Generally positive reception
- General agreement that it would be useful for integrative modeling etc.
- Discussion about what it would entail and how it could be done in practice (see slides 8-10 and comments therein)
- Next steps:
  - Will pick up discussion next time on 2/11/2022
  - Caterina will bring topic up at next IWG and gauge interest/feasibility
  - Would be advisable to figure out a specific type of experiment to start test feasibility of a common coordinate system for and then move forward with generalizing to other systems

January 14, 2022 - CXLD

December 10, 2021 CXLD - ANNUAL MEETING

November 26, 2021 CXLD - HOLIDAY

November 12, 2021

Agenda:

1. Short presentation: Chris Frick (AICS)
   a. SLIDES

2. Discussion about Phase 2 overarching goals

Minutes (Zoom recording link)

October 22, 2021

Agenda:

1.) **Topic**: *Method specific experimental perturbations of chromatin structure*
Discussion Leader: Andrew Belmont

Minutes (Zoom recording link)
11/26 meeting cancelled (day after thanksgiving)
12/10 meeting cancelled (4DN conference)

Thinking about effects of different kinds of artifacts on imaging versus omics comparisons
Low resolution omics ←→ low resolution imaging (may not exactly agree due to limited resolution of one or both approaches)
As we move toward high resolution for both…could see improvement…OR you could see a divergence as resolutions improve if OMICs and imaging are actually producing measurements of different structures.
Discussing differences in "biological replicates" among the two communities (imaging and omics)
Anecdote/third hand comment: took 6 groups 1 year to get the same omics results. They got different results due to different approaches to switching from low serum to high serum media.
Open question: What effect does cell detachment have on nuclear geometry? Nuclear bodies? (omics methods often require detachment while imaging is done live/fixed on substrate)

Caterina proposed that some effort toward bridging this gap could be the large joint effort for phase 2. Lacra proposed that for this to be really powerful for a phase2 large project we should identify how this affects biological conclusions.

ACTION ITEM:
We plan to continue this discussion again on the next meeting
Please contact IOWG organizers about speaking at 4DN conference.

DAVE's COMMENTS: What I took away from this is that everything matters. Given that, you can get depressed or you can just accept that as the eyes wide open part. Then you simply a) establish standardized conditions; b) carry those out in different labs; c) trust only the data that is reproducible and filter out the rest - unless your question depends on it and then you do more work.

Or another way to say it is - if everything matters then it is diminishing returns to worry about "everything"

October 8, 2021

Agenda:

1.) Continue discussing collaborative projects - see spreadsheet and minutes below

Minutes (Zoom recording link)
Bill and Susanne present slides for their collaborative project (Noble + AICS)
Also presentation by Ran Zhang from Noble lab

- Joint analysis of imaging and sci-Hi-C in WTC11 cell line.
- Aim to is to align cells from imaging and sci-Hi-C measurements
- Plan to use sci-Hi-C instead of single nucleus hiC. Single nucleus is more enriched in G1/S stage…sci-Hi-C can let us match across all cell cycles in interphase.
- This sci-Hi-C dataset has 600 cells. But also an order of magnitude less depth per cell than the Ren lab single nucleus scHiC.
- Using 3d shape modes of cells from Viana et al 2021 (AICS bioRxiv publication).
- Use contact decay profile to represent sci-Hi-C
    - G1, S, G2 can be determined from this.
- DNA volume can be used as a proxy for cell cycle (G1, S, G2) in imaging data.
- To do alignment, cell cycle is annotation is made as a continuous variable instead of discrete.
- MMD-MA algorithm to align data to a latent shared space.
    - Unsupervised alignment doesn't result in clear label correspondence.
    - Supervised MMD-MA with 10% cell cycle stage labels could align the remaining cells by cell cycle stage.

Huaiying Zhang and Frank Alber present slides.
Artificially induce telomere clustering in cells and measure chromosome contact changes that result. And then attempt to model how the genome is reorganizing based on this data.

Discussion of possibility of joint analysis working group in phase 2.
paraphrase : We want people to think about what they can accomplish. It could be many things. We are just asking for a few ideas about what could be a constructive broad collaborative effort.

We will continue this discussion at our next meeting.

September 24, 2021

Agenda:

1. Introduction of collaborative projects

   https://docs.google.com/spreadsheets/d/1IaCgqP7OgNQ9TTTzRm_jh6p-D7KCrJZZT5CC8-OV5_Q/edit#gid=0

Minutes (Zoom recording link)

Discussing collaborative projects that will report to this WG

See link here to spreadsheet

Lacra and Yin presenting slides for Shen, Bintu, Boettiger collaboration

- Imaging and sequencing analysis of 3D chromatin interactions in human neurons
- Excitatory neurons diffed via stably integrated Dox inducible neurogenin 2

Lacra, Katie and Benoit presenting second collaboration (@9:33) -- Bruneau, Pollard, Bintu, Boettiger

- Chromatin structure changes during hiPSCs to cardiomyocyte differentiation
- Type1 vs type 2 lads.
- Hypothesis is that type 2 might still be near lamina but moving toward the speckles
- Type 2 LADs seem to be cell-type dependent and dynamically change across diff states.

Yin presenting on 3rd project --Shen, Shu, and Alber

- Roles of phase separation in 3D chromatin structure changes
- Hp1 alpha is first chosen target (look at changes before and after condensate formation)
- [Question] can these condensates be induced to form at specific at specific location?
- [answer] we actually hope to dissolve these condensates, since HP1alpha is natively in a condensate
-

September 10, 2021

Agenda:

1. Talk by Ruochi Zhang (Jian Ma lab): “Single-cell 3D genome features and variability in WTC11”
2. Collecting information about all collaborative projects that report to the IOWG group:

https://docs.google.com/spreadsheets/d/1IaCgqP7OgNQ9TTTzRm_jh6p-D7KCrJZZT5CC8-OV5_Q/edit#gid=0

3. Proposal about the format of the forthcoming meetings.

ACTION:

- Please sign up for talks here
- Please sign up for leading a discussion session here [at bottom of document]
- Please put your collaborative project here in this google sheet

Minutes: (Zoom recording link)

Ruochi Zhang Presentation

- Higashi--method for imputation of missing contacts in scHi-C.
- Applied to WTC11 scHi-C from Ren Lab (50kb)
- Variability of A/B compartment has some correlation with the variability of transcription activity.
- Two types of variability at TAD-like boundaries:
    - On-off (prescence vs absence)
    - Sliding along genome (location of boundary is shifted from cell to cell)
    - TADs that occur more frequently have
        - Lower single cell insulation scores
        - More CTCF peaks
        - Higher average CTCF peak intensity
- [BING REN] how many boundaries did you find in total in group 1, 2 and 3?
- Differentially expressed genes identified from WTC11 RNA seq at 5 cardiac differentiation stages from Friedman et al., 2018
    - DEGs are near more variable domain boundaries.
- [Frank] what is the minimal number of contacts needed in scHiC data for the imputation to work well?
- [Ruochi] depends on the resolution that you want for analysis.
- [Bing] how did you call boundaries in individual cells? And how well do the boundaries match what you see in the bulk hiC?
- *[missed the answer...please see recording]*
- [Wenbo Li] how many cells in total and what are the average reads per cell?
- [Ruochi] 188 single cells. The data is from Bing Ren's lab. More details there.

Item 2 in agenda: Collecting information about all collaborative projects that report to the IOWG group and sign up for talks/discussions

Proposal:

- Once a month we have scientific talks
    - Please sign up for talks here
- Once a month we have dedicated discussion about specific topics
    - Please sign up for leading a discussion session here [at bottom of document]
    - Topics for discussion in integration methodology/technology development and standards
        - Need a discussion leader/volunteer for each of these discussions
        - Andy Belmont will lead the first discussion on 10/8
- Please put your collaborative project here in this google sheet

[Catarina] what is difference between the collaborative projects and the topics

[Susanne] the collaborative projects are part of our scientific agenda and will be presenting during the scientific talks in the future.

[Jian] maybe one of the outcomes after the first round of presentations of these proposed collaborative projects...if we can identify shared challenges we can then have ideas for the next round…[*i likely missed the full meaning of this comment*]

[Susanne] I hope this plan sounds good to everyone. We think this structure will be good.

[Bing] I think this is a very good structure. I would like the discussions to not just focus on technical challenges but also on the science and scientific questions.

[Susanne] that is a great idea. We should definitely do that. You could volunteer to lead that session.

August 27, 2021 - CANCELLED

August 13, 2021

Agenda:

4. Presentation by Ming Hu, title: "Preliminary analysis of WTC11 single cell Hi-C data"
5. Presentation by Ran Zhang (Noble lab); discussion about WTC11 ChIP data

Minutes: (Zoom recording link)

Ming Hu presentation

- Preliminary analysis of WTC11 single cell Hi-C data
- WTC11 derived clones
    - AICS-0036-006 (c6)
    - AICS-0036-028 (c28)
        - From notes below: “AICS-0036 which expresses cytoplasmic mEGFP from the AAVS1 safe harbor was chosen as the control cell line as no structure is tagged and we therefore expect the least perturbation from the tag. To allow for biological replicates of the controls, the Allen Institute for Cell Science released 2 clones of AICS-0036.”
- 94 cells in each clone, 188 cells in total, using optimized hi-C protocol from Ren lab.
- Use SnapHI-C method for calling chromatin loops
    - Uses all cells for calling loops and then look at population to see how many of those cells have loops.
- Used Higashi to identify A/B compartments and TAD-like domains (50kb) resolution.

Ran Zhang

- Imputation of HFFc6 & WTC-11 1D profiles using Avocado.
- Schreiber et al 2020
- Genomic positions factors, assay factors, cell type factors and then input missing profiles
- They want feedback from group to know how to expand the table to include more assay types for WTC11
    - Can genetically modified WTC11 cells be used?

[Yin] encode generated new WTC11 data that includes several different histone marks, and we have a new paper coming out in science advances that does CTCF chip-seq and …(more?)
[Bill] we don’t see the CHIP-seq data you referenced on the encode portal.
[Yin] ours are on the geo database.
(https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE166839)
[Dave] there is a lot of cut&run data available. That is something you should look for.

- Question2: are there other public available experiments that are not in ENCODE portal?

ACTION ITEMS: We want to continue with a couple of talks about work done in the last phase. Frank will reach out to get volunteers. We also think it would be great to get a couple

of short talks about the joint projects that we formed. And we also would like to have a discussion about subgroups again in the future.

July 23, 2021

Agenda:
1. WTC11 cell lines and which ones to use for what purposes in Phase 2 with context provided from decisions made in Phase 1.

Minutes: (Zoom recording link)

Goal of the meeting was to discuss which cell line(s) should be used as controls and to clarify updates/locations to the Allen Institute for Cell Science's cell culture SOPS.

The meeting started with a summary on the decisions the phase I teams had made for controls: Transcriptomic analysis showed that the transcriptome is more similar between gene-edited cell lines in the Allen Cell Collection than between the gene-edited cell lines and the parental WTC-11 cell line. This difference is most likely due to the difference in passaging numbers between the parental and the gene-edited cell lines. Therefore, the recommendation in phase I was to use a gene-edited cell line instead of the parental WTC-11 cell line as control since the Allen Institute for Cell Science doesn't distribute an older version of the parental cell line. AICS-0036 which expresses cytoplasmic mEGFP from the AAVS1 safe harbor was chosen as the control cell line as no structure is tagged and we therefore expect the least perturbation from the tag. To allow for biological replicates of the controls, the Allen Institute for Cell Science released 2 clones of AICS-0036. Data in phase I have been collected with AICS-0036 as control (2 replicates).

As we are moving into phase II, the following questions came up:

(1) What should be used as a control cell line in phase II?

(2) Can we mandate using 2 lines as controls since (a) the cell lines are expansive and (b) only two clones are available for AICS-0036 but not for the other gene-edited cell lines from the Allen Cell Collections (e.g. SON etc)?

(3) What line should be used as background for (additional) gene-editing?

Since not everyone on the phase II team has seen the transcriptome analysis for the gene-edited cell lines vs the parental WTC-11 line, the analysis should be presented again so everyone has a clear understanding how different the gene-edited cell lines are from each other and from the parental control.

Action item: Present transcriptomic analysis to phase II team at one of the next meetings

Stakeholders discussed that it makes sense to choose the control based on the experiment that is being performed and that using 2 control lines might not be feasible. We will provide a description of the different control choices which can be used as a guide for the different teams which control is the most appropriate for their experiment.

For example,

(a) if teams want to create dual edited cell lines, it makes sense to add the second edit on top of the first edit already present in one of the Allen Cell Collection cell lines. In that case, the respective lines from the collection should be used as background. If a new single edited cell line is being made, it makes sense to start with a younger cell line and therefore the parental WTC-11 cell line should be used as background.

(b) if imaging and genomic assay are being combined, it might make sense to match the lines used for both experiments as best as possible. For example, if the mEGFP tagged SON line is being used predominantly for imaging, it could be also used for the genomic studies. But if the a non-mEGFP expressing line is needed (for example for fixed imaging, immunolabeling etc) then the parental line might be the most appropriate line for both assays (imaging and genomics).

(c) if only a genomic assay is being performed, AICS-0036 might be the most appropriate line to use since its transcriptomic profile is similar to the one of the other gene-edited cell lines

(d) if the cells are being differentiated and differentiation potential of the gene-edited line might be impacted by the tag, the parental WTC-11 might be the most appropriate cell line to use.

Action item: Dave will write up a summary of the potential cell lines available for experiments and send the document to the appropriate stakeholders for edits prior to posting it on the wiki page.

The Allen Institute for Cell Science team clarified the updating cycle of their cell culture protocols. The protocols are being updated every few years and the most current version has a summary of the changes compared to the prior version at the beginning of the protocol. Most changes done to the protocol have been minor but there has been a change in reagents in both the cell culture and the cardiac differentiation protocol as well. Cell Science's website received a major reorganization in the last few months causing the wiki

page link to be broken. Such major reorganizations should not be happening again anytime soon but if they are, Cell Science will let the teams now.

The culturing protocols for H1 and H9 are not expected to be modified anytime soon but the Cell Science's cell culture protocol might be updated periodically. Stakeholders agreed to switch to the updated protocols as they become available to keep using the most optimized protocol possible.

In general, while there is a gold standard for type of media etc, it might not be possible to follow for all experiments. This is OK but teams should mark their datasets accordingly to specify what has been used to create the dataset.

It was discussed if it is possible to get notifications if changes to the wiki page have been made. Based on the Google drive functionality, only the file owner can get these notifications. "It will be overwhelming for all group members if the file owner forwards every file changes email to the group. Everyone should be able to check changes if they want (by logging in to the document)."

Teams were also reminded that the wiki page has an engineering section which contains a high-level summary, and a discussion forum for team members. There is also a list available for available and in progress gene-edited cell lines:

https://docs.google.com/spreadsheets/d/19cbxd_KFPNIaOp8MCxH7ViEGlqMRxftrY73HCbikIA/edit#gid=0

Combined these are great resources for team members to see what lines are being made so efforts are not duplicated and to exchange information and knowledge regarding the different cell lines (e.g. how the line differentiated etc). The experience so far has been that multiple editing does not affect differentiation potential of the cell lines.

July 9, 2021 - CANCELLED

June 25, 2021

Agenda:

1. Collaborative projects discussion.

Minutes (Zoom recording link)

- Huaiying Zhang presented [talk recorded in zoom recording link above]

- **Action items for this meeting and future meetings are here: https://docs.google.com/document/d/1Rp6Oc0tqcGrVGx1oID5FdBJfH7eMOghJ0D7oBsqGcvg/edit**

June 11, 2021

Agenda:

1. Discuss Collaborative Projects Brainstorming

Minutes: (Zoom recording link)

- Discuss google doc and items that we all discussed together since last meeting

[Susanne] I think we should start with section D
[Benoit] Lacra, Alistair, Katie and I have established a collaboration looking at Tbx5 and new flavor of heterochromatin that Katie is looking at.
[Susanne] Can you please update that on the list?
[Susanne] Bill wrote up a proposal that involves him and us about how he is working with us at AICS to match up single cell images with single cell genomics data. The initial goal is to test the two methods in his lab for integrating different single cell datasets with AICS imaging data of single cells.

[Yin] The project between Frank and I and Xiaokun Shu -- use chemical tool to perturb liquid-liquid phase separated condensates such as HP1alpha and Myc to understand how condensates change genomic structure. We want to look at this in WTC11, especially in neurons where the genome becomes more condensed. Frank's group can do modeling of data from these perturbed systems. Hi-C and cut-&-run data is planned to be collected.
[Huaiying Zhang] Are you accepting new members? I am interested in looking at similar phase separated bodies and their roles in genome organization. Maybe we can work together or at least in parallel.

[Susanne] Anyone else have items from the list that got nucleated into a half-page collaboration doc/white paper?

[Jian] It would be useful for this WG to come up with some short descriptions of the shared project in this working group.

[Andy] there is a paradox--B-globin (and other targets) very clearly become speckle associated in differentiation. However when we look genome wide at changes in speckle association we don't see such clear changes. How does nuclear body organization differ accross cell types is really important to explore. I would like to use Yin's differentiation system of WTC11 to do initial staining of nuclear bodies etc to see what differences appear.

[Wenbo and Benoit discussing cardio diff protocols--]

[Susanne] I will mark cardio diff protocols as a topic for discussion in the future.

[Dave] Any kind of SOPs would be handled in the cells WG, yes. Cells WG has made it clear that they won't micromanage. We will link SOPs and negotiate reagent costs.

[Jian] I wonder if its possible to have an item that involves everyone in the WG? Is there anyway we can put these together into a large item.

[Frank] WE have in topics B and C already the large scale ideas we all want to capture. These items in D are just the small items for this immediate collaboration write-up deadline.

[gap in notes]

[Susanne] we will propose a set of subgroups with some set of people that will meet with some cadence that will present/report in these meetings.

ACTION ITEMS

- **We would like to start having some presenters**
- **We will work on what are the subgroups and send out a sign up sheet [will take a couple of weeks]**
- **In the next two weeks and two weeks after that**
    - **Next meeting: it would be great to have someone present work/data updates from phase 1**
        - **Please email us if you have data updates to share!**
    - **4 weeks from now it would be great to start discussion on items in section C (related to bottlenecks and challenges)**

May 28, 2021

Agenda:

Minutes (Zoom meeting recording link)

Initial discussion

- Summary of Collaborative Projects Brainstorming doc (Google doc: **Collaborative Projects Brainstorming)**
- 3 main components

    - Scientific presentations (WTC11 continuing projects) and new collaboration projects (via subgroups)
    - Discussions of methodology, tech development, standards relevant to group
    - Continued development of infrastructure for data/results/sharing from phase 1
- Today we want to continue exploring topics for new collaborations and topics for further discussion

ACTION ITEMS FOR TODAY

- **Google doc**
    - Decide which topics which should stay on or go off
    - Decide which topic/subgroup you want to be a part of--this way groups will begin to nucleate and gain traction
        - NOTE: put your name down on those subgroups for which you absolutely do not want to miss. If you want to join other subgroups, you can always pop in. This will help with scheduling
        - NOTE: Any topics that don't have enough interested individuals we will drop
- **Continued presentations**
    - Those of have updates from phase 1 WTC11 work can you please reach out to Susanne, Yin, Frank

Starting on item B4 in google doc
[Susanne] What other subgroups could be of interest to others? Or other points?

[Frank] The topics under C, seem relevant for the B4 Subgroup (integration of imaging and omics)

[Susanne] Yes I agree with you, but C I was separating out as items for discussion. So we will definitely discuss those items.

[Andy] One theme from looking at images of cells [H1 vs fibroblast for example] is that some cell types are different but others are very similar (H1 vs K562(?)) ...and knowing why ES cells (H1) and non-ES cells (K562) are similar will be really interesting.

[Frank] Will subgroups meeting in-parallel? E.g. having subgroup meetings separately during this same time slot.

[Susanne] that could be a great idea. We meet twice a month so we can set one of those meetings for subgroups and one for the whole group.

ACTION: get vote on subgroups meeting in parallel?

ACTION: put your name down on those subgroups for which you absolutely do not want to miss. If you want to join other subgroups, you can always pop in. This will help with scheduling

[Yin] Lots of these projects overlap. Can we make them more concrete so people feel more comfortable choosing fewer subgroups?

[Susanne] Yes, we should try. I stuck Andy's idea to B1.

[Susanne] Maybe we can start with discussion meetings (see section C) and then some of how this fits with the different subgroups will fall out maybe.

[Yin] yes that sounds great.

[Andy] I think there is a semi-urgent thing about the collaborative projects--I wonder if we should elevate priority the discussion of which collaborations people are interested. All of the grants in year 2 need to have some aspect of collaboration. So we will have to work with program officers and describe specifically what we have in mind for collaborations.

[Susanne] so we can start actively figuring this out now.

[Frank] the subgroups will lead to collaboration, but perhaps later on

[Dave] I am imagining that we will be writing a white paper and that will be describe a collaborative effort between PIs on multiple grants. It's not clear how to describe the communication for the grant

[Ian] I don't want people to get super panicked about this. What Dave described is good. We want all the PIs …
[*see recording at the 9:30 am PST mark for this discussion*] .

[Susanne] so a group meeting around analyzing imaging data between H1 and H9 and WTC11 would work?
[Ian] Yes. That would work great.

[Dave] So for this urgent deadline we should choose the items that work now and won't take longer to crystallize into a clear vision. What susanne just described is a good idea for one that could crystallize quickly into a clear vision for this upcoming June deadline

[*gap]*

[Andy] I am very interested in looking at nuclear bodies in terminally differentated cell types (via staining) to begin looking

[Susanne] I am writing this down under “D”.

[Yin] I think the B2 project could also fit with Andy’s project. So andy if you are interested we would be happy to provide cells.

[Frank] The same for our lab.

[Benoit] Susanne, I am not sure what you have planned for imaging of cardiomyocytes but we have different cardio subtypes and mutants that we won’t have opportunities to image

[Susanne] unfortunately we are completely filled up on imaging capacity. So we can’t take on any new imaging tasks. WE also can only work with WTC11.

[gap]

[Lacra] we have a goal of looking at chromatin structure of specific regions during early diff to cardio and to neuronal types. I am curious as to what loci are most interesting to others, (Yin and Benoit) for us to image.

[Frank] Would it then be useful, now that these collaborative projects have crystallized, to present the ideas of these collaborative projects to see if others are interested and fit in in a logical way.

[Bill] Are the descriptions we submit in June going to be public to the consortium?

[Ian] No, we figured that these submissions will eventually be presented in other contexts.

[Susanne] at our next meeting.
AGENDA ITEM for next time: we should revisit all the items in D (the urgent items), just be ready and come prepared to share what is going on, so things can be finalized and everyone can have one more opportunity to think about joining in.
Please let Frank, Yin and Susanne know if you are nucleating around a group and have something to share.

ACTION ITEM: For the urgent items (in section D) , figure out what else you want on this list, and identify groups you are interested in.

[Dave] If you are interested in all of these groups, what do you do?

[Susanne] Identify the groups where you need to actively be in the subgroups. You can still participate in all the projects because all will present at the main session and you can catch up.

[Dave] Could we also think about sending people out into breakout rooms? It works for classes.

[Susanne] and for everyone, you are always able to join any subgroup you are interested in.

[Yin] If one group is involved in multiple collaborative projects, do they have to submit multiple submissions for the June deadline?
[Ian] Yes. That can work. [*discussion at 9:52 am PST time*]

[Jian] Is there additional money to support these collaborative projects.

[Ian] No, no additional available money is available

[Andy] I am personally looking into collaboration that are going to pursue scientific questions more directly, rather than large generally focused collaborations. So should we be a working group? or just report to a subgroup or a working group?

[Susanne] I think finding groups to present the work to makes perfect sense. Do others have thoughts on that?

[Frank] yes that is a good point. It's a continuum isn't it. The advantage of these groups is that we all get to see things presented that could be beneficial for those smaller projects,

May 14, 2021

Agenda:

1. Google doc: **Collaborative Projects Brainstorming**

MINUTES (Zoom meeting recording link)

Initial discussion

- Discuss the collaborative projects brainstorming document (here: https://docs.google.com/document/d/1Rp6Oc0tqcGrVGx1olD5FdBJfH7eMOghJ0D7oBsqGcvg/edit?usp=sharing)
- [lots of updates, comments and notes were added to the above doc during the meeting]

[Susanne] Bill and others from phase 1, do you want to continue the analysis and sharing that was being done in phase1?
[Bill] Absolutely,I think that makes sense. The goals are very much overlapped. And I don't think it needs to be a separate track. It can fit within the other tracks

[Susanne] Frank, Yin, and Chris, we should reach out to get presentations to get updates for people who weren't in this group in phase 1, so that they can get updated on what had been done previously.

[Bill] We also previously had infrastructure for sharing and storing data. It would be great to get people up to speed and have a short presentation (~10 min) that can show some of this and some data and then start some slack groups where people can access and analyze data together and begin using what we had previously in place.

[Susanne] Lets talk about other projects. Benoit suggested last time comparison of H1 and WTC11. (see doc for details). Does anyone want to spearhead this?

[Benoit] I suggested this because it would be good to see how similar are H1 and WTC11, because H1 has a lot of data and it would be good to know how generally replaceable H1 data is with WTC11 data as a proxy.

[Yin Shen] For the encode project when we started working on WTC11, we did a comparison between H1 and WTC11 with RNAseq, ATACseq. The majority of features in H1 are seen in WTC11. When you compare them among all other cell lines they are the most similar to one another compared to all others. So for encode we concluded that datasets from both lines can be interchangeable. (Note: couldn't catch all that was said, but it sounds like she said genomics data has also been compared)

[Benoit] So I guess that project has been done.

[Susanne] What do H1 cells look like? And how do they behave compare to WTC11? How similar are they in morphology.

[Yin] IN terms of tissue culture--they look exactly the same and behave the same--and they are handled the same in terms of passaging. And they are both male cell lines

[Susanne] Anyone know in terms of imaging?

[Dave] My comment is to say that H9 is female and also looks very similar to WTC11 morphologically. But I don't think there is much imaging data on H1. But we shold keep in

mind H9 since knowing differences between sexes is very important, and is a goal from the NIH.

[Susanne] I've heard that ESCs and iPSCs behave differently…

[Dave] There is a lot of literature on that and it is a bit controversial...but its generally hard to find differences. One difference that might be expected is that the iPSC lines might reflect their initial tissue context more.

[Susanne] Anyone else have ideas about imaging? I've heard that ESCs don't maintain pseudo-epithelial grouping (in contrast to hiPSCs).

[Benoit]

[Caterina] Was the comparison of structure of chromatin between H1 and WTC11 done with omics only or also with imaging? Also, I missed the comments on morphology

[Dave] There is not many people who have imaged embryonic stem cells. We have imaged PCNA in ESCs. But I don't think there is much literature on imaging ESCs. The reason everyone is silent is because I don't think people really know.

[Dave] In terms of basic morphology, we would look for the same morphology between ESCs and hiPSCs.

[Yin] Yes, under phase contrast on a regular microscope, we don't see any differences.

[Susanne] We cannot work on any non-WTC11 cells in our institute, but we can help anyone figure out how to collect this type of data. We also have images that are available to compare with. So please contact us if you want assistance / input/

[Dave] the strength of this project is to compare 3 different human pluripotent stem cells (WTC11, H1, H9). Which no one has ever done before. I think this is interesting as a comparison.

---gap ---

[Dave] Not many people are working on cell cycle in human pluripotent stem cells.

Moving on to differentiation

[Susanne] the lineages that people seem interested in-- neuronal, cardiomyocyte, early-diff (EMT like), and any others(?)

[Jian] The computational modeling side of this should stay central such that the analysis can feedback into determining what type of data should be acquired and what should be imaged. So I would like to propose to make sure that we keep modeling central in all this.

[Frank] Yes I hope that the modeling feedback emerges naturally in this context.

[Jian] Can we build something or a group to work on the speed of back and forth between imaging and modeling?

[Susanne] That sounds great. Can you add this to the document somewhere? We all share this desire. Sorry if this wasn't clear in the document.

[Bing] In the first phase of this working group we all discussed the bottlenecks of integration ...Bill did a lot of work to determine what type of data and what type of resolution was needed. Should we go back and revisit this?

[Susanne] Yes! See C1 in the document!.

[Frank] from a technical standpoint I have a question: we had originally thought that these topics might result in subgroups...does that seem like a good idea still with how these are developing? And how can we best coordinate among other groups? For example, if there is a topic that is a relevant to the predictive modeling group, is there a way we can bring them in easily?

[Susanne] regarding Frank's first point: The action item we have is: are there topics we have more or less interest in and who are the people who would want to be in a subgroup for that?

- Is that a topic that should stay on the list or go off?
- Are there people who want to tackle that challenge as a subgroup?

[Yin] I just want to reinforce Susanne's idea that these are teasers that allowpeople to join. So please sign up and start these small groups.
My colleague developed chemical genetic tools to manipulate LLPS. We are collaborating to integrate genome structure, with imaging and omics to manipulate phase separation. We will manipulate Myc to form condensates and look at the changes. This is an area that computational modeling is hugely lacking.

[Susanne] Yin, how would you call that?

[Yin] I would call it "Image Omic and Computational Analysis of Phase Separation".

[Susanne] back to differentiation...how many people are interested in the different diff types? Neuronal?

[Susanne] The Allen Institute is committed to collecting data for early-diff (EMT-like). So there will be data collected.

[Sussane] How much imaging and omics is taking place in the ROCCS (cell-cycle) group?

[Dave] Well we have Nicola, so we've talked about validating some of what we see with super-res methods.

[Susanne] so it might be that this could be worth forming a subgroup to discuss what could happen for cell-cycle in imaging and omics integration
[Dave] that sounds good--there is a lot that can be done…Frank, can you model the nucleus before it is built?
[Susanne] So this is perfect...it sounds like a subgroup could form to discuss the ins and outs of whether this will work.

[Susanne] In two weeks we will start again at point C. And we will give a brief summary of the above points at the start of the meeting.

[many] thanks for putting this all together Frank, Yin and Susanne!

April 23rd

Agenda:

Speakers:

- Caterina Strambio De Castillia

MINUTES (Zoom meeting recording link)

Initial discussion

- Plan to discuss at end of meeting how to move forward with this group
- We are thinking it could be best for this group to potentially break into ~5 subgroups centered around specific projects/goals/interests to improve synergy and move forward.

Caterina Strambio De Castillia talk -- research summary

- Towards community driven standards and tools for improved image data quality, reproducibility and value
- What would you definitively call a spot?
    - (image array of different signal-noise, intensity, background level)
- Rigorous method reporting, optical calibration and detector characterization enables superior, more reproducible analysis.
- Phase 1
    - Define common benchmark biological samples
        - Using the same sample in different labs gave completely different results…
- Imaging vs Quality and reproducibility: “Houston we have a problem”
    - Reproducibility of Cancer Biology Studies (elife 2019)
    - Study of how the morphology and concordance of 25 cellular structures varies with cell shape and volume (Viana et al 2021; from Allen Institute)
        - Both studies show that tracking instrument components and imaging parameters is essential
    - Imaging methods are vastly underreported in biomedical research (elife 2020)
        - Imaging metadata is inadequately reported in so many studies
- As a result, during Phase 1 it was decided to develop the following
    - Data exchange formats for commonly used data types emerging from imaging experiments
    - Community driven Microscopy metadata reporting guidelines
    - Easy-touse software tools to facilitate microscopy metadata
    - Recommended quality control and calibration procedures and tools
- How do we control for confounders and minimize batch effects?
    - Standard operating procedures
        - Cells WG and well designed and controlled experiments, shared sample prep procuedes, share dbiological source material. (and DCIC)
    - Good documentation of data provenance
    - Quality control and calibration
        - MetaMax (characterize illumination and detector)
    - Optical calibration
        - PSF, chromatic aberration, etc
    - Mechanical calibration
- How do we contain all this information/metadata?
    - Files, ome, jason swedlow
- Are there existing standards for light microscopy?
    - Yes, lots of previous standards (ISO)
    - Also OME data model, bio-formats library

- QUAREP-LiMi
    - Includes many groups-- standards organizations, manufacturers and different bio-imaging standards groups and consortia.
    - Tour of website
    - Anyone can join
    - Many different working groups on different subjects
        - Illumination power, detection system performance, uniformity of field, lateral and axial resolution, metadata, etc.

[Susanne] We of course want to combined images with ‘omics. But this talk is so important for us to know that we have a lot of work to do to even be able to properly integrate imaging data from different labs.

[Andy] Something you guys developed in phase 1 was the cell line with GFP-nucleoporins that you could use to calibrate your microscope. We would like to calibrate our microscope so that we can get an estimate the number of GFPs in a speckle. Is that cell-line still being distributed?

[Caterina] As I understand it, from phase 1, we determined that that cell line standard from phase1 was not sufficient/adequate. That standard was not developed by the working group but ws developed by EMBL by Jan Ellenberg. But imaging working group used it and evaluated it as a tools for comparison, but we decided against using it. Other optical controls were proposed as better. Multicolor beads were what people agreed upon as the best standard previously because of the quality /cost ratio.

[Caterina] metrology suitcase is being developed by some imaging standards consortia/groups. This can metrology suitcase can then be shared across labs to be used as a calibration standard.

[Andy] Do you remember why the cell-line didn’t work?

[Caterina] Without knowing the information under which the cells were acquired it was too difficult to use as a standard. But I wasn’t there for this standard comparison. Biut I don’t feel very comfortable saying much more than this since my characterization might not be 100% accurate.

[Bing] The major challenge of sharing imaging data is what level of data to be shared. If we are sharing raw-data the volume of data is simply too big to go across the internet. If we share at the level of spots, though, then its somewhat feasible. We used to have to save raw image data of sequencing, but now we don’t because standard sequencing call methods are trusted well-enough. Can we follow in those foot-steps and develop spot calling mechanisms that enable us to reach the same place where we then only need to share spots and not raw images.

[Nathalie] I just want to share a new preprint in which we are using FCS as a calibration for fluorescence intensity across samples and can then be used to determine number of fluorescent proteins in image. Concentration maps.

[Jian Ma] Some papers that come out recently have raw data available only upon request. And I think that is unfortunate. I think the goal is to come together and change that so that this statement is not made in any papers in 4DN. Spots are very informative, but the raw data is still so valuable, partly because spot calling has so much room for improvement. There are technologies that can make this happen too. Jason Swedlow's group has done these things for example.

[Caterina] I want to encourage people to join the imaging WG so that your voice can be heard in those meetings.
There are tools, like Jian said, to make raw data available. IT's possible but we still need to think about the best way of doing it. The idea of "tier system" used elsewhere in the 4DN could also be applied here potentially.
The imaging metadata and quality control need to be reported--that is most important.

[Nicola] the raw data, at least in the beginning, should be shared. Some of the key processing of the data is done with proprietary software (such as bruker instrument). And that makes things very difficult to reproduce. Developing an alternative pipeline will make this more reproducible eventually. BUt without opens source options, reproducibility is very difficult.

[Andy] A lot of the conversation has been so far about the stochastic imaging reconstruction methods where you are just reporting spots. We do a lot of stuff that doesn't fall into that category. We do a lot with segmentation, and this is difficult because there isn't a single segmentation workflow that can be applied in all cases. Segmentation workflows need to be included in reporting.

[Sarah] I definitely want to second Andy that there is a difference between localization for raw image based analysis. I want to add to what Nicola said. I am very often sitting down with a vendor talking about how software exports to OME-TIFFs. And some of these vendors have enough interest to work with us. Also, some of those bruker proprietary code are actually open source.

[Caterina] I definitely encourage people to talk with vendors and push them to make the information needed be automatic in their software

[Bing] I would suggest, a cloud server might be a good solution for sharing large amounts of data. Cloud based mechanisms have been used to share Terabytes of genomic data…

The plan originally today was to start nucleating topics of shared interests to start some subgroups in this WG. Those of you have thoughts please share your ideas with us via email. In phase 1, this WG was the NOFIC-AICS collab. Next week we will have a discussion around this. And then we will have a couple of weeks of talks that help us restart some of the work in this WG that was begun in phase 1 and is still continuing

[Andy] Quick question: has your group looked at the similarity or lack of similarity between H1 and WTC11?

[Susanne] some people have started to do that. So it might be in the notes from talks in phase 1.

[Bing] I know Bill had spearheaded some of that so it would be worth checking with him.

[Dave] I can say that we have definitely looked into that with the marker paper. But we are just getting started with the actual analyses in that marker paper

[Benoit] It would be nice to know who is comparing H1 with WTC11

[Susanne] Let's have this be a topic!!!

[Andy] When I saw WTC11 speckle images from AICS I thought they looked strange--but then we saw very similar appearance in H1.

April 9th

Agenda:

Speakers:

- Wenbo LI
- Andrew Belmont
- Caterina Strambio De Castillia

Minutes (Zoom meeting recording link)
Chat session

Wenbo Li talk -- research summary

- Core lab interest = RNA-mediated gene control and 3D genome organization.
- Main goals
    - Mechanisms--how does gene transcription really work, with precision in cell type, states, diseases
    - Genetic risk loci 00 can we use new mechanisms to explain noncoding mutations/variants
    - #3
- Motivation to be a part of IOWG
    - Contribute to efforts to connect genomics and imaging within the group
    - Ample other datasets already generated in WTC11
- Expertise:
    - Nascent RNA-seq -- PRO-seq, TT-seq, MINT-Seq, CLIP-Seq
    - Areas for collaboration
        - Optogenetic or chemical control of chromatin architecture/loops.
        - Imaging-omics of regulatory RNAs, enhancer RNAs
        - Live cell imaging of RNAs and RNA binding proteins in cell fate transition
- Overview of lab projects
    - (4DN funded project) -- trisomy 21 and genome organization in neurodevelopment disorder
- How 4DN changes underlie human diseases?
    - TAD border disruption (Hnsiz et al., 2016 for example, and others)
    - Enhancer hijacking (Northcott et al., 2014 and others)
    - Large size genome structural changes, such as those in a trisomy???
- Project plan
    - Conduct series of epigenome, transcriptome and 3d genome assays on iPSC (neural progenitors and neurons)
    - Also interested in studying this in cardiovascular types potentially
- Enhancer project
    - Many many enhancers are transcribed → eRNA.
    - Biophysical basis for eRNA function?
        - RNA sequence, RNA methylation, secondary structure?
        - Mechanisms-- RNA:protein binding? RNA stability? RNA/protein phase separation? 3d chromatin organization?
    - MINT-seq -- methylation inscribed Nascent transcript sequencing
        - A new and high sensitivity method to measure nascent RNA methylation
        - Primarily looking for m6A methylation
        - Modified form of TT-seq
        - Many many eRNAs are being methylated.

- m6A-eRNA recruits an epitranscriptome reader YTHDC1 to enrich at active enhancers
    - Lots of evidence (pull down, chip-seq)
    - YTHDC1 is required for stimulus-induced enhancer activity also for at least some targets
- CRISPR-based erasing of m6A on eRNA to assess effect on YTHDC1 recruitment
    - dCas13 was chosen because it can be used to effectively target eRNA
- HOW? Mechanism of m6A-eRNA and YTHDC1 in augmenting enahcner activity?
    - Phase separation? --YTHDC1 does participate in phase separation condensate formation.
- PROJECT3 -- introns harbo surprising regulatory elements
    - Intronic LINE-1 elements are highly marked by m6A modification in many cell types
    -
- RNA-methylated intronic LINE-1 (MILs) can act as transcriptional bumps in genes
    - These methylated intronic LINE-1 (MILs) are found frequently in brain and kidney (assessed across multiple cell types)
- PROJECT 4 -- nucleoporins in transcription and 3D genome control
    - Area under nuclear pore basket is generally observed to be open chromatin
- Rapid degron of several nucleoporins
    - Found that some regulate transcription directly, but did not affect 3D genome.

Questions:
[Andy] I thought a lot of enhancer elements were short, but you showed an eRNA of 10kb size or so that was also very highly transcribed
[Wenbo] eRNA are quite heterogenous---some are quite long, some are quite short. Median size is probably ~400 nucleotide. Some are more stable than others too. What we studied are likely a subset.
[Lacra] Question about m6A modification--can you recruit the reader protein to any sequence and it will work? Could you recruit YTHDC1 with cas9 and test its effects?
[Wenbo] We don't know currently. We should work on this together :)
[Lacra] which degron are you using? It looks really good.
[Wenbo] We are using HCT116 AID degron.
[Bill] Do you have a model or a guess about what is the function of this eRNA methylation?
[Wenbo] showed slide showing condensate formation/recruitment model
[Bill] have you looked closely at lncRNA interactions in all this?
[Questions in chat too]

Andy Belmont Talk

Untitled
Current state-- wrapping up U54, co-PI on UM1, has a U01 and R01
Focus of talk--original imaging experiments that caused interest in speckles

- Developed Lac Operon system in 2009. Hu et al, JCB (2009)
    - Multi-copy BAC arrays.
    - BAC based systems recapitulate localization of endogenous gene.
        - Beta-globin bac goes to nuclear periphery.
- Heat shock BAC
    - Both decondensation and gene movement
    - After heat shock induction--observed array was wrapping around the speckles.
    - 1-2 um per minute movement (toward speckles). Can move up to 4 um. Seems to stretch as if something is pulling on it (Khanna et al, Current Biology 2014)
        - Red CENPA, light-green speckles, bright-green is plasmid array tandem repeat.
        - PLasmid array is several hundred kilobase region
- Hsp70 BAC transgene induction during heat shock in live cells as function of speckle contact (Kim et al, JCB, 2020).
    - BAC seems to target closely to speckles even before heat shock.
    - red= mCherry-MS2 coat protein
    - Speckle association is important
        - Regions that are near speckles are producing several fold higher RNA transcripts.
- This all was motification for developing TSA seq.
    - Chen et al, JCB 2018
    - Some heat shock genes are always in the top1% in terms of TSA-seq measured proximity to speckles even before heat shock.
    - Some have induced localization that increases upon heat shock.
- Small shifts in SPADs correlate with gene expression changes.
    - A gene that is slightly above 95th percentile in H1 compared to gene that is slightly lower than 95th percentile in another cell type show significant alteration in expression.
    - Small shifts of 10% of genome correlate with gene expression changes
- P53 mediates target gene association with nuclear speckles for amplified RNA expression. (Alexander et al / shelley berger lab) recently published.

    - First sign that something like what is seen in heat shock genes is happening in another system, in this case p53.
- Expanding TSA-seq to map speckle, lamin, nucleoli, centromere association.
    - LAD to SPAD transition occurs sharply.
    - LaminB1 TSA vs SON TSA is inverse relationship scatter plot.
    - Things that move away from speckles move closer to lamins…
- Relative percentages
    - Only 10% of genome shifts relative to speckles
    - 40-60% of genome shifts relative to lamin.
- Lamin-TSA seq vs SON-TSA seq scatter profiles are interestingly different from one cell type to another
- HCT116, H1, K562, HFFc6--staining of nucleoli, speckles and lamin in these cells
    - Speckle and nucleolus organization, shape, size etc look different in these cell types.
- CRISPR/CAs9 knock in of TetO repeat for live cell imaging of endogenous loci
    - See also: Two-Color imaging of nonrepetititve endogenous loci in human cells
- PROJECT#2--Um1 related work
    - Mid-S to late-S movement of gene-amplified chromosome arm from periphery to nucleolus and bad. (Li, sudlow, belmont 198)
    - Do specific regions of the genome show these types of movements?
- Project#3--U01
    - Identify nuclear “dark matter” -- compartment(s) between chromatin???
    - Looking at proteins, genes, mRNAs and movements between compartments.
    - Speckles move in chromatin depleted regions (Kim et al 2019)
    - What speckle correlated proteins occupy these chromatin depleted regions?
    - New compartments?
- Project#4--high slope TSA-seq and high slope laminB1
    - Decondensed?
    - They did FISh and showed that they are extended (?)

[Susanne] In the p21 plots, how do you make sense of the other patters?
[Andy] It looks to me that the regions that are near speckles to begin with are activating faster, and the regions farther from speckles are increasing more slowly. So over time you see an increase in the far.

[Huaiying] We are looking at PML movement in telomeres. From your movies...the speckles are moving--but who moves to who? And what drives movement?
[Andy] with the plasmid study the most common thing is to nucleate a new speckle. Or the plasmid array moves to the speckle. It’s rare for the speckle to move to the plasmid array. In the BAC array study, we find the BAC moving almost exclusively. No speckle movement. And when we observe a speckle move--it looks like the moving speckle is the speckle that is

associated with the heat shock BAC. We also don't see long-range directional motion until after heat shock.
The motion could be blocked with nuclear actin or nuclear myosin inhibitions? [I believe that is what is said]. So they suspect some actinomyosin directed motion.
[Huaiying] That is very similar to DNA-damage related motion.

Friday, March 26th

Agenda:

Speakers:

- Lacra Bintu (presentation)
- Benoit Bruneau (presentation)

MINUTES (Zoom meeting recording link)

We will have a couple more meetings with lightning talks and then readjust in the future
Please check lighting talk sign up sheet and sign up!
Schedule for lightning Talks

Lacra Bintu talk

Measure perturb and model 4D chromatin associated with different dynamics of epigenetic memory in single cells

- Understanding and controlling the mammalian input-output transcriptional function
    - Measure and manipulate
- Ongoing projects
    - What are the rules of chromatin spreading?
    - Use nanobodies for chromatin-mediated gene regulation
    - What are the protein domains in human cells that can silence, activate and impart epigenetic memory?
- Todays topic: We built a synthetic reporter system to systematically measure the effects of chromatin regulators -- how much, how fast, how permanent?
    - Basic system published in bintu et al 2016 Science.
- EED (H3K27me3) turns genes off but at different times in different cells. Time in DOX alters the fraction of cells off. Release of DOX leads to an increased fraction of cells turning back on (again appears binary)
- They looked at EED, KRAB, Dnmt3b, HDAC4

    - For all of them they see on/off style behavior...but different strength (fraction of population), timing and memory
- Now they have expanded to test way more different epigenetic modifiers. Published in 2020 Cell (Tycko et al, 2020)
- Recruitment time or strength also affects the duration of memory (this was shown in Bintu et al 2016)
    - You can silence all cells in 1 day with KRAB domain. But only 10% will remain silenced (memory) after 10 days
    - However if you recruit KRAB for 5 days, then 50% will remain silenced. Memory
    - This can be modeled with a three-state system active←→ reversibly silent → irreversibly silent.
- What type of 3d chromatin structures are associated with different durations of epigenetic memory? [Is there a different structure for reversibly silent compared to irreversibly silent?]
- To measure chromatin structure--use ORCA (collaboration with Alistair Boettiger)
    - ORCA = Optical reconstruction of chromatin architecture // sequential hybridization walking along the chromatin fiber.
    - They plan to adapt the method to image with epifluorescence microscope so it can be integrated with time-lapse?
    - Track live, then fix, then image the 3d structures. They can then correlate 3d structure with the time a gene has been silenced.
- They also want to do this type of experiment (track then fix then image again) in differentiating WTC11 hiPSCs.
    - Possible lines
        - hiPSCs Sox2-GFP (Allen Cell Collection line)?
        - Dox-inducible neurogenin 2?
        - Other suggestions?
    - Also what are the best loci to look at in these processes?

[DAVE] Question: What is the half-life of the GFP-SOX2?

[Lacra] we don't know that yet. For our reporter system in previous publications we used H2B-mCitrine. But yes protein lifetime is very important. But our timescales are also very long in these experiments so its not so concerning for us..

[DAVE] A lot of people doing this with artificial reporters use an unstable FP to keep the timescale quick.

[Dave] Another weird thing is that Oct4 always seems to hang around long in differentiation experiments. Not sure why?

[Benoit] Oct4 is involved in other aspects of downstream differentiation.

[Benoit] Susanne you guys have a CRRIPRi with KRAB domain. I think that would be my approach to recommend for you Lacra. Then you can disentangle from the differentiation changes that might be occurring.

[Andy] How did you insert your reporters ?

[Lacra] in my postdoc work, we used a Human Artificial Chromosome. We inserted single copy in a landing pad region. The new system we have is AAVS1 with TALENs into the safe harbor locus in human cells.

[Andy] ONe thing to keep an eye on is that you can get different results depending on location in the nucleus/chromatin. So if you change location do you get the same results?

[Lacra] The rate of silencing and degree of memory does appear to change with cell types and loci. Do you have suggestions of other loci to explore?

[Andy] We are using a system where we can create lines where you can integrate into specific locations. (Talk with andy or dave for this system)

[Dave in chat] Putting AttP sites into strategic WTC-11 sties would be very interesting for this work and important for stem cell engineering applications in addition to basic questions of the basis of position effects.

[Wenbo] When you do the memory experiments, how much do you think the memory is affected by inability to completely remove the epigenetic modifier?

[missed Lacra's response]

[Dave in chat] We (or Andy I'm sure also now) can provide a construct that allows for promoterless selection after the first HR integration. So one crispr/HR and then after that positive selection for site specific integration without crispr.

Benoit Bruneau presentation

Genetic determinants of 4D genome folding in human cardiac development.

- Dynamics of cardiac differentiation
- Aim1: reference dataset across time course of cardiac and endothelial cell differentiation
    - 4 time points

        - Pluripotent, cardiogenic mesoderm, diff''ing cardiac precursors, cardiomyocytes
    - WTC11 reference line
    - Micro-C, Hi-C, Hi-ChIP
    - Matched epigenomic datasets
- Tbx5 and congenital heart disease?
    - Heterozygous mutations in human cause congenital heart defects (hole in heart and)
- Tbx5 ips cell model of congenital heart disease
    - They look at differentiation in mutant cells and find genetic interactors that affect cardiac development
        - PUBLISHED
    - H3K27Ac HiCHIP shoes new connections forming over time and different connections forming due to the Tbx5 mutations.
- Also looking at other chromatin remodeling factors (BRM)
    - BRM controls cell fate
    - The cells make normal mesoderm but instead of making CTnT positive cells they make neuronal cells.
        - They see alterations in chromatin accessibility at key TFs and alteration in chromatin architecture (hiC)
- Aim2: 3d organization of iPS cell models of congenital heart disease
    - Tbx5, Wdr5, GATA4, BRM, CTCF
    - All will be evaluated in time course of differentiation
    - HiC3, Hi-CHIP and matched with scRNAseq, ATACseq etc.
- AKITA: predicting 3d chromatin folding from sequence (fudenberg et al, nature methods 2020)
- Predicting chromatin folding in CHD
    - Compare control deletions vs CHD deletions
    - They find CHD mutations are more disruptive overall in 3d structure
- Aim3:
    - Build cardiomyocyte and endothelial specific Akita models based on iPS cell diff time course
    - In silico mutagensis of patient and synthetic mutations to predict most disruptive
    - Interpret the grammar of normal and disruptive folding
    - Validate predictions in iPSC derived cells with engineered deletions by Capture hiC.

[Andy] we are looking at lineages and where to stop and get homeogenous populations. Do you find precursors are pure state?

[Benoit] Its fairly pure--although not synchronous. The mesoderm stage is probably the most homogenous, but we are in mouse so I can't comment directly on human.

[Susanne] We are doing some of this analysis in our cells with scRNA seq also. We've done scRNA seq on cardiomyocytes at different stages (day 12 thru day 36). But we've never looked at changes in 3d genome organization. Are all the main changes you see in hi-C maps already changed? Or are they changing? How sensitive are these details to timepoint?

[Benoit] WE have not analyzed that time course yet. But Bing's lab published on that using H9s. If I recall tho, most of the changes happen during differentiation but not during the maturation/aging of cardios.

[Benoit] 20% of non-cardiac myocytes can still be in the dish from [?? fetal cardiac myocyte diff ?? missed this]

[Benoit] It would be nice to get your protocol Susanne. We are using a commercial kit.

[Susanne] Our protocol is on our website https://www.allencell.org/uploads/8/1/9/9/81996008/sop_for_cardiomyocyte_differentiation_methods_v1.2_200210.pdf

[Andy] We found BRM as a hit in drosophila study, can you tell us more about what you see in BRM defects?

[Benoit] We haven't really taken a deep dive into BRM yet and the specific 3d changes.

[Jian] I have I quick question about analyzing insertions rather than deletions using AKITA. How frequent are insertions mutations?

[Benoit] Insertions are not common. But inversions and duplications are observed. We focused on deletions only first. But those other variants are going to be interesting. You are right.

Friday, March 12th

Agenda:

Speakers:

- Yin Shen
- Alistair Boettiger

MINUTES (Zoom meeting recording link)

Yin Shen lightning talk

Functional Genomics Resources of WTC11

- WTC11 iPSC background
    - Derived from healthy donor, episomal methods with genomic data sharing consent
    - Whole genome sequencing available
    - Complete genomic phasing by Haplo-seq (Dixon and Shen labs, Song et al., Nature Genetics, 2019)
- WTC11 derivative cell lines
    - CRISPRn and CRISPRi lines for genome and epigenome editing
    - Published in Mandegar et al., 2016 Cell Stem Cell
    - Unpublished lines
        - WTC11 CAG-dCas9-krab knock in at CLYBL locus
        - WTC11-G3 (Ngn2) CAG-dCas9-krab knock in at CLYBL locus
- WTC11 differentiation system
    - rapid , robust, and synchronized differentiation
        - WTC11-NGn2 inducible --derive excitatory neurons (Wang et al 2017)
        - WTC11-LMN (ISL1, LHX3, Ngn2) inducible -- derive lower motor neurons (Fernandopulle et al 2018)
    - WTC11 growth factor induced microglia (Douvaras et al 2017)
- WTC11 reporter lines
    - Different from Allen Institute for Cell Science reporter lines
    - GFP or mCherry knocked in to monitor transcriptional levels. (usually has P2A cleavage) --these lines are available but not published.
- Genomics datasets
    - RNA-seq, ATAC-seq, pcHiC, Cut&RUN and H3K4me3 PLAC-seq in different cell lines. Some published and some unpublished.
- Coordinated functional characterization work in ENCODE consortium
    - WTC11 common loci (pluripotent genes)
    - WTC11 excitatory neurons (essential genes)
        - MPRA (massively parallel reporter assay)
        - CRISPR screens
    - Expected outcome: a catalog of functionally validated cis-regulatory elements in WTC11 and WTC11 derived neurons.
- Collaboration ideas
    - Biosample sharing (cell lines and reagents)
    - Providing datasets for integrative analysis
    - Sharing the information related to 3D regulatory interactions and CRE (cis reg) function
    - Differentiation system used for both imaging and sequencing analysis

[Benoit] we are deriving cardiomyocytes and soon endothelial cells. Also engineering deletions in WTC11 to alter 3D organization of genome. These lines are available.

[Susanne] Can you give a lightning talk so everyone can see this in slides?
[Benoit] Yin, it would also be really useful to know the protocol for the CRISPRi (how guides are delivered for example).

[Lacra] has anyone done CHIP-seq on WTC11
[Yin] Encode has done CHIP-seq on WTC11 (data coming soon???)

[Lacra] are there genes that people want to see how they change during diff in live cells?
[Yin] We tagged proteins using p2a (or t2a?) so you can't assess localization...but only transcriptional activity.

[Alistair] Is there a central repository for all the WTC11 tools.

[Susanne and Yin] I think we should definitely do that.

Data portal link: https://data.4dnucleome.org/4DN-AICS-Collaboration

[Frank] Lets expand that landing page to have a data section and a tool section.

[Susanne] Dave, Does Cells WG have a way of coordinating this?

[Dave] We have a google groups where people post this information including relevant validation data.

[Susanne] Is it organized cell line by cell line?

[Dave] If there is differentiation SOPS, we will post them on our wiki. We can also help negotiate reagent pricing for common reagents. The page is organized by whoever posted it first. The first person who posts has to post a very descriptive title so everyone can find it from there. Let me post the link so everyone can see it.

[Bill] I added the data availability links for this working group and for the encode data.
ENCODE data link: https://data.4dnucleome.org/joint-analysis

[Susanne and Bill] Getting one spot for all of this would be great.

Alistair Boettiger lighting talk

- Cell type specific genome folding
    - Structural differences between different genome regions
    - Structural differences between cell types
- Integrating Imaging + Omics

    - This imaging is a method of omics :)
    - These are not exclusive catagories
- Integrating IMaging + Next Gen. Sequencing
    - Third leg “T3 cells -- average distance in nanometers, change relative to silent cells
    - Abdomen “A1” cells and “A2” and “A3” cells
    - How do changes in epigenetic marks and 3d structure shape one-another and effect cell memory?
- Highly multiplexed Tissue analysis
    - Barcode tissues ahead of time before mixing them all into the same experiment.
- Integrating Imaging + Computer simulation
    - Finding predictive relationships in data with machine learning
    - Simulating polymers folding mechanism to compare distribution and distances (in addition to normalized contact-frequency)
    - Simulating the microscopy process itself to improve image analysis methods against a known ground truth polymer.

[Susanne] I want to ask you about the process for making a ground truth validation simulated microscopy image. What is your plan for this?

[Alistair] I suspect we have an easier challenge. There is a chain of point sources. We assume its a single chain of point sources although we know it is a pair of sister chromatids often. We are working

[Susanne] do you do this with 3D convolution with PSF?

[Alistair] we have only done it with forward modeling of convolution with PSF. But I think the deep learning approach could do a much better job.

[Susanne] Cool. I would like to talk more about that. This is such an important discussion for validating imaging data.

[Dave] Quick comment: on the subject of cell memory. In stem cells if we knock out RIF1 we lose the replication program. The epigenome is affected downstream of this. We are now diff’ing these cells to see how this disruption affects that process. Have you seen this story from us yet?

[Alistair] not yet

[gap in notes … apologies]

[Susanne] Chris and Brock volunteered to help to coordinate with 4DN OH and DCIC to get a WTC11 resource page (central location to list all of the data, diff SOPS, and tools available for WTC11.)

Frank presenting on mission statement.
We can designate a time in one of the next sessions to discuss the details and edits.
Questions and comments?

[Alistair] I'm wondering if there is an outreach to the pertubations group? We would love to have more degron lines in WCT11

[Frank] That would be great.

[gap in notes … apologies]

[Andy Belmont] I was struck some years ago in a talk by Ana Pombo--they see much longer range contacts in terminally differentiated neurons. Yin, ho hard is it to get these diff;d neurons? Can you get 10,000+ cells?

[Yin] Yes we have great success. We can get 100,000 to 1,000,000+ cells in our hands.

[Andy] one of the things we were shocked by was how conserved the distance to nuclear speckles is for all the cell tupes we have analysed. But these are all proliferating cells. So we would love to analyze these diff'ed cells to see what is different.

Friday, February 26th

Agenda:

Speakers:

- Jian Ma
- Mario Nicodemi
- Ming Hu

MINUTES (Zoom meeting recording link)

6 lightning talk slots are still available! One for next meeting

Jian Ma lightning talk:

- "Integrative analysis method for nuclear organization"
- Methods to identify compartmentalization states
- Important sequence elements and trans favcors

- Cell to cell variability
- SPIN (inferring spatial localization patterns): lamin and HiC + TSAseq as input, identify compartmentalization state
- CONCERT, prediction gDNA replication timing from sequence, machine learning based, predict repli-seq signal [in preparation]
- Integrative sc HiC analysis - Higashi (machine learning), study cell-to-cell variability. Input transformed into hypergraph (hyperedges), generate embeddings for scHiC data, from raw data to characterization of genome structures. Apply to WTC11, identify compartments, log2 of transcription variability [effects on function]. Also, scHiC from human prefrontal cortex.

[Frank] clustering → different compartments
[Allstair] Bias in the algorithm. If bias is homogeneous, different ot identify; if different technologies, bias can be removed

Mario Nicodemi lightning talk:

- Principled approaches from polymer physics into mechanisms shaping 3d structure and determinants: Strings&Binders (SBS) model: polymer chain with binding sites for diffusing molecules. TD phases identify the accessible configuration classes, coil v globule.
- Model predicts contact patterns, from polymer phase-separation (toy model, EPHA4 locus, WT and mutation)
- Compare locus superresolution microscopy and SBS model to gain insight on folding at single-molecule level: all v all comparison (experiments, coil and phase separate globule) using RMSD, SBS model conformations are a bona-fide representation of microscopic structures
- Variability in single-molecule conformations results from TD folding degeneracy; different binding sites correlate with distinct combinations fo epigenetic factors.

[allstair] Entropy v Energy, loop extrusion is non equilibrium, SBS is equilibrium (spontaneous). Can you match model and experiment, significantly and across a number of different features?
[Mario Nicodemi] Multiple mechanisms may coexist; also, how about mechanical features of chromatin?
[A Belmont] Intrinsic folding associated with histones (old literature), how does it fit in SBS? Binders loaded on the chromatin (like, direct interaction between histones): binders floating around are one way of implementing interactions.
[Mario] either by direct of mediated interactions, phase separation is universal

Ming Hu lightning talk:

- Preliminary sc HiC analysis in WTC11 (predict TADs and compartmnets)
- Sequencing depth: each cell, 00.9M intra reads, over 5k inter reads

- SnapHiC: convert matrix on each single cell from raw count into normalized contact probability, raw data (binary) → contact prob imputation w RWR → contact prob → distance stratified normalization → normalized, identify candidate chromatin loops
- SnapHiC identifies >5k loops from 188 cells, 10kb resolution
- Aggregated peak analysis: bulk v sc HiC data, HiCCUPS, SnapHiC loops
- Ex: EXT1 gene
- Future directions: Interagrate SnaphiC with Higashi, with scRNAseq from WTC11, with imaging data from WTC11

[Frank] Frequency of loops, that's related to cutoff
[Jian] Use analysis data to identify suitable locus candidate for further investigations

Discussion:

Shift from four to three talks
Updated mission statement left to discuss

Friday, Feb 12th

4 Lightning talks and discussions; each 5 minute presentation + 5 minute discussion

Schedule for lightning Talks

Speakers

- Bill Noble
- Dave Gilbert
- Susanne Rafelski
- Frank Alber

Feb 26 on the lightning talk sign up list is empty still -- pleas sign up!

MINUTES: (Zoom meeting recording link)

Bill Noble lightning talk:
- Posson-based algorithm for stable inference of DNA structure. (PASTIS)
    - Statistically rigorous and can model diploid genomes.
- MMD-MA algorithm --manifold alignment algorithm for aligning different datasets (scRNA-seq, scATAC-seq, scHi-C)
    - Should also be able to integrate with imaging data
- SCOT also performs multimodal single-cell integration (another mapping method for integrating different data types, but has fewer hyperparameters)

- What he hopes to get from working group:
    - The idea that we could try to combine imaging with sequencing data using this alignment algorithms his lab has.
    - Second, if you have a model like PASTIS--how do you take 3D imaging data and then align it with PASTIS-generated data to see how they align.
    - Generalizing PASTIS to work on single cells.

[Susanne] Do all the models work on the same type of data? That is if you compare your models to Frank's or Jian's models/methods.
[Bill] We do a consensus model--its not making a biologically complete picture of a population of cells--its making a median or average structure.
[Susanne] How does Jian's fit in?
[Jian] We have spent quite a bit of time on scHI-c. We have an approach for enhancing signal/quality of scHi-C (data imputation). As well as feature calling. Having the data processed in this way could make it more amenable to these different approaches.
[Susanne] This kind of discussion is what I hope will follow from these talks. So we can really see how to synergize best. Bill, for example, we have a new method for getting an average cell with average location of all structures that could synergize nicely with your approach.

[gap in notes here at 9:15 am]

Frank Gilbert Lightning Talk

- Replication drives the epigenome and 4D nucleome.
- High resolution repli-seq data.
    - Replication is really quite deterministic with some heterogeneity
    - We see more than just A/B compartments illuminated when we look at replication foci. These are structural domains of chromosomes.
    - Replication is the ultimate imaging/'omics liaison. :)
- Cis and Trans control of this Liason.
    - Early Replication Control Elements
        - You have to delete all of them
        - Upon deletion the region switches from early to late and then moves its spatial location also.
    - RIF-1
        - RIF-1 knockout leads to disruption of replication timing
            - Replication timing is upstream of epigenetics and organization.

        [Jian] It looks like there is still so*me pattern in RIF1? What do you think that could be?*

        [Dave] Good question. Not sure.
    - Alignment of replication initiation zones with nested corner dot TAD boundaries requires cohesin.

        - Not a ...
        - Rad21 focuses the timing of replication
- Imaging-- replication foci at high resolution in living cells (Jan Ellenberg study)
    - Replication domains are stable structural units
- Imaging-- stain early replication red and late replication green and then watch these domains reform after mitosis in G1.
    - They could isolate nuclei at different points in G1 and found that if you collect before a certain time, you get replication timing disrupted.
    - Timing decision point---the time at which in G1 that replication timing becomes re-established
- Suggested Dppa region as one to study in WTC11 cells.
    - Large scale reorganization of the Dppa domain during differentiation (ESCs -- early replicating // NPC--late replicating)
        - The domain moves to the lamina.
    - What would be cool is to identify ERCEs in human embryonic stem cells and then try to delete them and observe.

[Lacra] Does CTCF affect replication timing?
[Dave] CTCF data needs to be revisited to really say that with confidence. It could be altering initiation location?

Susanne Rafelski Lighting Talk

- First want to introduce the institute so you can have a knowledge of what we do and what type of work we will do in the next years.
- Holistic approach to understand cell organization and cell function
    - Imaging structures and organelles and working to integrate other data types
- Single cell imaging and analysis --the new spatio temporal omics
    - This is where we are working to make workflows and analysis and tools
- We can only work with hiPSCs and their differentiation derived cell types.
- Deeper dive into the nucleus:
    - What are the key nuclear structural landmarks--is there a refernece system?
    - How does the 3D genome fit in?
    - How does nuclear organization vary from cell to cell? Through time? From cell state to cell state?
- Many cell lines expressing fluorescently tagged nuclear structures.
- Example of how imaging and omics can be combined
- Example of integration in time (imaging of replication foci via GFP tagged PCNA)
- Final slide showing status of our lines, their availability and their imaging data availability

[Frank] how far along is the imaging of cardiomyocytes? Is that feasible in phase 2?

[Susanne] its not something we are doing in a global way or a pipeline way. Its very huge amount of work and often very difficult to get them to cooperate on glass. Not reproducible enough for a large-scale pipeline, but its fine for smaller datasets. We have some beautiful initial data looking at how things change during differentiation.

Frank Alber Lightning Talk

- Integrating omics and imaging data
    - Multiple datasets of genomics (hi-C, DamID, Sprite, HIPmap, TSA-seq, scHiC)
    - Use scHiC to prime initial structures for modeling using the population level datasets.
    - Now using imaging segmentations from Allen Institute for Cell Science to perform modeling within segmentations--initial dataset is nuclear envelope, nucleoli, nuclear speckle segmentations.
    - OUTCOMES OF THIS APPROACH:
        - Improve the accuracy of models
        - Increase the nominal resolution of imaging by inferring missing chromatin regions not imaged
        - Study the influence of nuclear shape on genome architecture
        - Identify functional state of single cells from genome models
- This approach is formulated as a probability maximization problem
    - Data-driven genome population modeling
    - Modeling a population of structures to reproduce bulk/ensembl data.
    - No closed-form solution--its iterative optimization with addition of restraints.
- Model Quality has quite good predictive value (examples from other cell lines)
    - The models can predict data not used for modeling--such as Gpseq (radial position), Speckle location via TSA-seq.
    - Integrating multiple data types improves accuracy of predictions.
- 3D structure-function relationships.
    - They can predict cell-cell variability of different regions and lots of other structural features
    - They can also look at how these structural features are distributed along different chromatin states (PCA subcompartments or SPIN states)
    - This can be used for prediction of nuclear bodies, structure function correlations, and comparative genome structural analysis
- Projects
    - Cell differentiation, Cell cycle, Study time-series data from live-cell imaging

[Alistair] Can you use the cases where the model is struggling (e.g. areas where there is more degeneracy--multiple solutions are possible nothing specific gets predicted) to

determine what types of regions we need to image or data to collect to then better model these regions and accurately constrain them in models
[Frank] That would be very useful and very interesting.
[Susanne] That is great. I really like that idea.
[Jian] I think it will be great to think about what data can be further incorporated to add to improve models (such as chromosome tracing data).

Friday, January 22th

Agenda

1. Brief overview of phase 1 NOFIC-AICS working group
2. Discussions about the general direction of the working group
   We want to discuss a proposal to expand the scope of the working group to the integration of imaging and omics rather than a focus on one specific cell type.

   Mission statement proposal:
   Integrating Imaging and Omics Working Group (IOWG)

   The working group's efforts will be devoted to developing an integrative framework for combining various imaging modalities—in particular live-cell imaging—with single cell and ensemble genomics data to study the changes in global cellular organization during biological processes, such as cell cycle, differentiation or perturbations. An explicit goal of this working group is to provide a platform and foster interactions between researchers from different disciplines—imaging, genomics and computational modeling—to work jointly on this common goal. The working group will develop strategies for data integration, common practices and potentially coordinate joint efforts in data generation for the benefit of a joint project.

   The working group is a continuation of an effort in phase 1, which focused on WTC11 cells as a target system to integrate live-cell imaging with genomics data for studying the temporal dimension of 3D genome organization. The goal was to understand the principles by which cells reorganize in 3D space as they move from state to state in response to the cell cycle, differentiation, or changes in environment. Although WTC11 will still be a primary target, the working group will foster all projects devoted to the integration of live-cell and other imaging modalities with omics data.

Minutes: (Zoom recording link)
Meetings will be recorded (link to recording should go here and on wiki)

(Bing) *Just want to paste what I wrote in the chat window -*

1. *The main goal of this working group could be to break barriers in the integration of OMICS and Imaging datasets, for the ultimate goal of generating predictive 3D models of chromatin.*
2. *Deliverables - a). generating imaging and omics data in a coordinated manner and for a common cell system; b) developing strategies and evaluating existing ones for integrating the two modalities.*

Proposal for the direction of this working group. (Presented by Susanne Rafelski)

- History of NOFIC-AICS collaboration working group.
    - The group was primarily about integrating genomics data with imaging data.
    - WTC11 was chosen as the common line to use for these purposes.
- In phase 2, this working group was renamed WTC 11 WG. Kept the same mission statement as before.
- The point people/organizers realized this group would be stronger if it was broadened to include more lines than WTC11, and retained the central mission of integrating 3D imaging with 'omics data to understand dynamic nuclear organization in mammalian cells.
- Proposal is to change the name and mission statement. (mission statement is included above in the agenda).
    - One major reason is that no other working group addresses the integration of imaging and omics in general.
    - This WG would enable overlap between disciplines which is not occurring significantly in other WGs.

Discussion:

[Olivier]: Something that is different in phase 2 compared to phase 1 is beginning to look at biology and disease. Two questions:1. might we want to consider looping in people interested in using WTC11 to diff cells into different systems? 2. Also, how feasible is 3d culture imaging?

[Susanne]: Great questions. First, other stem cell lines could be used for differentiation and it would be valuable to have them included. Second, 3D imaging would be great. We are not actively pursuing it at AICS, necessarily. But its definitely possible in WTC11 and in other cell lines.

[Benoit]: Should we standardize differentiation protocols?

[Dave]: The Cells WG is handling these type of decisions. In phase 1 we decided standard conditions. Now we want to make it so groups who are interested in a specific differentiation process would get together and decide the SOPs and then report back to the Cells WG.

[Dave]: Would having other cell lines detract from the focus of this group? Are we trying to standardize methods? We need a group to combine imaging and omics, but will that cause this group to be too severely diluted in focus.

[Bing and Bill in chat]: WG don't need to establish standards.

[Olivier]: Working groups should have deliverables.

[Caterina Strambio]: I am currently working on the imaging WG. It would be great to have a working group that talks about integration of imaging with 'omics. We could have an interest group to discuss new ideas, and a WG to tackle more practical issues.

[Bill]: The most useful aspect would be looking at comparisons of different methods for integrating omics and imaging.

[Job]: A working group needs to be somewhat limited in scope to be honest, in order to accomplish the goals. For instance if you want to integrate imaging and omics, you need to have the data. It's a challenge to get a richness of data for any cell type to actually do this type of work. That is why WTC11 is so good. I worry if you open it up to too many types of cells, then you might get bogged down by too many instances of different cell types with datasets that are either too limited to actually accomplish integration in a meaningful way or they are different types of imaging or genomics.

[Bing]: This working group could be a super WG that brings other groups together to discuss how to break the barriers in integrating these two very different data types. The solution is to generate all of the data we are interested in within one cell type to so it can all be compared in a standardized way. This also allows us to generate data in a coordinated manner. Having data alone is not sufficient -- we actually have to work together to develop new methodologies. Hopefully this working group will generate these kind of discussions to occur. We absolutely need a working group like this to accomplish this. And I am arguing the WTC11 WG is positioned to break this barrier--and I think changing the name makes sense. But it does not mean we should abandoned WTC11. Using WTC11 is one of the most powerful parts of this group because of the powerful way it allows for comparison and working together.

[Jian] WTC11 does seem to be an ideal system. There could be good interface with the predictive modeling working group. The data, the expertise, and resources from this working group could facilitate really good interactions with other WGs.

[Caterina]: The idea of changing the name is good, even though I think we agree WTC11 is the place we want to start for collecting the data. This will enable others who are interested in this integration to join the conversation even if we stay tightly focused on wTC11 initially. Also, its worth noting that WGs can evolve over time.

[Caterina]: The second aspect I want to talk about is that it would be great if we could coordinate how to use the standards…
There is a common metadata proposal being developed that AICS and other 4DN groups will start using.

[Susanne]: Nathalie will probably join the imaging WG…

[Caterina]: ok great!

[Susanne]: What we would be creating as an output is to look at all the datasets we collect and try to integrate, and then share with others what are the minimal sets that we find are usable for integration so others have direction in which data to collect.

[Dave]: It seems like there is a consensus building here: 1. We all agree on changing the name to broaden the interest and include more people. 2. We all seem to believe that focusing on WTC11 is the best way to start. And then organically as the years go on, there will be differentiation and other systems and those can develop as the years go on.

[Susanne]: thanks Dave. Is there anyone who disagrees? Job?

[Job] I think this works absolutely. My only worry was that opening it up too wide means you get too few data sets and data types for integration. One other thought is that within the current budget structure there are funds for collaboration… so there are opportunities to see further data sets get collected through funding collaboration.

[Susanne] Shall we vote? How do we do that in a WG?

[Dave]: Silence means yes :)

[Dave and Susanne]: We propose to have 10 minute talks (5min presentation+5min discussion) with one person per “research group” representing best what they want to get out of this working group so we get to know their goals etc. We will have 5 talks per WG meeting (1 hour) so we have time at either end to recap/ask questions/chat. We will have these presentations over the next 2-3 meetings. The goal would be to hear from groups who have already been in this WG from phase1, those coming in new to this WG in phase 2 and those who are interested in WTC11 or integration of imaging and omics. We want to get to know everyone who is working on WTC11 data and integration and those interested in seeing where these integration efforts are headed.

[Susanne]: Chris Frick (scientist at AICS who will be helping to coordinate this WG) will reach out to you to get these 10 minute talks organized.

[Susanne]: Also please let us know who should be or might want to be part of this WG so we can gather that together and loop in everyone.

Friday, January 8th (First WTC 11 meeting)

Agenda

1. Analyzing the replication timing of repetitive regions (Nicholas Skvir / Neretti lab)
2. Discussion of mission statement

Minutes: (recording)