

# Integrating Imaging and Omics WG

The working group's efforts are devoted to developing an integrative framework for combining imaging modalities 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.

Co-chairs: Frank Alber, Susanne Rafelski, Yin Shen

IOWG-IWG Inter-WG partnership

**Development of a Nuclear Common Coordinate Framework (CCF) and integration with the HuBMAP Human Reference Atlas CCF**



Frank Alber  
UCLA



Susanne  
Rafelski  
AICS



Yin Shen  
UCSF

4DN Integrating and  
Imaging and Omics WG



Lacia Bintu  
Stanford



Caterina  
Strambio DC  
UMass Chan

4DN Imaging  
WG



Quan Zhu  
UCSD



Bogdan Bintu  
UCSD

Benchmarking  
Datasets



Katy Borner  
Indiana U.

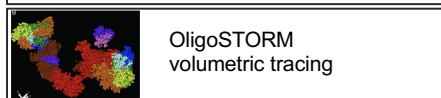
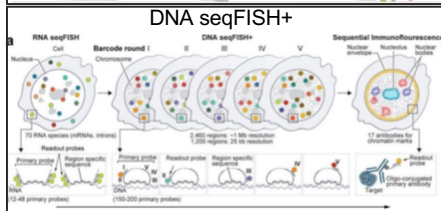
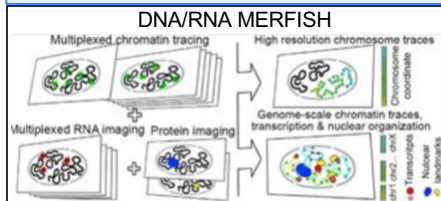
HuBMAP HRA  
CCF



# Development of a Nuclear Common Coordinate Framework

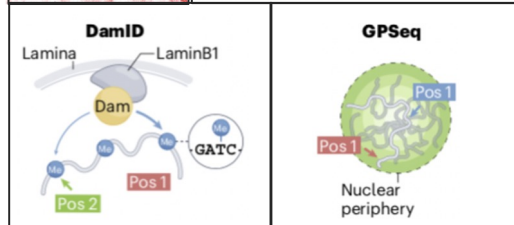
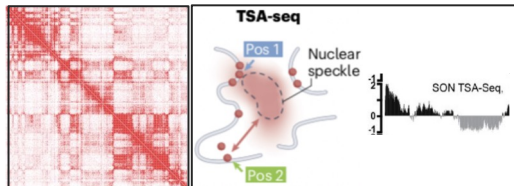
IWG  
FISH-Omics  
Format for  
Chromatin  
Tracing

**Imaging  
FISH-Omics**  
(multiplexed FISH)  
and other imaging



etc

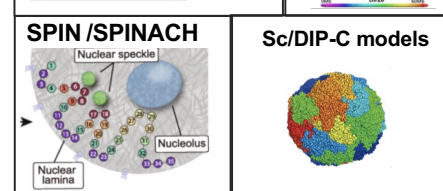
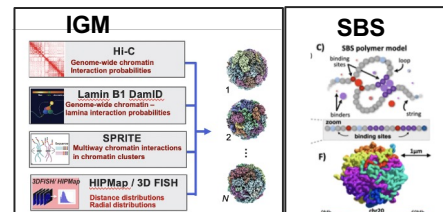
**Single cell and bulk  
genomics data**  
Cytological mapping data



NAD-seq, SPRITE, etc.

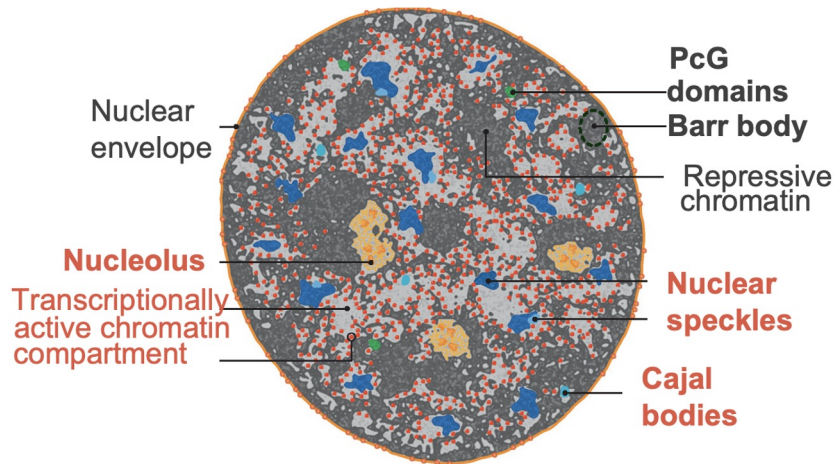
etc

**Computational  
models and  
methods**



etc

# Why do nuclear coordinates matter?

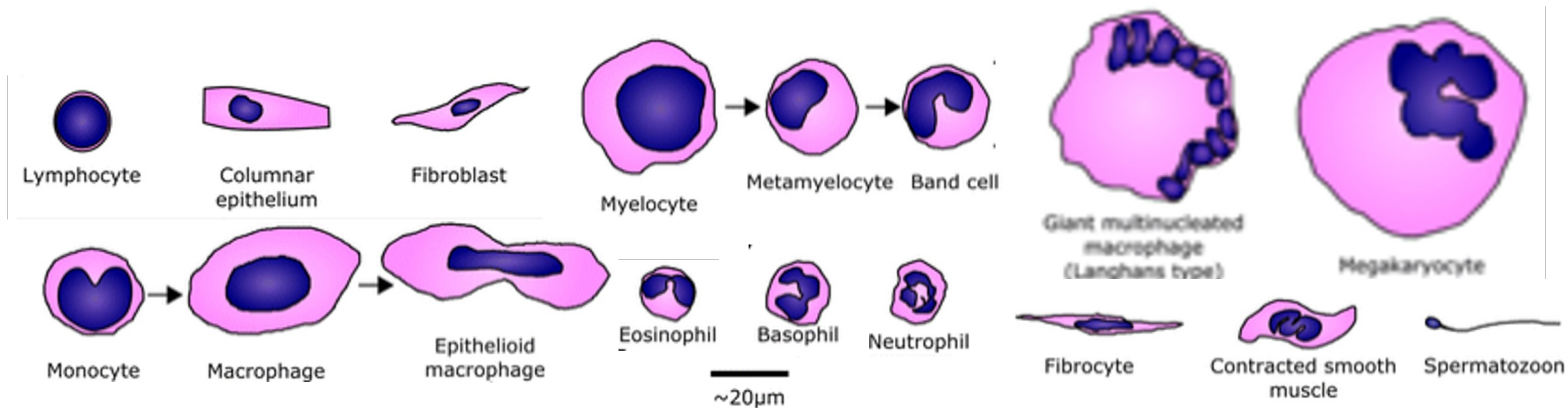


Caudron-Herger et al., Curr.Op.Gen.&Dev. 2012

The nucleus is organized into functional compartments. The spatial proximity to nuclear compartments and nuclear bodies matters.

# Why do nuclear coordinates matter?

The nucleus **varies widely across cell types, tissues, and differentiation states**. This affects the overall shape, size, and internal nuclear compartment organization.



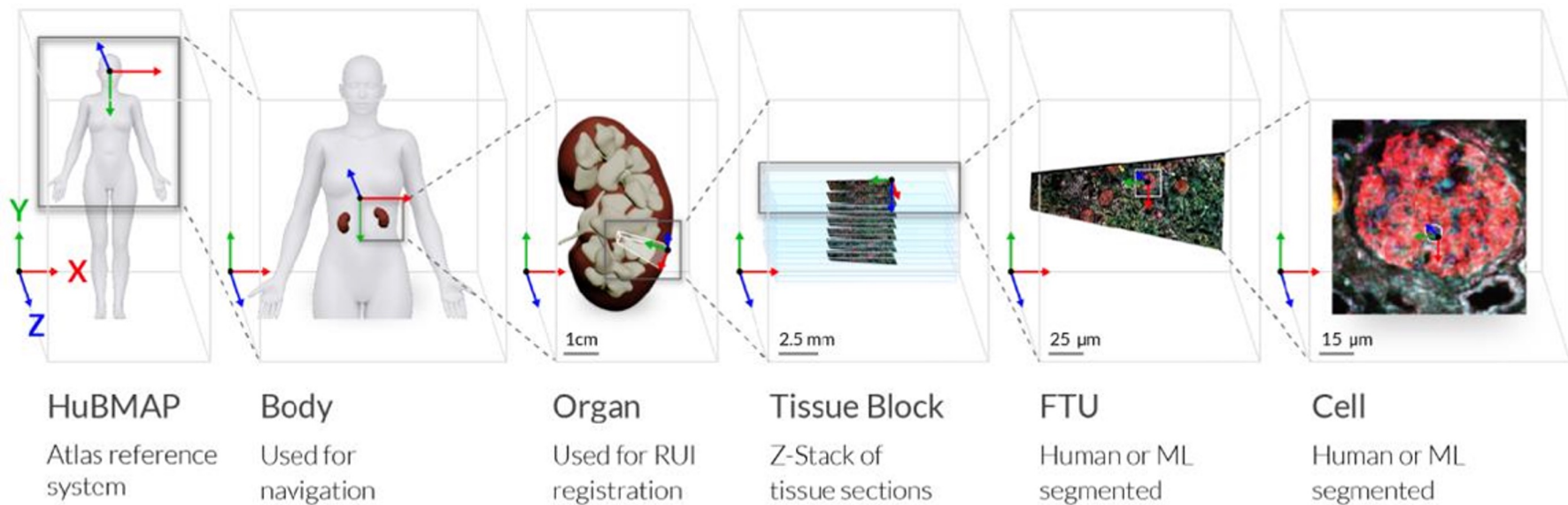
Skinner et al. Chromosoma 2017

- 1) How do we compare data across different cell types and conditions?
  - We need a **shared and systematic way to describe nuclear topography**
  - We need a **reference system to quantitatively characterize describe the nuclear landscape**
- 2) What are the **minimum requirements for a nuclear landscape reference system** (e.g., with respect to the location of nuclear compartments and landmarks)?



# What is a Common Coordinate Framework?

**A standard spatial coordinate system** is used to integrate spatial and molecular data across different laboratories, bio-samples, specimens, and conditions and **move past the use of single standardized samples**. The HuBMAP Human Reference Atlas effort developed a recent example.



**Borner et al. (2020)**

<https://doi.org/10.48550/arXiv.2007.14474>

**Borner et al., (2022)**

<https://doi.org/10.1038/s42003-022-03644-x>

**Herr et al., (2023)**

<https://doi.org/10.1038/s41597-023-01993-8>

**Borner et al., (2024)**

<https://doi.org/10.1101/2024.03.27.587041>



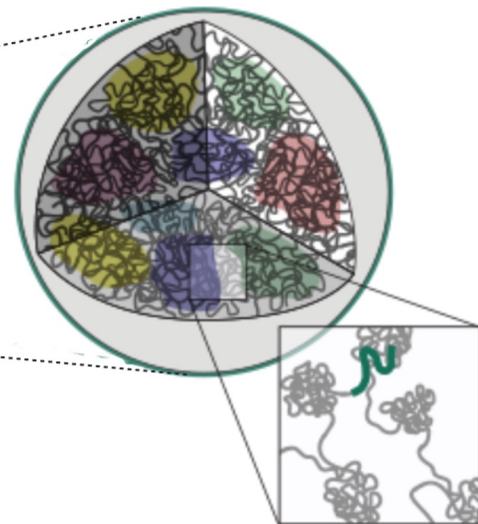
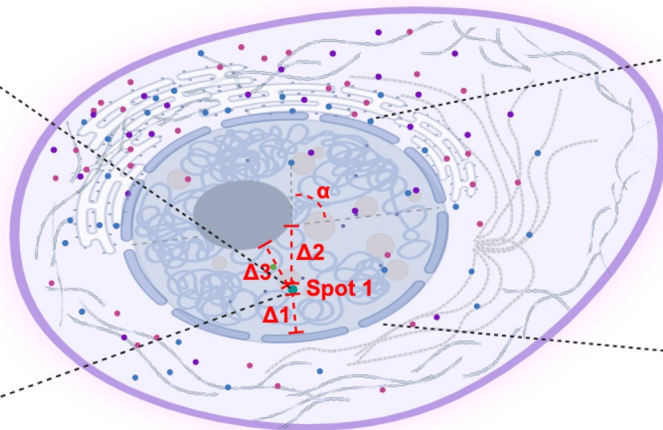
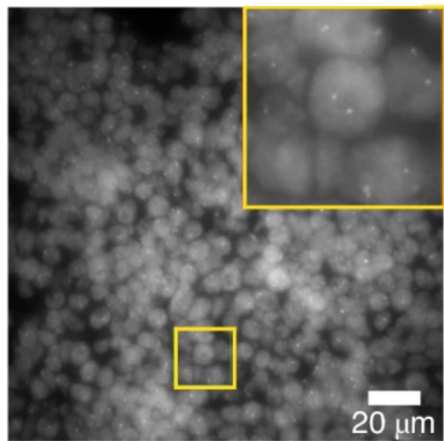


# 4DN Nuclear Common Coordinate Framework (CCF)

The goal is to have a set of recommendations how to measure and store nuclear information together with locations of loci.

Models from genomics data (Hi-C)

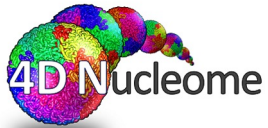
Multiplexed FISH



Spot 1 ( $\Delta 1$ ,  $\Delta 2$ ,  $\Delta 3$ ,  $\alpha$ )

Liu, M., Yang, B., Hu, M. *et al.* (2021). 10.1038/s41596-021-00518-0

An **underlying common ‘language’ for describing and indexing the data in a spatially explicit and semantically consistent way to integrate knowledge** from diverse data types (i.e., multiplexed FISH and 3C methods) and sources and build coherent predictive models of 4D Nucleome structure and function



# Advantages of CCF

- Allows us to **compare data across different cell types, tissues conditions**,
- The CCF **helps reusability and sharing (easily exchangeable)**,  
It **increases usability** and usefulness image data sets (and structure models).
- It would provide rich **information for modeling** to identify which topographic **information is functionally most relevant**
- The CCF would **help research projects to connect** and to allow **more efficient integration** of information across different data modalities (different imaging methods, genomics data, whole genome modeling.)



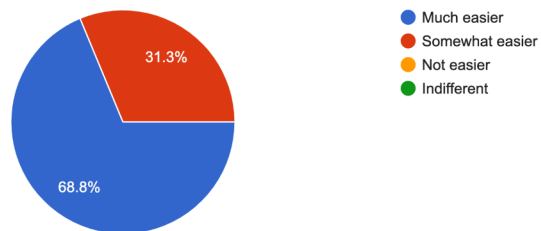




# Spring 2024: CCF interest survey among researchers developing downstream analysis pipelines

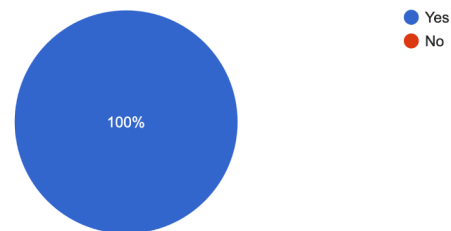
Would a common coordinate framework (CCF) for the nucleus make it easier for you to reuse 4D Nucleome microscopy data (e.g., analysis, integrative modeling, etc.)?

16 responses

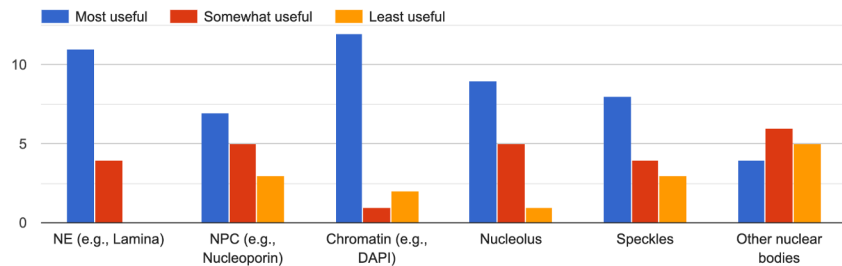


Would best practices or shared tools to represent the boundary of the nucleus in a broad range of 4D nucleome microscopy datasets be of value to you?

16 responses

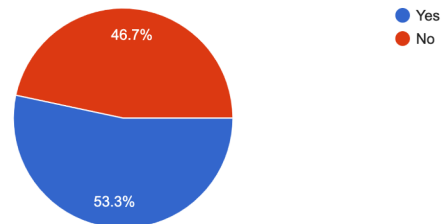


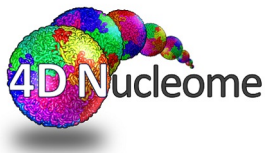
If the existence of a CCF would make it easier to reuse 4D Nucleome microscopy data, what nuclear markers would be the most useful? Please ...E, nuclear envelope; NPC, nuclear pore complex.



Are you currently including nuclear boundary markers in the analysis of microscopy datasets?

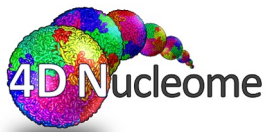
15 responses





# **Recommendations for Nuclear CCF best practices: should be minimally intrusive and widely applicable**

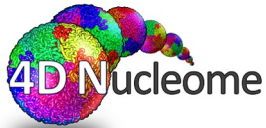
- **A nuclear boundary marker is necessary (but not sufficient):**
  - Examples: DAPI, Nucleoporin, Lamina (LaminA/C, LaminB)
- **Other markers are required for triangulation and breaking symmetry:**
  - Examples: Nucleoli, Nuclear speckles, Histone epigenetic markers, RNA polymerase, mRNA transcripts
- **Key requirements:**
  - Easy-to-use,
  - Different options for different experimental designs
  - Consider methods that do not require the use of fluorescence markers and use Machine Learning to predict nuclear markers localization:
    - Brightfield images
    - Fluorescence background (from DNA/RNA FISH-Omics probes)
    - Autofluorescence



# Recommendations for Nuclear CCF best practices: should be minimally intrusive and widely applicable

- **A nuclear boundary marker is necessary (but not sufficient):**
  - Examples: DAPI, Nucleoporin, LaminA/C, LaminB
- **Other markers are necessary for breaking symmetry:**
  - Examples: RNA markers, RNA polymerase
- **Key requirements:**
  - Easy-to-use
  - Different options
  - Consider methods that use fluorescence markers and use Machine Learning to predict nuclear markers localization:
    - Brightfield images
    - Fluorescence background (from DNA/RNA FISH-Omics probes)
    - Autofluorescence

Different methods have to be tested to develop best practices for Nuclear CCF



# NIH-Supplement

to support generation of benchmarking datasets for testing CCF strategies

**Title: Expediting the development of a Common Coordinate Framework for integrating multiplexed FISH datasets from different data production centers**

PIs: Strambio-De-Castillia, Rafelski, Bintu, Alber, Shen, Zhu

**DELIVERABLE 1: Produce benchmarking Multiplexed FISH datasets to assess** the opportunity and **feasibility** of different **experimental methods** to extract nuclear topology information (i.e., sample preparation, image analysis and segmentation, distance calculation, and model integration.)

**DELIVERABLE 2: Integration of 4DN Nuclear CCF specifications into FOF-CT:** The core of FOF-CT reports the XYZ coordinates of bright spots corresponding to the location of genomic segments with respect to the image frame. Currently, FOF-CT does not provide specifications for how topographical mappings should be obtained and reported. The goal of this proposal is to integrate specifications into the Spot Biological Data table of FOF-CT.



# Ongoing: acquisition of benchmarking datasets @ Center for Epigenomics, UCSD



Quan Zhu  
UCSD



Bogdan Bintu  
UCSD

- **Optimization**

- Tested different nuclear markers
- Tested Chromatin Tracing probe library

- **Model System**

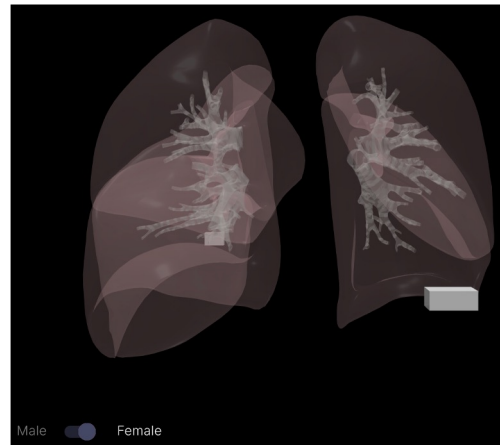
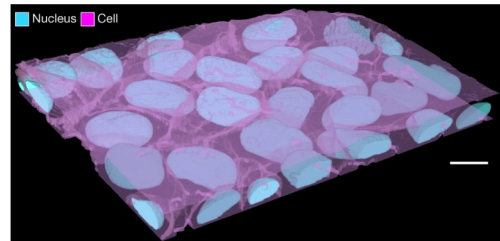
- WTC-11 hiPSC
- Human adult lung sections (from HuBMAP)

- **Ongoing experiments:**

- RNA and DNA MERFISH
- Multiple markers: DAPI, Lamina, NPC, Nucleoli, RNA Polymerase II, SC35, Histone epigenetic markers, PCNA
- Brightfield image

- **Questions**

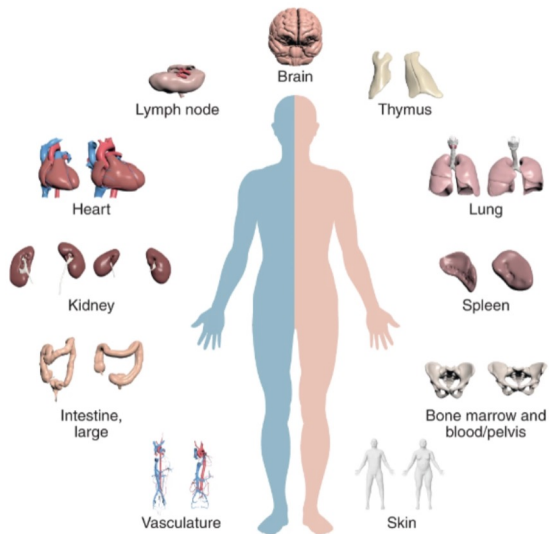
- What combination of markers are necessary and sufficient?
- What protocol should be used for
- Can we use machine learning approaches to predict the position of nuclear landmarks in stain-free transmitted light, background fluorescence, or autofluorescence images?







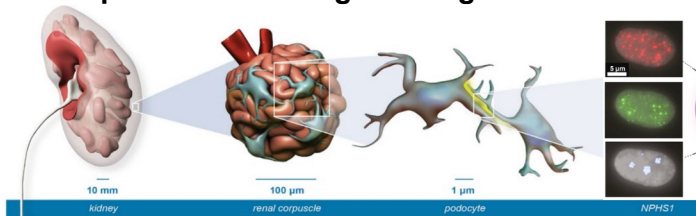
# Next Steps: Integration of Nuclear CCF with the HuBMAP Human Reference Atlas



## Questions:

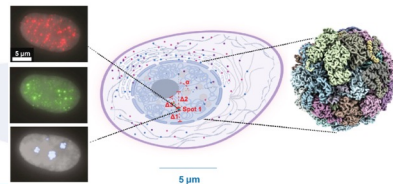
- What is the role of the chromatin organization and gene expression regulation in determining the organization of microenvironments in healthy and diseased Functional Tissue Units (FTUs)
- Determine how the molecular and cellular functions for a given cell type compare across organs (for example, genes essential in water transport across the kidneys, intestines, and lungs)

## Common Coordinate Framework for Spatial and Ontological Integration



1500 anatomical structures

## Integration of 4DN FISH Omics



267

Donors



2358

Samples



3080

Datasets



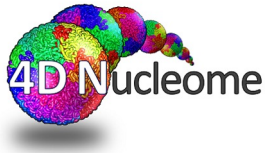
31

Organs



18

Collections



**Thank you to all CCF subgroup participants!**  
**Please join our IOWG meetings**

---