NIH Common Fund
HuBMAP / SCAP Mini Workshop
Neuroscience Center, Bethesda, MD

WEDNESDAY, JUNE 28, 2017

12:20 pm **Introductory Remarks - Robert Star (Director Division of Kidney, Urologic, and Hematologic Diseases National Institute of Diabetes and Digestive and Kidney Diseases, HuBMAP Co-Chair)**

12:30 – 2:30pm Challenges in collecting and pre-analytical processing of tissue

Moderator: Robert Star (NIDDK)

There are many tissue collection and processing factors that influence data quality, from length of ischemia time to storage conditions and collection method. These factors influence the distribution and degradation of biomolecules at different rates. Therefore, it is critical to match the choice of tissue source, collection method and preservation technique with the types of biomolecules being studied by different downstream assays.

The purpose of this session is to identify some of the challenges in collecting, preserving, and annotating high quality human tissue that will be used for downstream analytical techniques in the HuBMAP program. These techniques include single cell RNAseq, FISH, immuno-fluorescence as well as emerging techniques such as MERFISH, FISSEQ, seqFISH, MIBI-TOF, and 3-dimensional high-end imaging. Through the discussion, we hope to have a better understanding of the challenges HuBMAP might face in collecting and pre-analytical processing of tissue specimens and how this processing will impact the quality of data collected by different single cell, tissue, and imaging assays.

A number of components add to these challenges. One component is to record the spatial orientation of samples relative to anatomical landmarks (and build this into the sample management pipeline). A second component is the analysis, then integration and iteration of data from multiple imaging and omics assays to develop comprehensive molecular (and omic) profiles of the cells within the tissue, including location information. A third key component is to understand when sources of variability are biologically relevant (within tissue samples from same patient, across multiple tissues, and across multiple donors) or artifacts of the collection and processing of the samples.

Questions for the breakout session to consider include:

- Quality: What are practical quality measures for assessing the impact of tissue collection methods and the degree of degradation? How does the magnitude of ischemia signatures compare with collection, dissociation or storage signatures? Is there a common set of quality biomarkers that can be used across all tissues and that are compatible with downstream assays?
- Metadata: Beyond SPREC 2.0, are there common data elements describing collection and processing that are relevant to mapping DNA, RNA and proteins biomolecular distributions in tissues?
- Assay Workflow: What are best practices for assessing the impact of single cell (liberase) and tissue (LCM, super-resolution, imaging MS/MS) based tissue “dissociation” methods on assay measurements? Can tissue sections be used for multiple assays (RNA in situ, then protein, then routine stains)?
- Collection: For what assays and tissue types do tissues need to be collected from live donors? Rapid autopsy protocols?
- Staining: Do common stains (e.g. H&E, trichrome, toluidine) influence the sensitivity and specificity of downstream assays?
- Orientation: How do we preserve orientation of a tissue specimen through the processing chain?

- Fixing, clearing and embedding: Are there tissue stabilization techniques that can be used before or during collection? For current and emerging fixatives/preservatives of excised tissue, which biomolecular species do they preserve with good fidelity (not only nucleic acids and proteins, but how effective are these techniques at preserving metabolites or carbohydrates), what compatibility issues are there with different tissue types, cell types, dissociation techniques and assays? What are some of the challenges associated with clearing techniques?
- Sectioning: What are tissue-specific considerations in preparing tissue sections? How does the choice of tissue size and format influence ischemia and preservation timing and in term the quality of the tissue for different downstream assays?
- End-users: What format, quantity, and quality level is needed for: RNAseq, DropSeq, MERFISH / FISSEQ / seqFISH, immuno-florescence, MIBI-TOF and CyTOF approaches?

2:30 – 3:00pm Break

3:00 – 5:00 pm Data Analysis, Standards, and Benchmarks for Single Cell Analysis

Moderator: Junhyong Kim (University of Pennsylvania)

Because of the difficulty of obtaining measurements at the single cell scale, the field has been driven by technological advances, including various RNA/DNA sequencing technologies, high-resolution proteomics and metabolomics, multiplexing strategies, cell handling technologies, etc. Despite these technological advances, single cell measurements remain difficult and is fundamentally challenged by the fact that the units of measurement, each cell, has no replication. It has been extremely difficult to assess the efficiency of measurements, establish benchmarks or controls, agree on protocols for data analysis, and coherently define standards for reporting experiments and data analysis. An especially important challenge is placing single cell data in their organismal context, including spatial coordinates.

Questions for this breakout session to consider include:

- Is there benchmark data to compare new experimental or computational methods?
- How do we establish material standards such as specific cells or spike-in RNA?
- What metadata about calibration is important to know?
- What information is important to collect about the sample and its preparation?
- How can we work together with manufacturers to build standards into their methods?
- Does an ontology need to be established for single cell analysis?
- How can we associate single cells to tissue orientation information? More generally, how can data be organized from the single cell scale to whole organism scale?
- What are the common data elements between imaging and sequencing assays? Is there a common header we can use for all data, similar to FITS or DICOM?

5:00 pm Closing Remarks

Suggested background reading for these breakouts:

- Unhale, S. A., Skubitz, A. P., Solomon, R., & Hubel, A. (2012). Stabilization of tissue specimens for pathological examination and biomedical research. Biopreservation and biobanking, 10(6), 493-500. [http://online.liebertpub.com/doi/abs/10.1089/bio.2012.0031]
- Hubel, A., Spindler, R., & Skubitz, A. P. (2014). Storage of human biospecimens: selection of the optimal storage temperature. *Biopreservation and biobanking*, *12*(3), 165-175. [http://online.liebertpub.com/doi/abs/10.1089/bio.2013.0084]
- Hubel, A., Aksan, A., Skubitz, A. P., Wendt, C., & Zhong, X. (2011). State of the art in preservation of fluid biospecimens. Biopreservation and biobanking, 9(3), 237-244. [http://online.liebertpub.com/doi/abs/10.1089/bio.2010.0034]
- Chung, Cho H, Hewitt SM (2016). The paraffin-embedded RNA metric (PERM) for RNA isolated from formalin-fixed, paraffin-embedded tissue. Biotechniques. May 1;60(5):239-44 [http://www.biotechniques.com/BiotechniquesJournal/2016/May/The-paraffin-embedded-RNA-metric-PERM-for-RNA-isolated-from-formalin-fixed-paraffin-embedded-tissue/biotechniques-364401.html]
- Carithers, L. J., Ardlie, K., Barcus, M., Branton, P. A., Britton, A., Buia, S. A., ... & Guan, P. (2015). A novel approach to high-quality postmortem tissue procurement: the GTEx project. Biopreservation and biobanking, 13(5), 311-319. [http://online.liebertpub.com/doi/full/10.1089/bio.2015.0032]

NIH Common Fund

Single Cell Analysis Program (SCAP)

Human BioMolecular Atlas Program (HuBMAP)

Tissue Acquisition and Metadata Standards Workshop

Robert A. Star, MD
NIDDK

Disclosure

My laboratory is involved in research to improve pre-analytic processing steps after tissue biopsy.

I am an inventor on a provisional patent application for a **tissue transfer device** that reduces tissue damage after biopsy.

I am an inventor on a provisional patent application for a **chemically engineered fixative** (BE70G) that does not contain formaldehyde, and improves molecular analyses.

If NIH successfully commercializes the inventions, I **may receive royalty payments** using standard NIH formulas.

NIH HuBMAP Working Group

Co-Chairs:
Gary Gibbons, M.D. (NHLBI)
Roderic Pettigrew, Ph.D., M.D. (NIBIB)
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Common Fund Program Leader:
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José M. Velázquez, Ph.D. (NIA)
Yong Yao, Ph.D. (NIMH)

HuBMAP: Background

- In past, cells classified by structure, function, location in tissue, histologic staining
- Opportunity:
  - Massively parallel single cell analysis (genomics) **assays**
  - Computational **algorithms** to find types, sub-types, states, transitions;
  - **Imaging** at growing scale and resolution
- New paradigm: Classify cells and tissue components based on molecular omic profile

- Critical questions (for example)
  - How do cells vary within structure; within tissue; across tissues, systems, and organs (vasculature, supportive cells).
  - Are there undiscovered sub-compartments, rare cells
  - How do cells interact (ligands, receptors)
  - How do cells influence health and disease

National Institute of Diabetes and Digestive and Kidney Diseases

HuBMAP Goals

The overall goal of HuBMAP is to catalyze the development of **a comprehensive atlas of cellular/tissue organization** in human tissues that will elucidate the principles of organization-function by:

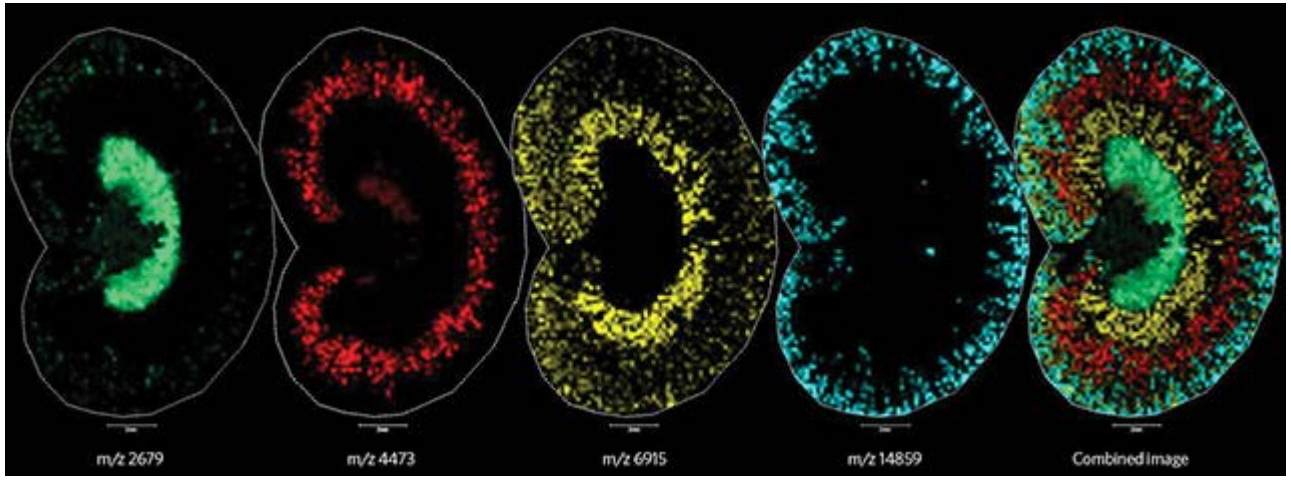
- accelerating **development of tools** for constructing comprehensive spatial tissue maps and integrating data types,
- building and **generating tissue maps** from validated high-content, high-throughput imaging and omics assays,
- **coordinating and collaborating** with other funding agencies, programs and the biomedical research community,
- rapidly making **data findable, accessible, interoperable, and reusable** in standardized formats.

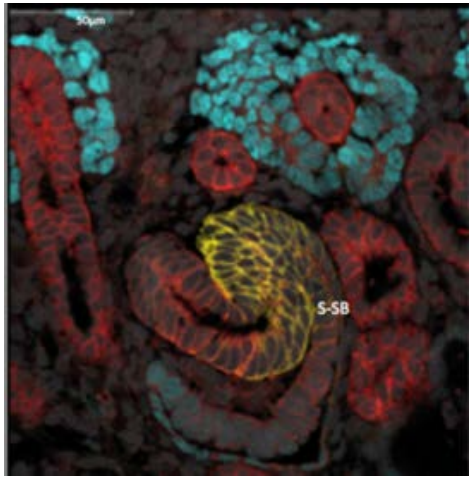
HuBMAP Vision

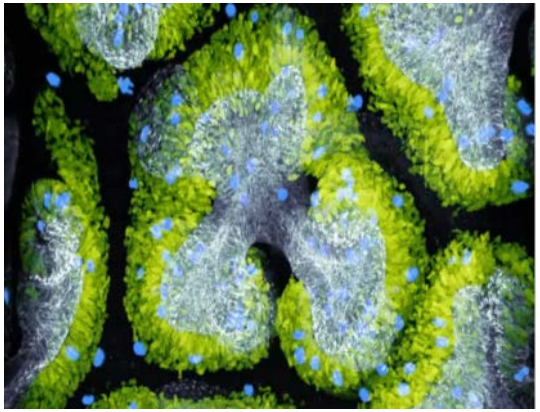
If successful, this program will lead to a data resource like "**Google Maps" for tissues** in the human body

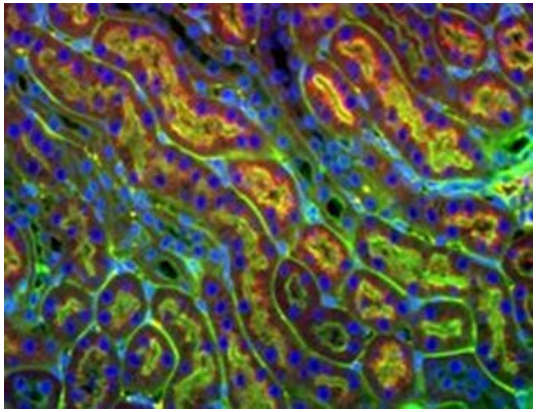
that will *give rise to* new insights **into inter-individual variation and tissue changes across the lifespan,** and serve as baseline for **understanding disease**.

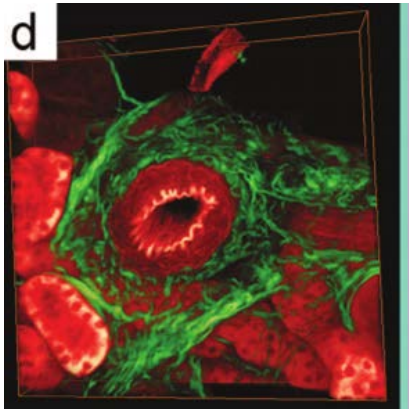
What is a tissue atlas?
Kidney pathology of the future

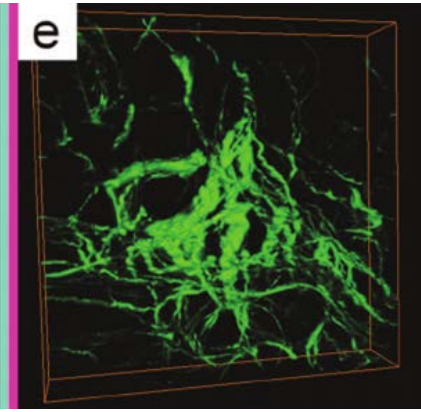










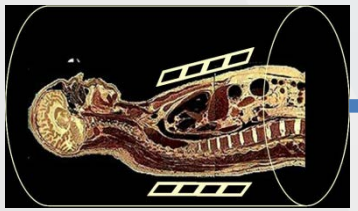


Analyze single cells/tissue to find **tissue markers** (cells, and interstitial areas between cells) then **paint** cells, structures, cell trajectory (healthy, injured, repair, regen), activated pathways

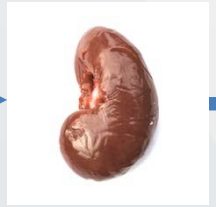
Understand heterogeneity between regions, neighboring cells

Even better, use 3D imaging to better see interstitium, glomerulus

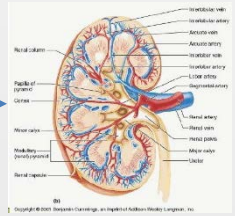
Generating Tissue Maps



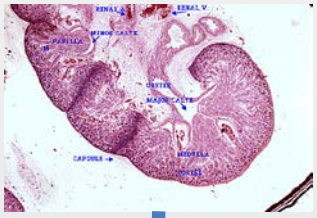
In vivo Imaging
(if possible)



Tissue
Collection



Reference
Atlas



Histology,
Fiducials &
Annotation

Preservation
and QC

Key Elements

- **Comprehensive profiling**
- **3D Spatial coordinate system**
- **Iterative imaging and omics**

Species: DNA, RNA, Protein, Metabolites …

Methods: WGA, LIANTI, RNAseq, ChIPseq, MALDI, ATACseq, CyTOF…

Dissociative

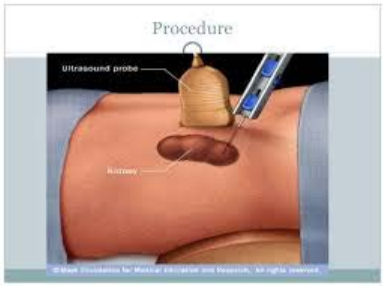
Laser capture, Spatial encoding, On tissue processing …

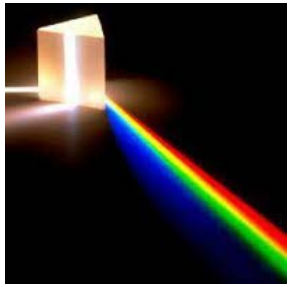
Species: DNA, RNA, Protein, Metabolites …

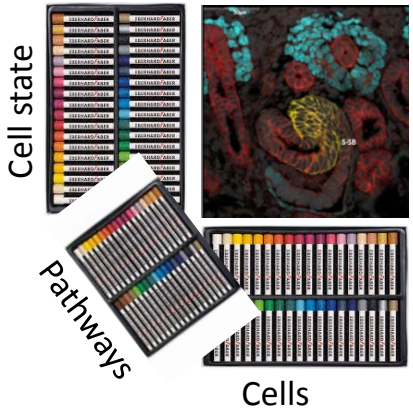
Methods: IHC, FISH, seqFISH, MERFISH, FISSEQ, MIBI-TOF…

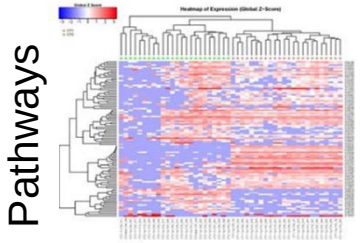
In Situ

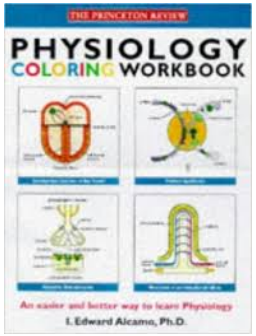
Kidney Precision Medicine Project workflow (modified for HuBMAP)











Obtain tissue

Learn from tissue
Find components
Find markers

Evaluate markers on tissue

Define dark areas

Find cell pathway markers; tie to Patient outcomes

Create Kidney Tissue Atlas

National Institute of Diabetes and Digestive and Kidney Diseases

Identifying Key Areas in a Human BioMolecular Atlas

Planning Workshop
June 15, 2016

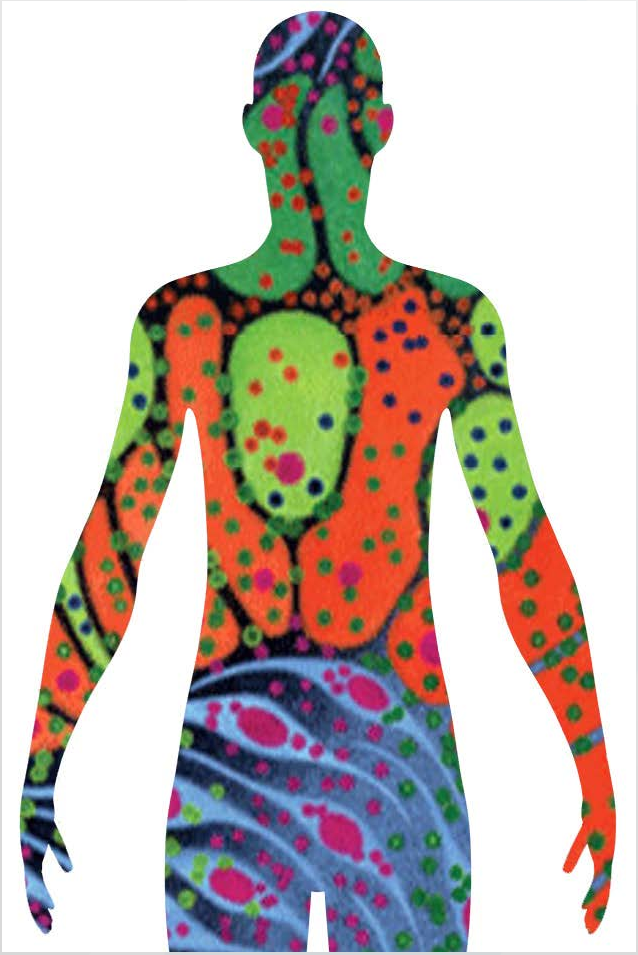
Areas with challenges and opportunities for investment by the NIH:

1. Sourcing high quality tissue from multiple organ sites
2. Processing and preserving tissue for multiple imaging and omics assays
3. Quality control, validation and variation in data generation
4. Data coordination across multiple acquisition techniques
5. Annotation, curation and archiving of the data
6. Browsing, visualizing and searching the data
7. Building statistical and analytic techniques and models for nonlinear analysis of highly multidimensional data
8. Community engagement

Challenges in collecting and pre-analytical processing of tissue

Data analysis, standards, and benchmarks for single cell analysis

Questions?



What's next?

- FOAs this Fall
- Program rolled out in phases
- Mini workshop June 28, 2017
  - Pre-analytic processing
  - Metadata
- Single Cell Analysis Investigators Meeting, June 29-30, 2017

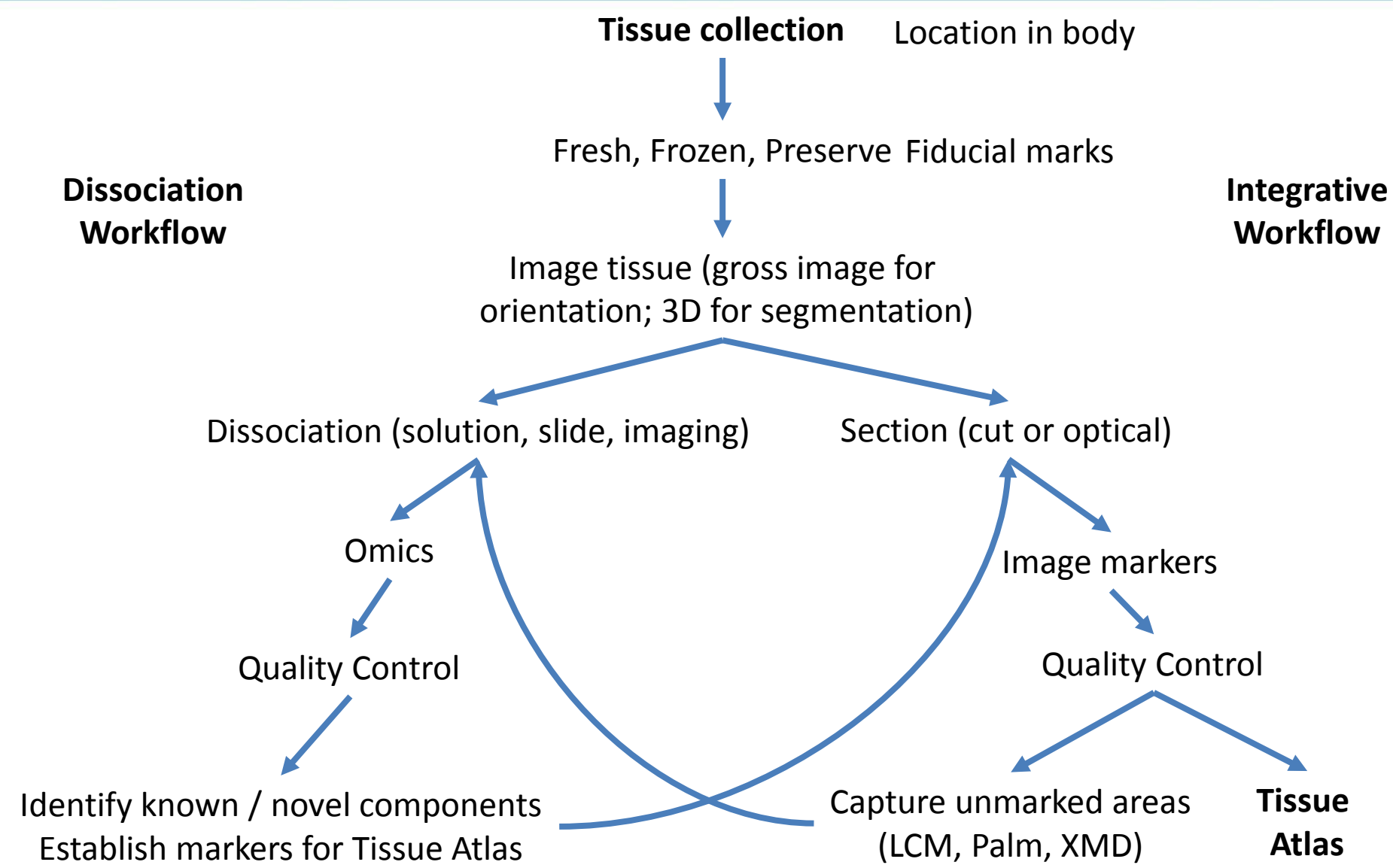
Challenges in collecting and pre-analytical processing of tissue

Robert A. Star, MD
NIDDK

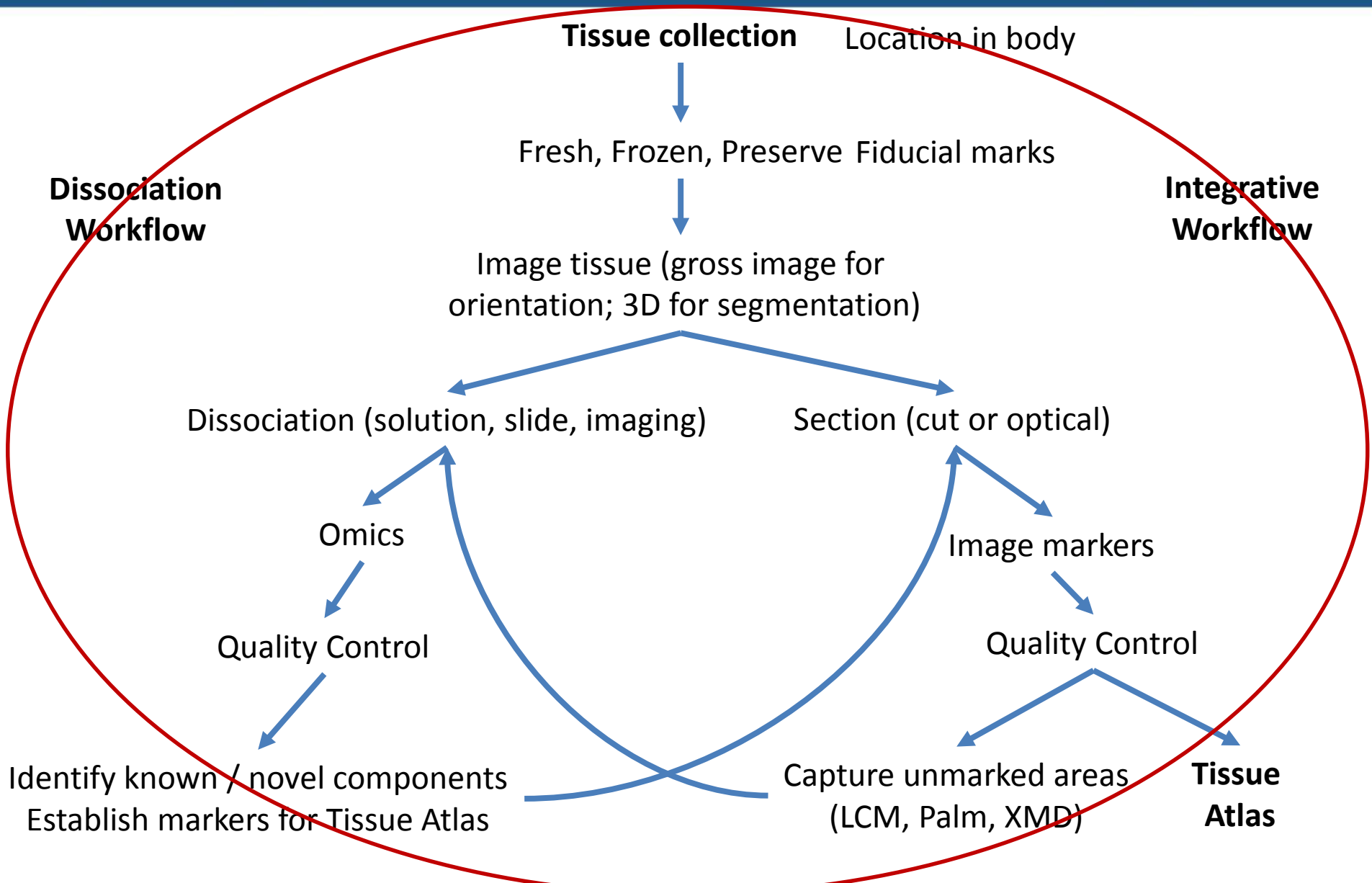
Pre-analytic processing of tissue samples

- Tissue collection and processing influences sample quality and composition. Distribution and degradation of biomolecules occur at different rates. Critical to match initial steps to downstream uses
- Optimize
  - Tissue source, collection method: Live donor vs warm autopsy vs ??
  - Initial preservation and storage
  - Initial processing: clearing, embedding, sectioning
  - Sample/assay workflow
  - Quality control for each step
- Are there overarching considerations to maximize quality, minimize artifact?
- Dark matter

Simplified HuBMAP Workflow



Many areas for Improvement



Examples of dark matter

Tissue preservation / fixation step

- Formalin bad for RNA, some proteins (‘antigen retrieval’)
- Frozen bad for histology
- Fresh tissue is fragile
- Need better preservation steps that match preservation to intended downstream uses (fit for context)

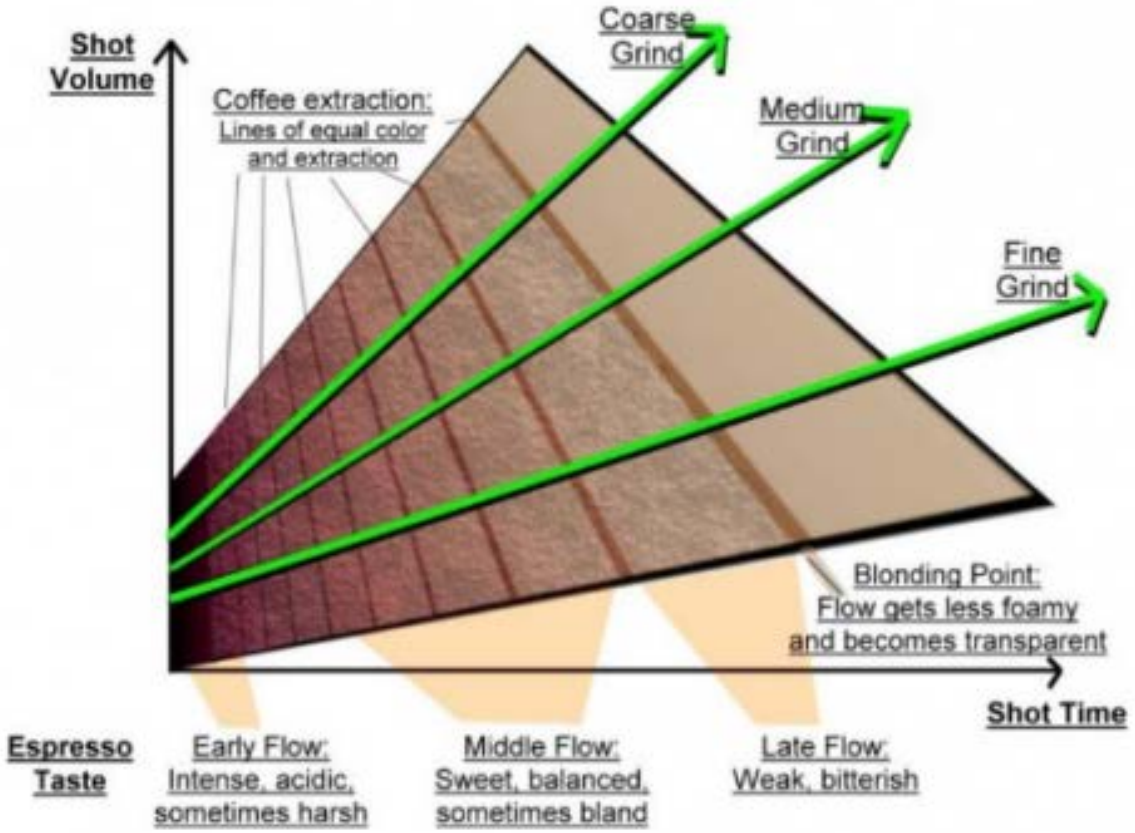
Single cell dissociation step

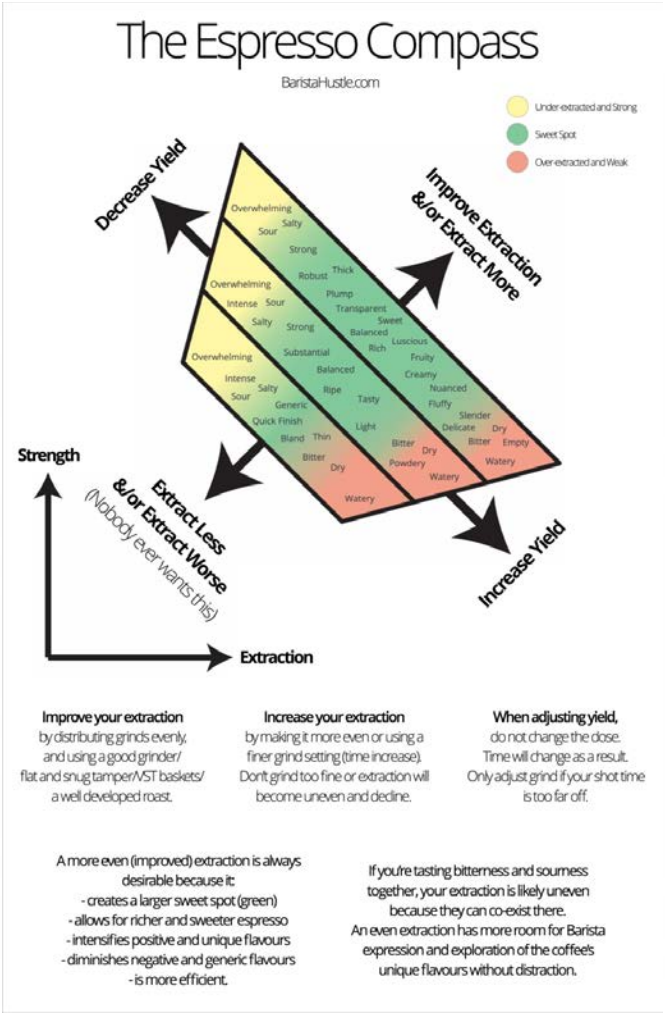
- Digestion (37^{o}C) leaves mRNA signature
- Selective for mobile inflammatory cells
- Not many cells analyzed
- Very few structural (organ) cells analyzed
- Need less destructive dissociation technologies

How to make espresso

How avoid sour or bitter espresso?

Grid, volume, temperature influence extraction, and taste. Need optimization process





Bottom line: difficult, need balance multiple factors

Innovation: Look for alternative

National Institute of Diabetes and Digestive and Kidney Diseases

- **Orientation**: How do we preserve orientation of a tissue specimen through the processing chain?

- **Fixing, clearing and embedding**: Are there tissue stabilization techniques that can be used before or during collection? For current and emerging fixatives/preservatives of excised tissue, which biomolecular species do they preserve with good fidelity (not only nucleic acids and proteins, but how effective are these techniques at preserving metabolites or carbohydrates), what compatibility issues are there with different tissue types, cell types, dissociation techniques and assays? What are some of the challenges associated with clearing techniques?

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- **Collection**: For what assays and tissue types do tissues need to be collected from live donors? Rapid autopsy protocols?

- **Staining**: Do common stains (e.g. H&E, trichrome, toluidine) influence the sensitivity and specificity of downstream assays?

Why are we here?

- What is working, working well?
- What are the weak links in chain?
- What tools, techniques are needed before going into production phase?

National Institute of Diabetes and Digestive and Kidney Diseases

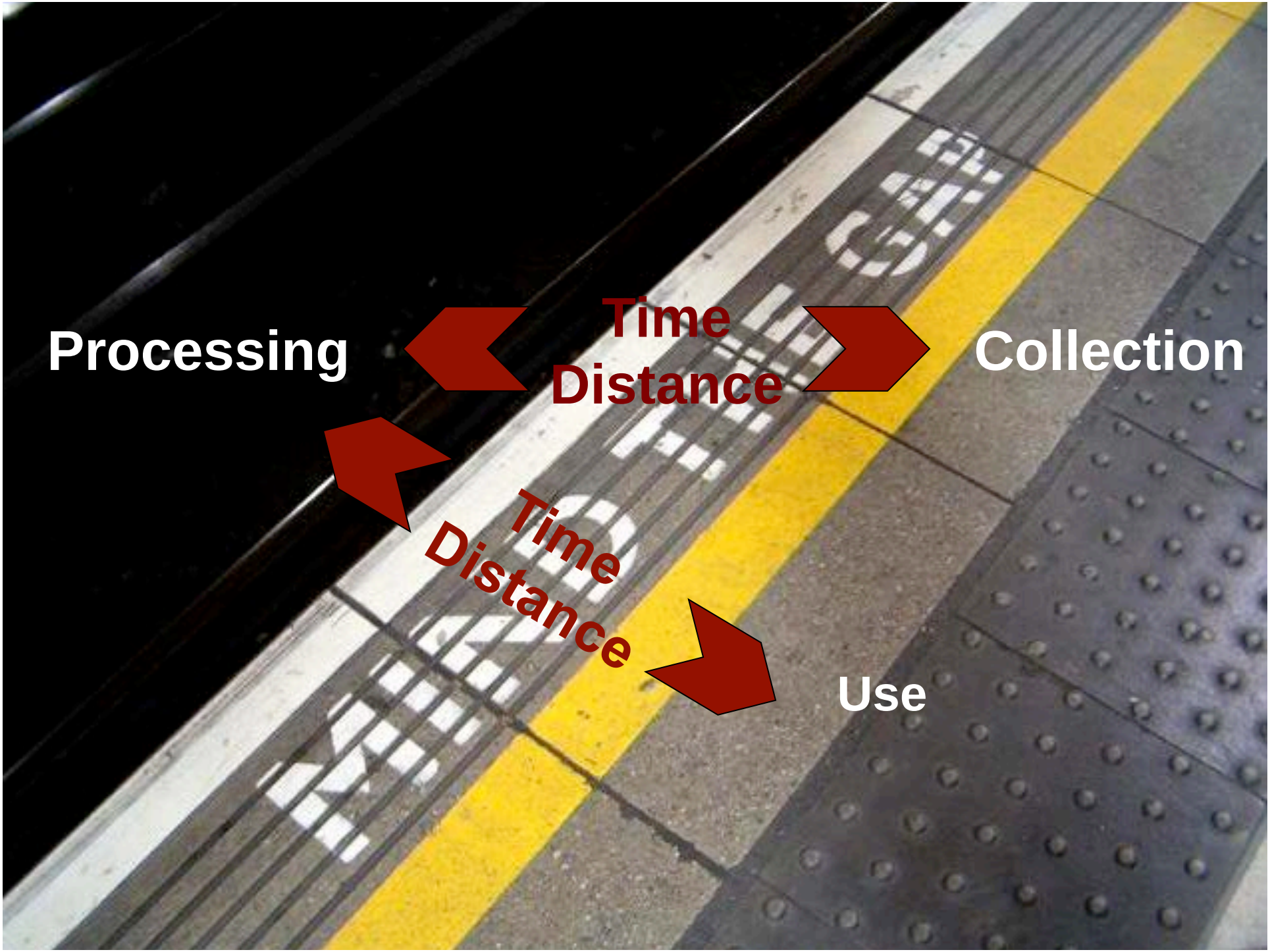
National Institute of Diabetes and Digestive and Kidney Diseases

Advancing the preservation of tissue biospecimens

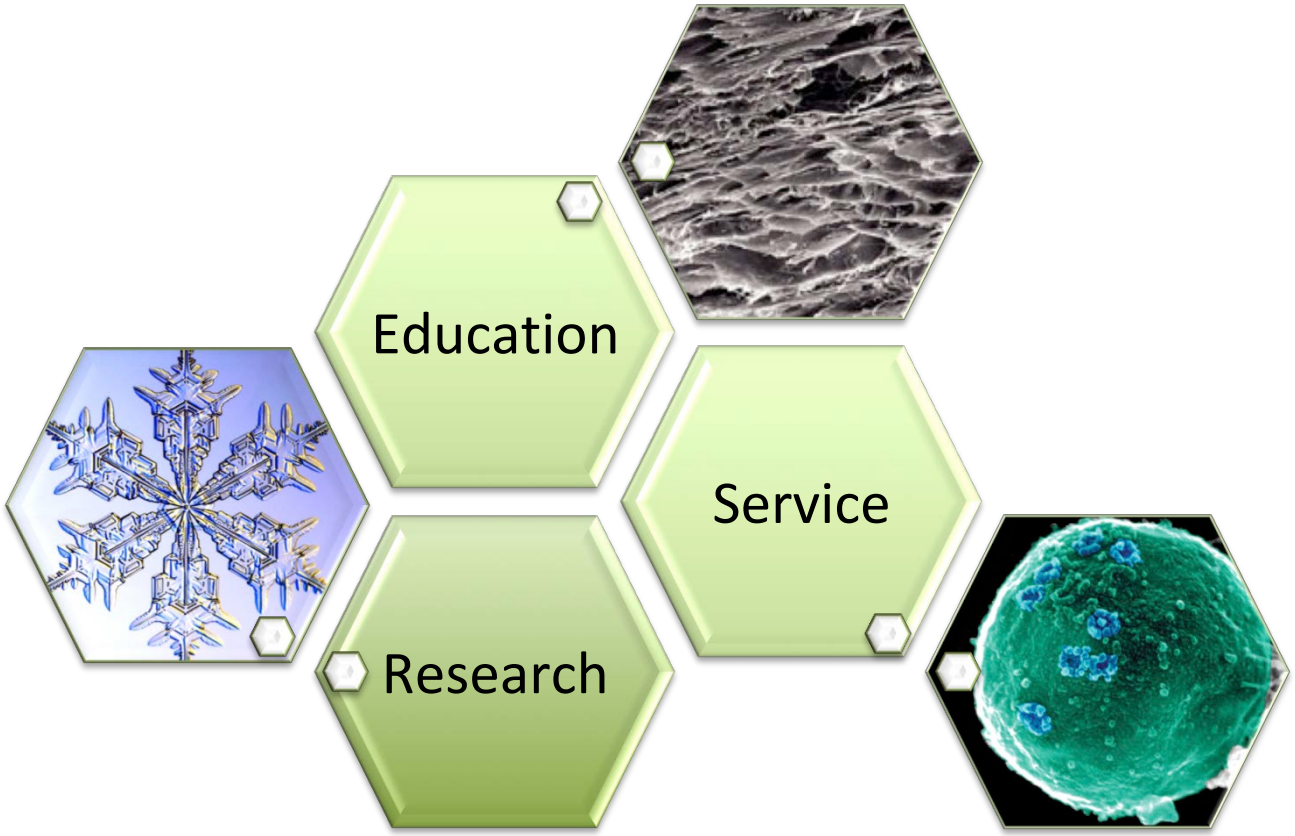
Allison Hubel, PhD

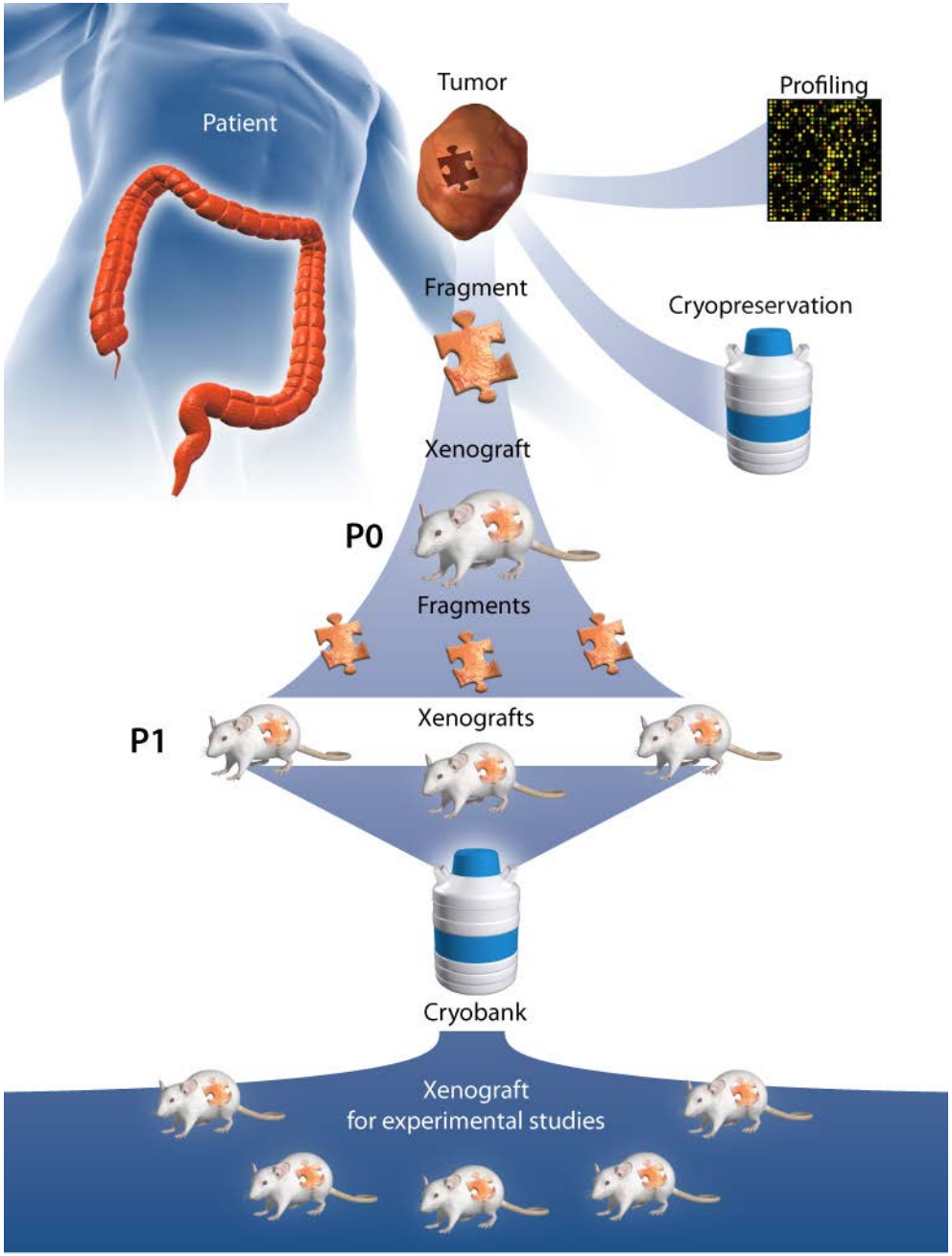
Biopreservation Core Resource (BioCoR) Univer

sity of Minnesota


Processing
Time
Distance
Collection
Time
Distance
Use

BioCoR Resources





InTechOpen.com

BioCoR Service

- PDX models have complex work flow
- Viability must be maintained along this workflow

<u>BioCoR tasks:</u>

- Develop short term storage solution suitable for resected tumors
- Develop effective cryopreservation protocols for xenografts

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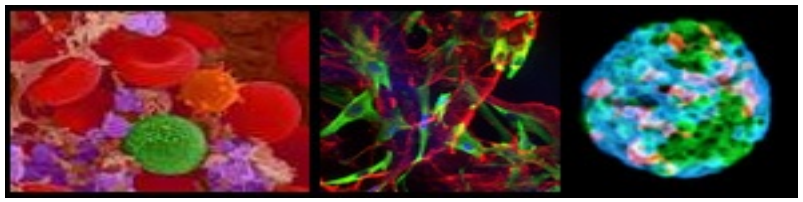
Education Resource

BioCoR library



Online short courses in preservation

NHLBI training grant

Monthly newsletter

www.biocor.umn.edu

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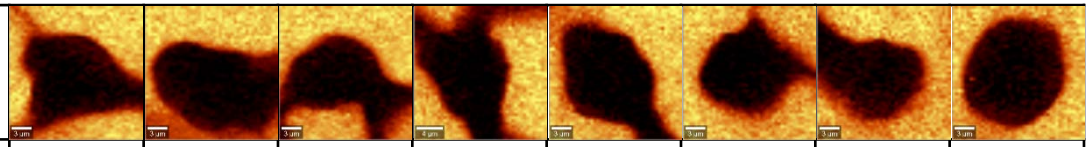
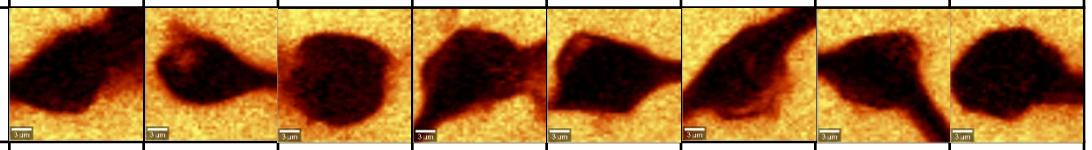
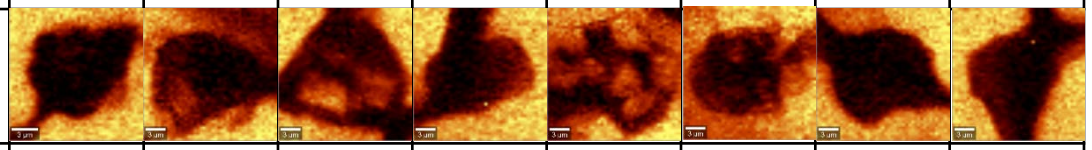
BioCoR Research

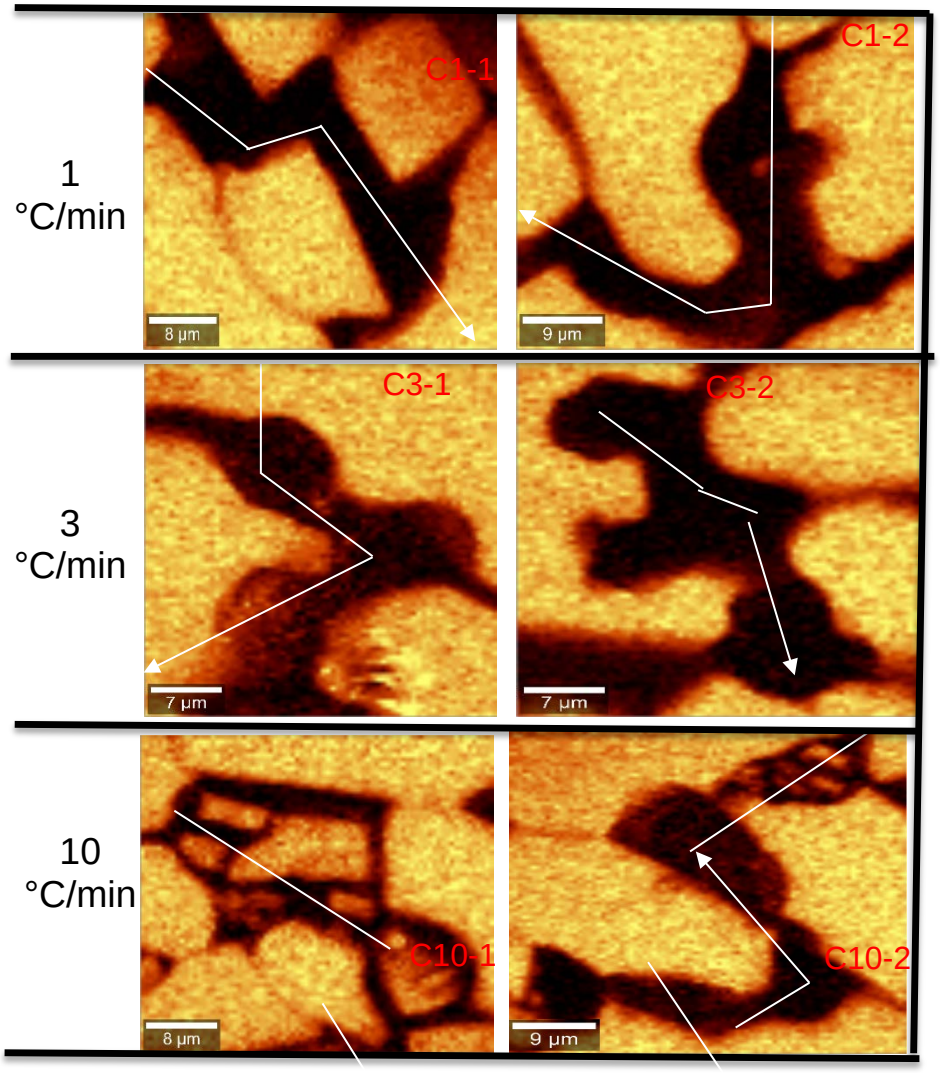
Why do tissues respond poorly (compared to cells isolated from the same tissue)?

Freezing response of isolated iPS cells (Imaged at -50°C)

1 °C/ min	Ice								
	Area/μm^2	104.8	105.8	71.6	94.5	114.7	81.5	111.8	138.5
	Aic-s	0.15	0.07	0	0	0	0	0.12	0.16
3 °C/min	Ice								
	Area/μm^2	113.2	74.6	160.2	129.0	117.8	118.2	132.1	119.8
	Aic-s	0.08	0.26	0.37	0.42	0.15	0.24	0.33	0
10 °C/min	Ice								
	Area/μm^2	148.3	159.1	223.6	128.6	252.6	189.1	157.7	132.3
	Aic-s	0.11	0.49	0.67	0.43	0.65	0.46	0.20	0.29

Scale bar: 3 μm

BioCoR Research



- We can interrogate
  - small aggregates (3-5 cells)
  - Full sized colonies
- A wide range of signals can be detected
  - Water (liquid or ice)
  - DMSO
  - Cryoprotective agents
  - Proteins
  - DNA
- These signals can help answer the central questions

Five to ten years from now…

- Dispel the myth of the ‘cold black box’
- Improve and disseminate preservation protocols
- Improve preservation of tissue

Acknowledgements

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Disclosures

- "Employee" Of The US Federal Government
  - Inventor On Multiple Technologies For Which The Intellectual Property Is Assigned To The Federal Government
    - For Those Inventions That Are Licensed, I Am Eligible To Receive Royalties, As Stipulated In US Code, Title 15, Chapter 63.
- Editor-in-Chief, *Journal of Histochemistry & Cytochemistry*
- Chair-holder, Subcommittee on Immunohistochemical Assays, Clinical & Laboratory Standards Institute
- Food & Drug Administration, Center For Devises & Radiological Health
  - Consultant, Hematology & Pathology Devices Panel

Quality Is Everything

Quality Remains Subjective

- Tissue Quality
  - Histology
  - Proteins
  - Nucleic Acids
- Clinical Data
  - Complete
  - Detailed
  - Defined

Development of A Tissue Handling Protocol

- Historic Perspective
  - Histology & Protein – FFPE
  - Nucleic Acids – Frozen

Small Study- Frozen

Large Study – FFPE

- Recommended Strategy
  - Fit-For-Purpose

Define Goals

Physiology Is Biology

Specimen Preservation Is Chemistry

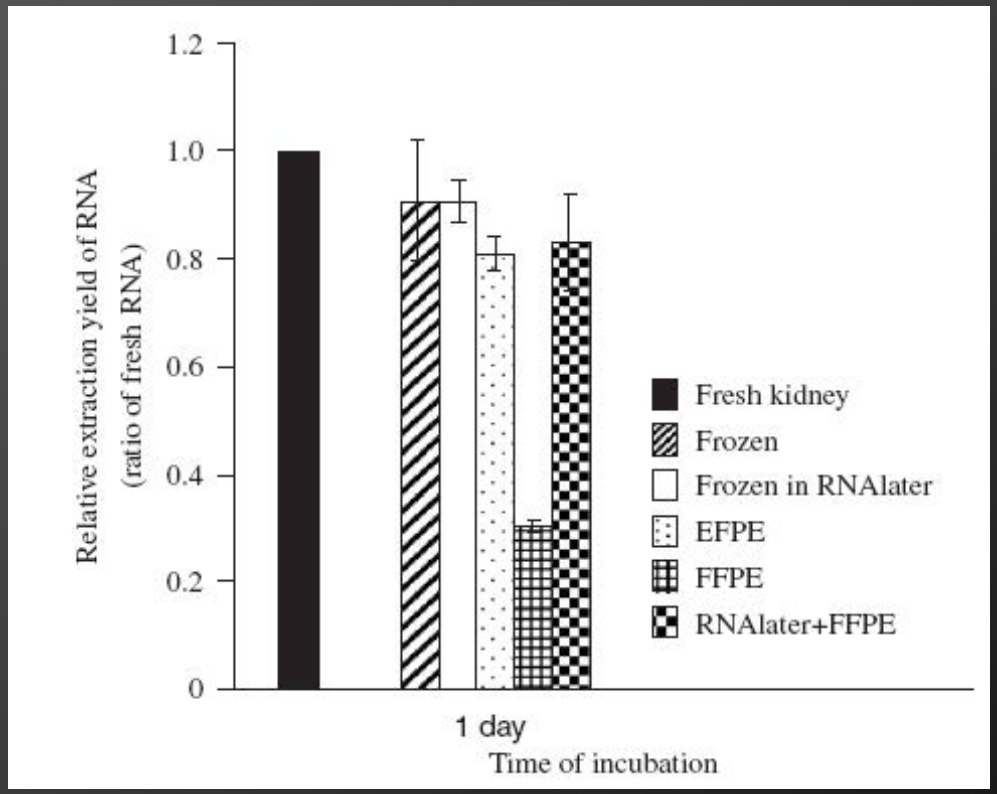
Chemical Preservation

- Formalin
- PaxGene
- Ethanol
- …….

- Lack Of Scientific Underpinning
- Two Broad Classes
  - Acid/Aldehyde Fixatives
  - Coagulative Fixatives
- Underlying Critical Factors
  - Ischemia
  - Size
  - Time Temp?
  - Impregnation
  - Storage

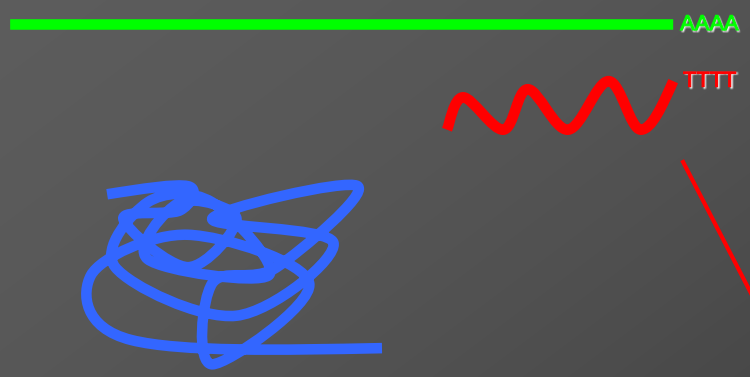
RNA Recovery - Quantity

- First Study To Address RNA Recovery Based On Equal Volumes Of Starting Material
- FFPE Demonstrates A 30% Recovery
- Ethanol-fixed, PE Has A Recovery Of 80%
  - Formalin Contributes To the Majority Of The Damage

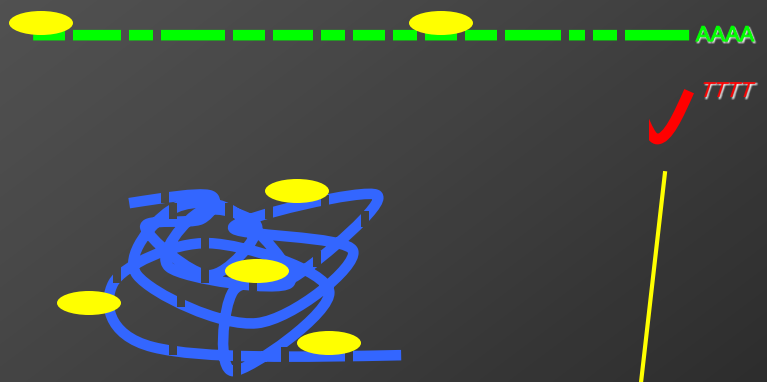


RNA Recovery From Tissue

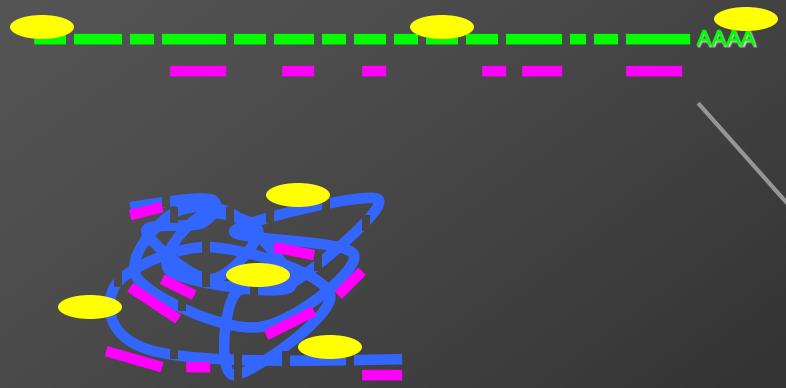
RNA In Frozen Tissue



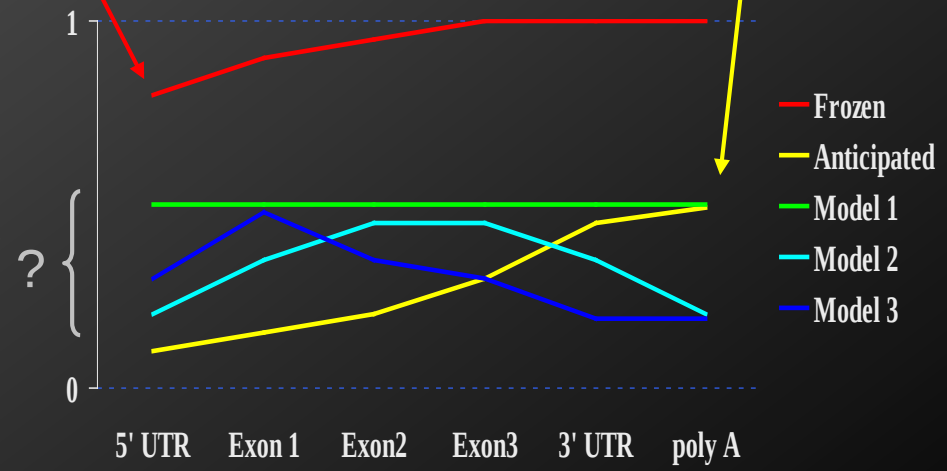
RNA In FFPE -Anticipated



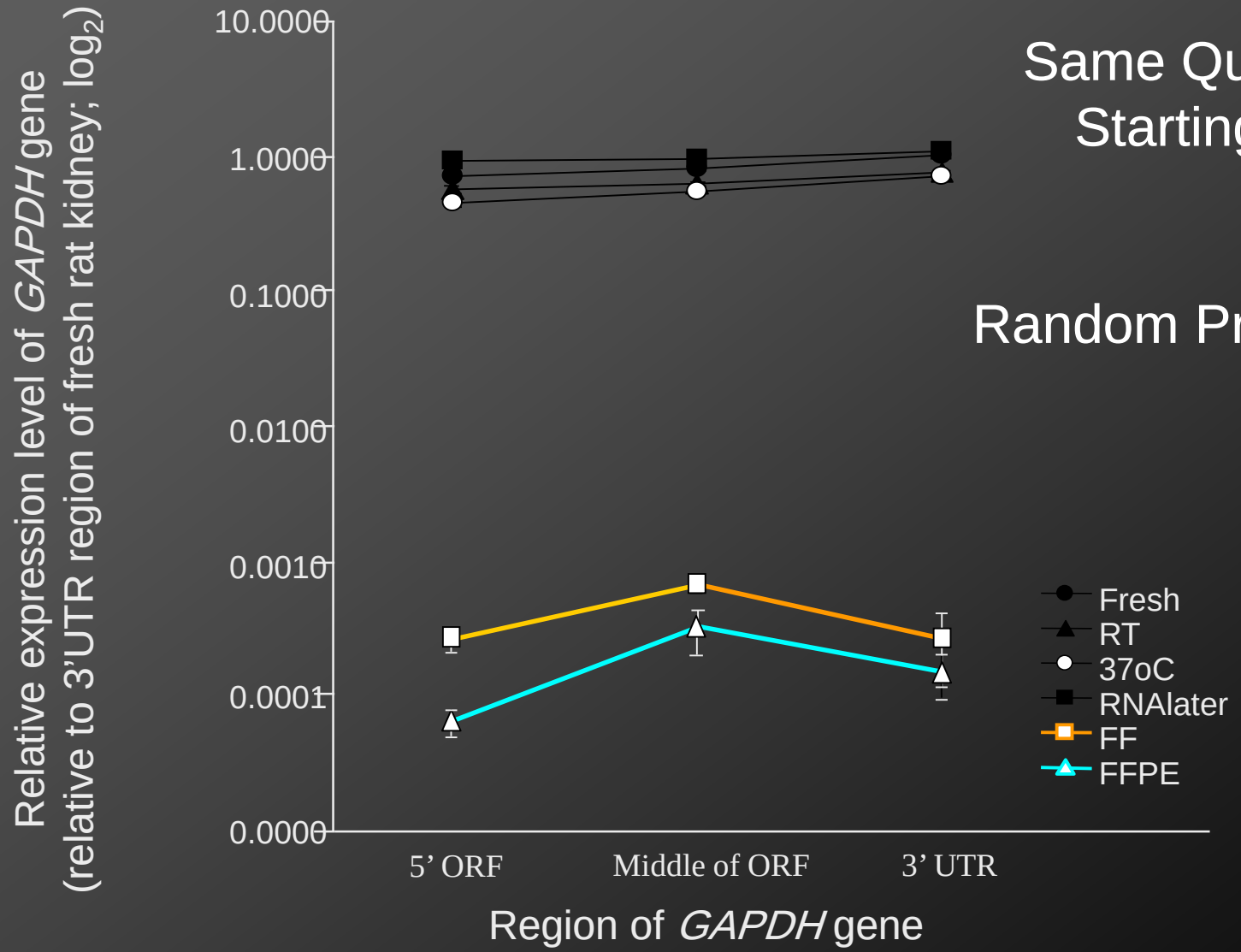
RNA In FFPE -Revised



Results Of RT-PCR



Quantitative Amplification Based On RNA Source & Primer Location

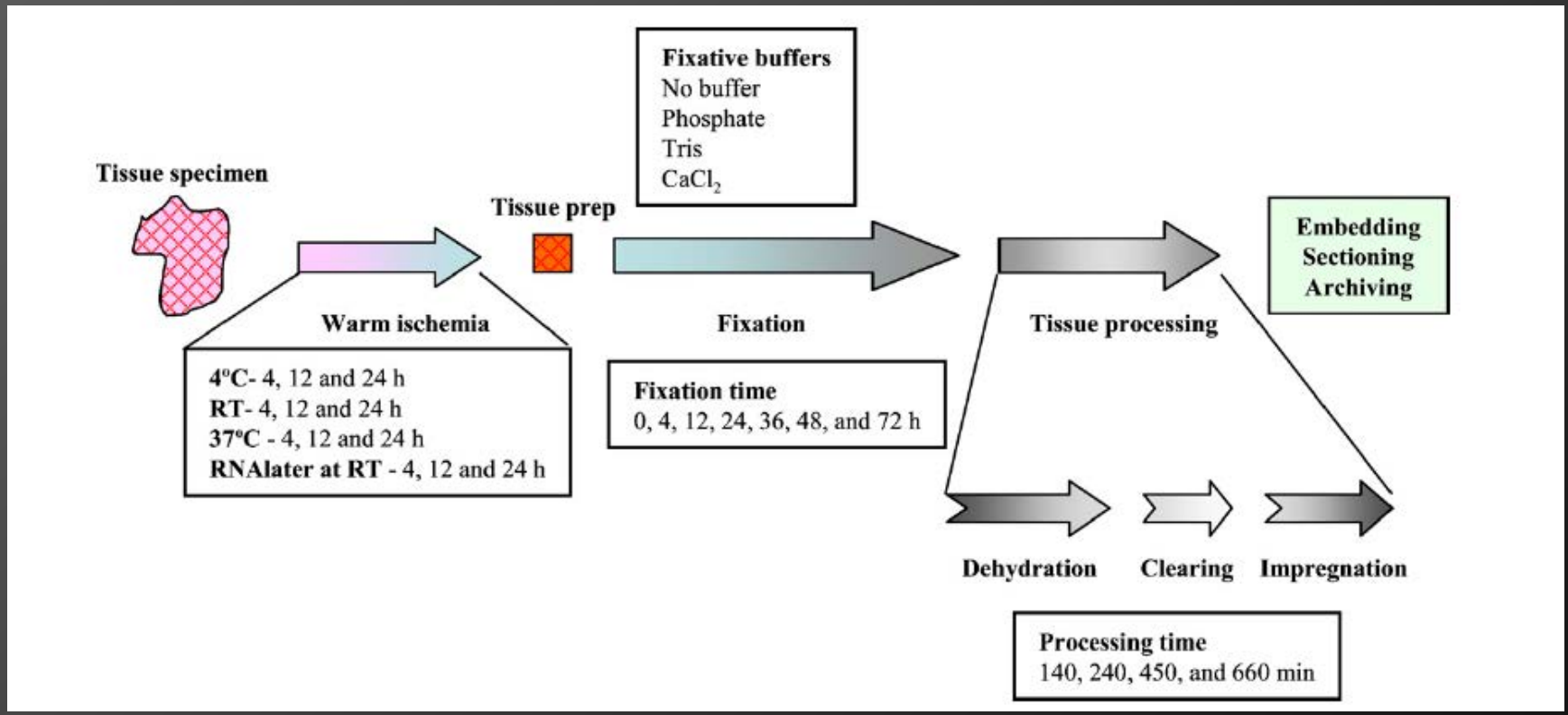


Same Quantity of Starting RNA

Random Prime cDNA

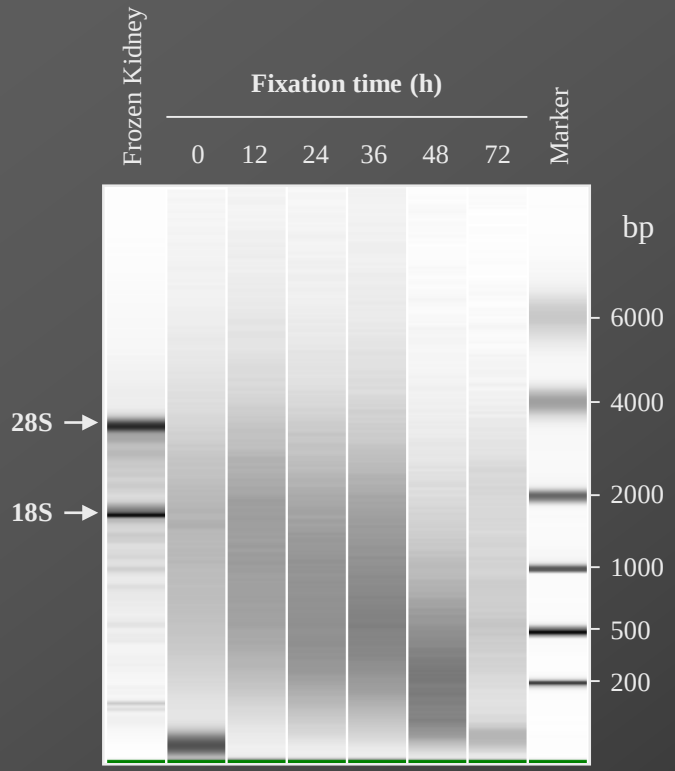
Tissue Collection, Handing & Processing

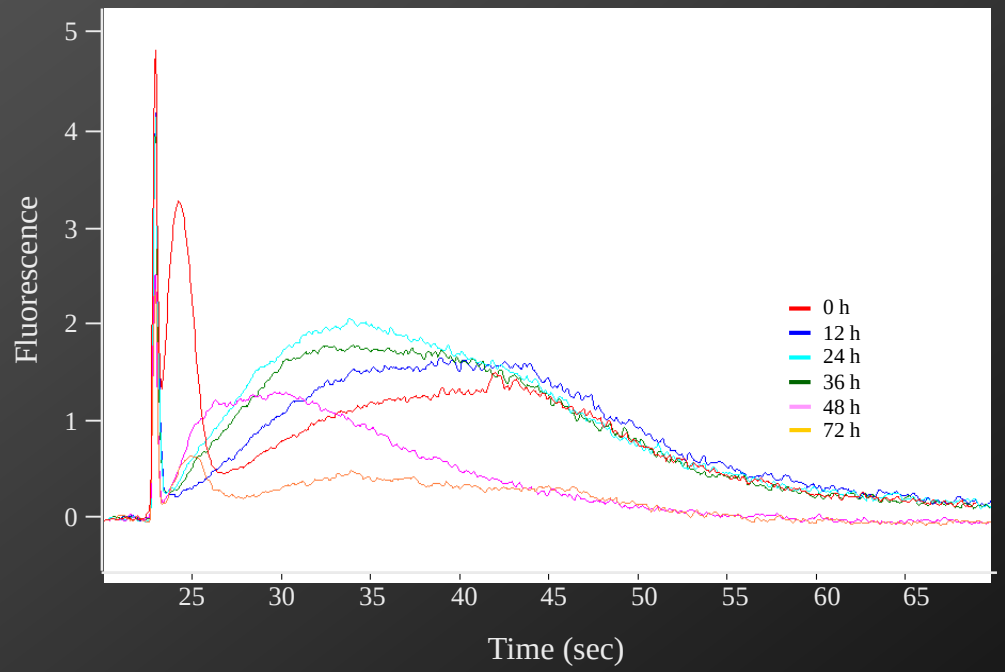
- No Such Thing As "*Standard Protocol*"
- Multiple Steps, Multiple Parameters



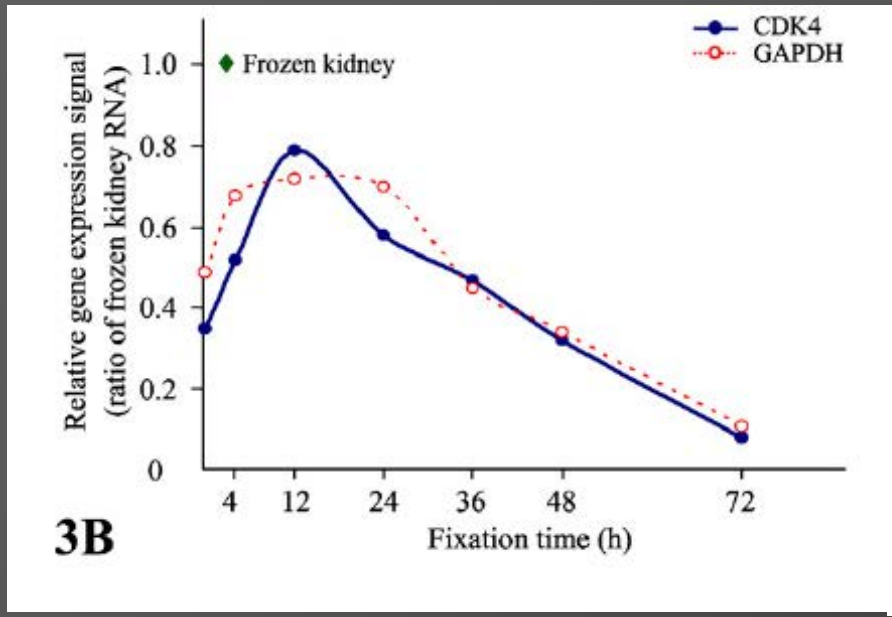
Effect Of Fixation Time On RNA Quality

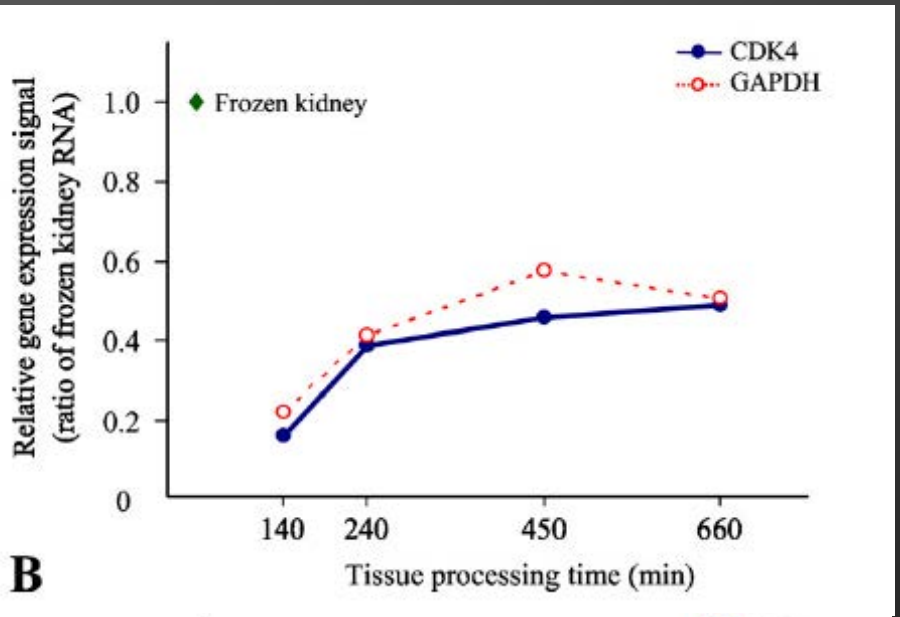
B



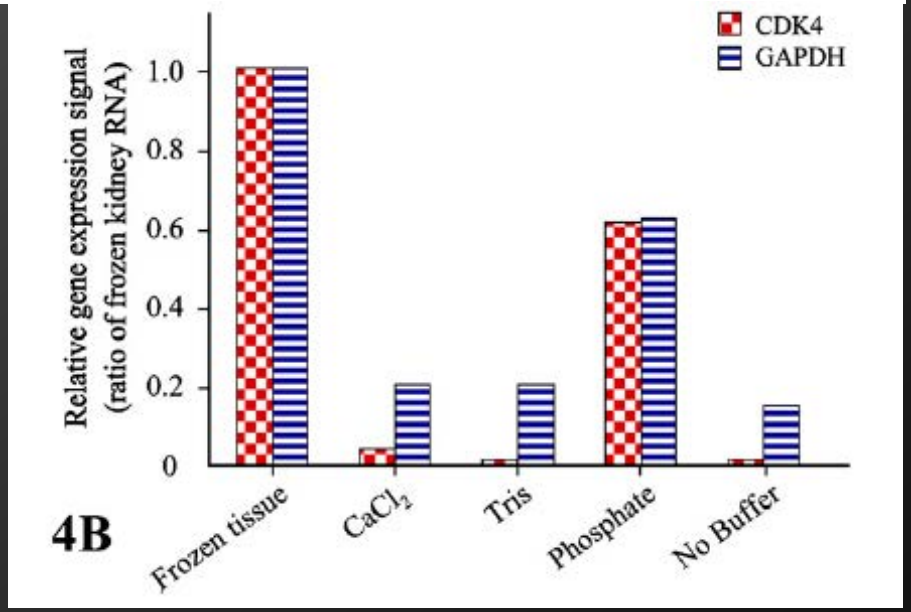


Measuring The Effects Quantitatively



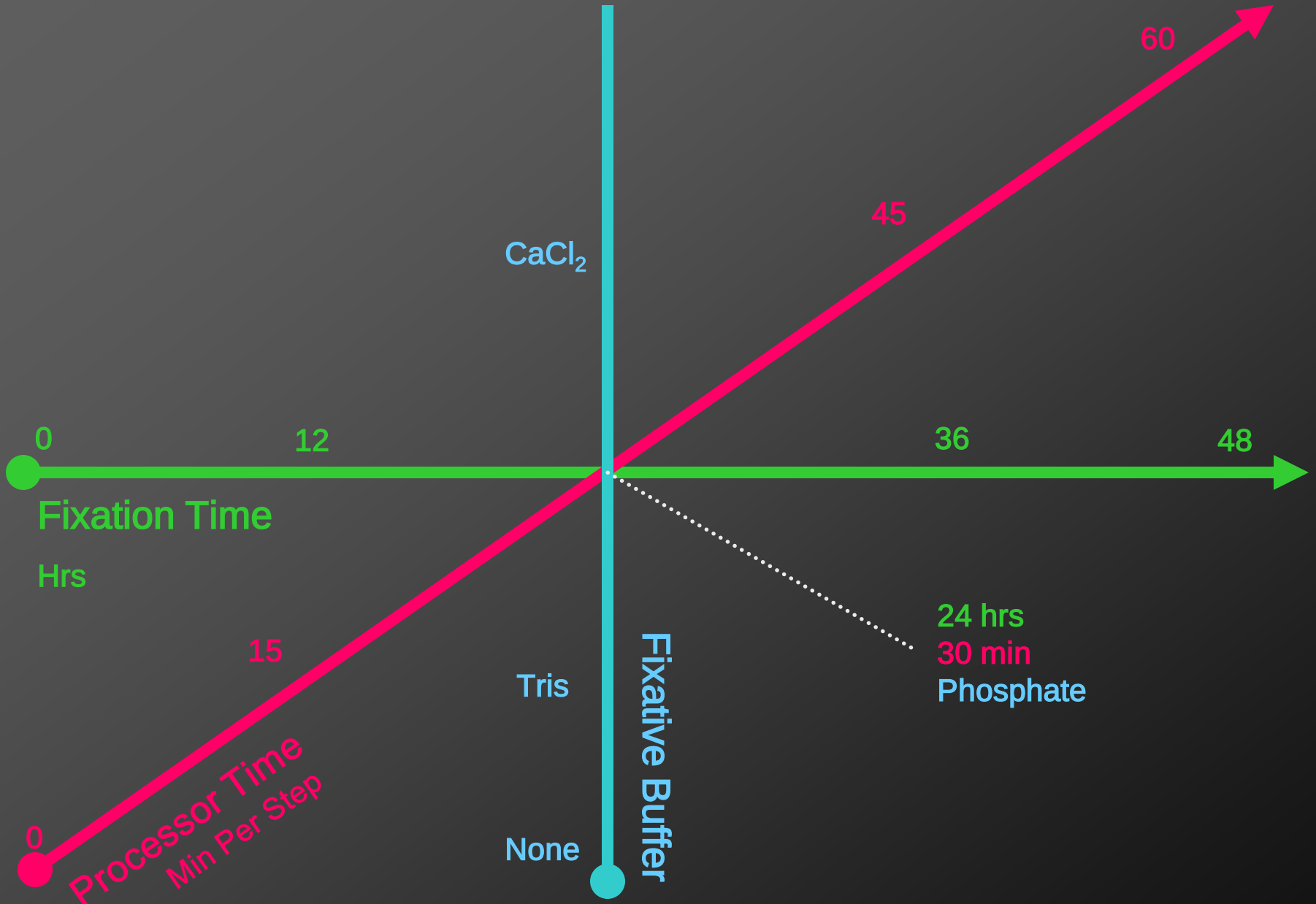


- Branch DNA Based Assays
  - Nucleic Acid ELISA
  - No Enzymatic Steps



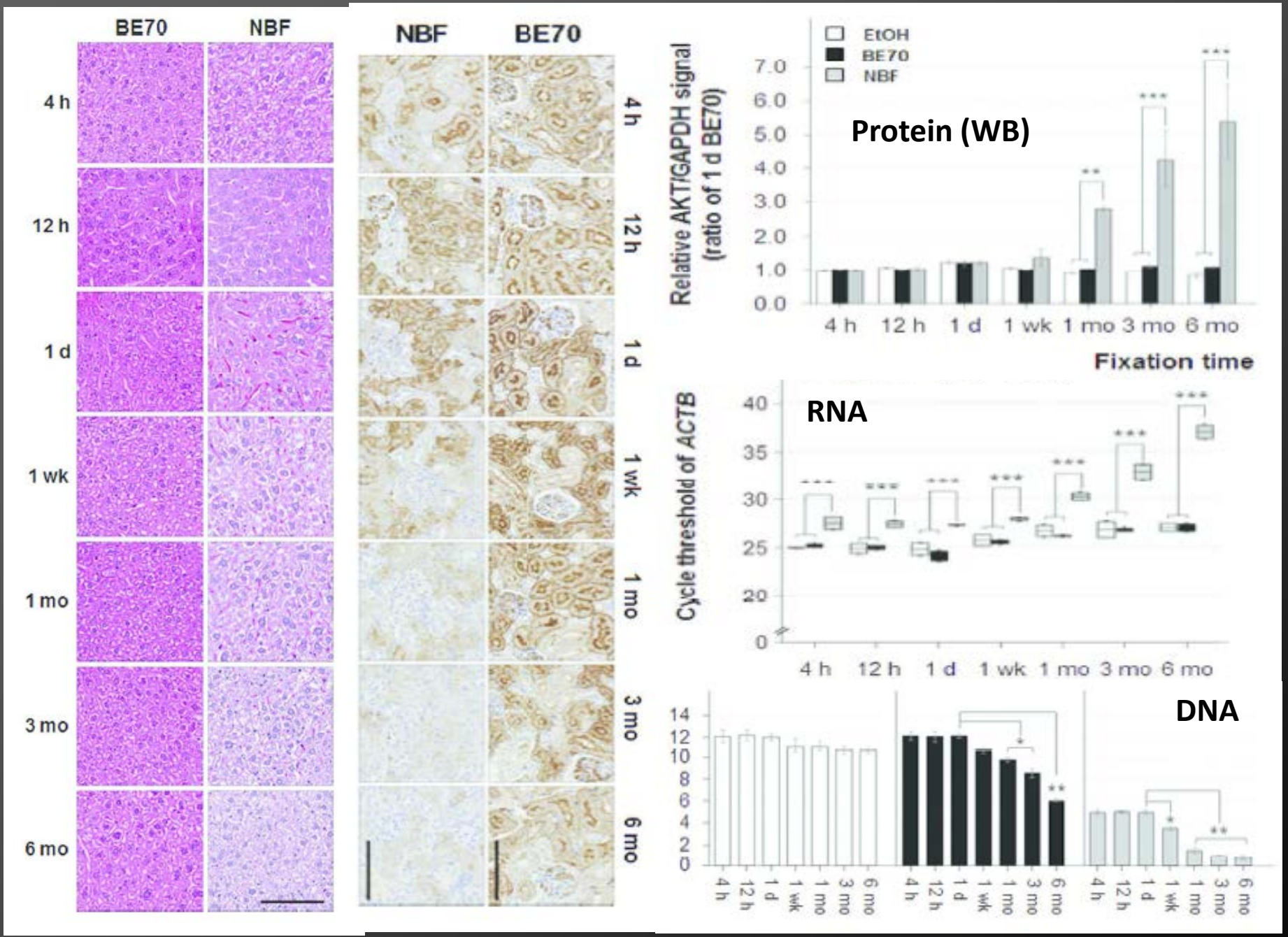
Schematic Of Process

Formalin Fixed Paraffin Embedded Tissue



Revised Model Of Chemical Fixation

- Tissue Hypoxia & Switching To Glycolysis - "*Drowning*"
  - RNA stores consumed, Alterations in Phospho-Proteome
- Infiltration & Inhibition Of Glycolysis & Oxidative Phosphorylation
  - Halting Of Most Biologic Process
- Chemical Reactions Crosslinking Proteins and Nucleic Acids
  - Halting Of Remaining Enzymatic Activity


BE70
NBF
4 h
12 h
1 d
1 wk
1 mo
3 mo
6 mo
NBF
BE70
4 h
12 h
1 d
1 wk
1 mo
3 mo
6 mo
EtOH
BE70
NBF
Relative AKT/GAPDH signal
(ratio of 1 d BE70)
Protein (WB)
Fixation time
RNA
Cycle threshold of ACTB
DNA

Chemical Fixation

- Aldehyde Fixatives Are Two-Step Fixatives
  - Coagulative
  - Acid/Aldehyde Crosslinking - Degradation
- Alcohol Fixatives Are Single-Step Fixatives
  - Coagulative
  - No Acid-Base Degradative Chemistry

- Coagulative Fixatives Are More Stable & Result In Improved Biomolecular Analytes

Experimental Pathology Laboratory

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Center For Device & Radiologic Health

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Icahn School of Medicine at Mount Sinai

Challenges in collecting and pre-analytical processing of tissue: the human arterial wall

Chiara Giannarelli, MD, PhD

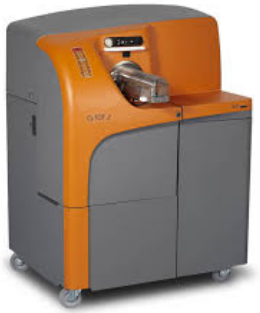
Assistant Professor of Medicine, Cardiology

Assistant Professor of Genetics and Genomics Sciences

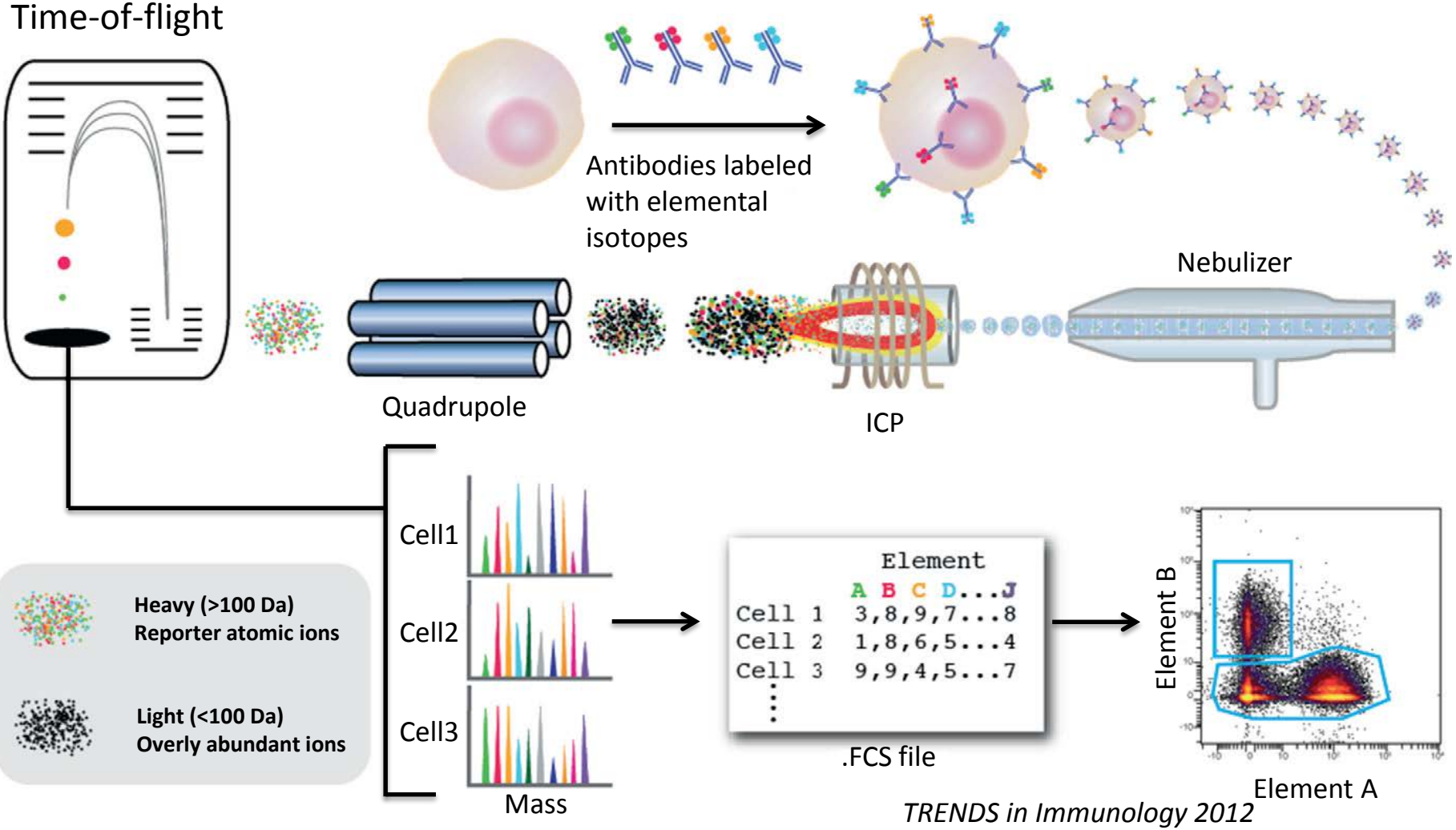
Icahn School of Medicine at Mount Sinai

NIH Common Fund
HuBMAP / SCAP Mini Workshop
June 28, 2017
Neuroscience Center,
Bethesda, MD

Time of Flight Mass Cytometry (CyTOF)

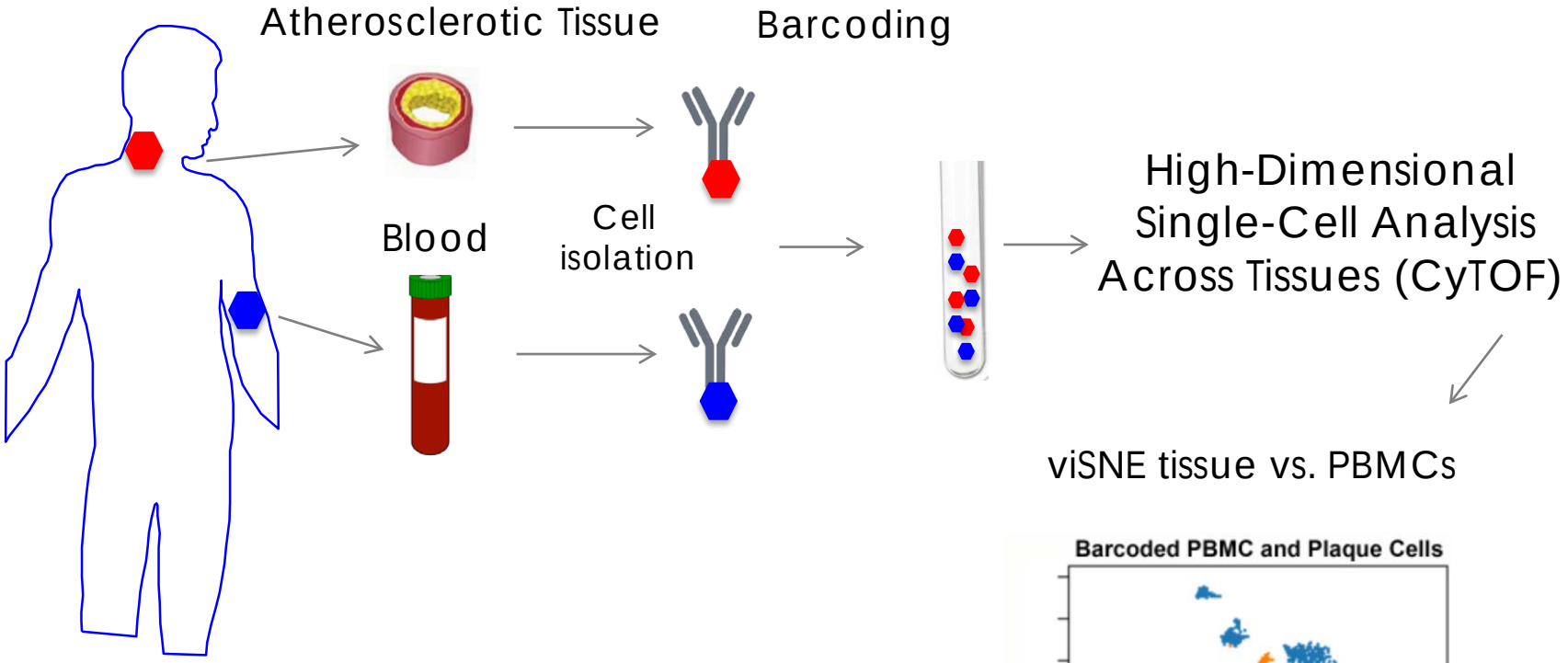


Time-of-flight



TRENDS in Immunology 2012

Experimental Pipeline
CyTOF Single-Cell Analysis



Lai, Cytometry 2015.
Amir, Nature Biotechnology 2013

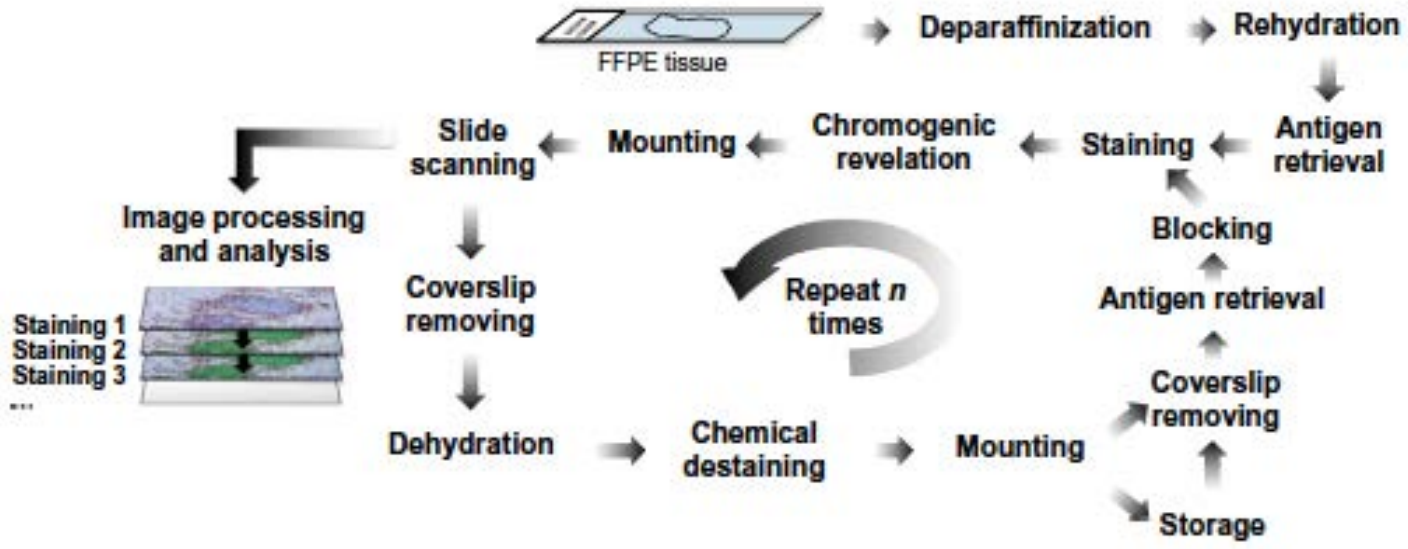
SCIENCE IMMUNOLOGY | RESEARCH RESOURCE

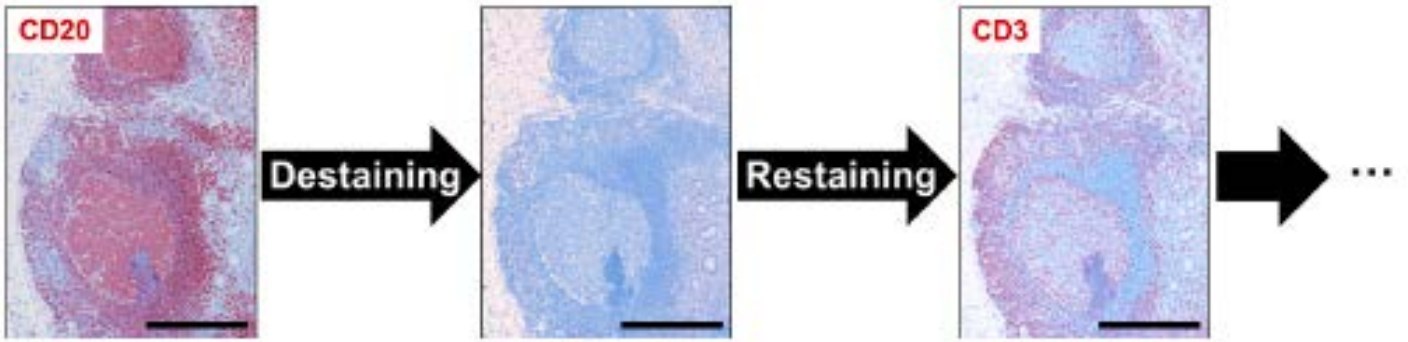
IMMUNOTHERAPY

In-depth tissue profiling using multiplexed immunohistochemical consecutive staining on single slide

Romain Remark,[1] Taha Merghoub,[2] Niels Grabe,[3] Geert Litjens,[3] Diane Damotte,[4,5] Jedd D. Wolchok,[2] Miriam Merad,[1*†] Sacha Gnjatic[1*†]

Multiplexed IHC for Validation and Tissue Discrimination

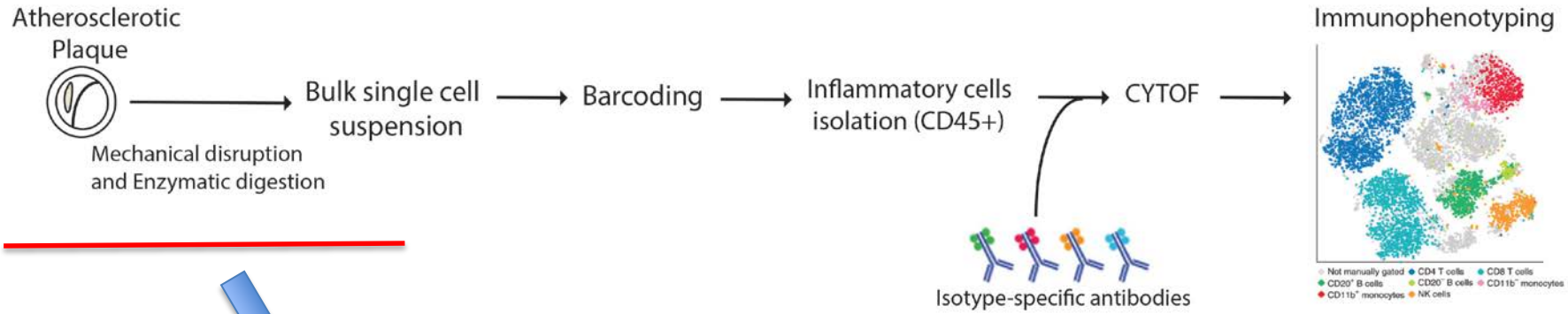




Experimental Workflow: challenges

?

In the lab



90% of the challenges

1. Alteration of surface markers
2. Alteration of functional state
3. Incomplete or too harsh digestion
4. Minimize the digestion time: live cell recovery
5. Tissue debris (collagen, elastin, calcium deposits)
6. Blood contamination

Future Directions

5-years goals

1. Map the human immune system in the diseased human arterial wall

2. Extend our pipeline to other cell types: i.e. VSMC, ECs, fibroblasts

3. Map the healthy arterial wall

4. Understand the impact of age, gender, cardiovascular risk factors, treatments on cell diversity in health and disease

Future Directions

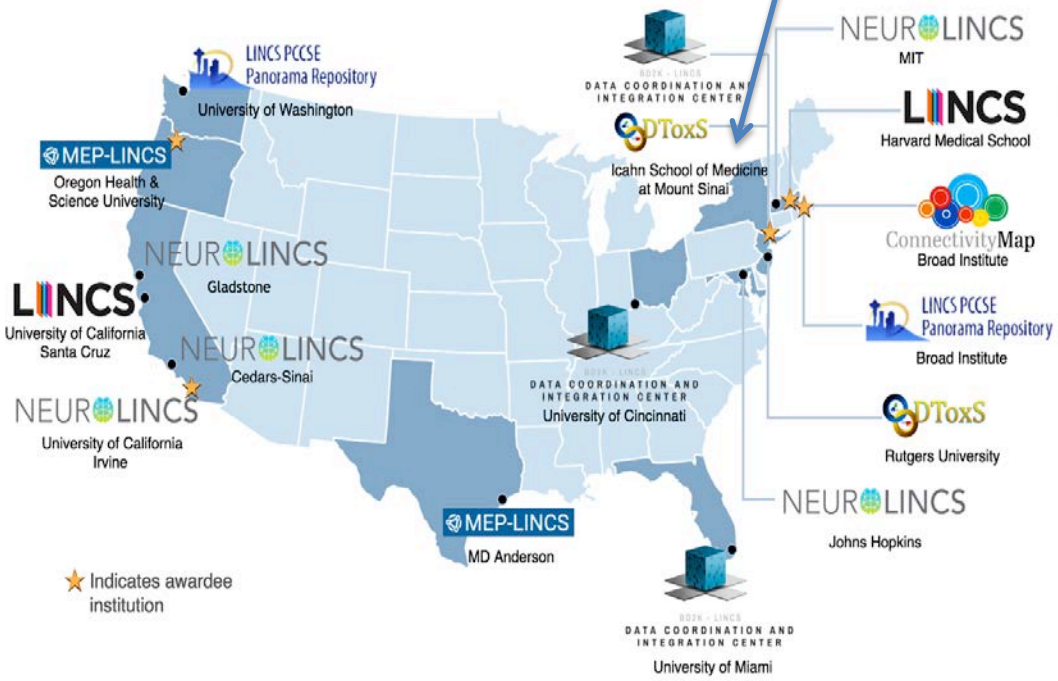
10-years goals

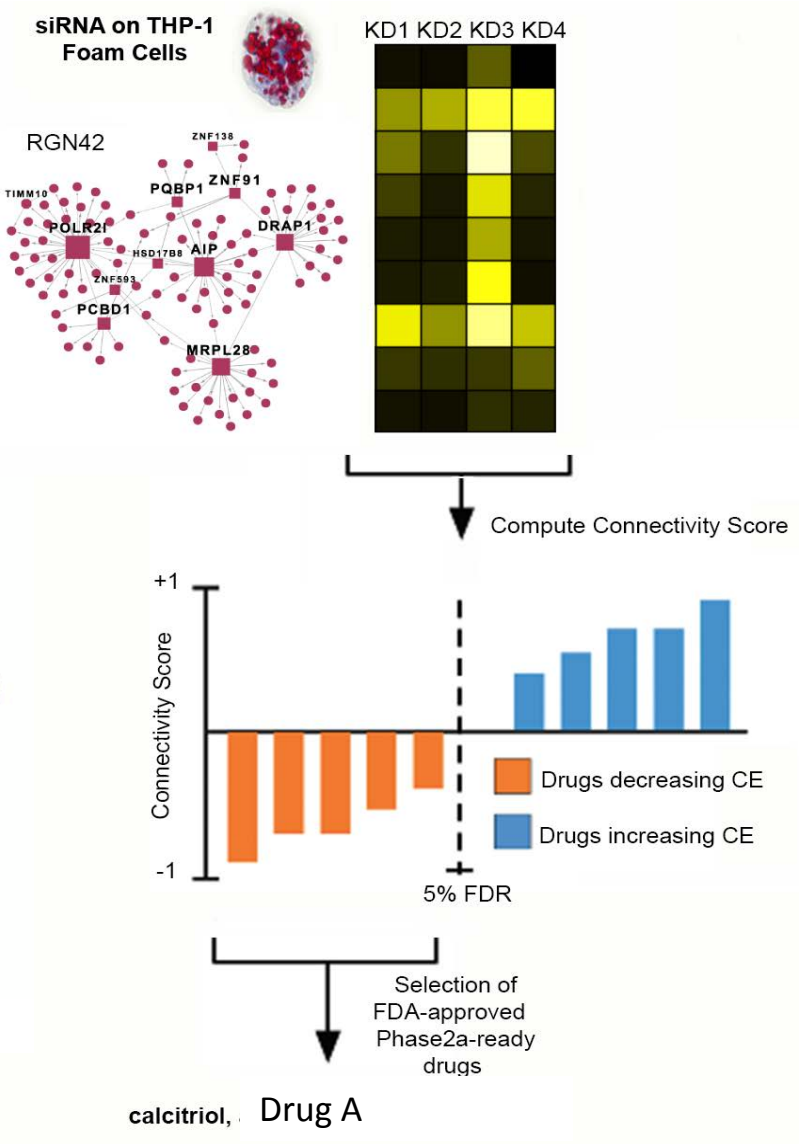
To identify of tissue-specific immune and other cell type variations to provide new mechanist insights for the rational design of immunotherapies in atherosclerosis and to preserve vascular health

Network-driven drug repositioning approaches to treat CAD

NIH LINCS Program

LIBRARY OF INTEGRATED NETWORK-BASED CELLULAR SIGNATURES





Schematic representation of the computational workflow for the repositioning approach used to identify candidate drugs targeting RGN42.

National Center for Advancing Translational Sciences

R21TR001739

Future Directions

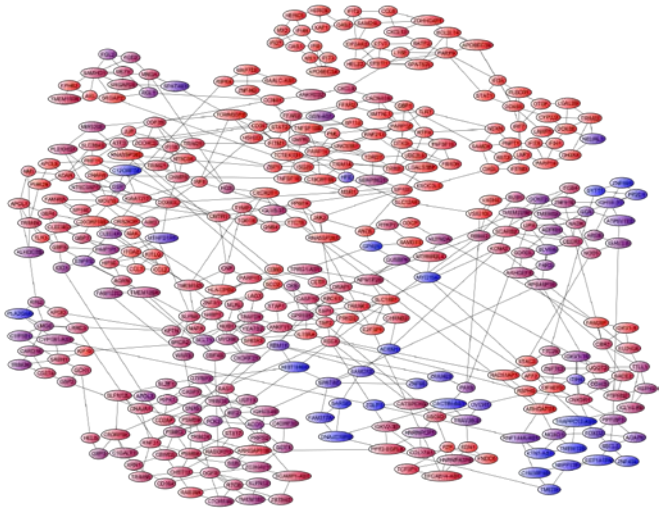
10-years goals

To identify of tissue-specific immune and other cell type variations to provide new mechanist insights for the rational design of immunotherapies in atherosclerosis and to preserve vascular health

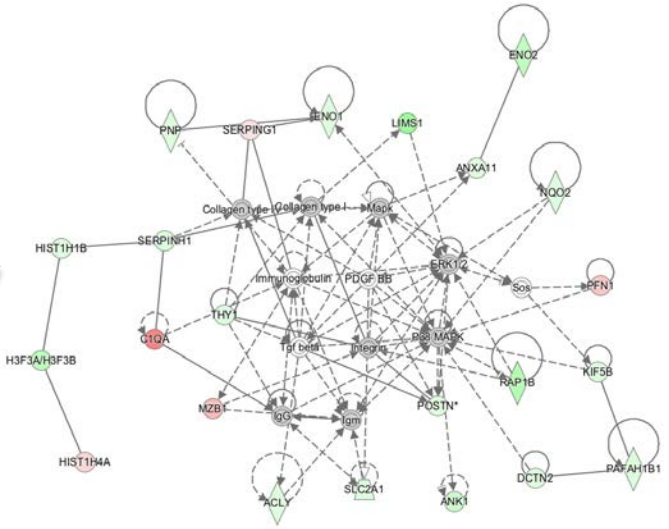
To integrate non-invasive imaging modalities for precision diagnosis and personalized treatments

Systems Biology of Human Atherosclerotic Arterial Wall

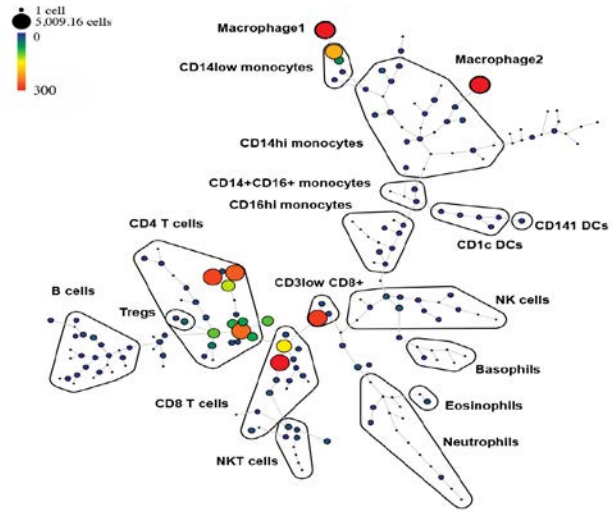
Gene networks



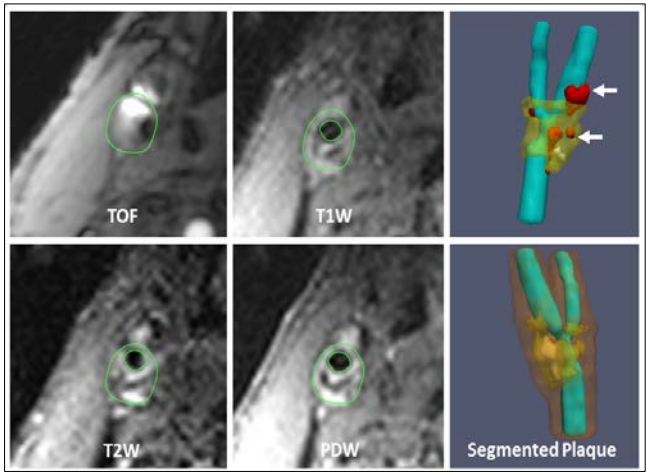
Protein Networks



Cellular Networks



Imaging



Drs. Fayad and Calcagno-TMII

How to build an ideal future state

1. Standardized SOP across different lab
2. Data sharing policy to build a human atlas
3. Establish collaborative multidisciplinary environment for investigators
4. Rigorous collection of health and disease information for each individual
5. Interdisciplinary working groups (bioinformatics, biology, medical background)

Thank you !

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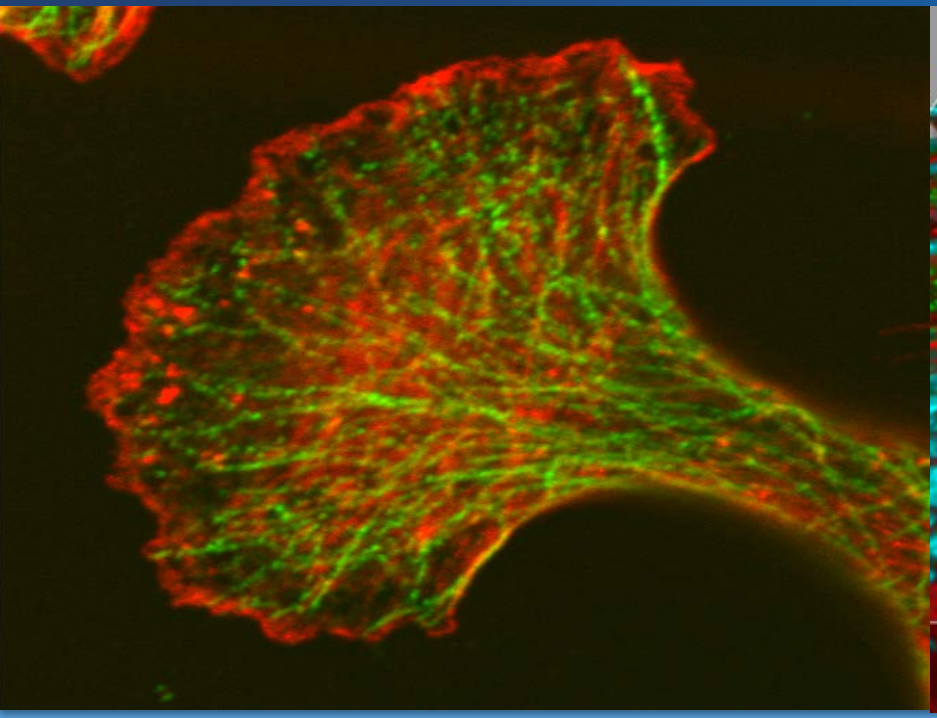
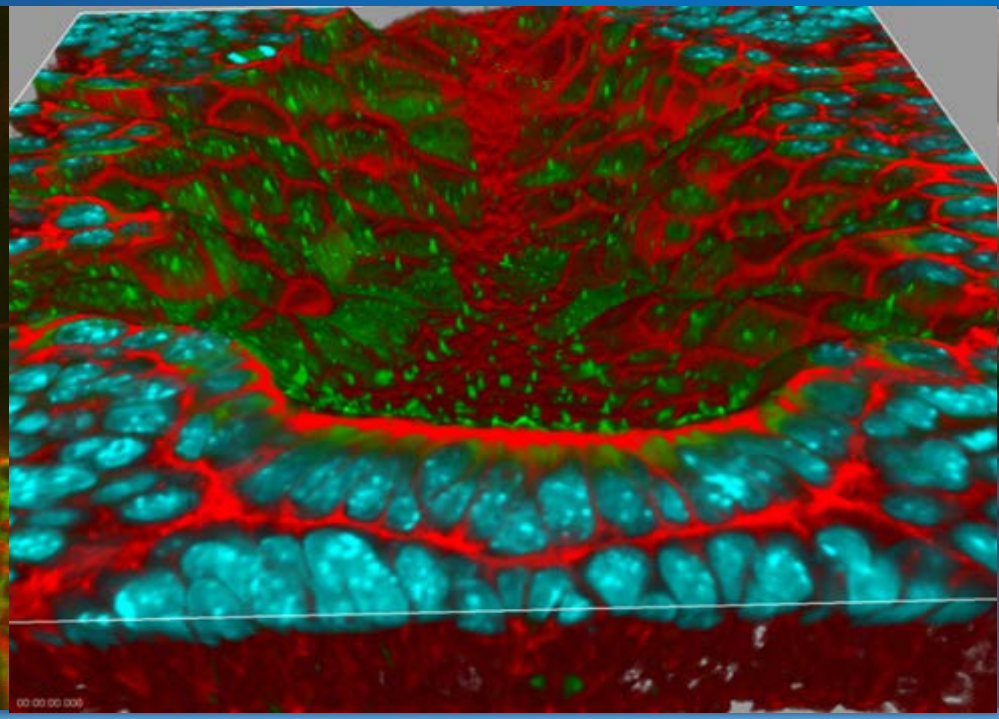
Frederick National Laboratory
for Cancer Research





Optical Microscopy and Analysis Laboratory

Stephen J. Lockett

OMAL
Optical Microscopy and Analysis Laboratory

https://confocal.cancer.gov/cores/optical-microscopy-and-analysis-laboratory

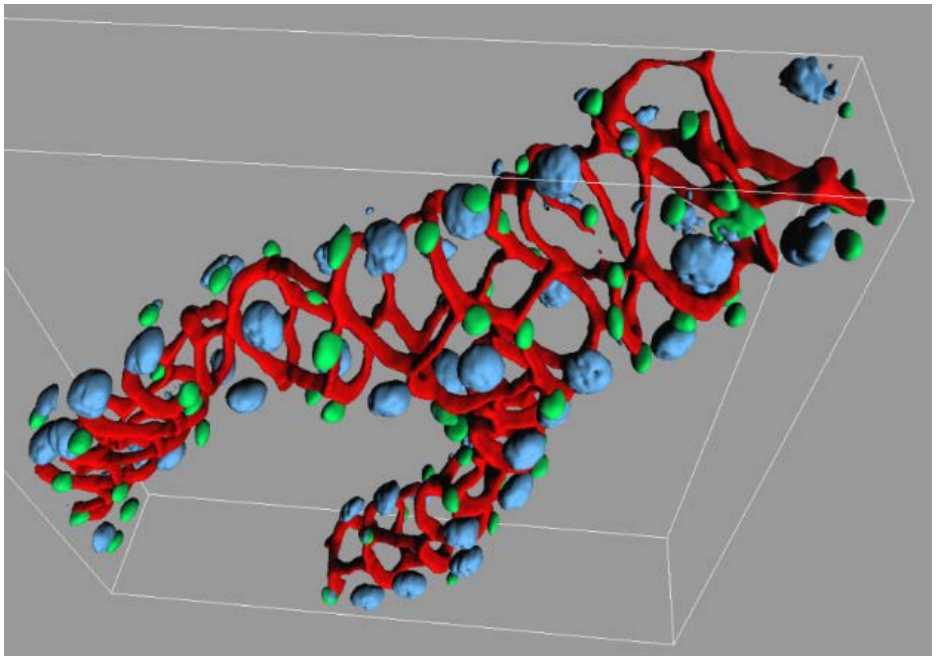
The Frederick National Laboratory is a Federally Funded Research and Development Center operated by Leidos Biomedical Research, Inc., for the National Cancer Institute
DEPARTMENT OF HEALTH AND HUMAN SERVICES • National Institutes of Health • National Cancer Institute

Capabilities

Frederick National Laboratory for Cancer Research

Standard:

- 3D confocal microscopy
- Live cell imaging
- High content
- Fluorescence Recovery After Photobleaching (FRAP)
- Förster Resonance Energy Transfer (FRET)
- Structured Illumination Microscopy (SIM)
- Software for cell segmentation, colocalization, deconvolution, visualization



Advanced:

- Photo-activation Localization Microscopy and Stochastic Optical Reconstruction Microscopy
- Fluorescence Correlation Spectroscopy (FCS)
- Intravital animal imaging
- Tissue clearing
- Lightsheet microscopy
- Fluorescence life-time imaging (FLIM)
- Multi-functional and multi-focus microscopy (MFM)
- Total internal reflection fluorescence (TIRF) microscopy
- Customized image analysis, e.g particle tracking

Drosophila Kidney, Dr. Steven Hou, CCR

OMAL
Optical Microscopy and Analysis Laboratory

High Quality Tissue and Cell Imaging

Frederick National Laboratory for Cancer Research

	Criterion	Method
Sample	Physical tissue damage Good overall morphology	Visual assessment
Labeling	Correctly localized protein	Visual: in the correct cells, compare FP to antibody
Microscope	Spatial Resolution, Spherical aberration, chromatic aberration	Visual, quantitative measurement with fluorescent beads
Microscope	Spectral bleedthrough	Visual, spectral unmixing
Image Analysis	Segmentation accuracy	Use well-known algorithms: thresholding, watershed and compare to ground truth

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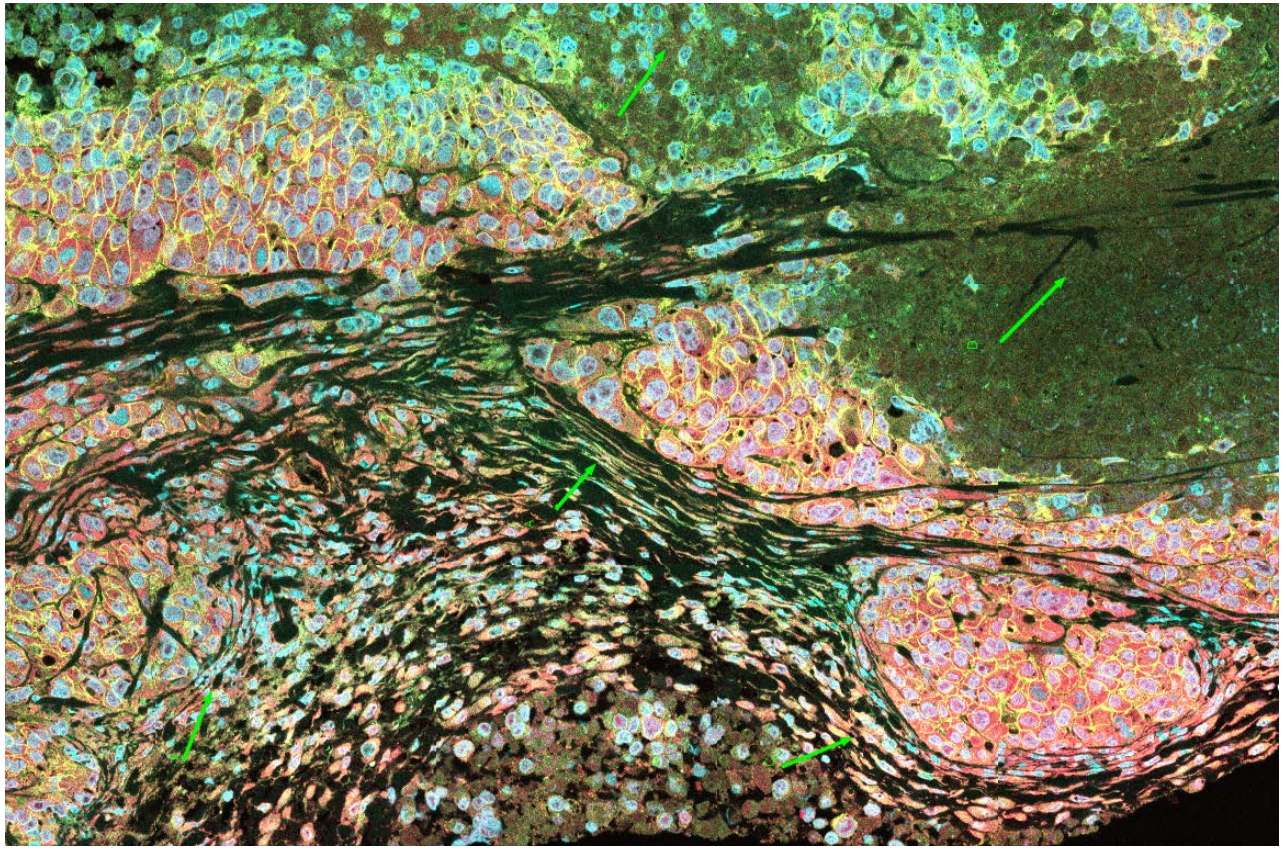
2D and 3D Cell and Nucleus Segmentation

Frederick National Laboratory for Cancer Research

Basic Research: Some interaction generally OK

Drug screening: Automatic. Some degree of error can be tolerated

Pathology: Mixture of automatic and interactive. Inherent sample heterogeneity.



EMT panel on mouse xenograft of human gastric cell line, MKN45. In collaboration with Dr. Robert Kinders, Leidos / DCTD

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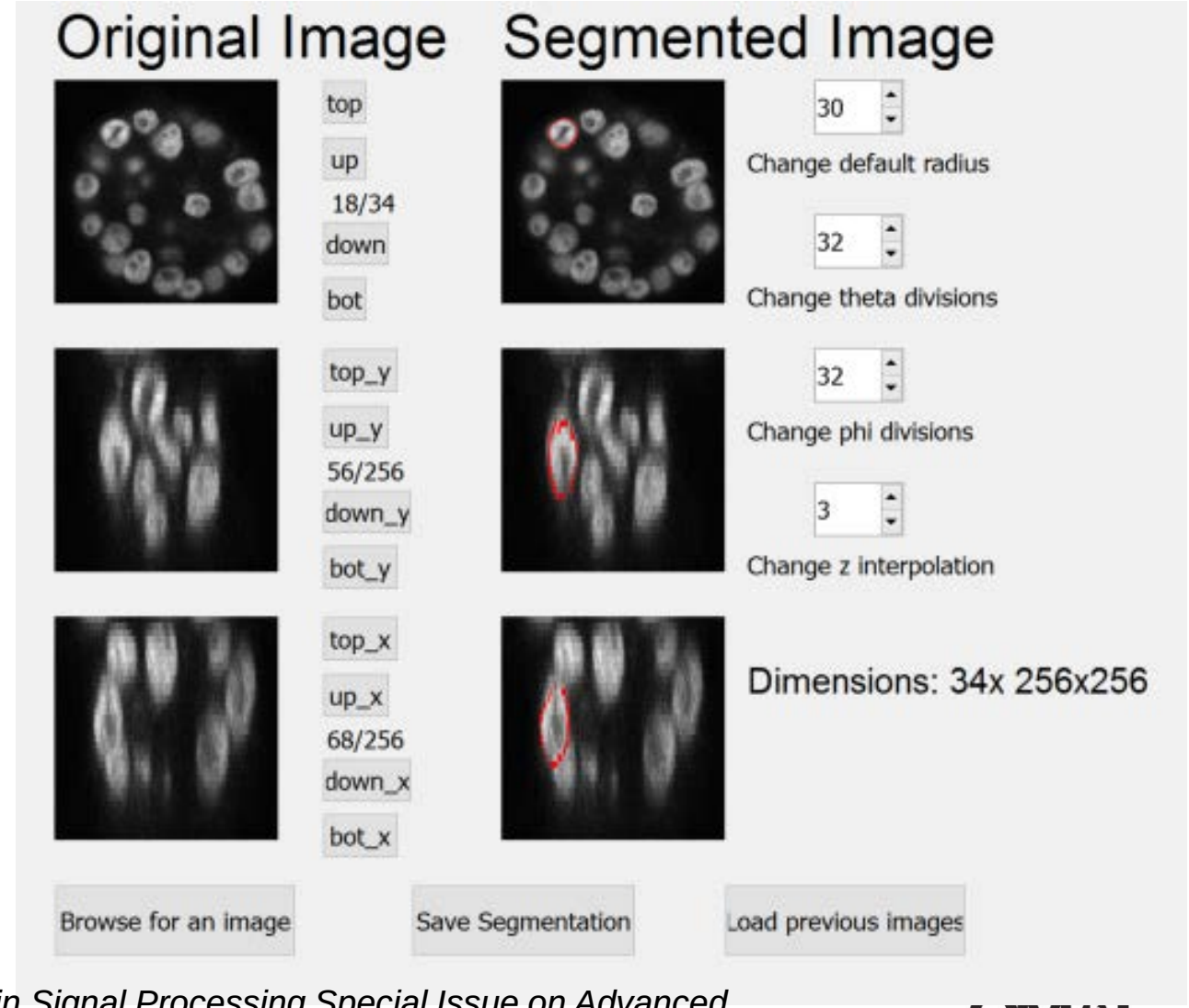
Algorithms for 3D Segmentation

Frederick National Laboratory for Cancer Research

Graph cut segmentation:

- **One click per nucleus**
- **Plus correction points**
- **Finds optimal surface**



Nandy et at, IEEE Selected Topics in Signal Processing Special Issue on Advanced Signal Processing in Microscopy and Cell Imaging. 2016

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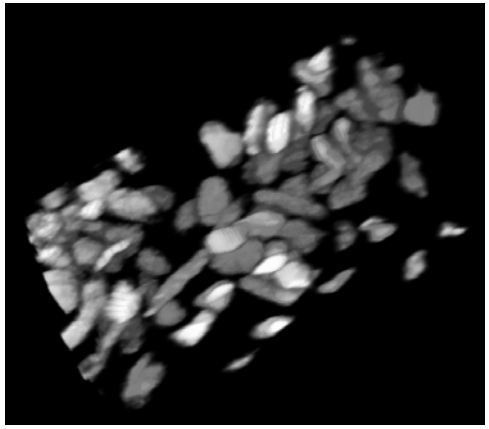
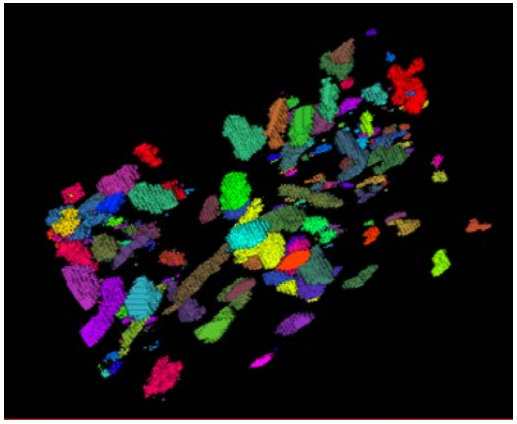
3D Segmentation Results

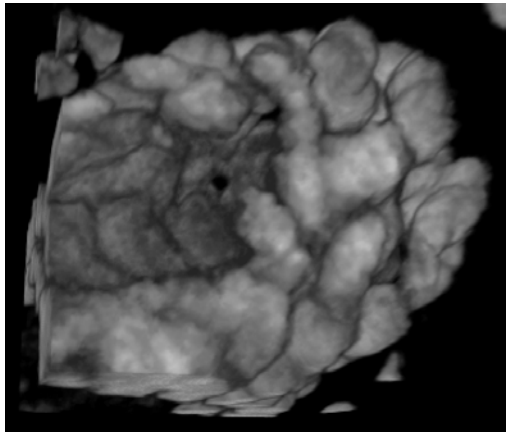
Frederick National Laboratory for Cancer Research



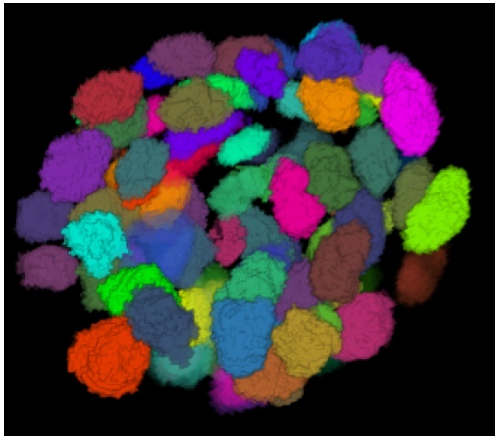
Volume rendering of low density of nuclei



Automatic segmentation can be trusted



Volume rendering of high density of nuclei



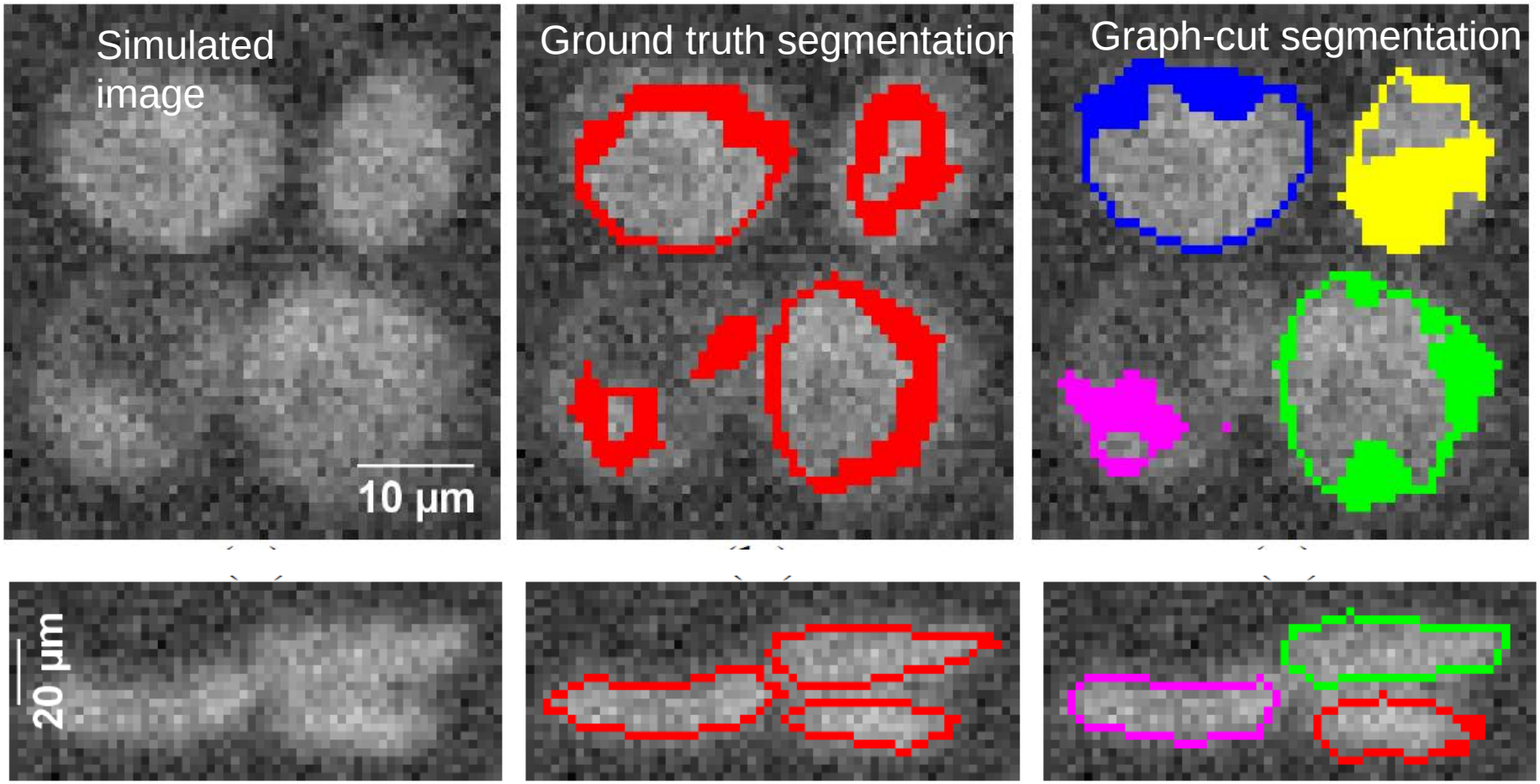
Need semi-interactive segmentation

OMAL Optical Microscopy and Analysis Laboratory

3D Ground Truth for Validation

Frederick National Laboratory for Cancer Research

1) **Segment actual 3D images as accurately as possible.**
2) **Treat the segmented image as the "perfect" image.**
3) **Artificially reintroduce the distortions of 3D microscopy: 3D PSF and noise, then use this image to test segmentation methods**

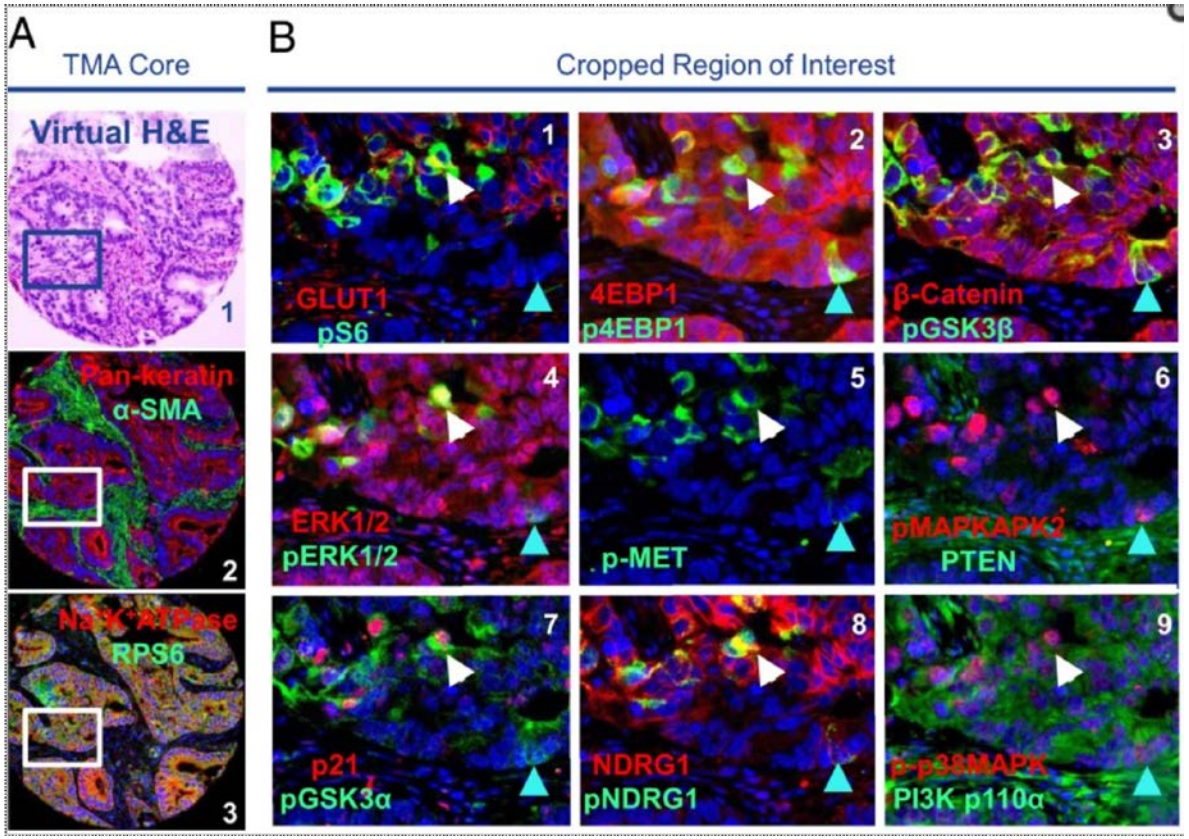


Where we are now

Frederick National Laboratory for Cancer Research

Where we are at:

Grind and bind -> single, disaggregated cell analysis -> *in situ* molecular profiling



Gerdes et al, PNAS, 2013

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Five Years from Now

Frederick National Laboratory for Cancer Research

In situ molecular profiling -> tissue / cell / nucleus structure -> phenotype

SOPs for: - tissue collection
- staining
- clearing
- 3D image acquisition
- 3D image analysis
- visual representation of results

190 µm

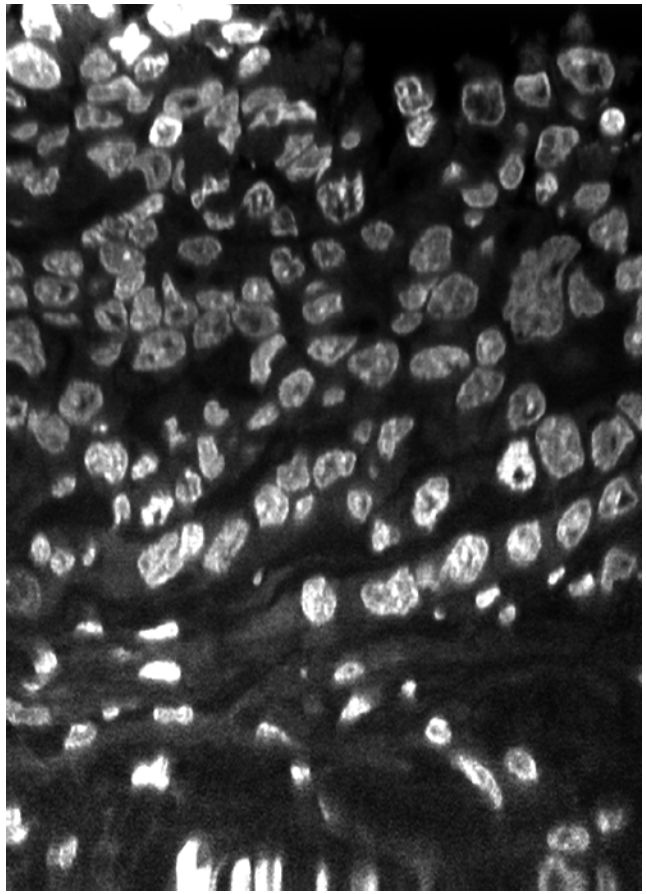

Image acquired with two photon (2P) 3D microscopy of TDE cleared tissue

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2017 HuBMAP Mini-workshop: Data Analysis, Standards, and Benchmarks for Single Cell Analysis

Junhyong Kim
University of Pennsylvania

Questions

- Is there benchmark data to compare new experimental or computational methods?
- How do we establish material standards such as specific cells or spike-in RNA?
- What metadata about calibration is important to know?
- What information is important to collect about the sample and its preparation?

Questions

- How can we work together with manufacturers to build standards into their methods?
- Does an ontology need to be established for single cell analysis?
- How can we associate single cells to tissue orientation information? More generally, how can data be organized from the single cell scale to whole organism scale?
- What are the common data elements between imaging and sequencing assays? Is there a common header we can use for all data, similar to FITS or DICOM?

Agenda:

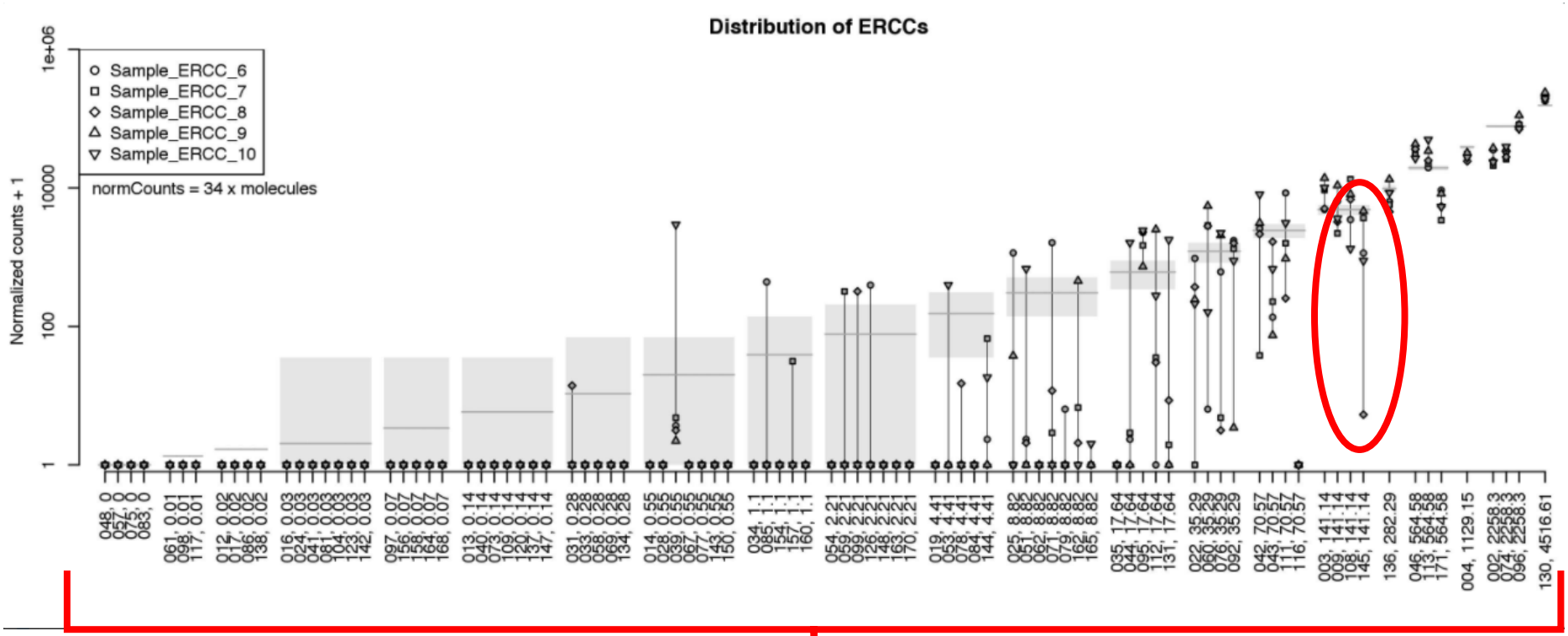
- Overview (3:00-3:15)
- Breakout sessions (3:20-4:00)
- Summary of breakout sessions and synthesis (4:10-5:00)

Breakout Sessions

- Material Standards and Benchmarks
- Calibration, QC, and Instruments
- Experimental Designs, Ontologies, and Metadata
- Data Integration, Scale Alignment, and Data Analysis

Material Standards and Benchmarks

- Standard Cells?
- Control RNA?
- Compendium Data as Benchmarks?



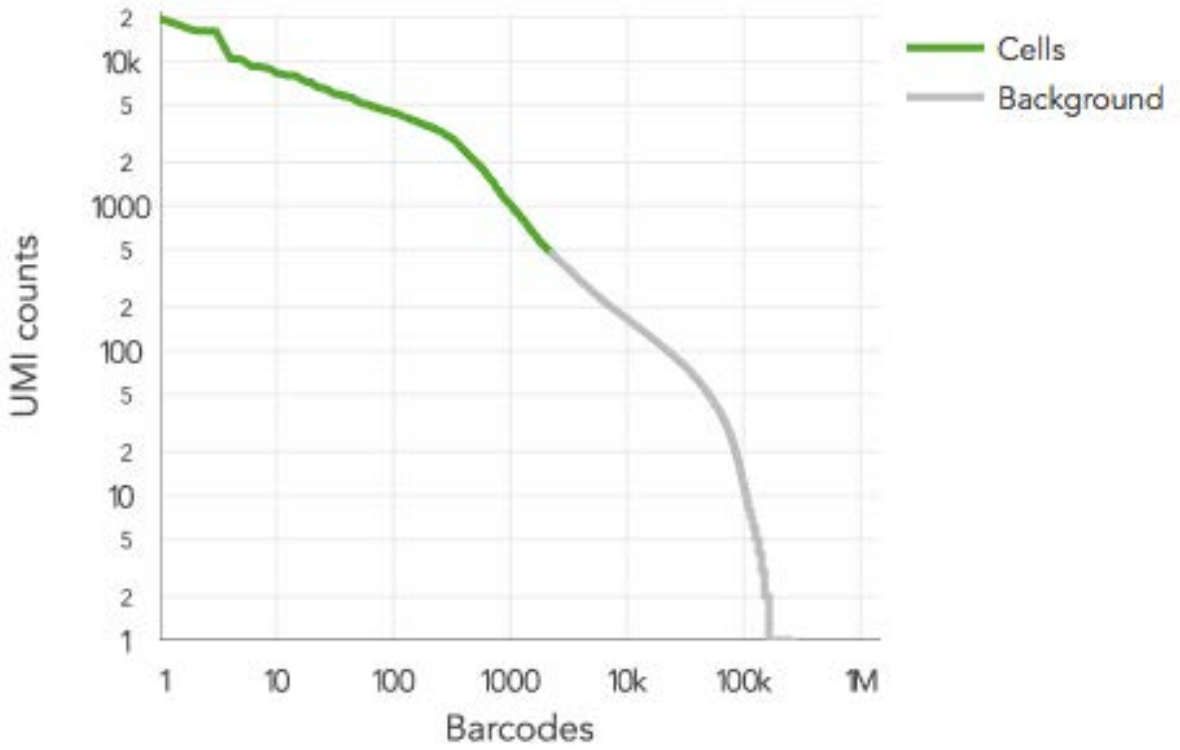
ERCC probes: 20 levels spanning 10^6 range

Calibration, QC, and Instruments

- Can there be an instrument calibration protocol?
- Quality Control protocol?
- Commercial instruments: standards and comparisons?

Cells

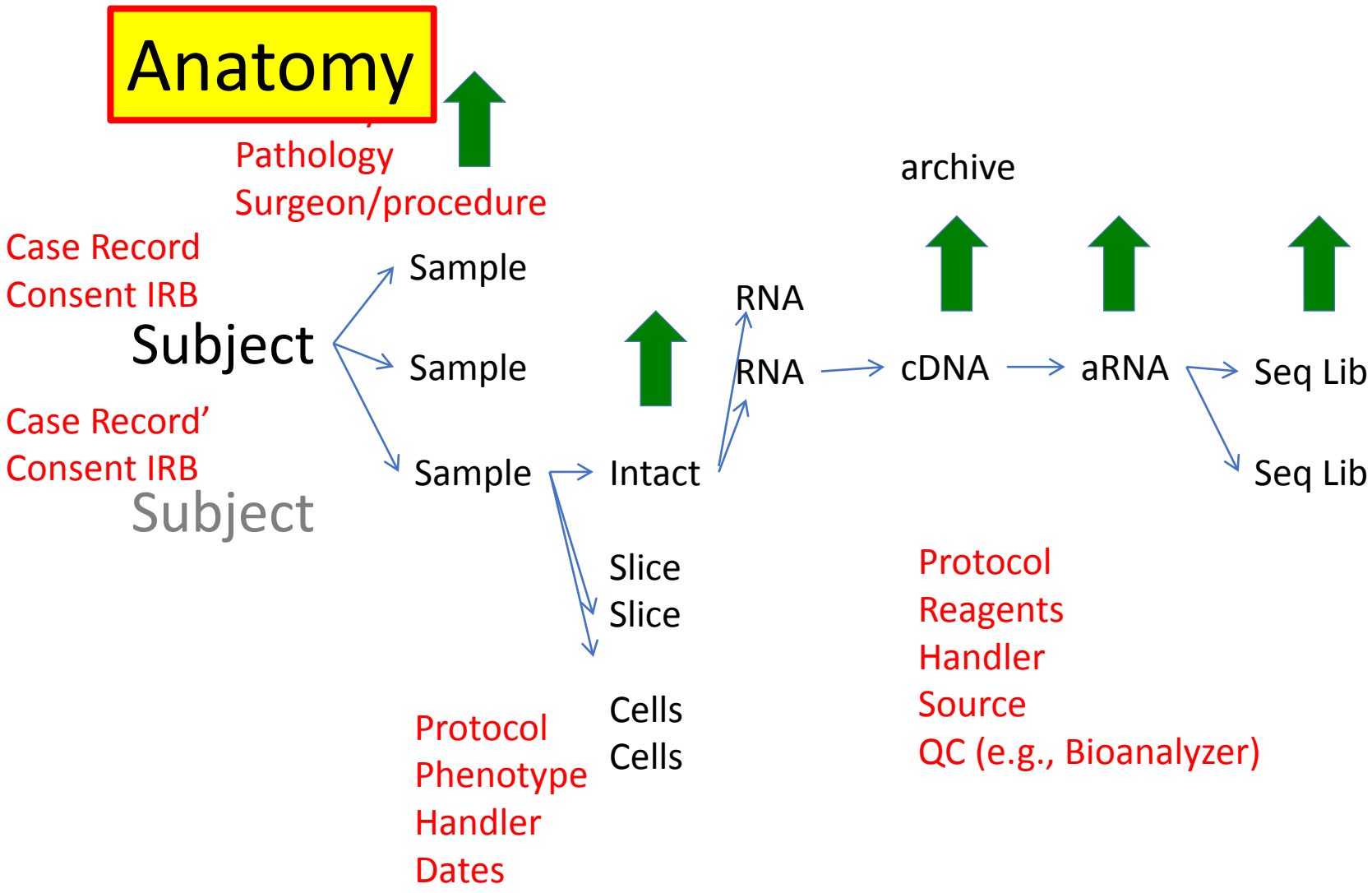
40-fold



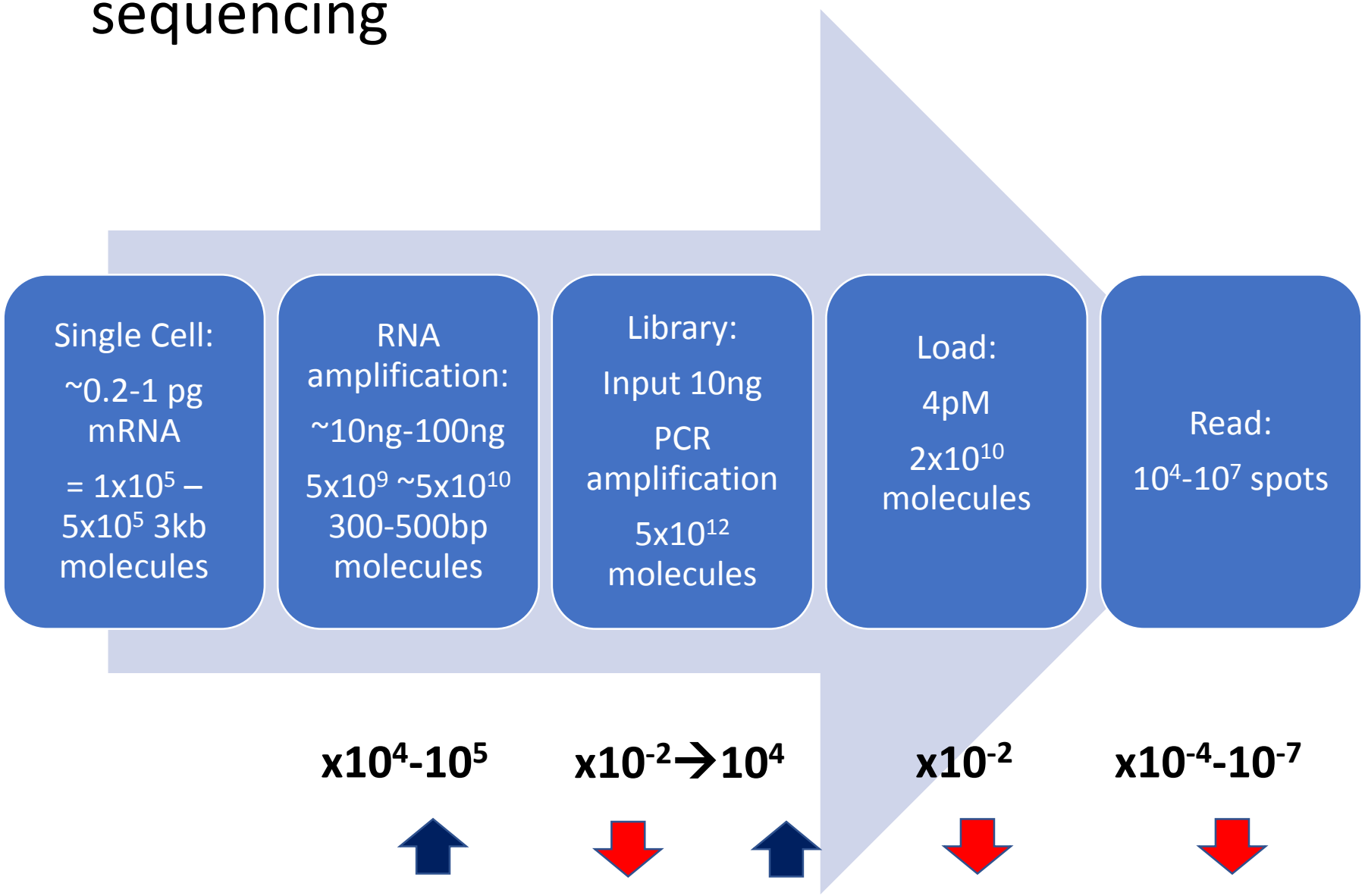
Estimated Number of Cells	2,262
ce WS257 Estimated Number of Cell Partitions	1,482
cb WS257 kona Estimated Number of Cell Partitions	827
ce WS257 Fraction Reads in Cells	22.0%
cb WS257 kona Fraction Reads in Cells	60.0%
Mean Reads per Cell	22,466

Experimental Designs, Ontologies, and Metadata

- Single cell sampling?
- Molecular biology?
- Standardized information models (ontologies) and information capture for provenance?


Anatomy
Pathology
Surgeon/procedure
Case Record
Consent IRB
Subject
Case Record'
Consent IRB
Subject
Sample
Sample
Sample
Intact
Slice
Slice
Cells
Cells
Protocol
Phenotype
Handler
Dates
RNA
RNA
archive
cDNA
aRNA
Seq Lib
Seq Lib
Protocol
Reagents
Handler
Source
QC (e.g., Bioanalyzer)

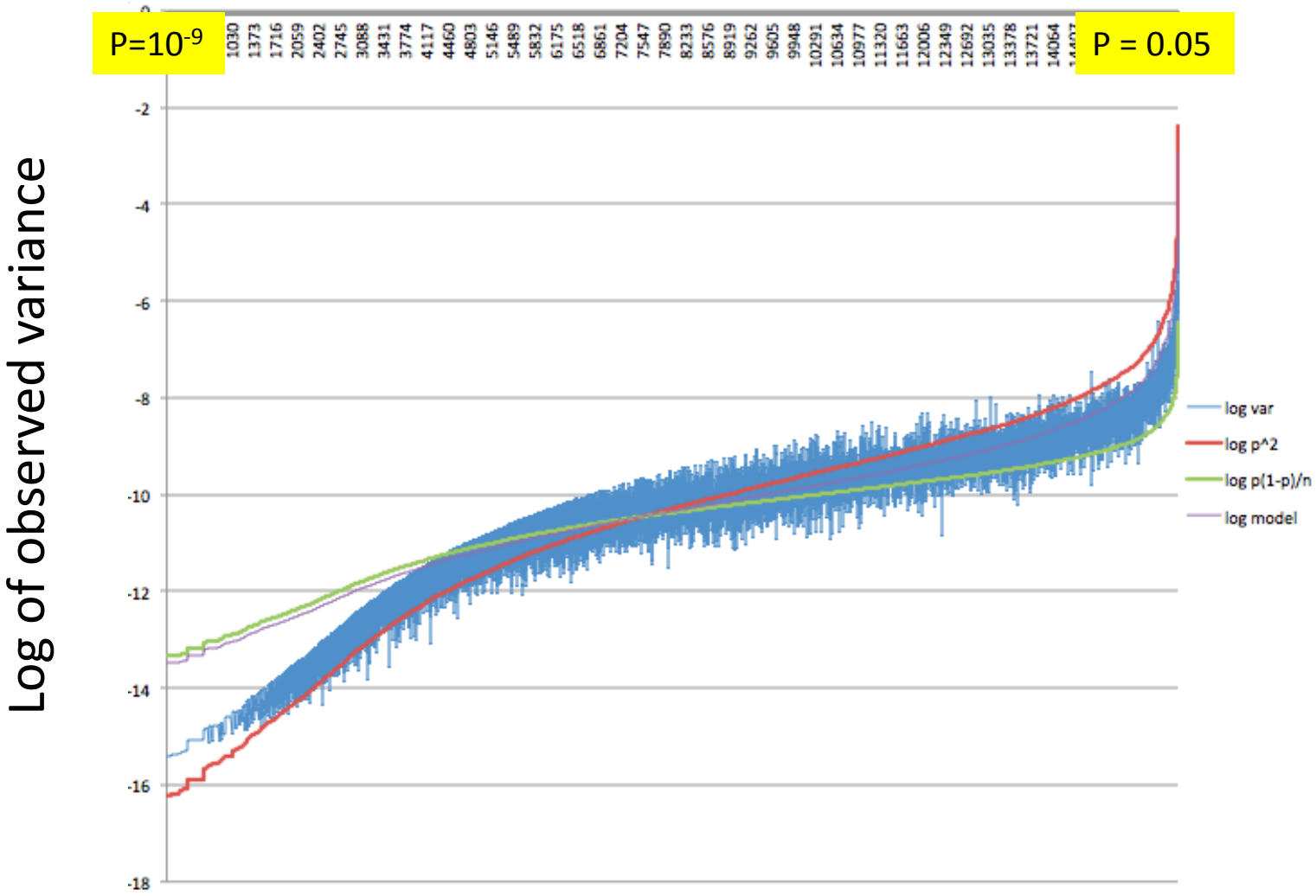
Sampling Variance Associated with RNA sequencing



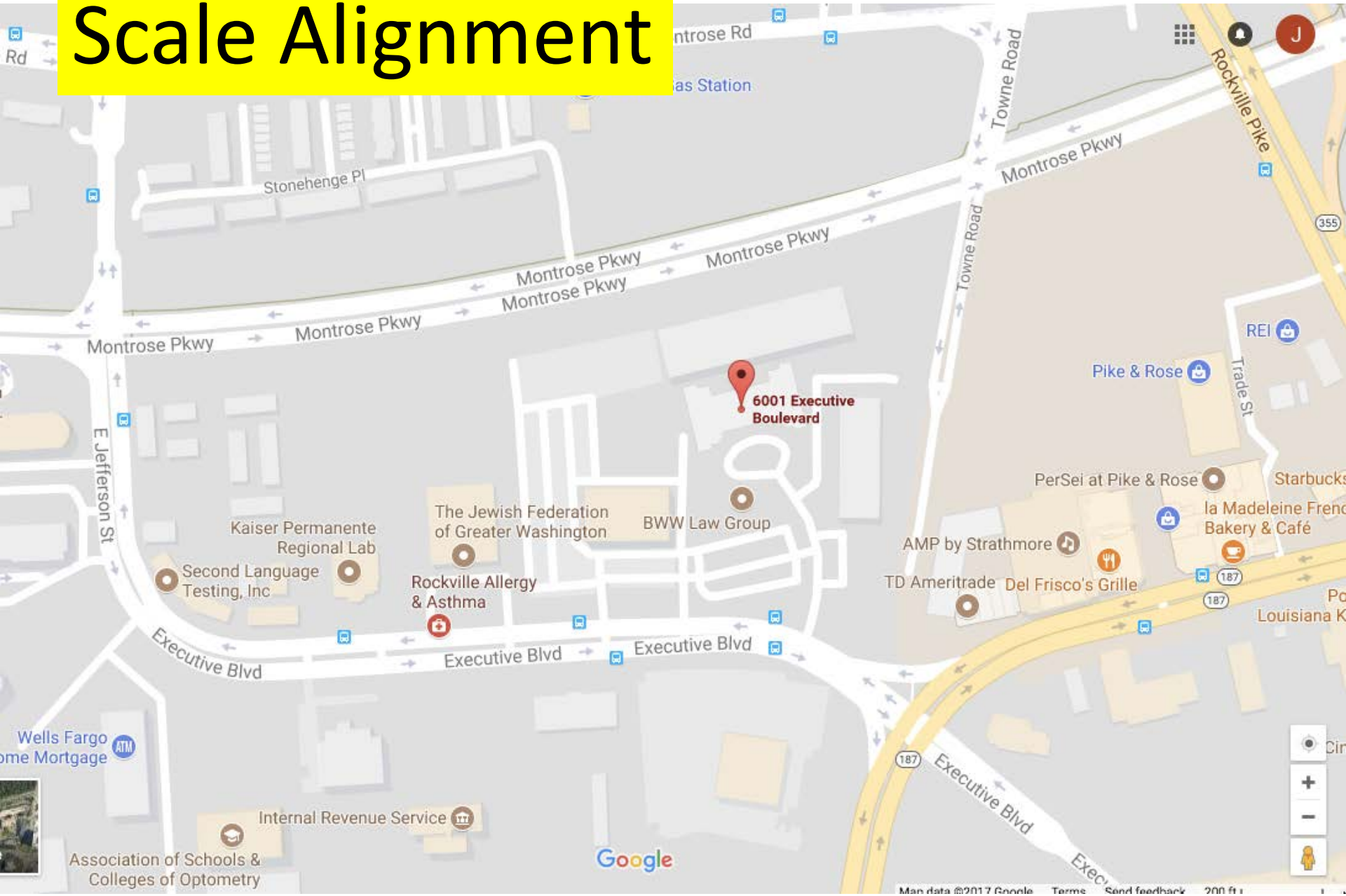
Data Integration, Scale Alignment, and Data Analysis

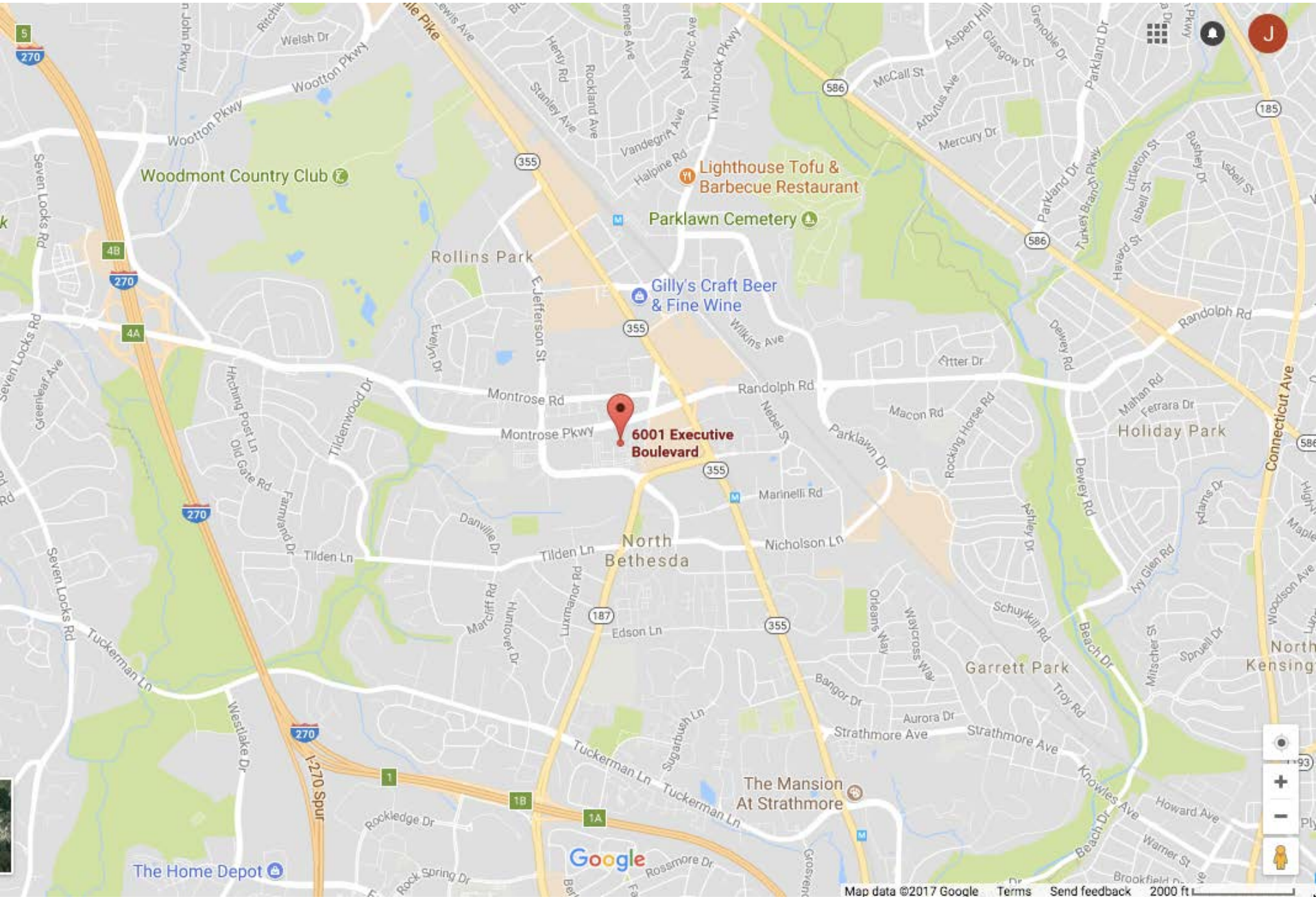
- What are the common data elements between imaging and sequencing assays?
- How do we integrate information from different modalities?
- What are the signal to noise characteristics of various single cell platforms?
- How do we align information from single cell scales, to tissues, to organs, to whole bodies?

Replicate variance as a function of expression levels



Scale Alignment




6001 Executive Boulevard
Woodmont Country Club
Lighthouse Tofu & Barbecue Restaurant
Parklawn Cemetery
Rollins Park
Gilly's Craft Beer & Fine Wine
Montrose Rd
Montrose Pkwy
Randolph Rd
Wootton Pkwy
North Bethesda
Garrett Park
Holiday Park
The Mansion At Strathmore
The Home Depot
Tuckerman Ln
Tilden Ln
Nicholson Ln
Marinelli Rd
Edson Ln
Strathmore Ave
Aurora Dr
Bangor Dr
Rockledge Dr
Rock Spring Dr
Rossmore Dr
Parklawn Dr
Connecticut Ave
Seven Locks Rd
Wootton Pkwy
Welsh Dr
Halpine Rd
Wilkins Ave
Randolph Rd
Macon Rd
Marinelli Rd
Nicholson Ln
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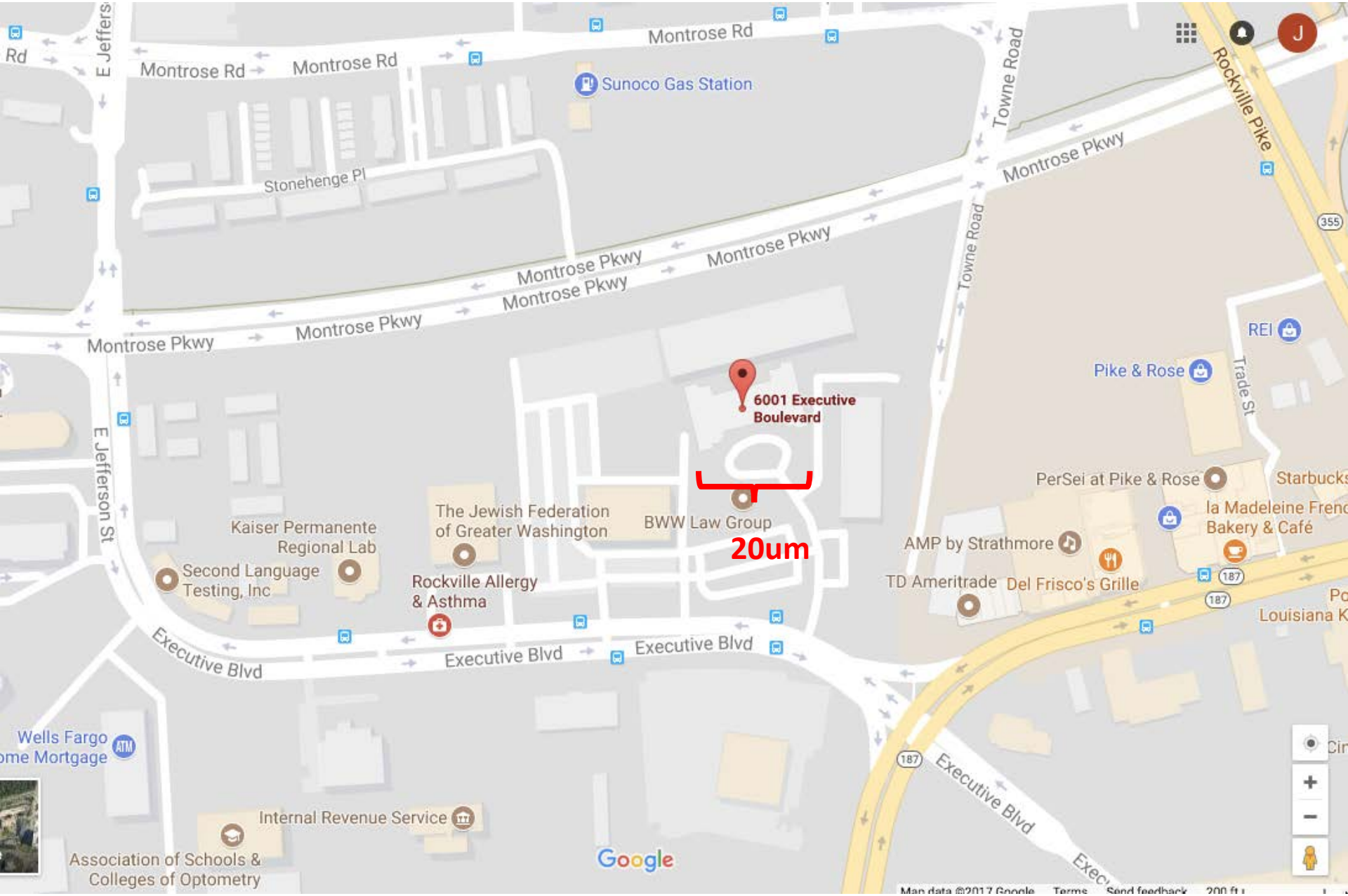
Montrose Rd
Montrose Rd
Montrose Rd
Sunoco Gas Station
Towne Road
Rockville Pike
Stonehenge Pl
Montrose Pkwy
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Montrose Pkwy
Montrose Pkwy
Towne Road
355
REI
Pike & Rose
Trade St
6001 Executive Boulevard
E Jefferson St
PerSei at Pike & Rose
Starbucks
la Madeleine French Bakery & Café
The Jewish Federation of Greater Washington
BWW Law Group
20um
Kaiser Permanente Regional Lab
Second Language Testing, Inc
Rockville Allergy & Asthma
AMP by Strathmore
TD Ameritrade
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Executive Blvd
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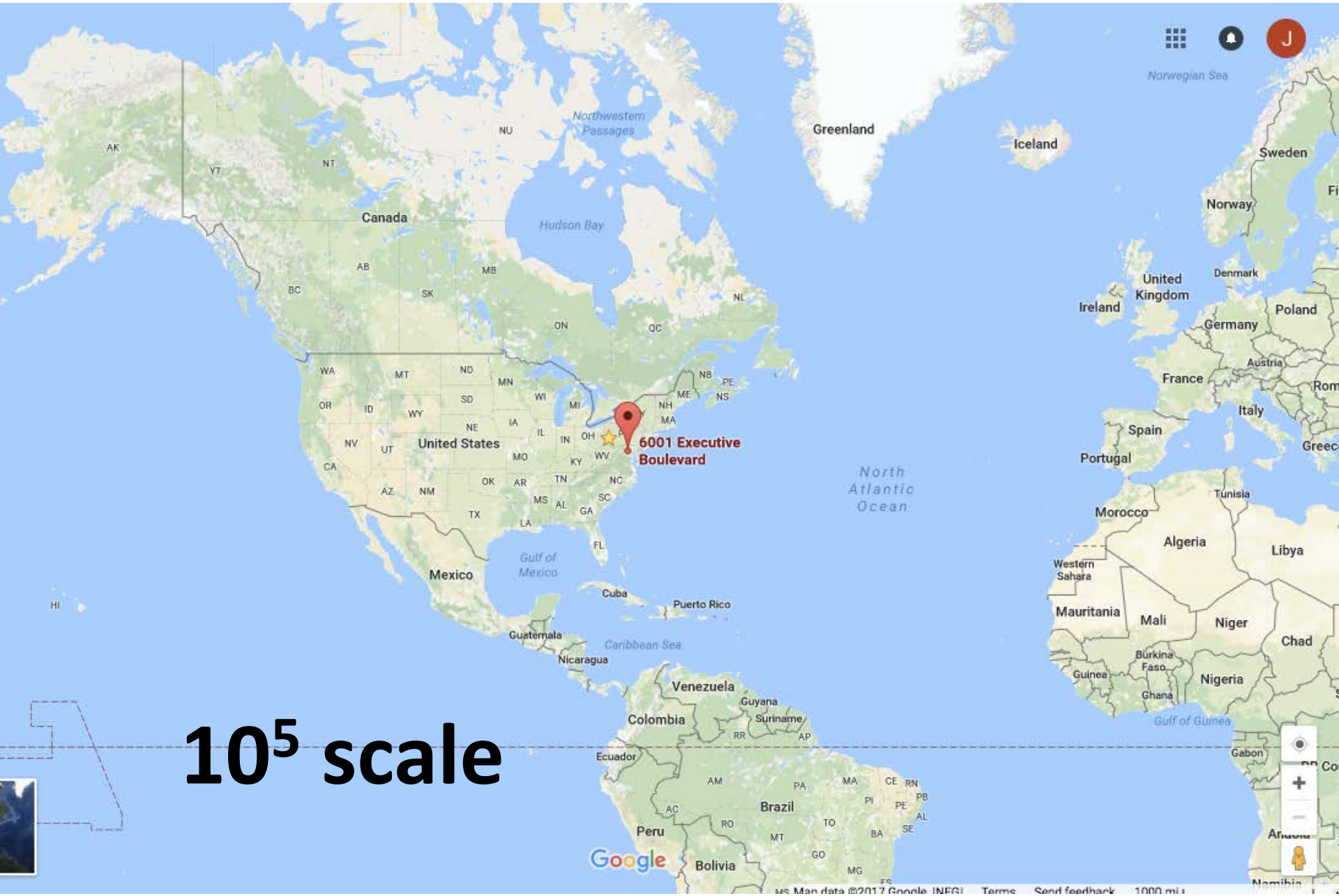
Wells Fargo
Internal Revenue Service
Association of Schools & Colleges of Optometry
Google


6001 Executive Boulevard
United States
Canada
Mexico
Greenland
Iceland
Norwegian Sea
Sweden
Norway
Ireland
United Kingdom
Denmark
Poland
Germany
France
Spain
Portugal
Italy
Austria
Morocco
Algeria
Libya
Tunisia
Western Sahara
Mauritania
Mali
Niger
Chad
Nigeria
Guinea
Burkina Faso
Ghana
Gulf of Guinea
Gabon
Cuba
Puerto Rico
Guatemala
Nicaragua
Caribbean Sea
Gulf of Mexico
Venezuela
Guyana
Suriname
Colombia
Ecuador
Peru
Brazil
Bolivia
North Atlantic Ocean
Northwestern Passages
Hudson Bay
Google

10^5 scale

Breakout Sessions

- Material Standards and Benchmarks
- Calibration, QC, and Instruments
- Experimental Designs, Ontologies, and Metadata
- Data Integration, Scale Alignment, and Data Analysis

http://discourse.singlecellbiology.org