Why we need Precision Nephrology

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Disclosures

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.

Nephrology 2019

Fluids

Time

ACE/ARB

SGLT2 ?

AKI

CKD

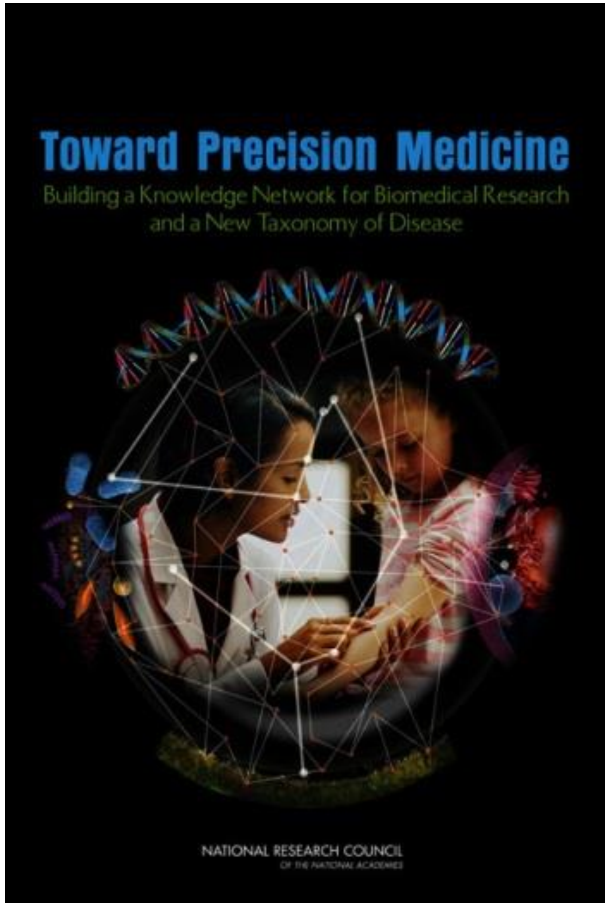
Whitespace

Dialysis

Depressing

Patients want to know

- What do I have?
- What will happen to me?
- What can I do about it?
- What does it mean for my family?



Prefer precision medicine approach

- Right intervention for right patient at right time

Why a Kidney Precision Medicine Project is needed

AKI and CKD are currently defined by **serum creatinine** and **urine protein**. However, they **fail to cluster patients** into distinct mechanisms of diseases.

Development of drugs for AKI**s** and CKD**s** has been hampered by:

- **Animal models fail to replicate** the human disease phenotypes
- **Poor molecular understanding of heterogeneity** of human AKIs and CKDs
- Inability to identify and **prioritize high value human targets**
- **Lack of human kidney biopsy tissue**, unlike cancer

Need human tissue to improve human health

Goals of the Kidney Precision Medicine Project (KPMP)

Understand and treat *human* kidney diseases

- Ethically and safely obtain **kidney biopsies** from participants with AKIs or CKDs
- Create a **kidney tissue atlas**
- Identify critical cells, **pathways and targets** for novel therapies
- Find disease subgroups to **stratify patients**
- Devise **individualized** treatments
- Improve scientific knowledge base
- Improve pipeline

KPMP Sites and Organization

Participant Collaborators

Recruitment Sites (RS)

AKI

- Columbia University
- John Hopkins - Yale
- University of Pittsburgh

CKD

- Cleveland Clinic
- UT Southwestern
- Harvard University

Tissue Interrogation Sites (TIS)

- Indiana-OHSU – 3D tissue cytometry & LCM
- Broad-Michigan-Princeton– single cell transcriptomics
- UCSF-Stanford – tissue mIFISH and cytof
- UTHSA-PNNL-EMBL – tissue metabolomics
- UCSD-Wash U– single nucleus RNAseq & DART-FISH

Central Hub (CH)

Data and sample Coordinating Center (DCC)

- Clinical protocol development and statistical calculations
- Standard clinical assessments
- Collect, curate, aggregate, store, distribute, and ensure quality control

Data Visualization Center (DVC)

- Digital pathology
- Kidney tissue atlas to classify and locate different cell types and interstitial components in health and disease
- Website for sharing

Administrative Core (AC)

- Administrative and meeting support
- Establish working groups
- Patient input and feedback
- Opportunity Pool to form new partnerships

University of Washington, University of Michigan, Mt. Sinai

KPMP Is Unique in Precision Medicine

- The entire community - patients, clinicians, scientists, ethicists - is included
- Patients are equitable partners with an active voice in the entire research enterprise
- The culture promotes priorities and safety of patients first
- Address the big public health problems in kidney diseases
- Clinical orientation of the kidney atlas
- Focus on kidney pathology and deep biological profiling

Community Engagement in the KPMP

- **Who is the KPMP Community?**
  - Patients
  - Clinicians
  - Scientists
  - Ethicists

- **What is our purpose?**
  - Educate and inform about chronic kidney diseases and acute kidney injuries
  - Develop equitable partnerships in the KPMP
  - Cultivate an ethical approach to kidney biopsy for research purposes

Participant concerns about a research kidney biopsy

- **Ethics**
  - Limited benefit and some risk to patient, large benefit to society
  - Ethical, if reasonable expectation of useful information, and risks are reasonable in relation to anticipated benefits to subjects and society
  - Informed consent critical
- **Safety**
  - Discomfort, bleeding; small risk of death
- **Costs**
  - Complications
    - No fault harm insurance
  - Compensation for lost work

Want the **research biopsy** to provide enough information to become **clinically useful to guide diagnosis, prognosis**, and **therapy**

→ Preform **routine clinical biopsy that guides care (change culture)**

KPMP Participant Collaborators (Cohorts)

Acute Kidney Injuries (AKIs)

Types of AKIs
- AKI with ATN or AIN
- Open Biopsy during laparotomy

Key Inclusion Criteria
- Baseline eGFR > 45 mL/min/1.73 m^2
- Serum Cr > 1.5x baseline

Chronic Kidney Diseases (CKDs)

Types of CKDs
- Associated with Diabetes
- Associated with HTN
- Unknown cause

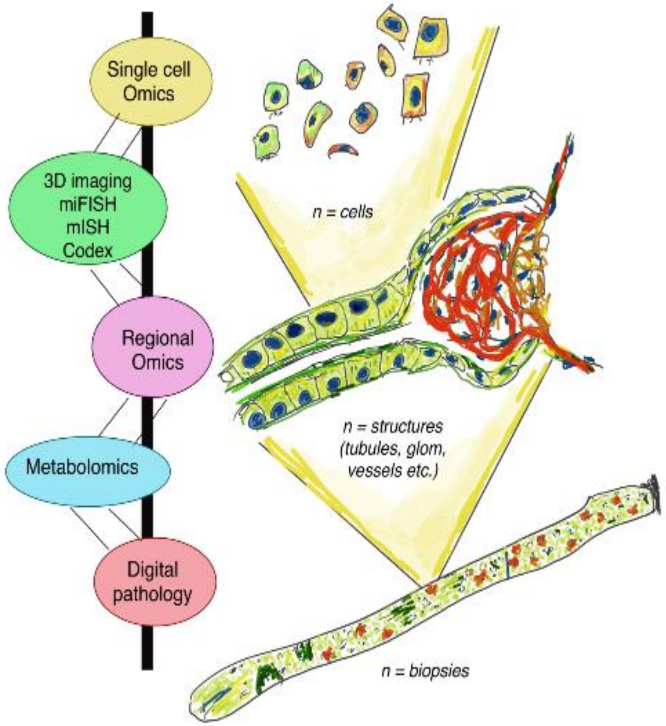
Key Inclusion Criteria
- DKD: Type 1 or 2
- HTN: BP >140/90 3 times within 1 month

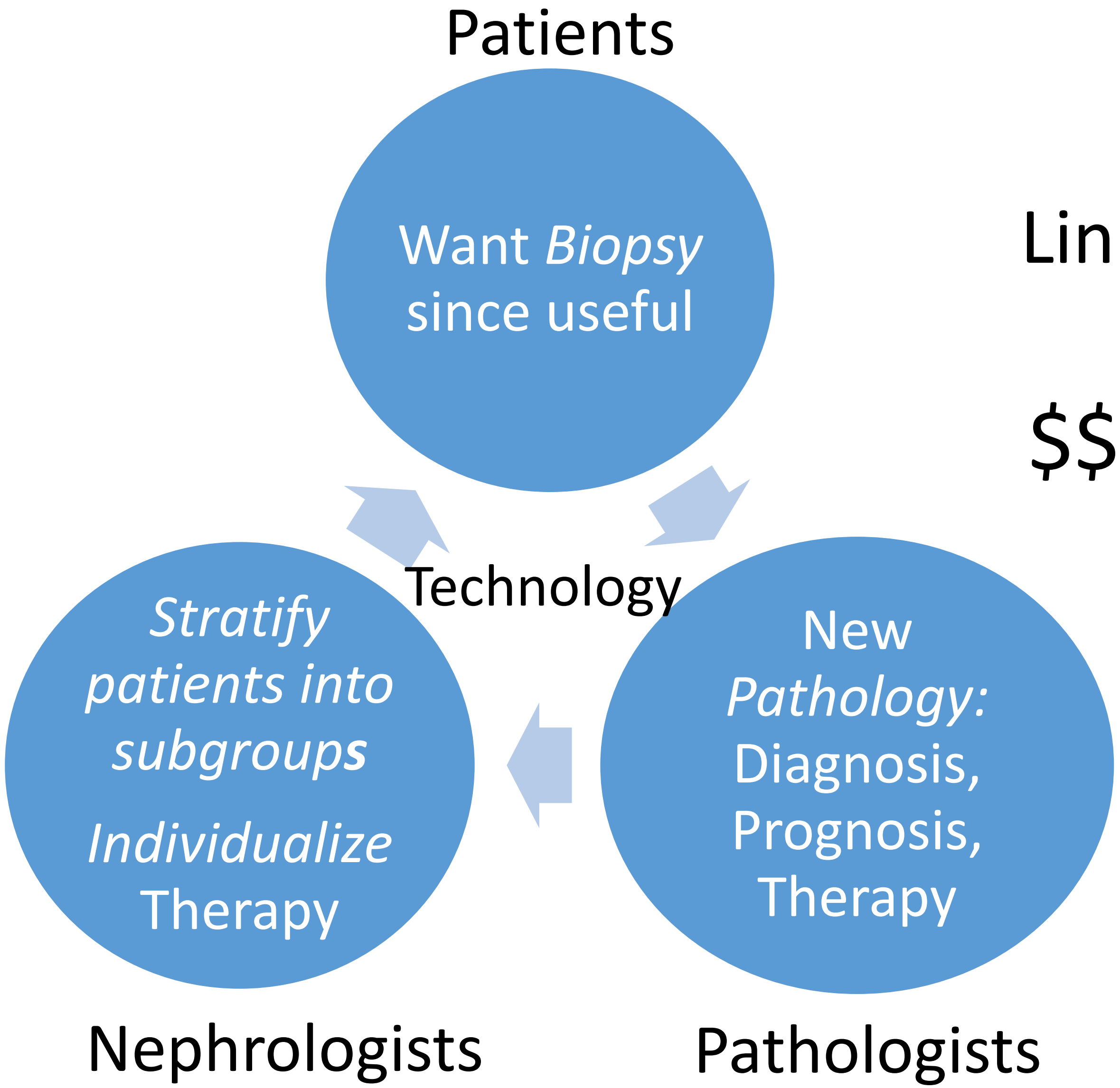
Common Methods/QC
- Research biopsy
  - 3 cores
- Common sample handling
- Standard clinical report
- Longitudinal cohort
  - Baseline data
  - Follow-up data

Implications for KPMP
- Better understand heterogeneity of human AKIs & CKDs
- Improve massive public health burden of AKI & CKD

Common protocols increase rigor and reproducibility

Difficult parts of project



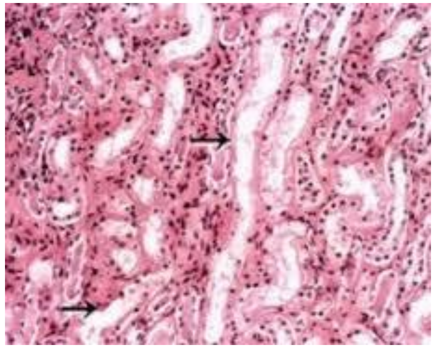


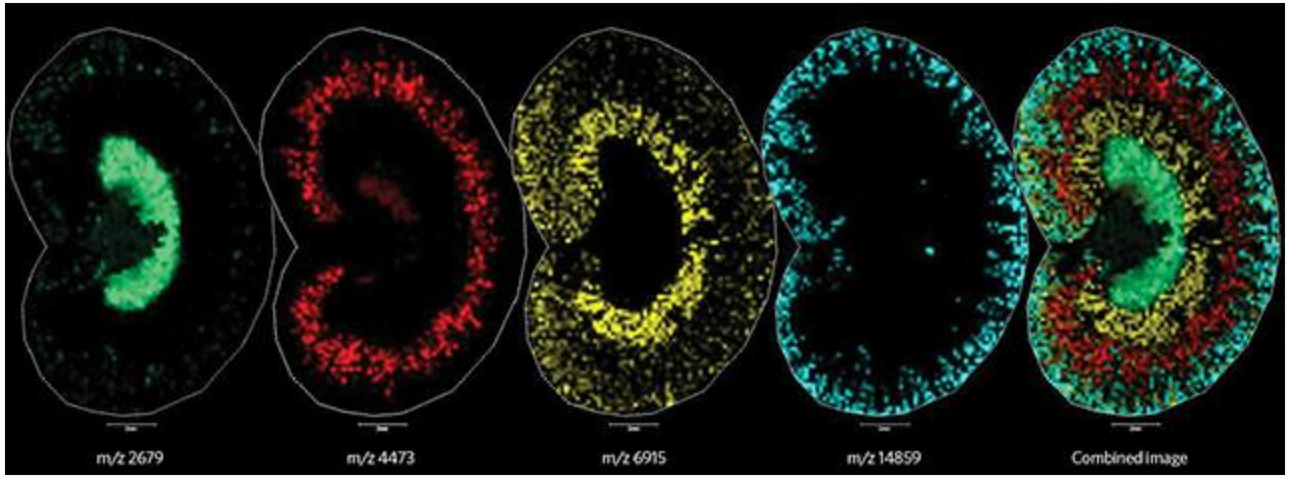
Linked

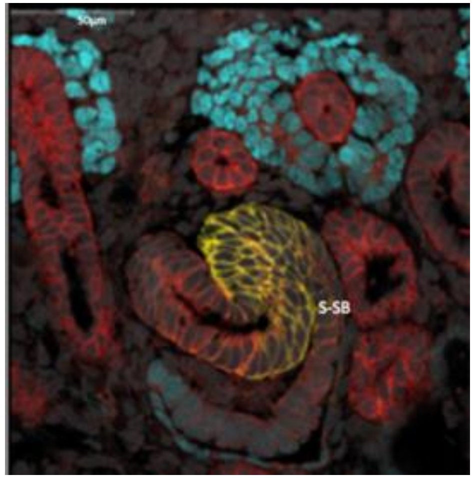
$$$

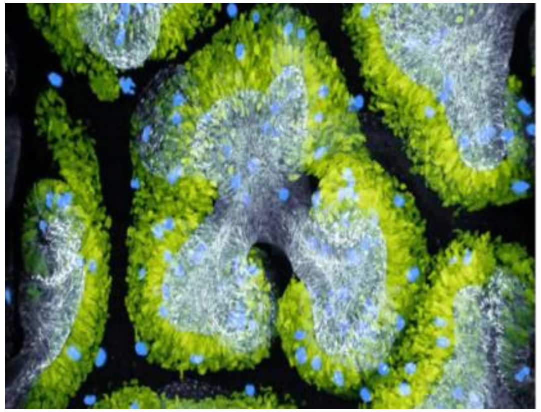
Culture changes

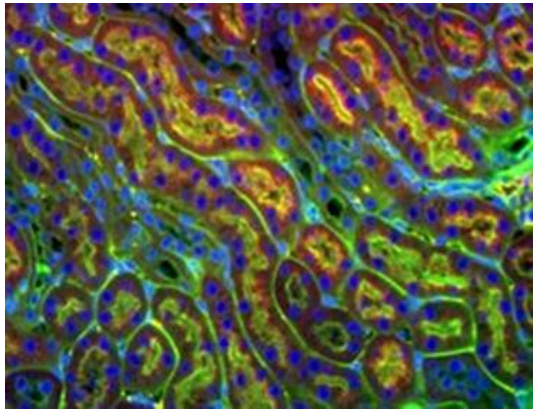
Kidney pathology of the future

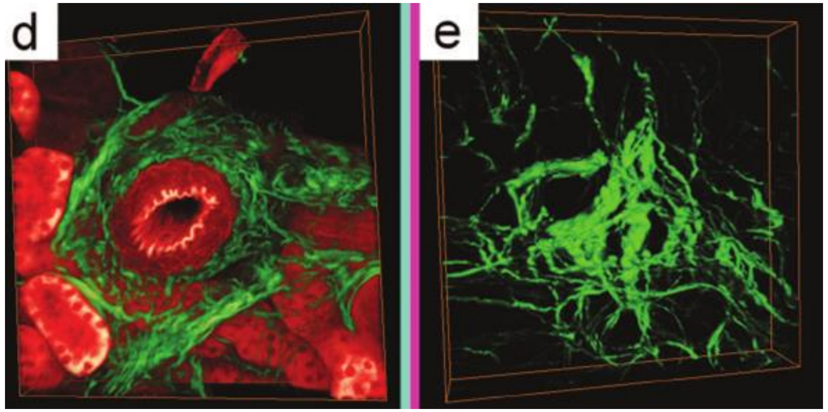












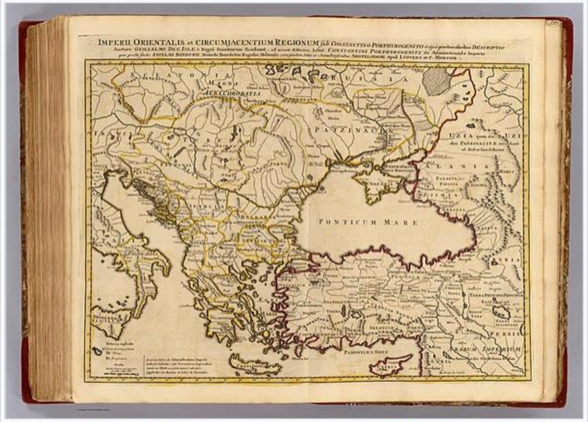
Harness new techmology. Analyze single cells to find tissue markers (cells, and interstitial areas between cells) then paint cells, structures, cell trajectory (healthy, injured, repair, regeneration), activated pathways

Understand heterogeneity between regions, neighboring cells

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

What is an Atlas?

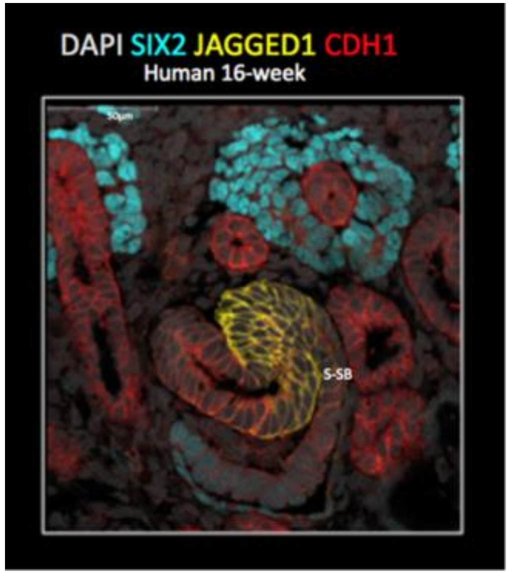
An **atlas** is a collection of **maps**; typically a bundle of maps of the Earth or a region of the Earth. **Maps** show **geographic features** and political boundaries, but can show **geopolitical, social,** religious, and **economic statistics**



Imperii Orientalis et Circumjacentium Regionum by Guillaume Delisle (1742) (en.wikipedia.org)

KPMP **Kidney Tissue Atlas** will have multiple **maps**

- Digital Histology images
- 2-3D tissue architecture makers (kidney/immune cells, vasculature, *extracellular matrix*, etc.)
- Consensus tissue diagrams
- Cell state markers, pathway markers
- Cell structures (transporters, receptors)
- Cell-cell interactions
- Physiologic characteristics (metabolites, etc.)



Using 4 markers, want 30-40 markers

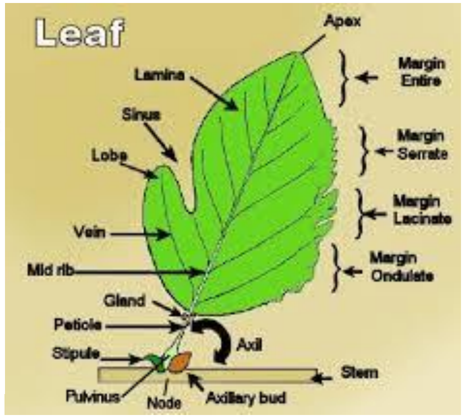
Tracy Tran (McMahon lab)

Content will improve over time

Kidney Tissue Atlas will be constructed with two types of information

What is where?

What kind of leaf? What will happen to me?
What can I do about it?



Dx: Poison Ivy
Px: Itch
Rx: Steroids

Visual display of spatial data

Help make decisions

How to construct the Kidney Tissue Atlas

Multi-dimensional, Organized / Tagged (Ontology), Open, Accessible, can Query

Discovery
Validation

Tissue

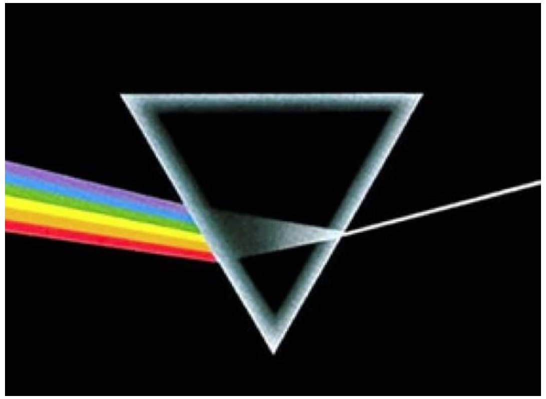
What is there?
What is where?
GUDMAP
HuBMAP
Human Cell Atlas

What changed?

Disaggregation
Anchors / Markers
Map to tissue

Visual display of spatial data

Stratify patients
Implementation



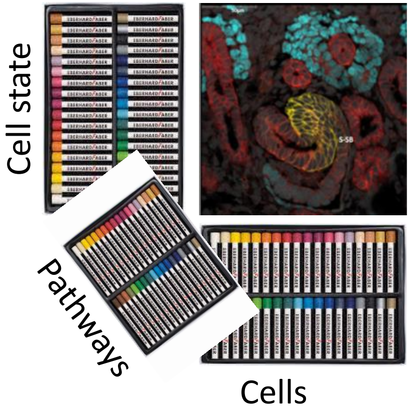
Individualized:
Diagnosis
Prognosis
Treatment

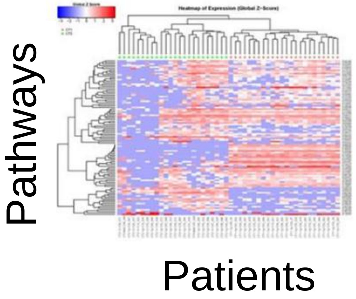
Correlate with
Clinical outcomes
Model systems

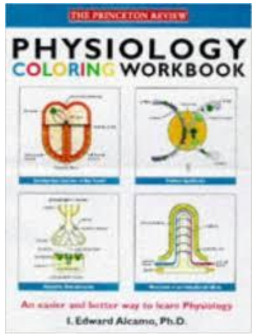
Stratification markers for clinical decisions

First step easier than second step

Kidney Precision Medicine Project
Initial Integrated Workflow







Use banked tissue;
Ethically and
safely obtain
kidney biopsies

Learn from
Tissue:
Single cell/nuclei
Find markers

Evaluate
markers on
biopsies in
2-3 Dimensions

Find cell pathway
markers; ML
Tie to Patient
outcomes

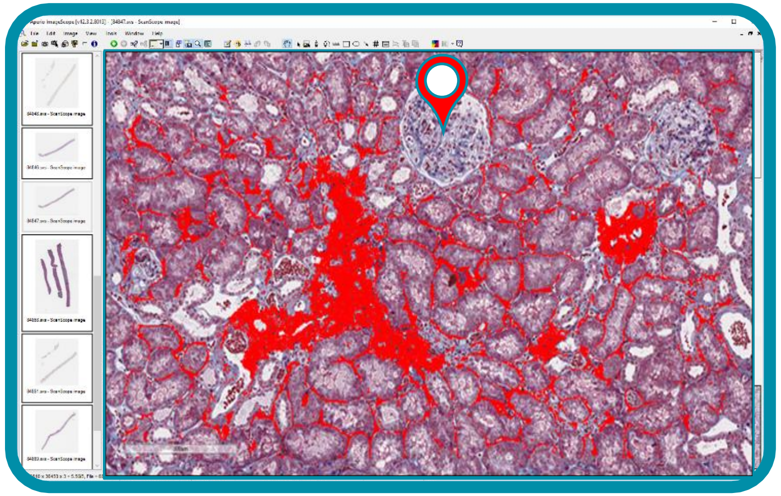
Create
Kidney
Tissue
Atlas

Define dark areas
Find new cell types

This will change as new technologies develop

Major Deliverable: KPMP Data Visualization Core

Digital Pathology

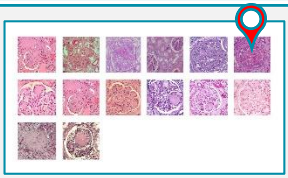


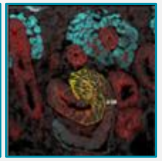
Old: Snap picture, put in repository
New: New image-query and image analysis tools will allow interrogation of histology slides using **known** patterns, and find **new patterns** that inform classification, prognosis, pathways, and therapy

Kidney Tissue Atlas

Matched Morphological Content

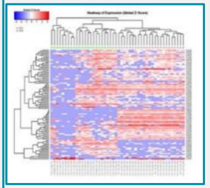
Histology
Molecular Markers
Structural Elements
Structural Ensembles

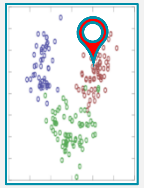




Matched Molecular Content

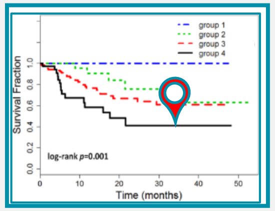
Expression Patterns
Linkages to Phenotypes
Molec. Def. of Disease
Novel Targets





Matched Clinical Content

Demographics
Comorbidities
Outcomes
eGFR
Exposures
Medications



Implications for the KPMP Project

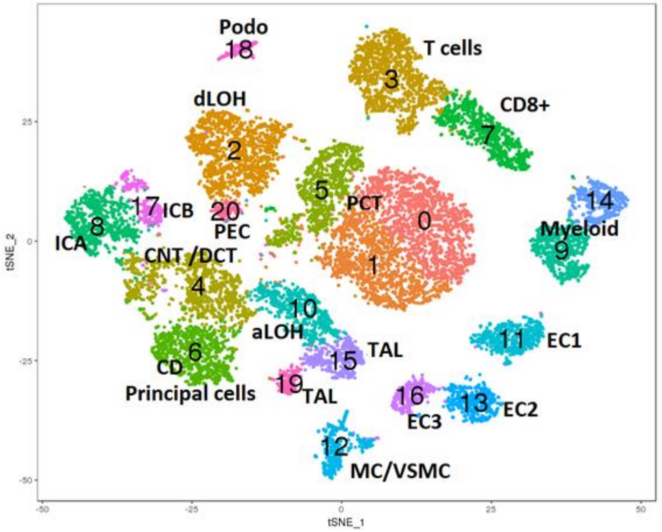
- **Link** human kidney architecture with disease and repair pathways in specific cells in specific patients
- Use image analysis, machine learning, and multi-omic analytics to **predict meaningful outcomes** that are important to patients, and inform drug discovery

This will evolve over time

Major Deliverable: Clinical Assays

Assay	Fruit Smoothie	Fruit Tart	Fruit salad
Technology	Blender	Slice, Bake	Pick, Cut
Information	% composition	What is where	What in each piece
2-3D Location	None	Preserved	None

Major Deliverable: Clinical Assays



	Single cell /nuclei sequencing
Technical complexity	Difficult
Fibrotic tissue	May be difficult; nuclei easier
Sample size	Entire core ??
Information depth	Limited to highly expressed
Location information	
Examples	???

Fruit salad

Fast moving field

Disruption likely

Unique and challenging aspects of KPMP

- **Participant Collaborators** intrinsic to research effort
  - Maintain ethical, safety, and rigorous quality control standards
- **Research biopsies** in people with **common kidney diseases**
- **Large longitudinal 5-10+ year biopsy cohorts** for *AKIs* and CKDs
  - Need large cohorts to create personalized medicine metrics
- Finding out what **we do not know** (dark matter)
- Create **routine clinical assays** for use in clinical practice
  - Initially, better use of routine histology; then 20-40 markers; then ??
- Develop **new patient categorizations (endophenotypes)**, find **disease pathways**

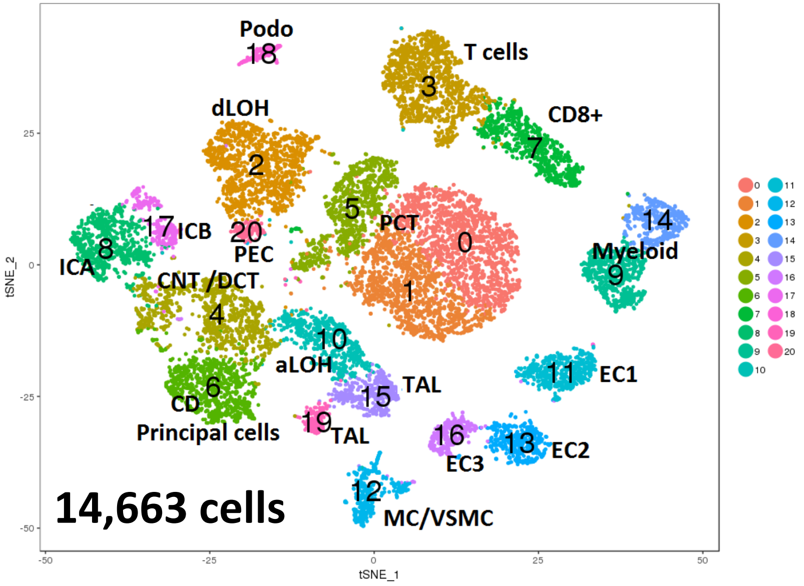
KPMP.org

National Institute of Diabetes and Digestive and Kidney Diseases

Discovery – single cell resolution

- Single cell RNAseq
- Single nuclei RNAseq
- Metabolomics – 20µ resolution

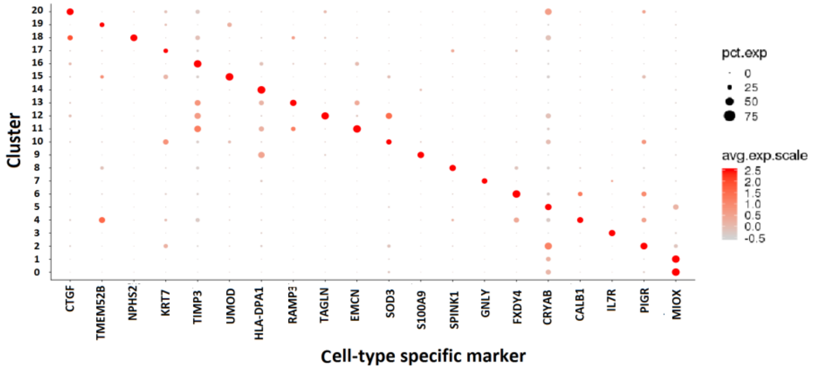
Cell clusters from the combined analysis of 15 samples



- aLOH Ascending Loop of Henle
- CD Collecting duct
- EC Endothelial
- TAL Thick aLOH
- CNT Connecting tubule
- DCT Distal convoluted tubule
- ICA Intercalated alpha
- ICB Intercalated beta
- MC Mesangial
- PEC Parietal epithelial
- VSMC Vascular smooth muscle
- dLOH descending loop of Henle
- PCT Proximal tubule

Cluster	No. of Cells
0	1504
1	1348
2	1321
3	1285
4	1065
5	958
6	926
7	791
8	760
9	646
10	633
11	531
12	451
13	451
14	448
15	436
16	311
17	302
18	188
19	177
20	131

Top cluster-specific markers



University of Michigan / Broad

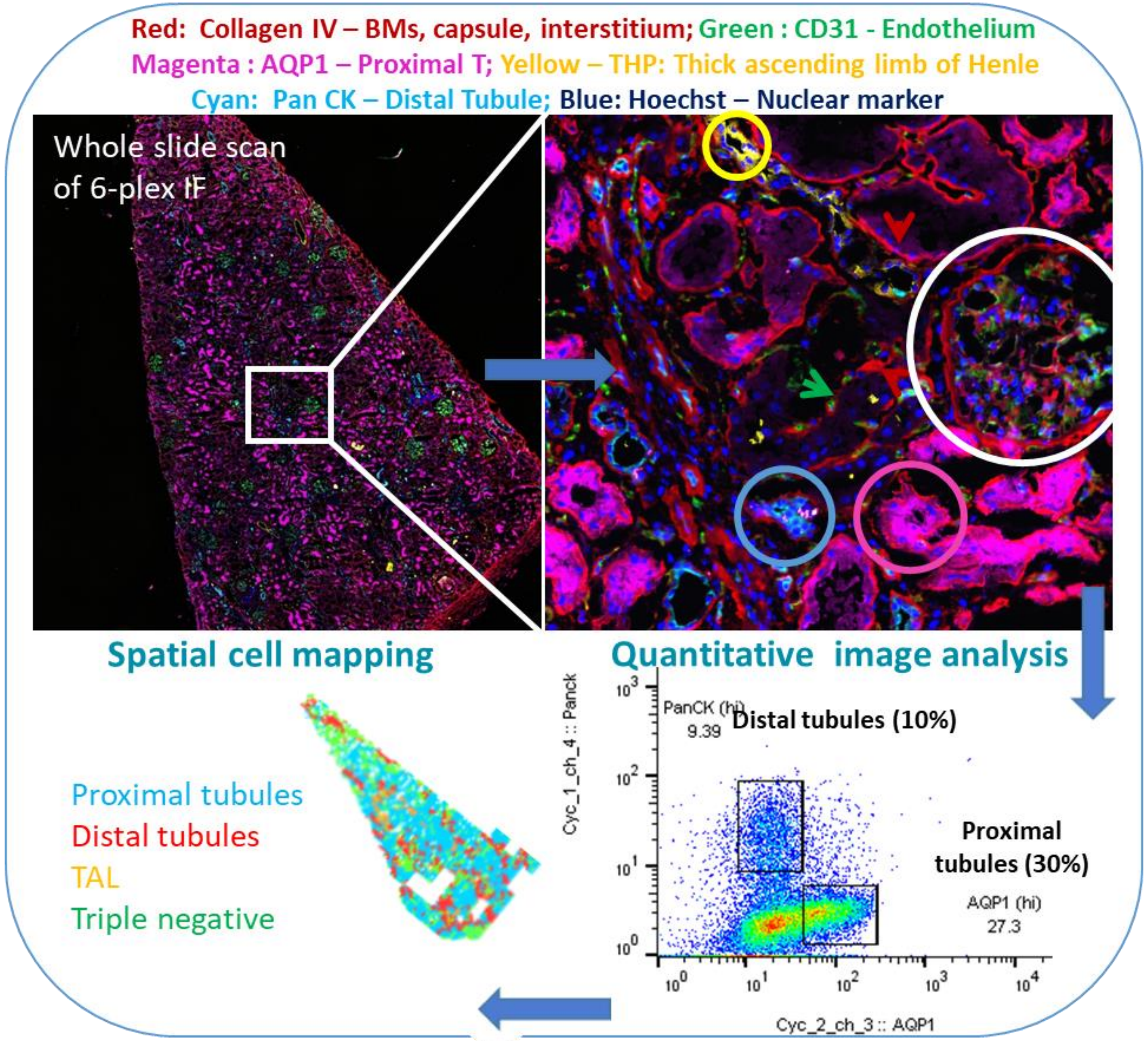
Single cell resolution to find rare cells/spaces/events

2-3 Dimensional Imaging

Method	Site	Technology	# probes
3D Tissue Cytometry	Dagher	IF protein	8-10
mIFISH	Kretzler	In situ RNA	3
mIFISH	Laszik	In situ *and* IF	2 ISH + 8 IF
CODEX	Laszik	IF protein	~28 (to 40)
DART-FISH	Zhang	In situ RNA	Up to 300?
MALDI-MSI	Sharma	Imaging Mass Spec	

Classification by probe color (flow cytometry)

Current issues: throughput, sensitivity, specificity



CODEX: UCSF/Stanford

Imaging to define “Where in tissue”

Other "Atlas" initiatives

ALLEN INSTITUTE for CELL SCIENCE

The BRAIN Initiative®

GUDMAP
GenitoUrinary Molecular Anatomy Project

HuBMAP
The Human BioMolecular Atlas Program

FANTOM
FUNCTIONAL ANNOTATION OF THE MAMMALIAN GENOME

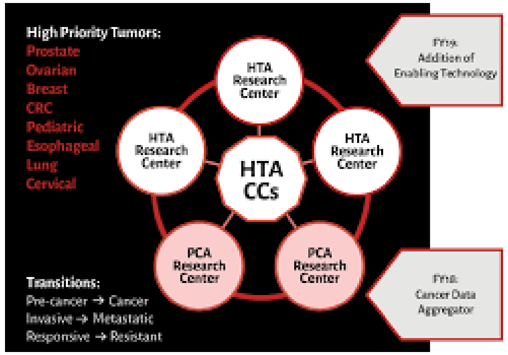
HUMAN CELL ATLAS

KPMP

NIH THE CANCER GENOME ATLAS
National Cancer Institute
National Human Genome Research Institute

International Cancer Genome Consortium

Human Tumor Atlas Network (NCI HTAN)



ReBuilding a Kidney

IVY GAP
IVY GLIOBLASTOMA ATLAS PROJECT

Share technologies, approaches, growing pains