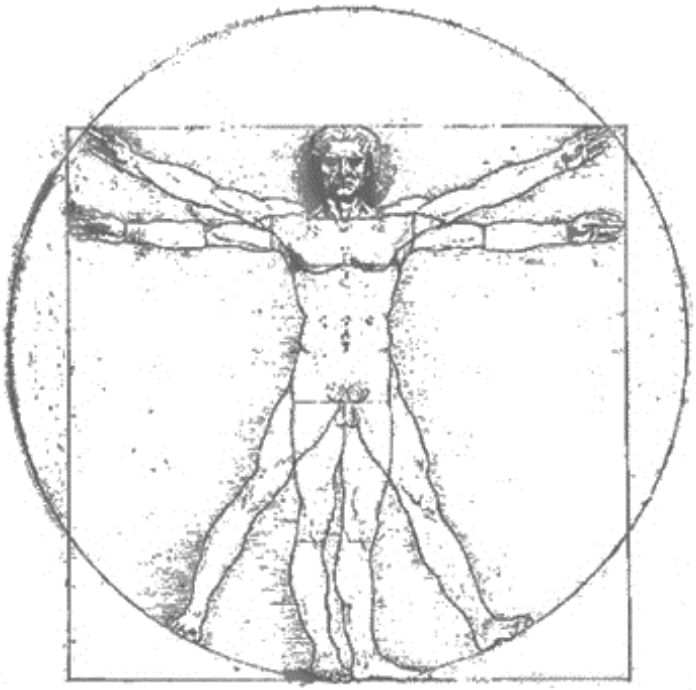
EnCON2017
Advancing Technology for Humanity
November 10, 2017



"Mapping the Human Body: Splitting, Lumping, and the Rubik's Cube Dilemma"

Zorina S. Galis, Ph.D.

Chief, Vascular Biology and Hypertension Branch
Division of Cardiovascular Sciences
National Heart, Lung, and Blood Institute (NHLBI)

National Institutes of Health

NIH National Heart, Lung, and Blood Institute

Conflicts And Other Disclosures

- **No** financial conflicts
- **Note**: The opinions presented are personal, they do not necessarily represent the opinions of the NHLBI.

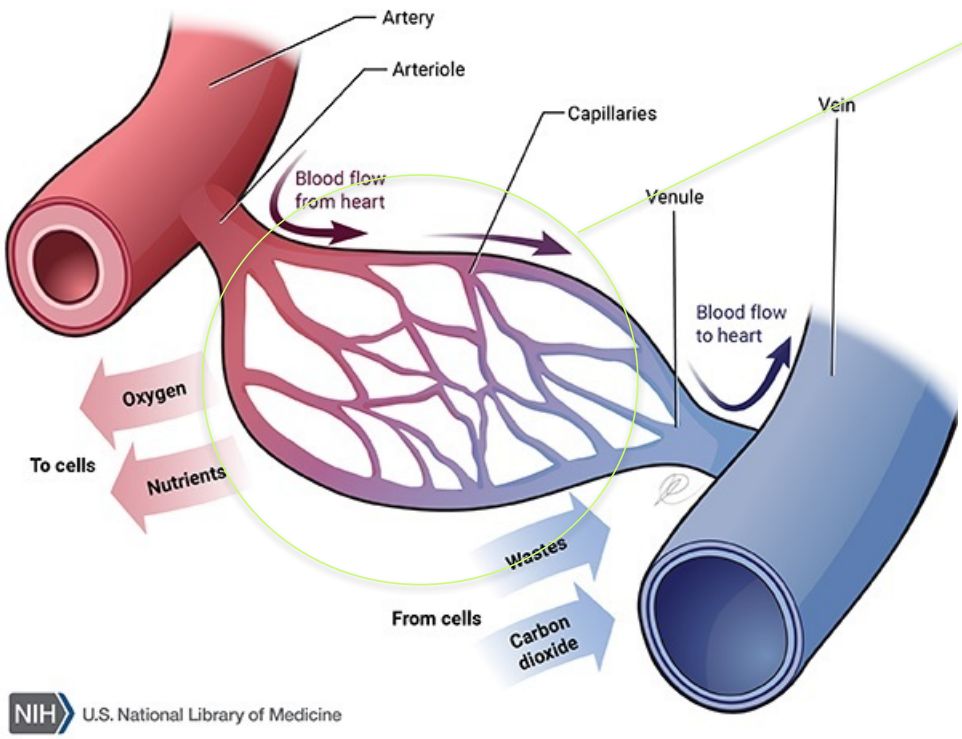
Topics

- "What is *the problem*?"
  - Example from the cardiovascular world
  - Expanding to the human body
- Some current/emerging solutions for:
  - "Splitting"
  - "Lumping"
- The Rubik Cube Dilemma?
- Challenges harbor opportunities!

Could YOU help?

The Human Vascular System

When small… gets BIG!



NIH U.S. National Library of Medicine

- **In Health**

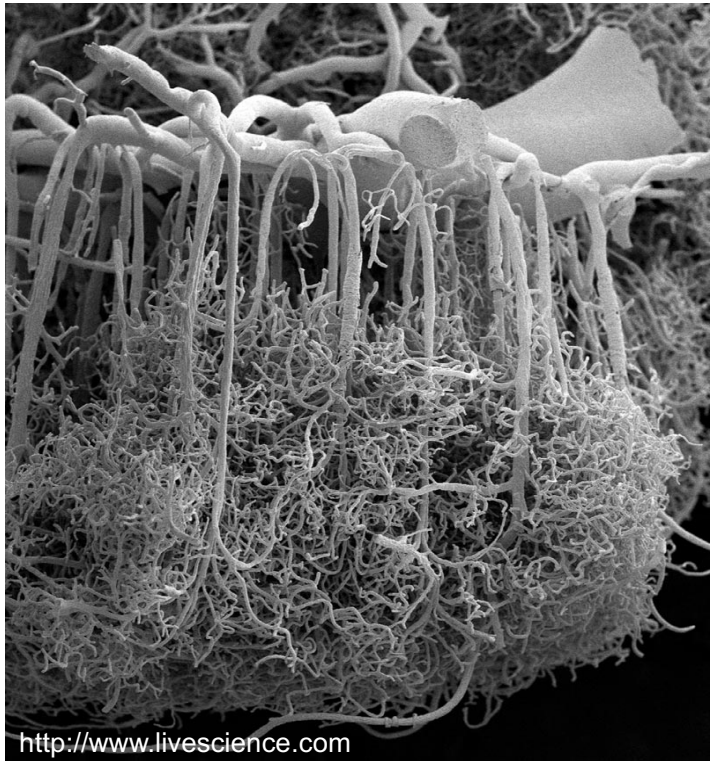
Essential for survival and normal function of ALL tissues/organs
Major site for local and systemic exchanges, sensing, integration and dynamic response to signals

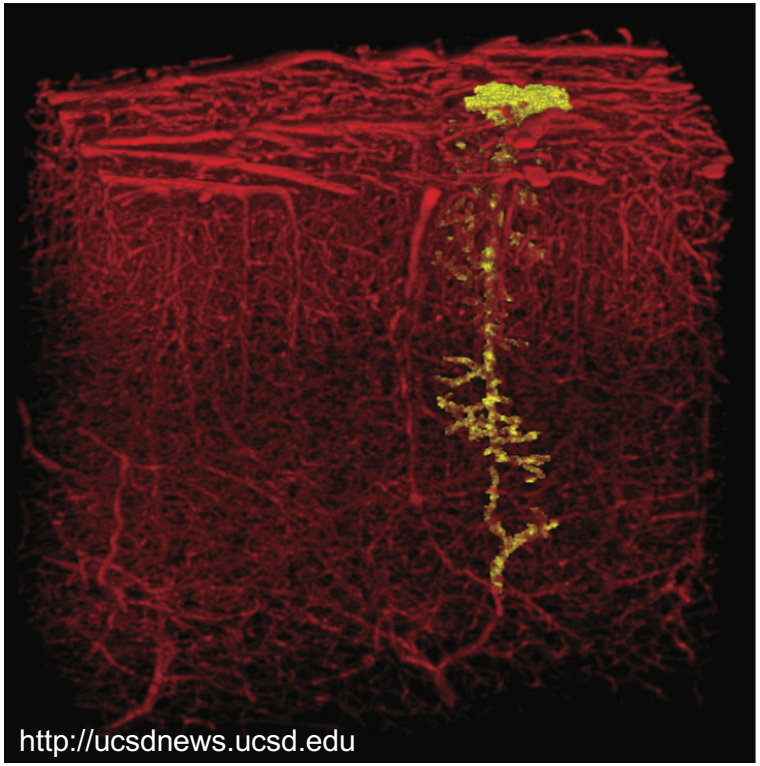
- **In Disease**

Local dysfunction <=> organ and systemic diseases

NIH National Heart, Lung, and Blood Institute

Brain Small Blood Vessel Disease

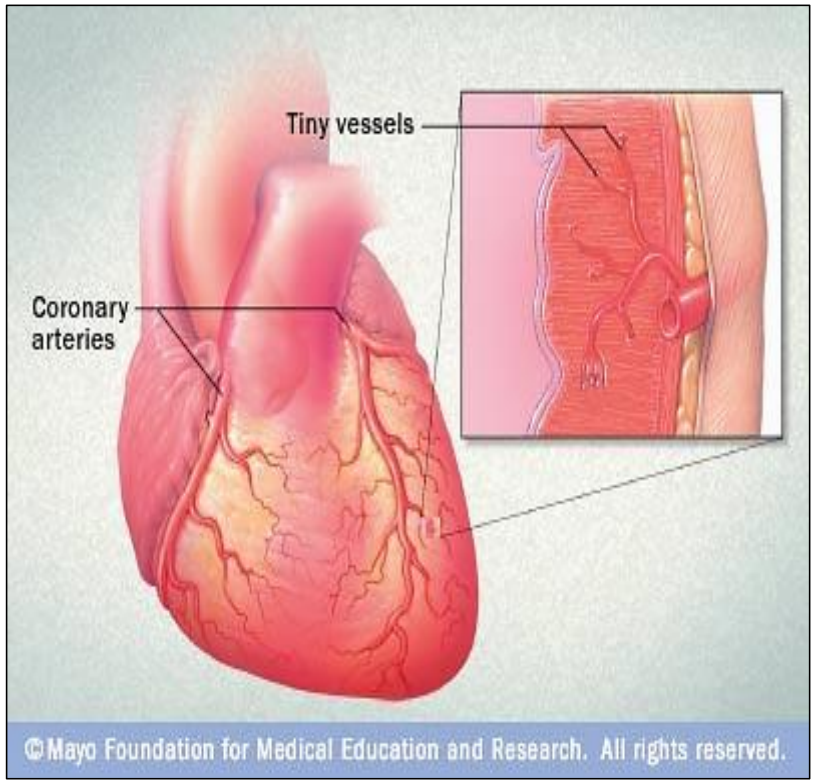




Brain infarctions/hemorrhage, associated with small strokes, vascular cognitive impairment, Alzheimer's

NIH National Heart, Lung, and Blood Institute

"Small Vessel Heart Disease"



- **Hard to diagnose, can cause:**
  - **Coronary Artery Spasm**
  - **Heart Attack**
  - **Sudden Cardiac Death**
  - **Heart Failure**
- **Risk factors:**
  - **Tobacco use, High cholesterol, High blood pressure, Obesity (body mass index of 30 or higher), Inactive lifestyle, Diabetes, Insulin resistance, Female sex, Polycystic ovarian syndrome, Age (> 45 y in men and >55 y in women)**

Small Vessels Dysfunction Implicated In…

Lacunar stroke

Lymphedema

Retinal vasculopathy

Dementia

Hemorrhage

Aging

Hypertension

Coronary microvascular disease

Diabetes

Leukoclastic vasculitis

Malaria

Pulmonary hypertension

Kidney disease

Literally from head to toe, from local to systemic diseases

Preeclampsia

Scleroderma

Wound healing

Alcohol and substance abuse

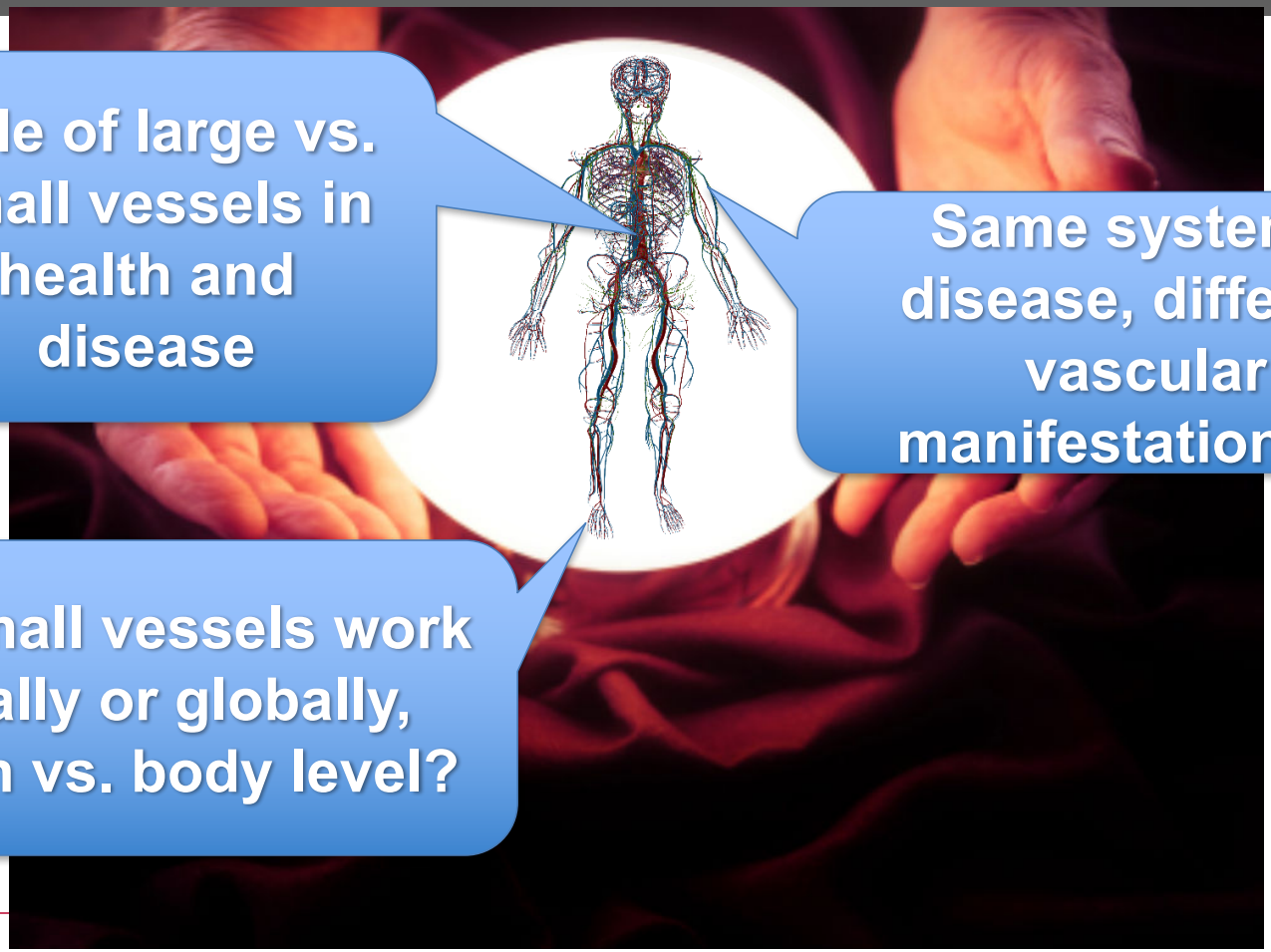
Cancer

Systemic inflammation

Sepsis

NIH National Heart, Lung, and Blood Institute

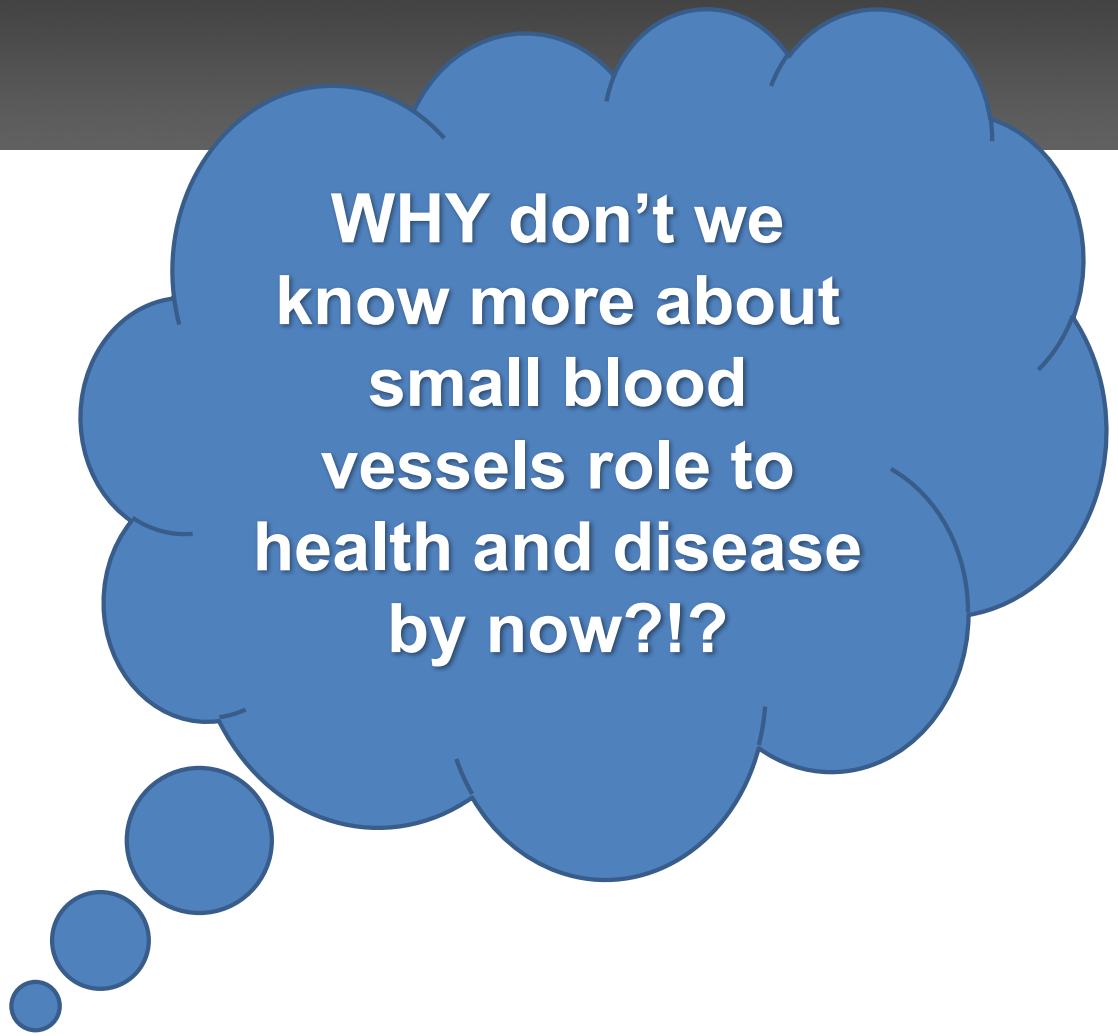
Enduring Human Health Mysteries…



Role of large vs. small vessels in health and disease

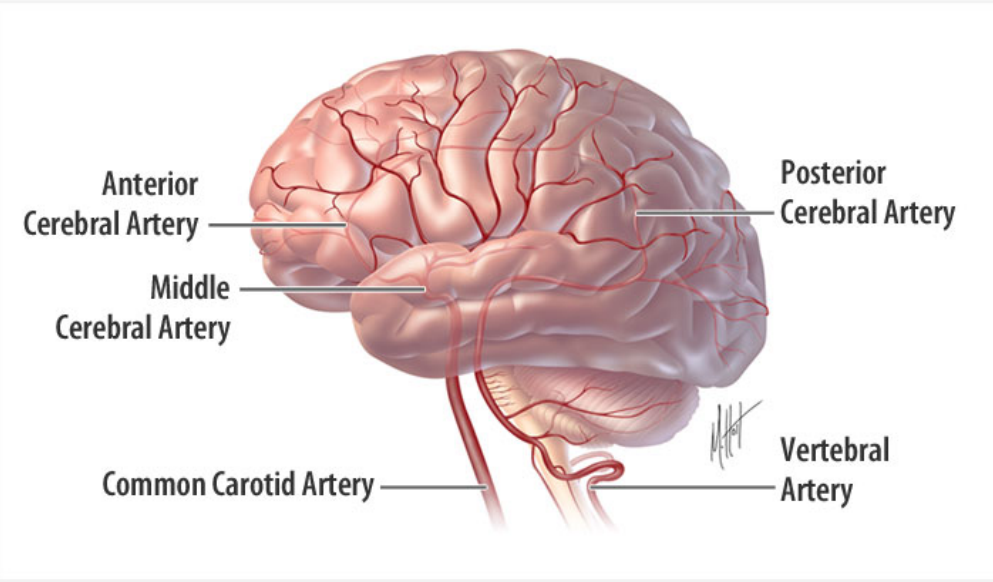
Same systemic disease, different vascular manifestations …

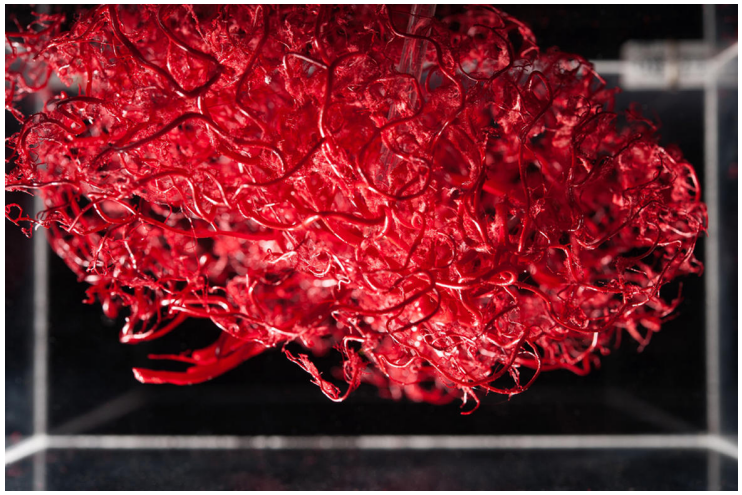
Do small vessels work locally or globally, organ vs. body level?


WHY don't we know more about small blood vessels role to health and disease by now?!?

Small Blood Vessels Have A….

SIZE problem

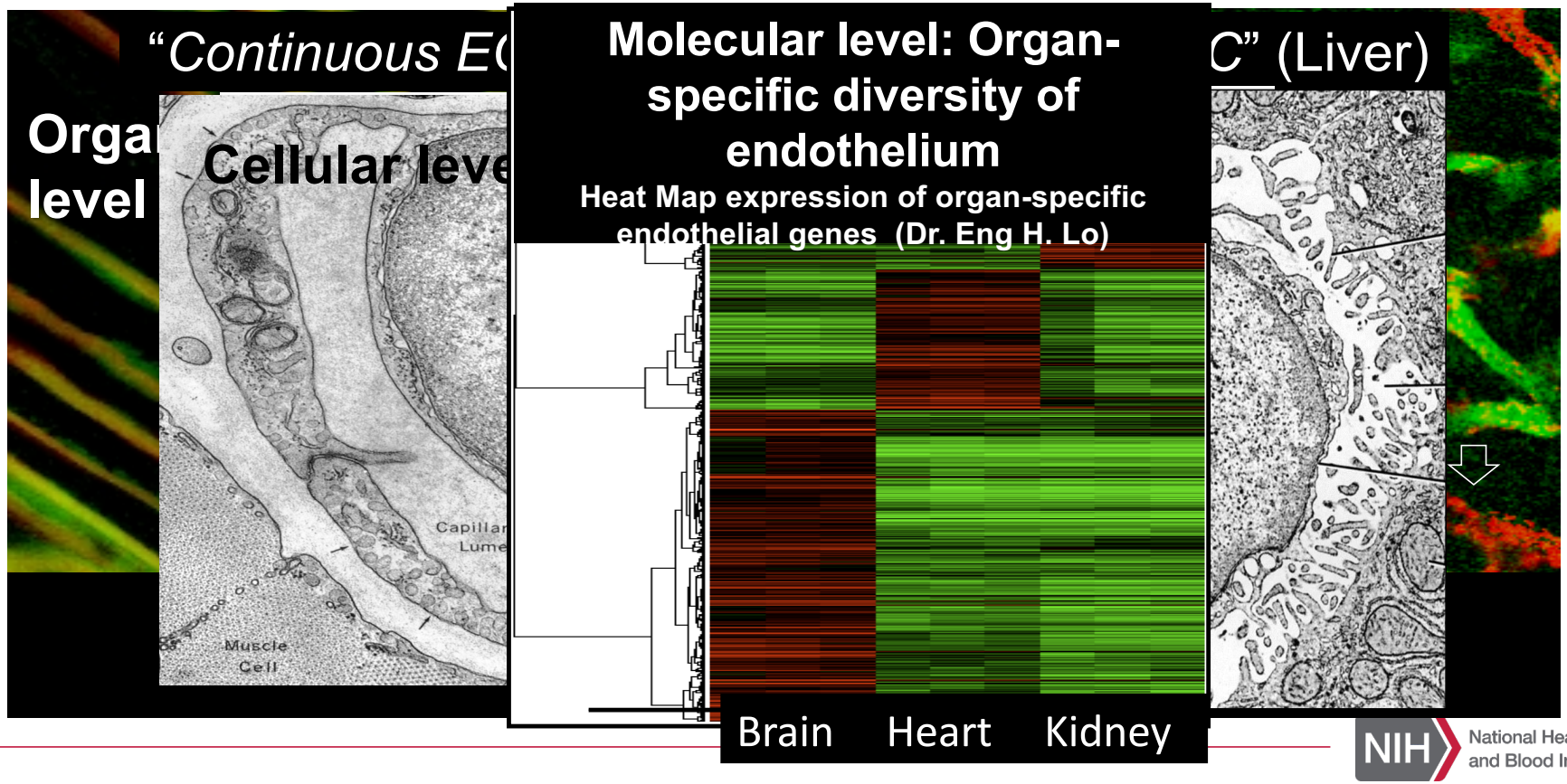




Out of sight, out of mind!

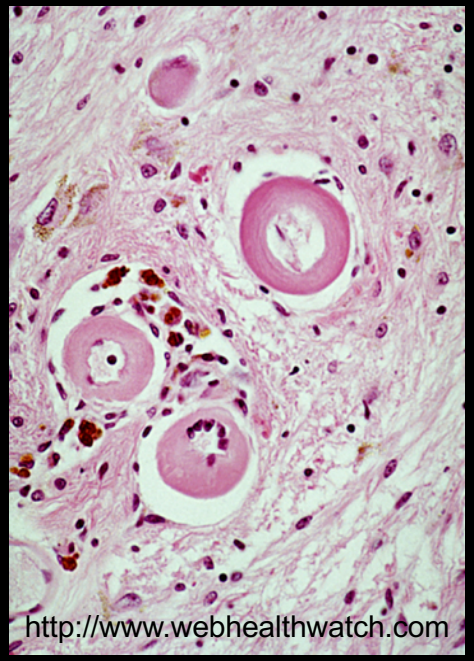
Small Blood Vessels Have a Complexity Issue

Structural/functional Diversity, e.g., Endothelial cells (EC)



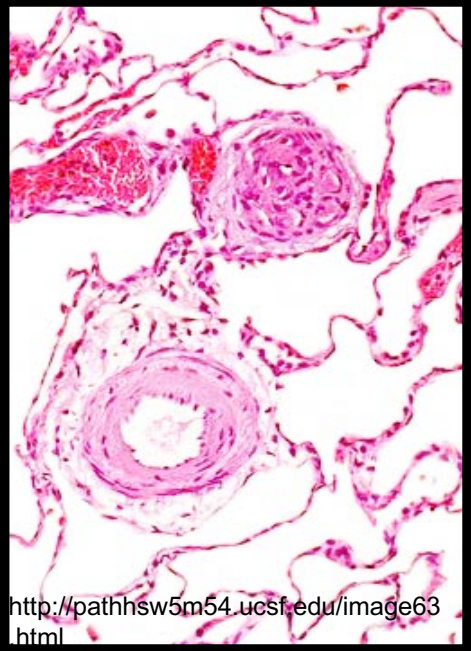
Organ-Specific Small Blood Vessel Pathology in Hypertension

Brain



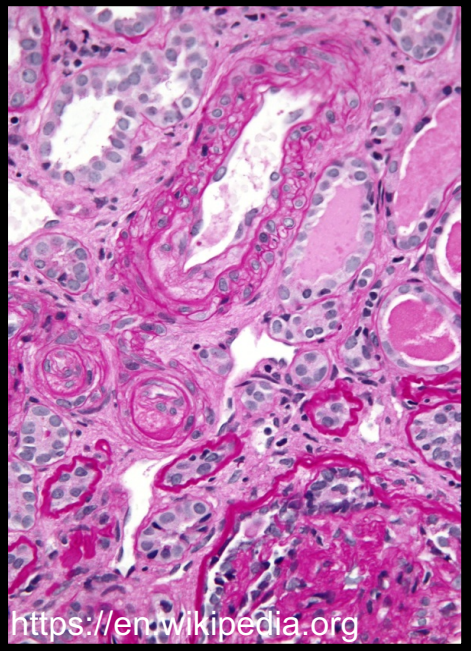
http://www.webhealthwatch.com

Lung



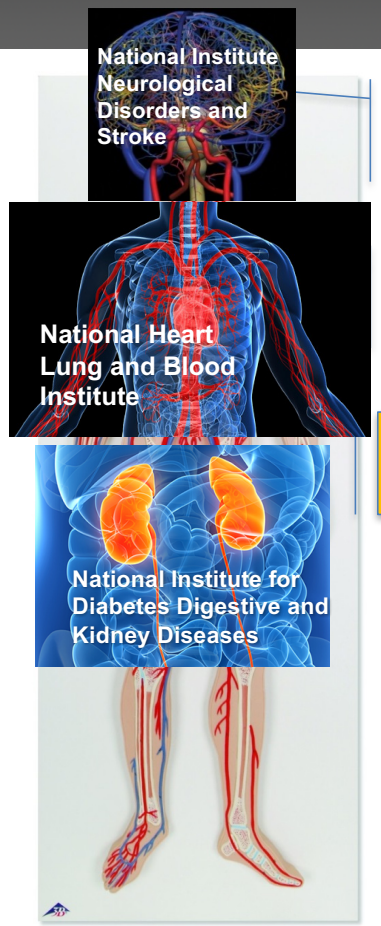
http://pathhsw5m54.ucsf.edu/image63.html

Kidney



https://en.wikipedia.org

Small Blood Vessels Have A….



Neurology

Cardiology

Nephrology

“KNOWEDGE FRAGMENTATION” problem!

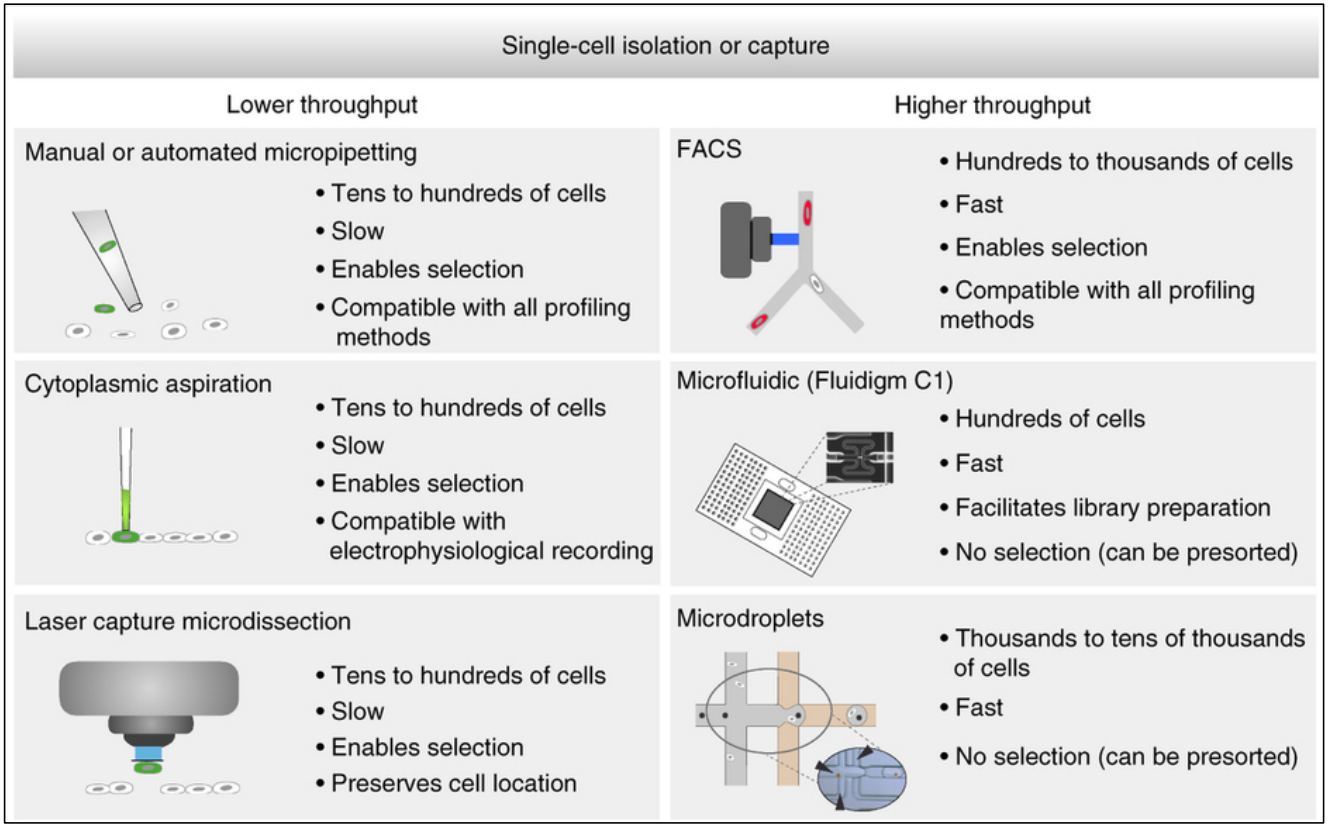
“Knowledge silos”

- **Training, specialization**
- **Professional life**
- **Funding**

Major….“ENGINEERING” problem

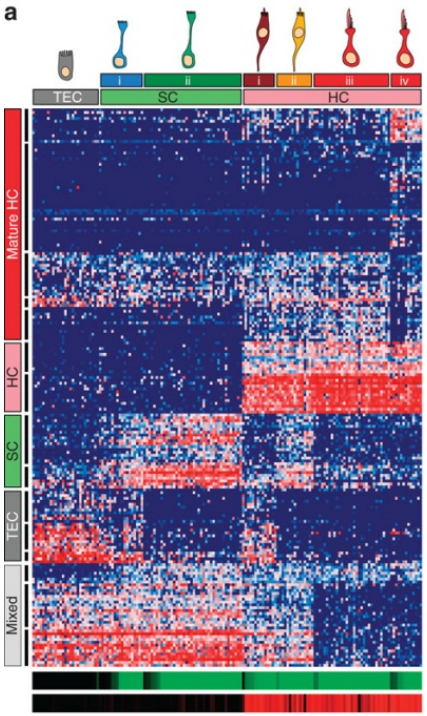
"Splitting"

- **Individual cell = smallest functional unit**
- **Emerging opportunities:**
  - **Single cell analyses "–omics" and imaging (RNA, DNA, protein)**
  - **The "in situ" analysis challenge**

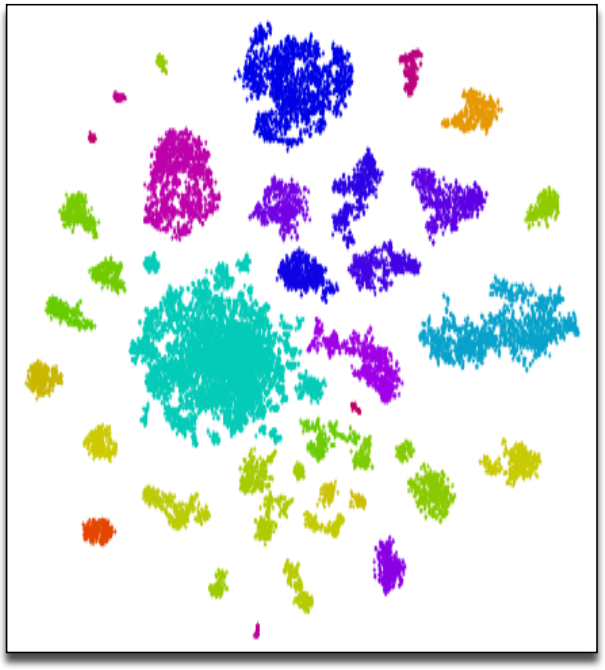


Poulin et al, Nature Neuroscience 19, 1131–1141, 2016

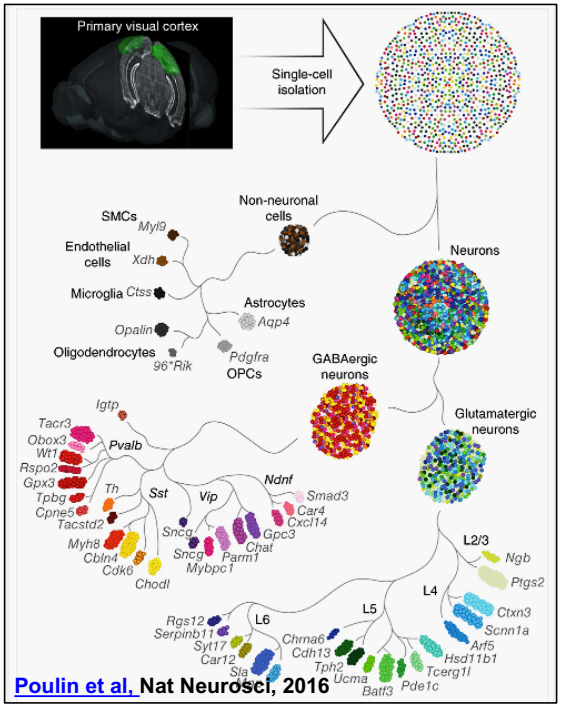
Single Cell Technologies:
New Era in Human Body Exploration!



RNA-Seq identifies unique cell types in mouse utricle (Kelley Lab)



Retina Drop-Seq (48,808 cells) – Identification of 3 new cell types (Regev Lab)



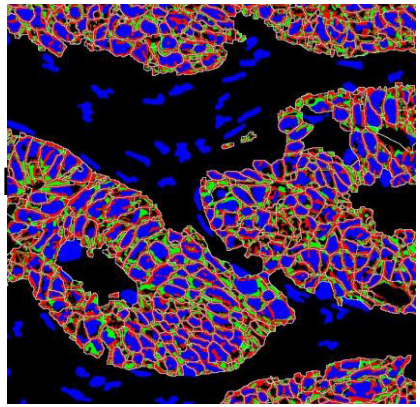
[Poulin et al,](#) **Nat Neurosci, 2016**

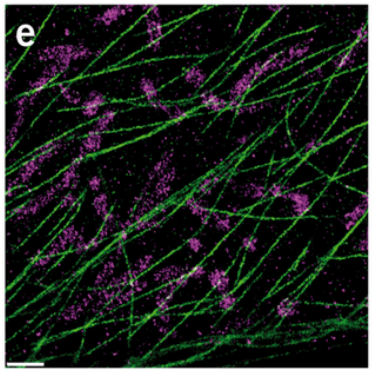
Disentangling Neural Cell Diversity Using Single-cell Transcriptomics

NIH National Heart, Lung, and Blood Institute

NIH Single Cell Project (SCAP)
Innovation In Single-cell Proteomics And Metabolomics

>60 Proteins then DNA FISH
Gerdes, GE Global Research
SCAP Project: R01CA173377

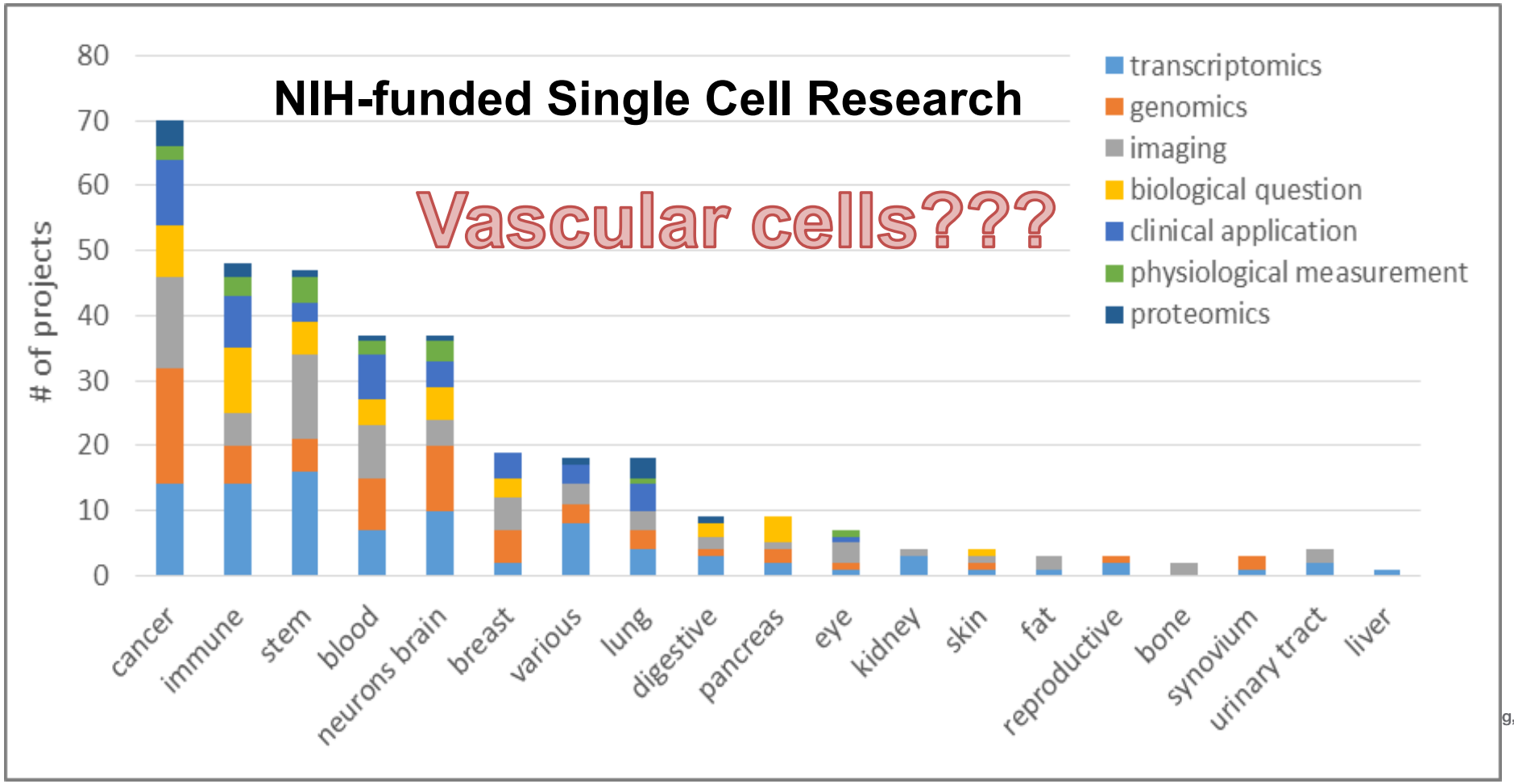




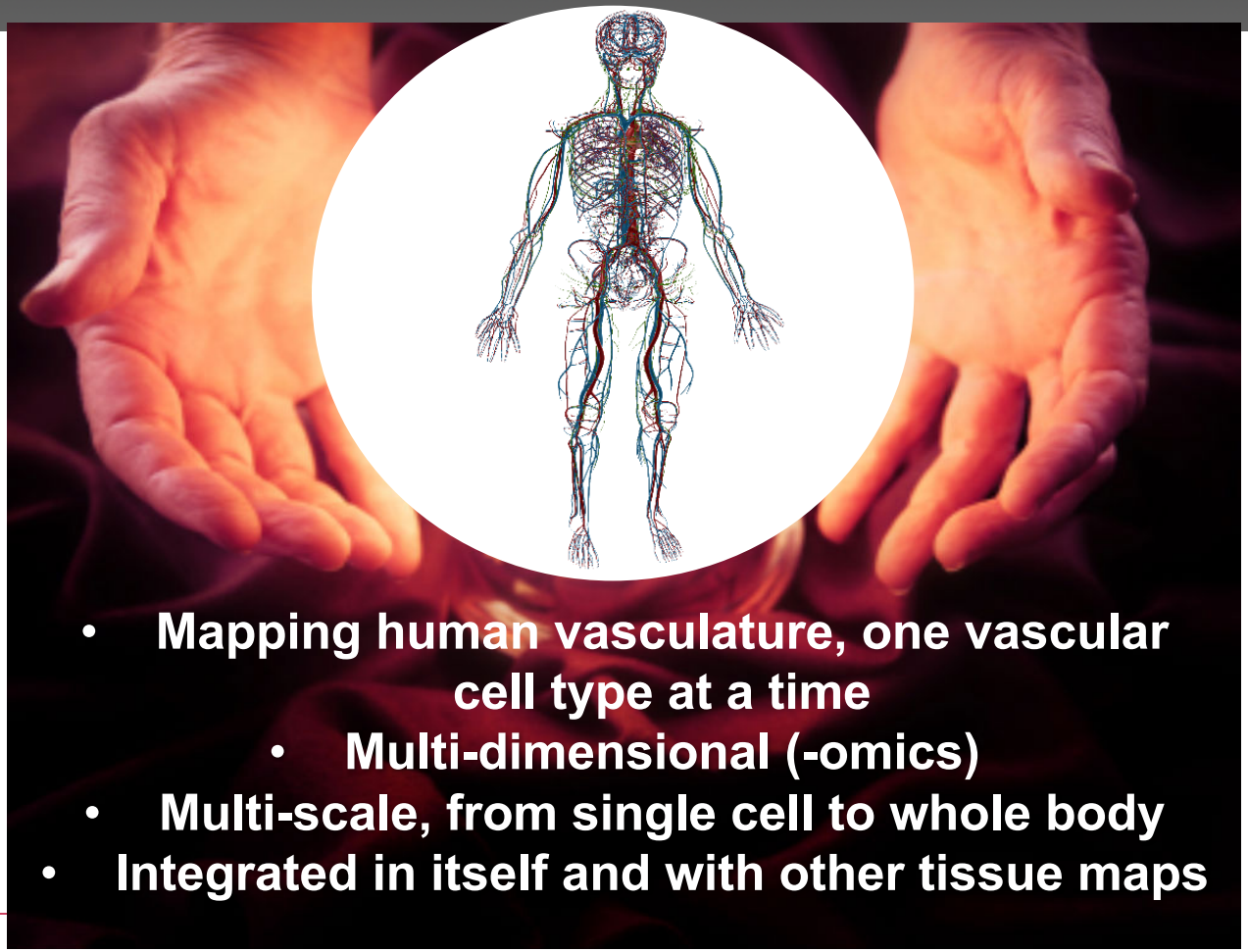
Multiplexed cellular super-resolution imaging using DNA-PAINT and Exchange-PAINT, Yin et al., Nat Methods. 2014 Mar;11(3):313-8.

Imaging lipid metabolism in live C. elegans using stimulated Raman scattering imaging. Cheng et al. SCAP Project: R21 GM114853

New technologies > New Biology!



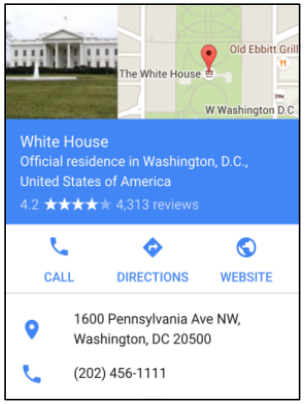
Time to Build "*The Vasculome?*"

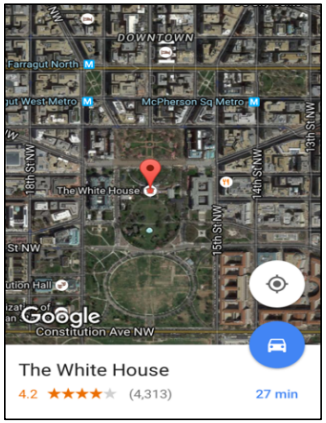


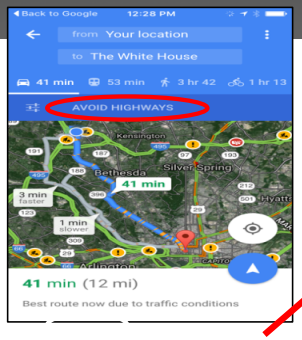
- Mapping human vasculature, one vascular cell type at a time
- Multi-dimensional (-omics)
- Multi-scale, from single cell to whole body
- Integrated in itself and with other tissue maps

A Google Map for the Human Vasculature?

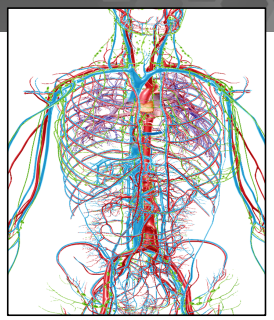
Distance (Scale)







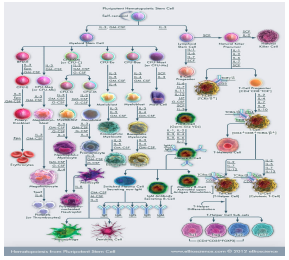
Human BODY



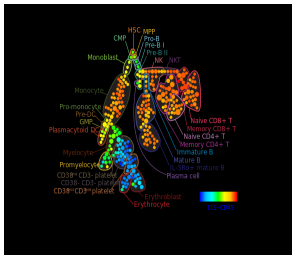
Understand Principles

Tissue

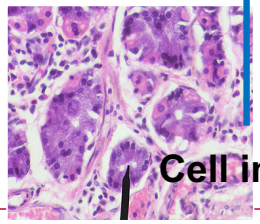
Understand Patterns



Neighborhood



Understand Relations



Cell in-situ

Amount of data (dimension)

NIH: Human BioMolecular Atlas Program "HuBMAP"

The vision: https://commonfund.nih.gov/hubmap

Catalyze development of a comprehensive atlas of cellular organization in human tissues to elucidate principles of organization-function by:

- **accelerating tool development** for 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 research community
- **rapidly making data findable, accessible, interoperable, and reusable (FAIR)** in standardized formats

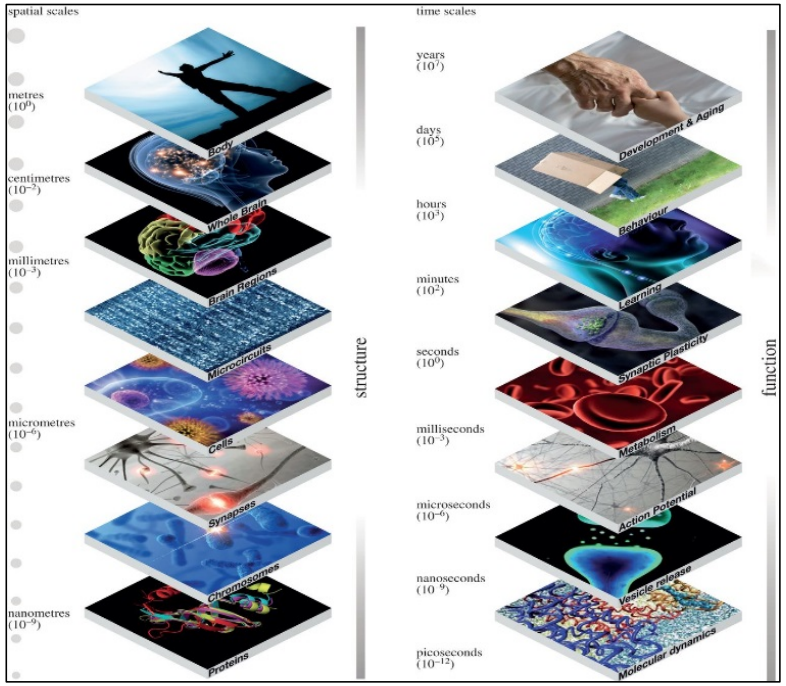
To begin funding in FY18!

NIH National Institutes of Health
Office of Strategic Coordination - The Common Fund

https://commonfund.nih.gov/

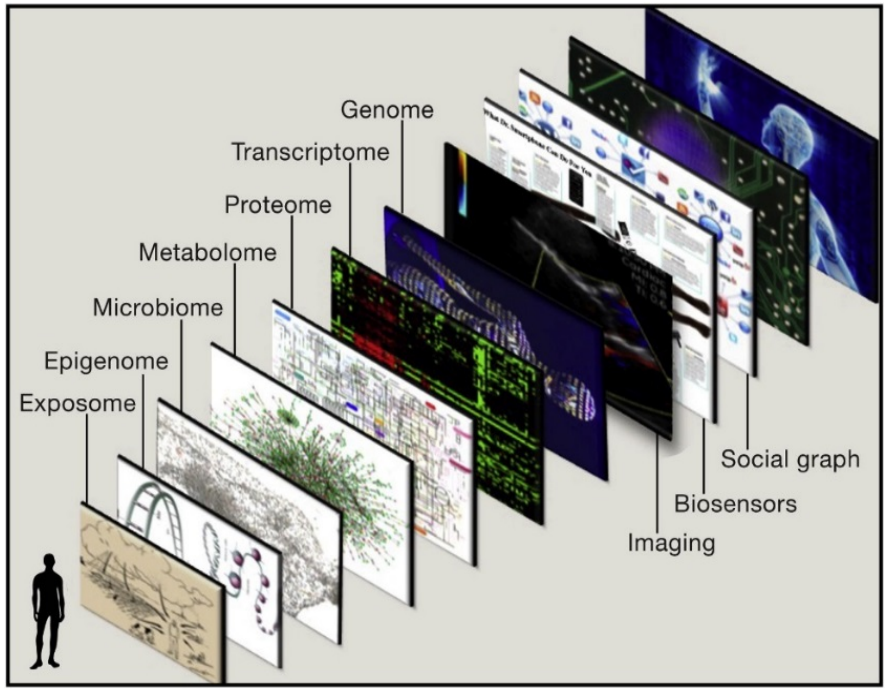
Vision for NIH HuBMAP

Multiscale



Frackowiak et al., Phil Trans R Soc B (2015)

Multimodal



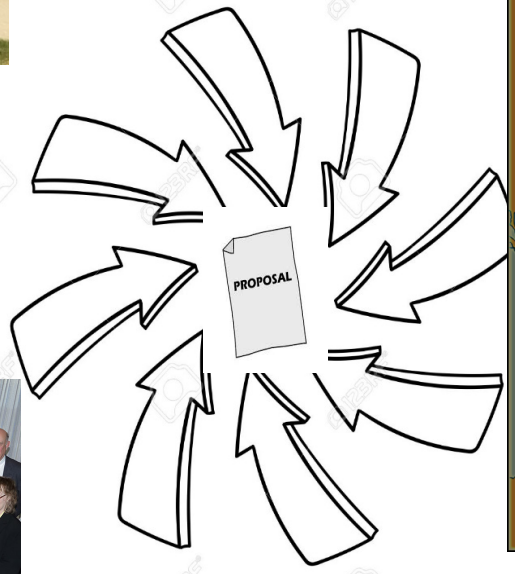
Topol, Cell (2014)

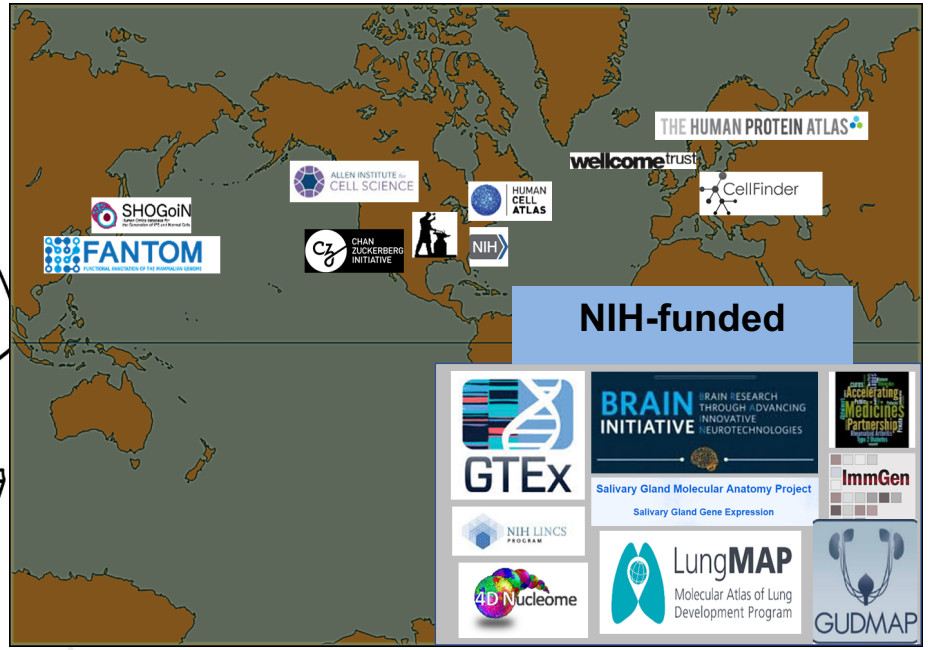
Reality check..

Identifying Key Areas in a Human BioMolecular Atlas (HuBMAP) WS, June 15, 2016

NIH RePORTER

PubMed.gov





NIH Staff HuBMAP Working Group

Co-Chairs:
Gary Gibbons, M.D. (NHLBI)
Roderic Pettigrew, Ph.D., M.D. (NIBIB)
Robert Star, M.D. (NIDDK)

Working Group Leaders:
Zorina Galis, Ph.D. (NHLBI)
Deborah Hoshizaki, Ph.D. (NIDDK)

Common Fund Program Leader:
Richard Conroy. Ph.D., M.B.A. (OD)

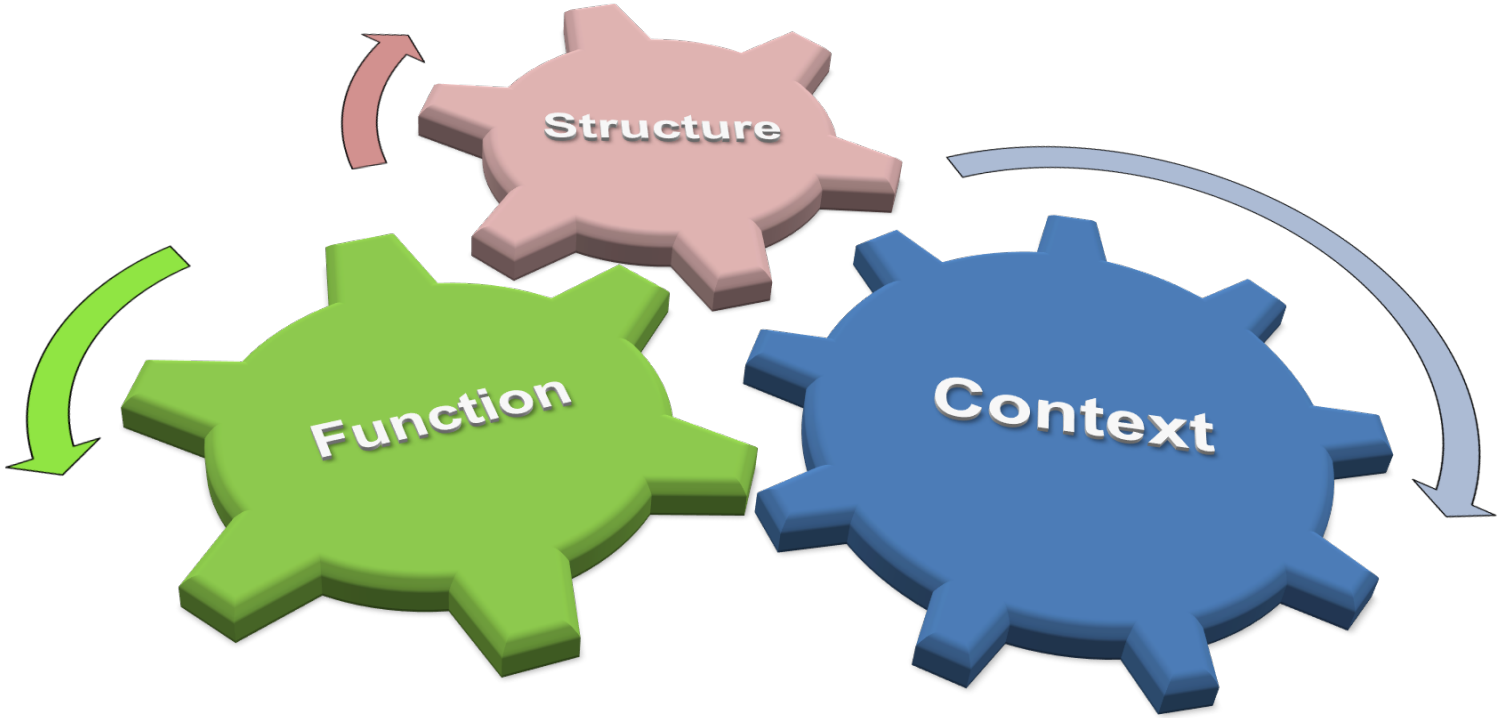
https://commonfund.nih.gov/HuBMAP/index

Members:
David Balasundaram, Ph.D.(CSR)
Jenna Baker, Ph.D.(NIDDK)
Andrea Beckel-Mitchener, Ph.D. (NIMH)
Francesca Bosetti, Pharm. D., Ph.D. (NINDS)
Katarzyna Bourcier, Ph.D. (NIAID)
Robert Carter, M.D. (NIAMS)
Tony Casco (OD)
Elizabeth Church, Ph.D. (NIAID)
Jennifer Couch, Ph.D. (NCI)
Sarah Dunsmore, Ph.D. (NIGMS)
Daniel Gilchrist, Ph.D. (NHGRI)
Joseph G. Gindhart, Ph.D. (NIGMS)
Patricia Greenwel, Ph.D. (NIDDK)
Jill Heemskerk, Ph.D. (NIBIB)
Shannon Hughes, Ph.D. (NCI)
Halonna Kelly, Ph.D. (NIAID)
J. Randy Knowlton, Ph.D. (NCI)
Lillian S. Kuo, Ph.D. (NIAID)
Jerry Li, Ph.D. (NCI)
Sara Lin, Ph.D. (NHLBI)
Margaret Ochocinska, Ph.D. (NHLBI)
Aaron Pawlyk, Ph.D. (NIDDK)
Ajay Pillai, Ph.D. (NHGRI)
Ipolia Ramadan, Ph.D. (NINDS)
Krystyna Rys-Sikora, Ph.D. (NIDDK)
John Satterlee, Ph.D. (NIDA)
Tonya Scott (OD)
Salvatore Sechi, Ph.D. (NIDDK)
Kentner Singleton, Ph.D. (NIAID)
Jessica Smith, Ph.D.(OD)
Pothur Srinivas, Ph.D. (NHLBI)
Reiko Toyama, Ph.D. (NICHD)
José M. Velázquez, Ph.D. (NIA)
Yong Yao, Ph.D. (NIMH)

"Lumping"

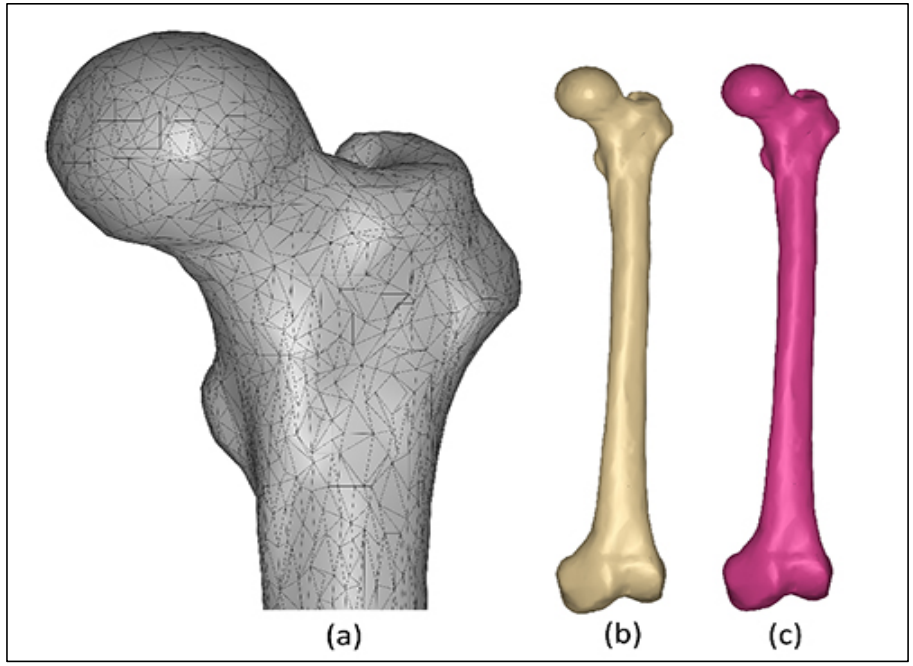
- **Assembling the puzzle pieces**
- **Integrating across scales**
  - **Individual cells > tissues> organs> human body**
  - **Key organizing principles?**
  - **Coordinates to use for the human body?**
  - **Filling in the blanks?**

Some Current/Emerging Approaches



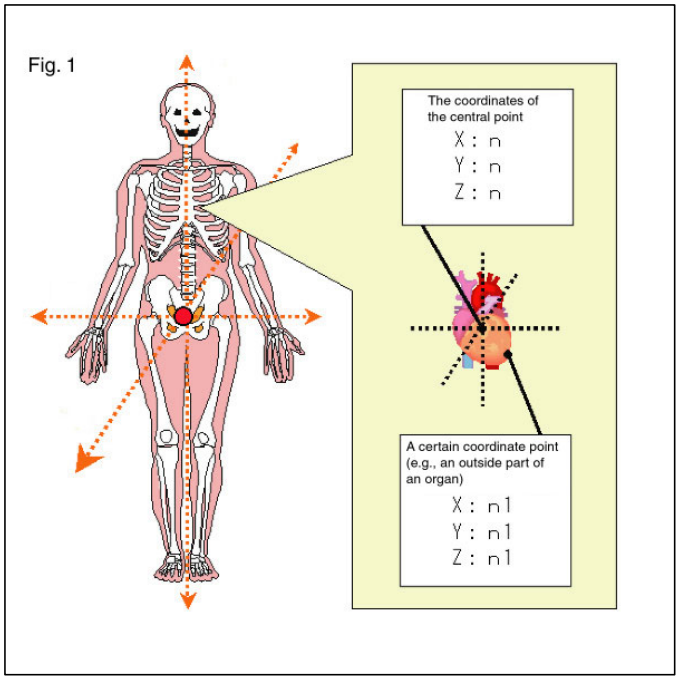
Coordinates For The Human Body Map?

Local level integration



Vector surface component of the left femur
http://www.geospatialhealth.net/index.php/gh/article/view/375/423

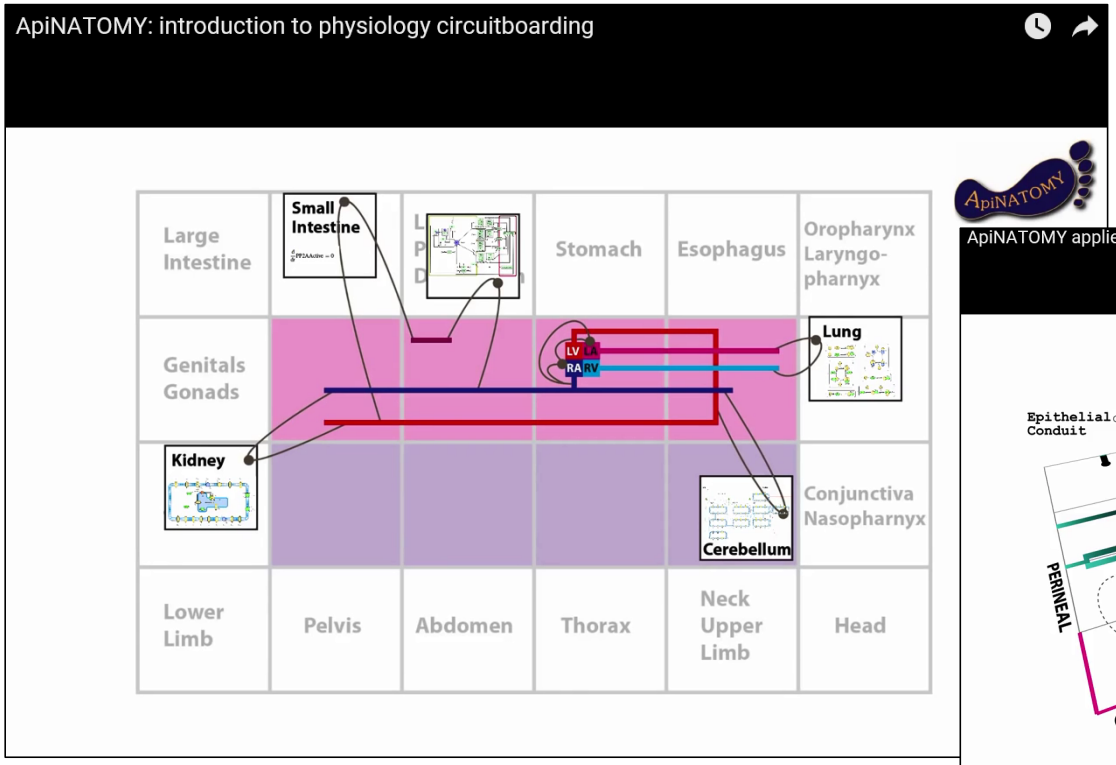
Central coordinates

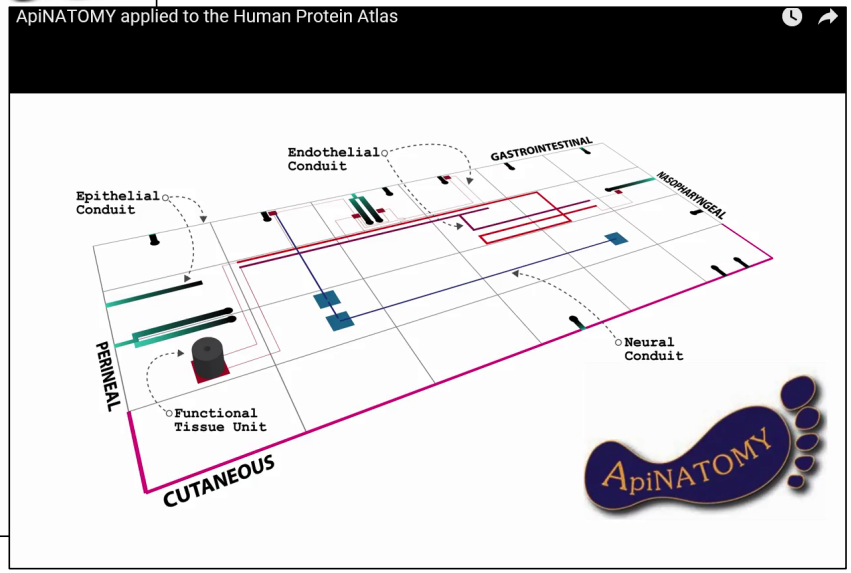


http://www.natureinterface.com/e/ni04/P056-059/0104_058+01.jpg

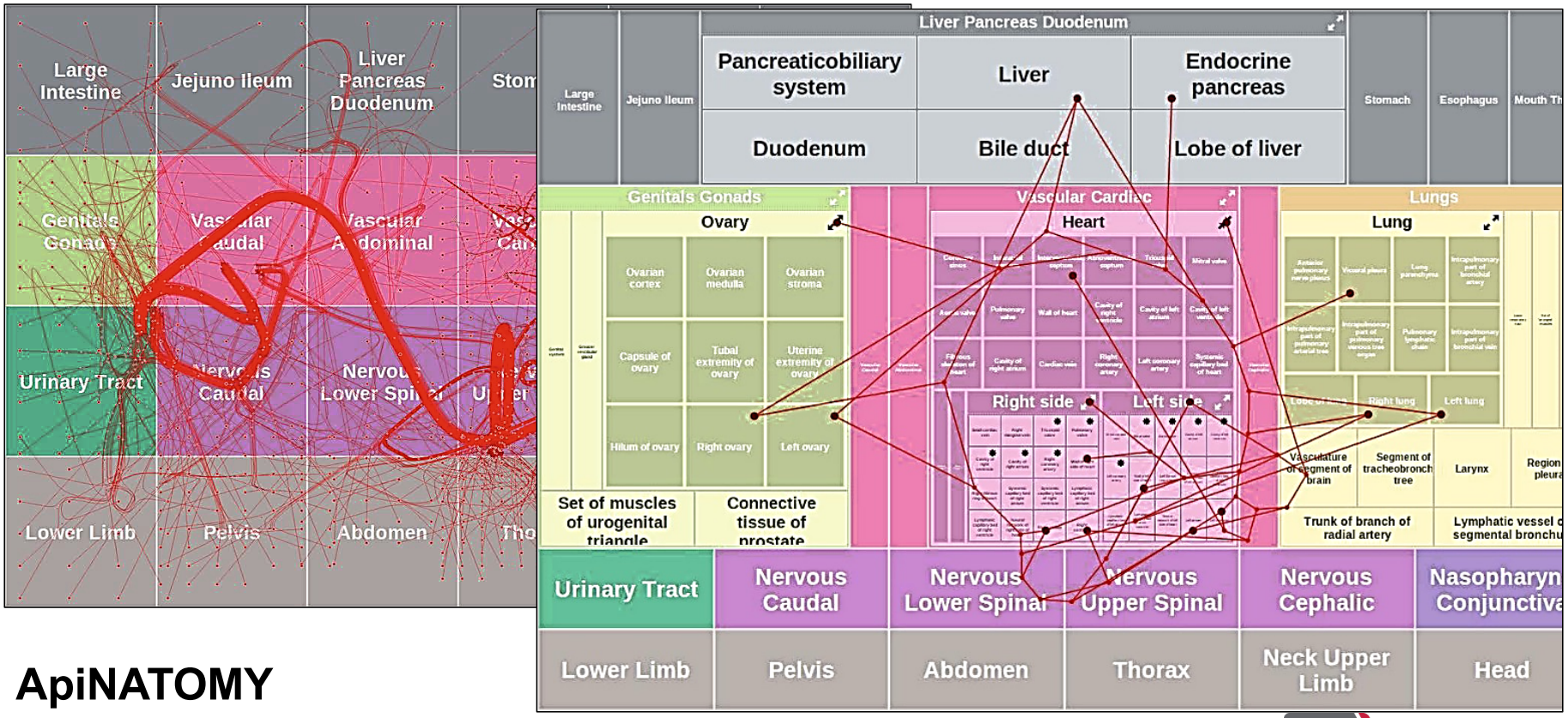
NIH National Heart, Lung, and Blood Institute

Functional Integration Of The Human Body: A Circuit Board Approach - “ApiNATOMY”



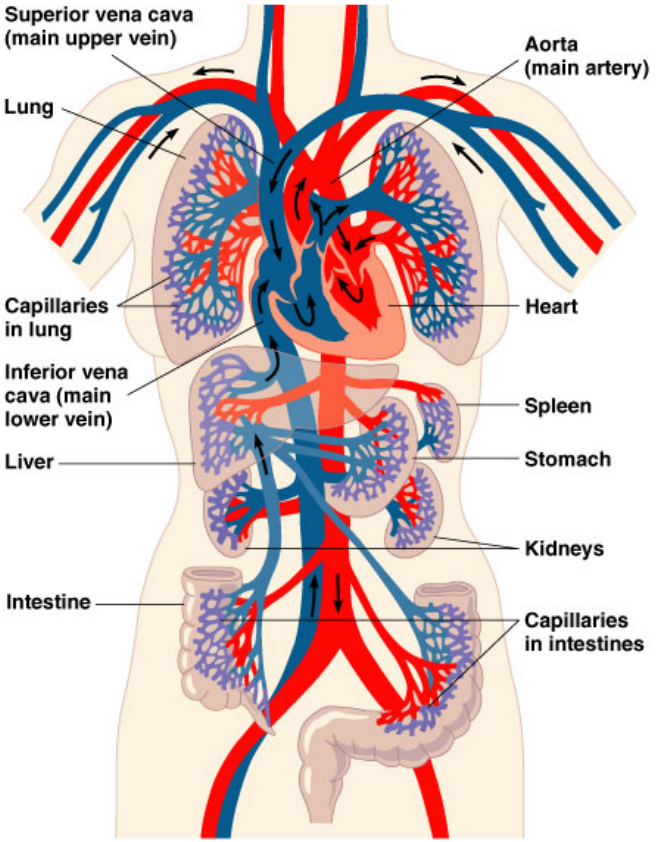


Vascular Conduits Are Used To "Wire" Together Different Organs

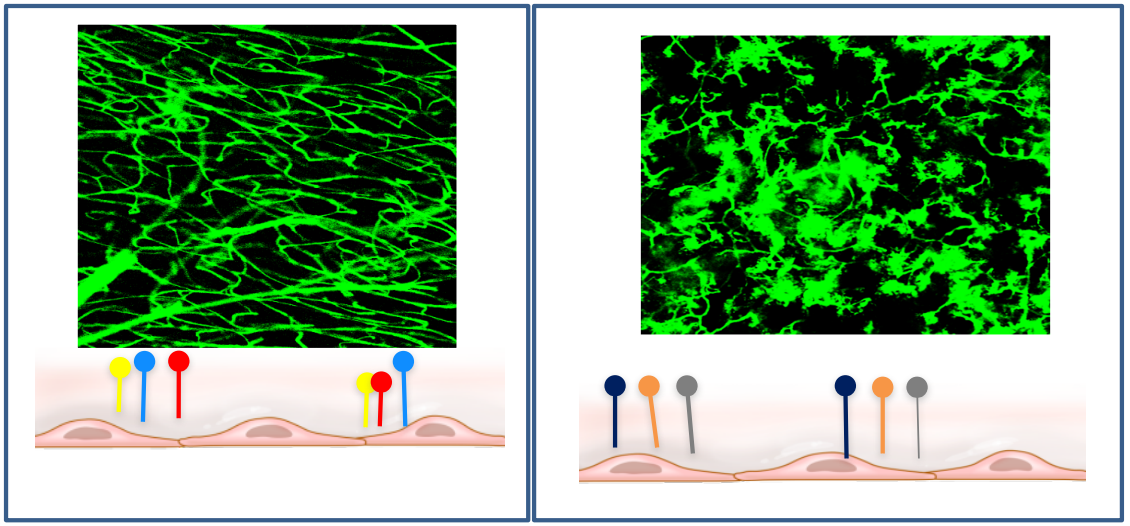


ApiNATOMY

Blood Vessels:
Tissue Organization, Integration, and Navigation

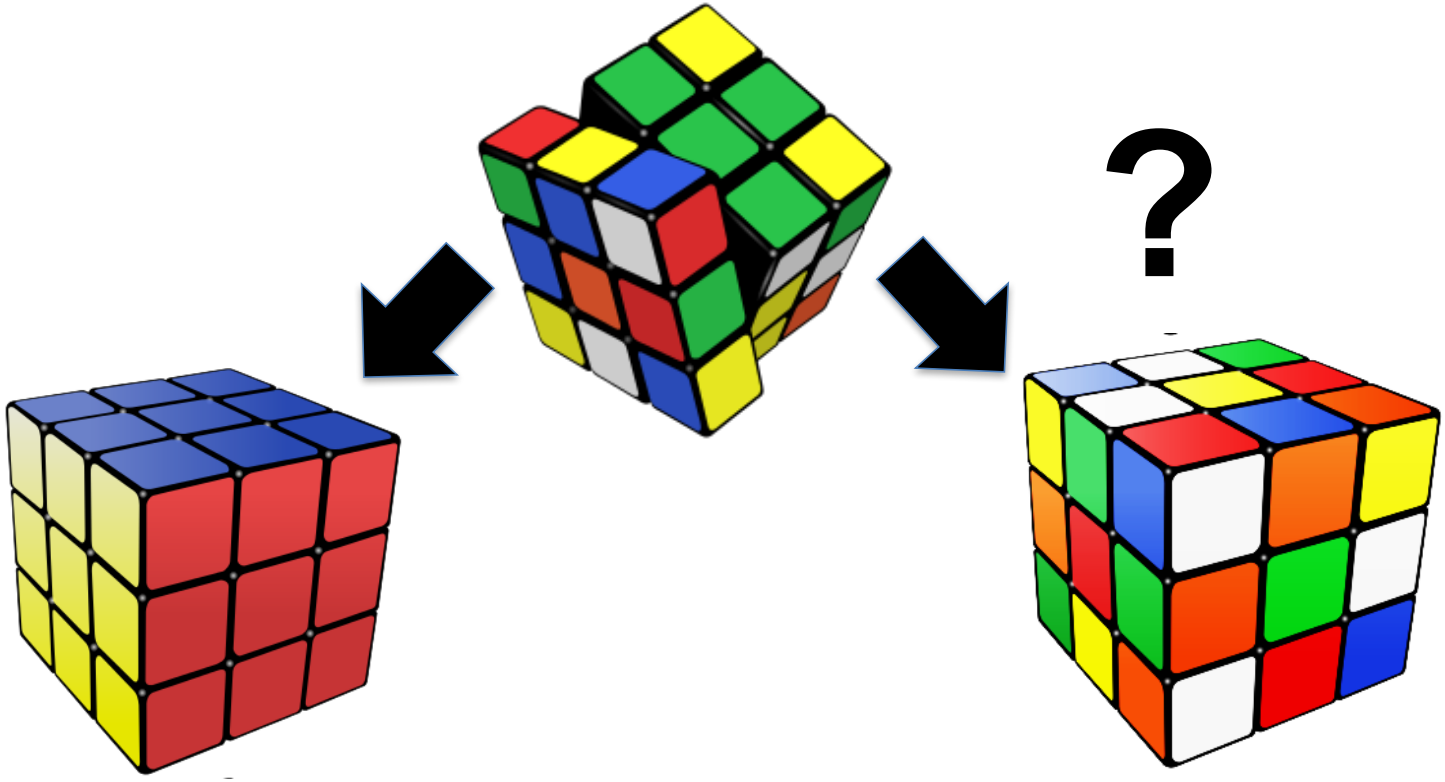


Organ-specific Vascular "Zip Codes" (Narasimhan 2002)



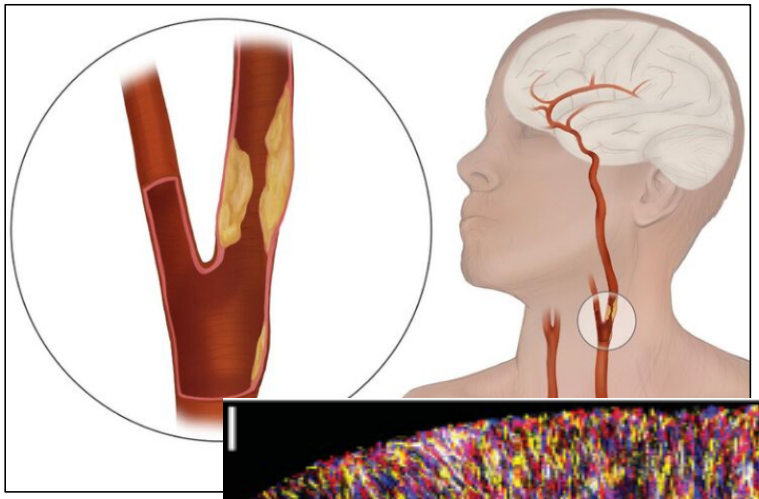
Brain: "02215" **Spleen: "10013"**

The Rubik's Cube Dilemma?

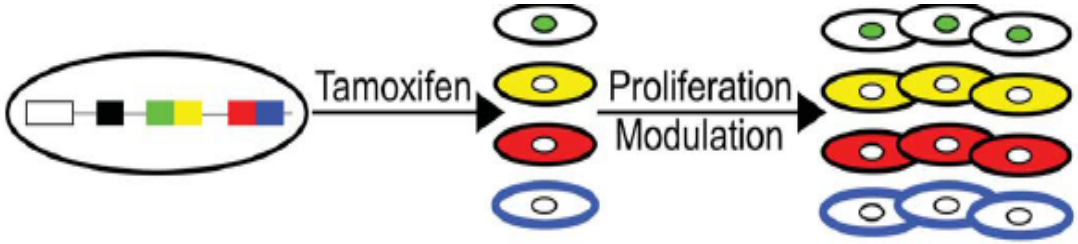


https://en.wikipedia.org/wiki/Rubik's_Cube

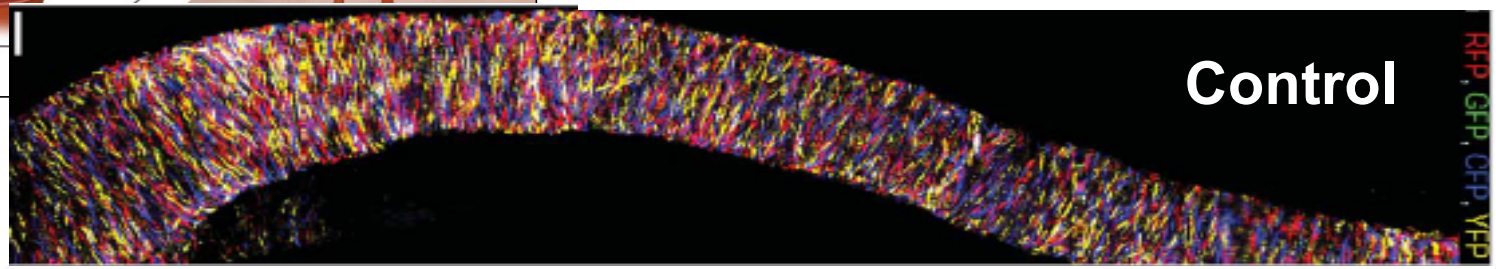
Carotid Artery: Diversity of Vascular Smooth Muscle Cells



Experimental Multi-color cell labeling in "Confetti mice"

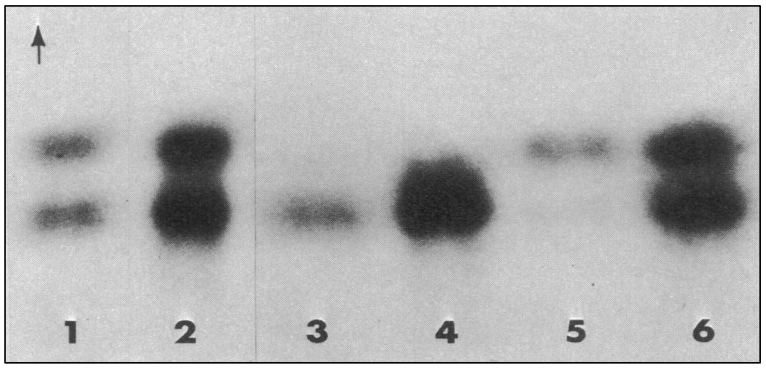


Chappell et al, *Circ Research* online, Sept 28, 2016



New Technologies Explain Previously Reported Vascular Smooth Muscle Cell (SMC) "Curiosities"

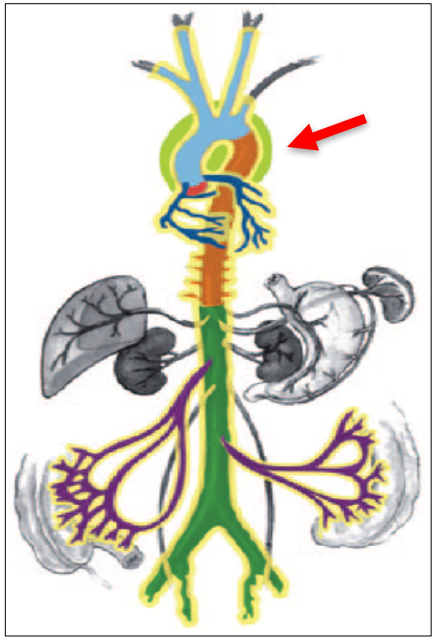
Benditt *PNAS* 1973: Discovery of SMC "Clones"



Normal artery

Atherosclerotic plaques

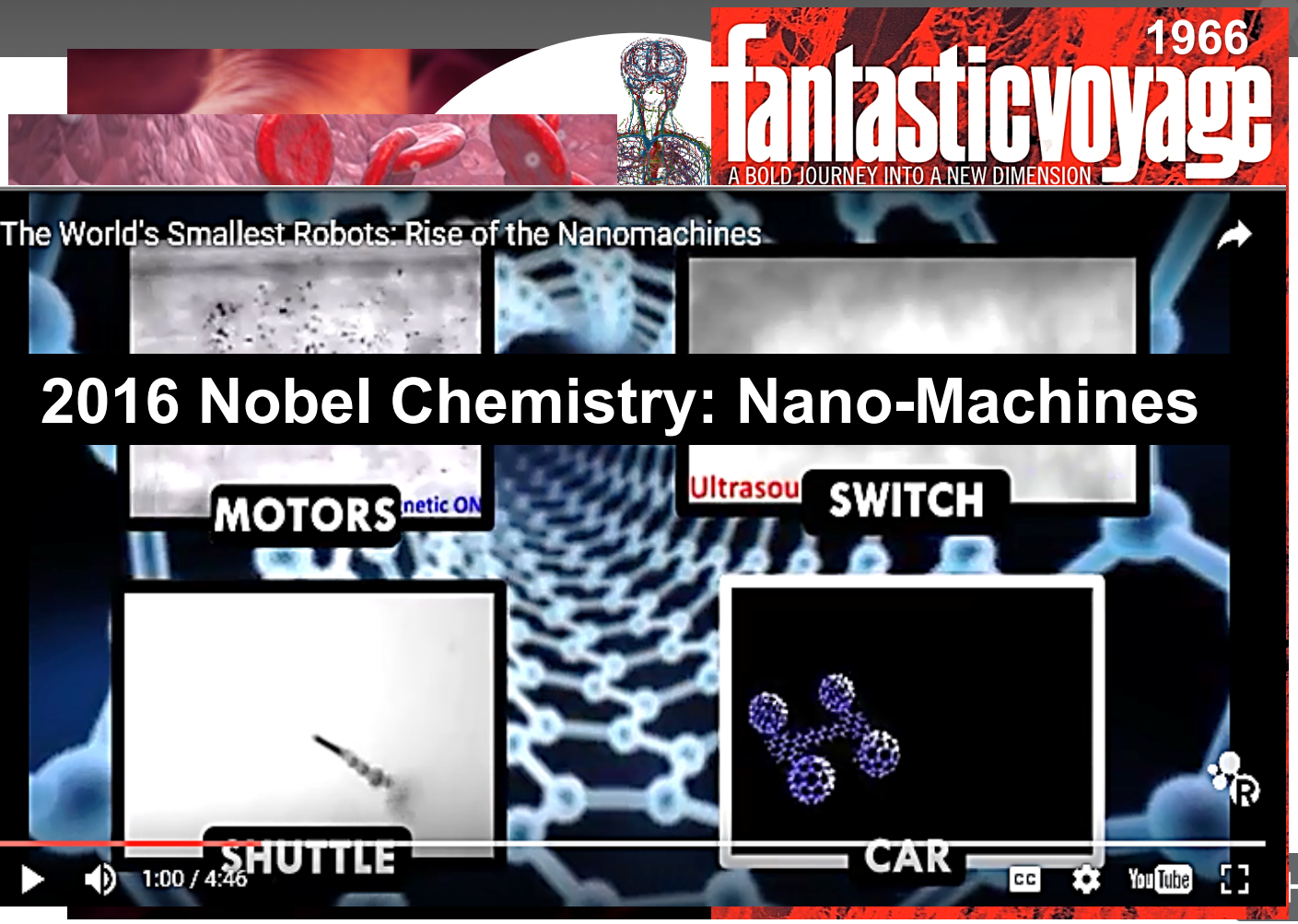
Majesky *ATVB* 2007: Different Developmental SMC Origins



Site of Aneurysms!

Understanding SMC diversity may hold the key to unsolved vascular mysteries

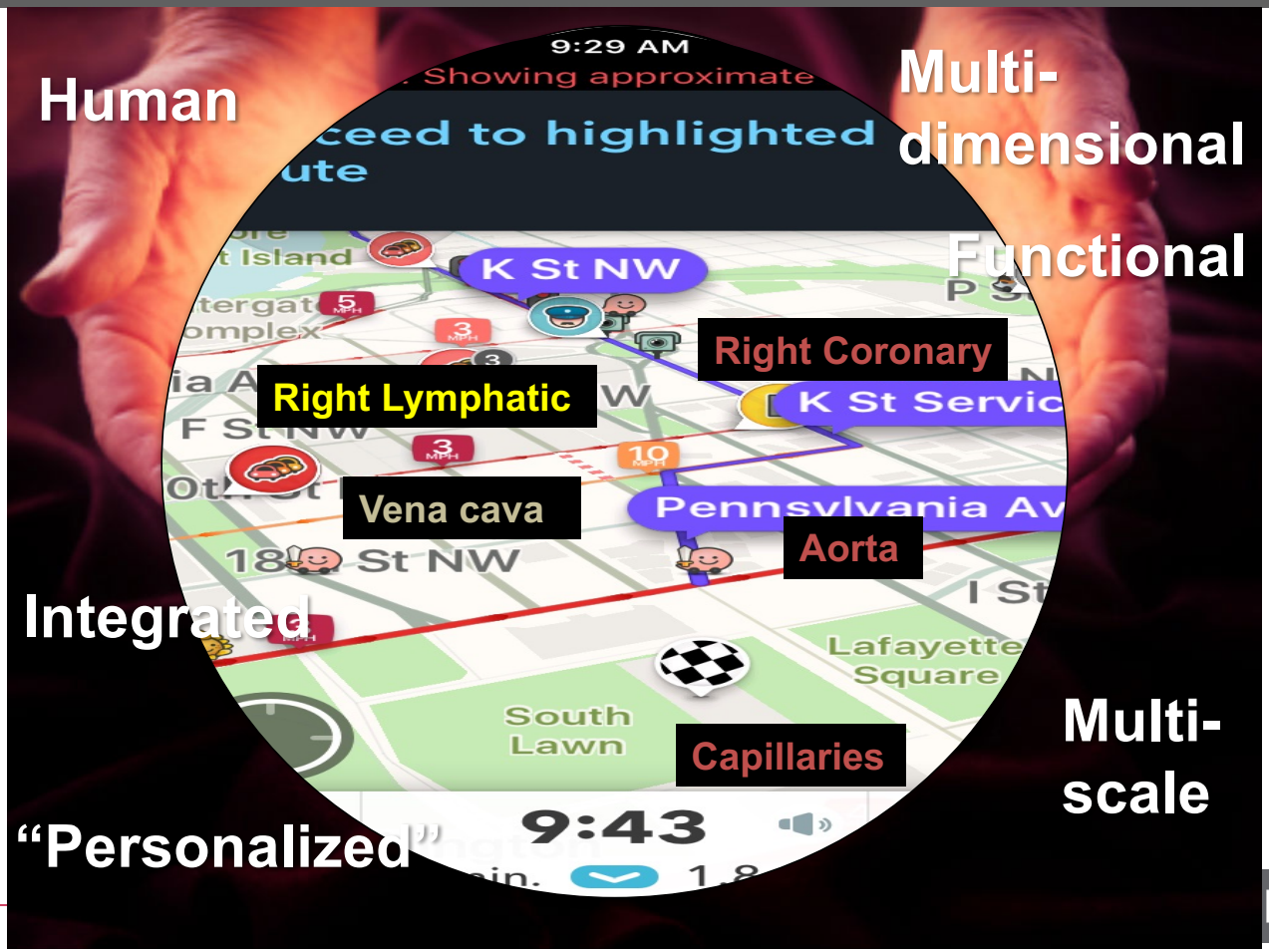
Vasculome-Specific Challenges and Opportunities?



The “Vasculome” for HuBMAP Success

- Needed to complete any individual human tissue map
- May provide anatomically relevant coordinate system to organize any tissue architecture
- May serve as a prime example for body-wide integration of local heterogeneity of distributed systems
- May become the road map for the body “Google Map,” used to connect and navigate within and between tissues and organs

The Vasculome for HuBMAP…



NIH National Heart, Lung, and Blood Institute

Questions?
Suggestions?

The Human BioMolecular Atlas Program (HuBMAP)

https://commonfund.nih.gov/hubmap

NHLBI Funding Opportunities And Operating Guidelines & Strategic Visioning

NHLBI webpage: www.nhlbi.nih.gov

Research Portfolio Online Reporting Tools (Re-PORTER)

http://projectreporter.nih.gov/reporter.cfm

When in doubt…. Google us!

NIH National Heart, Lung, and Blood Institute

Backup slides

HuBMAP Consortium Expectations

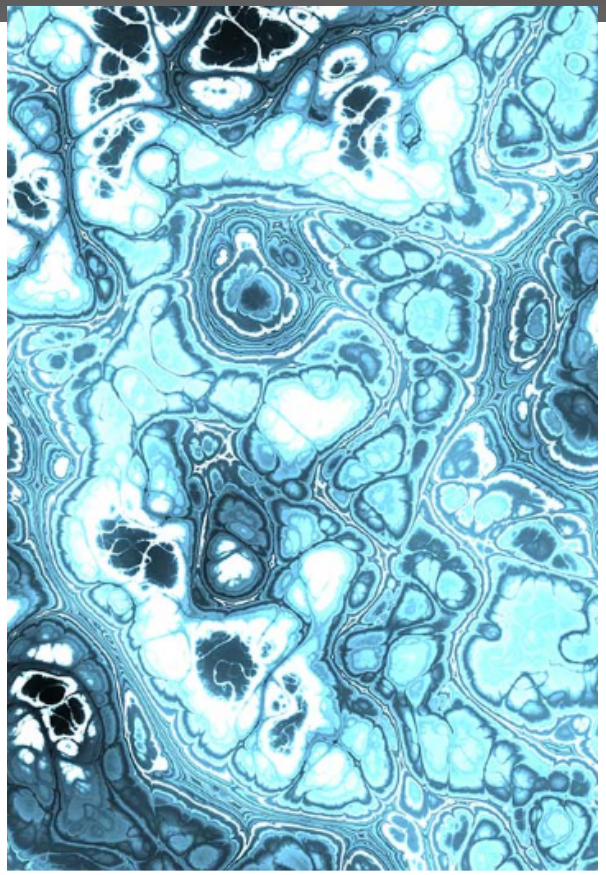
- **Membership: all successful applicants**
- **Purpose: enable groups to effectively collaborate with each other to maximize the chances of overall success of the program**
- **Expectations:**
  - **complete own research goals**
  - **work collaboratively for development of SOPs, data and metadata standards, metrics for data generation**
  - **participate in cross-site studies**
  - **engage in cross-training**
  - **guide development of data analysis and visualization tools that can be used by the broader scientific community.**
  - **attend an HuBMAP Kickoff meeting, as well as annual investigator meetings and regular teleconferences with Network members and NIH Staff for the duration of the funding cycle.**

Transformative Technology Development

Purpose: to solicit transformative technologies that will significantly expand throughput, multiplexing and discrimination of biomolecules in human tissues for comprehensive mapping of individual cells and their context in human tissues

Phases: The initial two year UH2 phase will support development and demonstration of feasibility of these emerging technologies for human tissue mapping. The subsequent UH3 phase is to support initial validation in human tissues, optimization and scale-up, and generation of production level data.

Tissue Mapping Centers



Purpose: to solicit transformative technologies that will significantly expand throughput, multiplexing and discrimination of biomolecules in human tissues for comprehensive mapping of individual cells and their context in human tissues

Tissue Mapping Center Structure:

- Coordination Core: The Coordination Core will be responsible general administrative duties and for coordinating
  - 1 core, required; 6 pages; plus 6 pages for Overall
- Organ Specific Projects: The Organ-Specific Projects will be responsible for generating high quality tissues maps
  - Can propose up to 4 projects, at least 1 required; 6 pages to describe each
- Data Analysis Core: The Data Analysis Core will be responsible for data annotation, curation, and analysis.
  - 1 core, required; 6 pages

The "HIVE"
HuBMAP Integration Visualization and Engagement

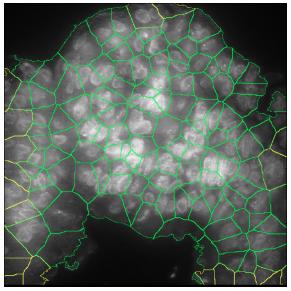
The HIVE Structure:

- Coordination Component –responsible for coordinat… collaboration with the other funded components of the HuBMAP Consortium and the wider research community;
- Infrastructure Component –responsible for building and optimizing the data ingestion and archiving platform and support the internal and external facing IT tools for the Consortium;
- Mapping Component - responsible for developing mapping pipelines and frameworks for analyzing data in the archive;
- Tools Component - responsible for developing search, analysis and visualization tools for HuBMAP data or enable adoption and usage of relevant ones from the community

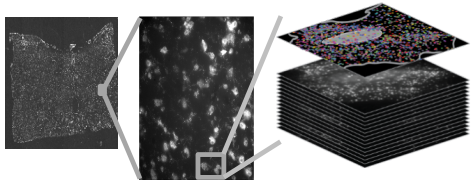
Why HuBMAP?

	HuBMAP	GTEx	GUDMAP	LungMAP	BRAIN	SGMAP	HPA
Primary Species	**Human**	Human	Mouse moving to Human	Human / Mouse	Mouse	Mouse	Human
Tissues	**Phase 1: ~10 Phase 2: ~40**	~53	Kidney / Prostate	Lung	Brain	Salivary glands	~44
Focus	**Inter-individual variability**	eQTLs	Early development	Early development	Cell census	Early development	Proteome
Tech	**FISH, RNA-Seq, IMS**	RNA-Seq	FISH, RNA-Seq	FISH, RNA-Seq, MS, CT	RNA-Seq	Microarray / RNA-Seq	60,000+ Antibody
Single cell focus?	**Yes**	No	Yes	Yes	Yes	No	Moving towards
Spatial?	**Yes**	No	Yes	Yes	No	No	Yes
Across Body?	**Yes**	**Yes**	**No**	**No**	**No**	**No**	**Yes**

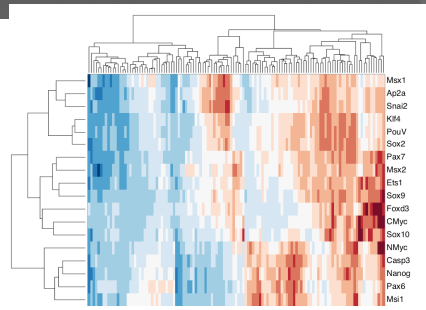
Emerging In-situ Technologies



FISH Imaging

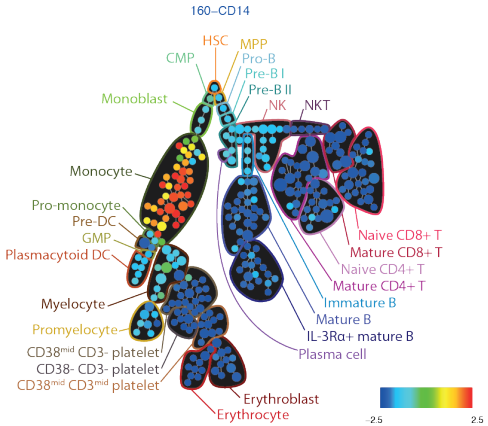


MERFISH – Imaging 1000+ genes in tissue (Zhuang Lab, 2016);



SeqFISH– Sequential barcoding, 100+ parameters, single molecule sensitivity (Cai Lab)

Mass Spec & CyTOF



CyTOF – 30+ parameters, high throughput, <5 Ab sensitivity (Nolan Lab)

The Human Cell Taxonomy Project ('Periodic Table' of Cells)

MIBI-TOF – up to 50 parameter imaging, down to 20nm (Angelo Lab, 2016)

	smFISH	Padlock probes and RCA	Branched FISH	LCM	Microtomy sequencing	TIVA	ISS	FISSEQ	Imagi… mass …
Sample	Fixed cells and tissues; purified RNA	Fixed cells and tissues; purified DNA or RNA	Fixed cells and tissues; possibly purified DNA or RNA	Fixed tissues	Fixed and fresh tissues	Live cells	Fixed cells and tissues	Fixed c…ls and …issues	…issues
Target	RNA	DNA; RNA	RNA	RNA; DNA; proteins	RNA; possibly DNA and proteins	RNA	RNA	NA	Proteins
Type	Targeted	Targeted	Targeted	Targeted or non-targeted	Non-targeted	Non-targeted	…rgeted	Non-targeted	Targeted
Variable measured	Abundance; SNVs; fusion transcripts; splice variants; subcellular localization	Abundance; SNVs; fusion transcripts; splice variants; subcellular localization	Abundance; subcellular localization	Abundance; possibly SNVs, fusion transcripts and splice variants	Abundance; possibly SNVs, fusion transcripts and splice variants	Ab…da…ce; p…sib… S…Vs, …s… …ar…ripts …nd …plice variants	Abundance; possibly SNVs, fusion transcripts and splice variants	Abundance; possibly SNVs, fusion transcripts and splice variants	Abundance; protein modifications
Single-cell?	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Spatial resolution	Subcellular	Subcellular	Subcellular (except the nucleus)	Anatomi… or cellul…	…natomical	Cellular	Cellular	Cellular	Subcellular
Morphology assessment	Yes	Yes	Yes	…s	No	Yes	Yes	Yes	Yes
Throughput (number of cells)	Low to medium	Low to medium	Low to me…	M…dium	High	Low	Low to medium	Low to medium	Very high
Throughput (number of genes or proteins)	Low to medium	Low to medium	…e…	High	High	High	Low	High	Low
Estimated efficiency	~90%	…3…%	NA	NA	~5–10%	NA	NA	NA	NA
Readout	Micro…copy; flow …	M…roscopy; flow cytometry	Microscopy; flow cytometry	Microarray; RNA-seq; MS	RNA-seq; possibly MS	RNA-seq	Microscopy	Microscopy	MS
Technic… difficulty	…sy	Easy	Easy	Moderately easy	Moderately easy	Moderately difficult	Difficult	Difficult	Difficult
Refs	23,53–58, 60–63	64–68	70,71	72,74,76–78	79–81	82	83	85	87,88

FISH, fluorescence *in situ* hybridization; FISSEQ, fluorescent *in situ* RNA sequencing; ISS, *in situ* sequencing; LCM, laser capture microdissection; MS, mass spectrometry; NA, not available; RCA, rolling circle amplification; RNA-seq, RNA sequencing; smFISH, single-molecule RNA fluorescence *in situ* hybridization; SNV, single-nucleotide variant; TIVA, transcriptome *in vivo* analysis.

Crosetto, Nicola, Magda Bienko, and Alexander van Oudenaarden. "Spatially resolved transcriptomics and beyond." *Nature Reviews Genetics* 16.1 (2015): 57-66

Assays	Cell capture strategies	cDNA amplification strategies	Target RNAs	Poly(A) minus RNA detection	Number of cells	UMI
scRNA-seq	Mouth pipette or FACS	Polyadenylation followed by PCR	Full-length mRNAs	No	1–100	No
Quartz-seq	Mouth pipette or FACS	Polyadenylation followed by PCR	Full-length mRNAs	No	1–100	No
Smart-seq/Smart-seq2	Mouth pipette or FACS	Template-switch followed by PCR	Full-length mRNAs	No	1–100	No
MALBAC-RNA	Mouth pipette or FACS	MALBAC	Full-length mRNAs	No	1–100	No
PMA	Mouth pipette or FACS	Rolling circle amplification	Full-length mRNAs	No	1–100	No
SMA	Mouth pipette or FACS	Semi-random priming followed by PCR	Full-length mRNAs	No	1–100	No
SUPeR-seq	Mouth pipette or FACS	Random priming followed by PCR	Full-length mRNAs	Yes	1–100	No
Fluidigm C1	Microfluidic system	Template-switch followed by PCR	Full-length mRNAs	No	100–1000	No
Microfluidic scRNA-seq	Microfluidic system	Polyadenylation followed by PCR	Full-length mRNAs	No	100–1000	No
STRT-seq	Mouth pipette or FACS	Template-switch followed by PCR	5′ end of mRNAs	No	10–100	Yes
CEL-seq	Mouth pipette or FACS	In vitro transcription and linear RNA	3′ end of mRNAs	No	10–100	Yes

Wen, Lu, and Fuchou Tang. "Single cell sequencing in stem cell biology." *Genome Biology* 17.1 (2016)

National Heart, Lung, and Blood Institute

Assays	Cell capture strategies	cDNA amplification strategies	Target RNAs	Poly(A) minus RNA detection	Number of cells	UMI
MARS-seq	Robotics and automation	CEL-seq	3′ end of mRNAs	No	100–1000	Yes
CytoSeq	Bead-based	CEL-seq	3′ end of mRNAs	No	>1000	Yes
Drop-seq	Droplet- and bead-based	Template-switch followed by PCR	3′ end of mRNAs	No	>1000	Yes
inDrop	Droplet- and bead-based	CEL-seq	3′ end of mRNAs	No	>1000	Yes
TIVA	In vivo mRNA capture based on photo-activation	In vitro transcription	Full-length mRNAs	No	10–100	No
FRISCR	FACS or fixed cells	SMART-seq2	Full-length mRNAs	No	10–100	No
Patch-seq	Aspiration through patch-clamp pipette	STRT-seq/SMART-seq2	5′ end of mRNAs or full-length mRNAs	No	10–100	Yes/no
FISSEQ	In situ RNA sequencing	Rolling circle amplification	Full-length mRNAs	No	100–1000	No

Wen, Lu, and Fuchou Tang. "Single-cell sequencing in stem cell biology."*Genome Biology* 17.1 (2016)