Architecture as defined by organization of niches and INTER-CELLULAR relationships

PATHOLOGY From the Atomic Scale ‘ON-UP’

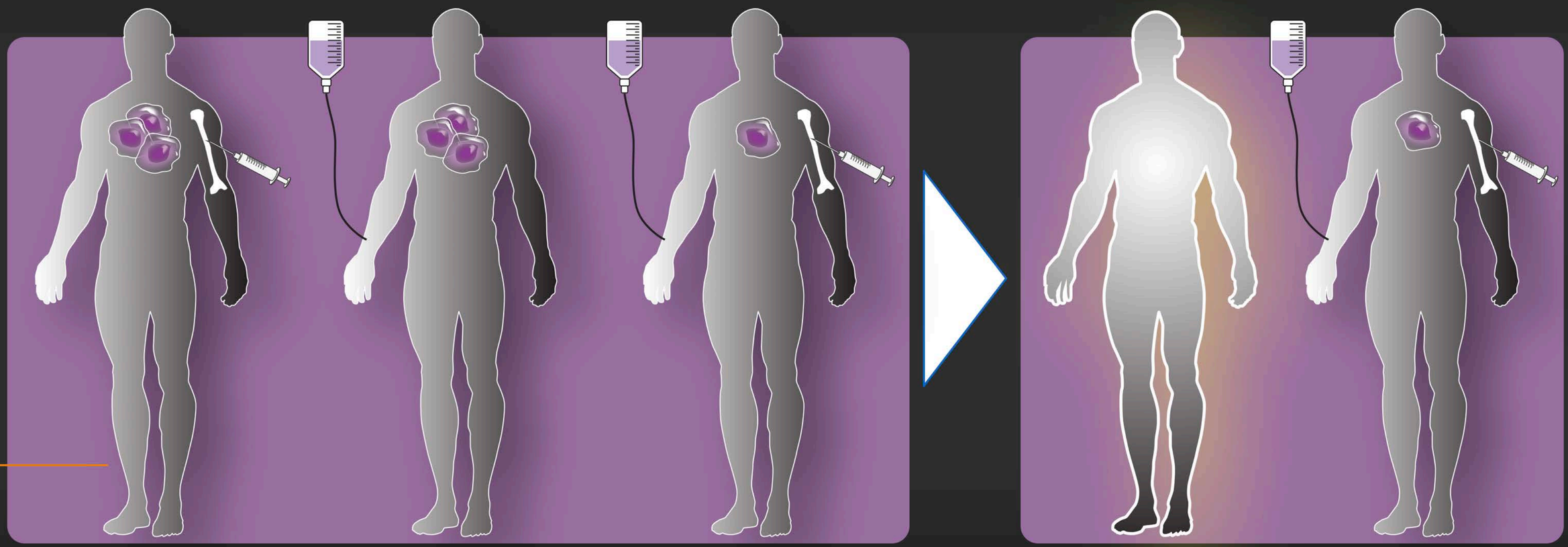
Garry P. Nolan, Ph.D.
Rachford and Carlota A. Harris Professor
Dept. of Microbiology & Immunology
Stanford University

Parker Institute for Cancer Immunotherapy

ORDERING IN PSEUDOTIME

Can ordering help us with predictors of relapse?

B-cell precursor acute lymphoblastic leukemia (BCP-ALL)



Diagnosis

Treatment

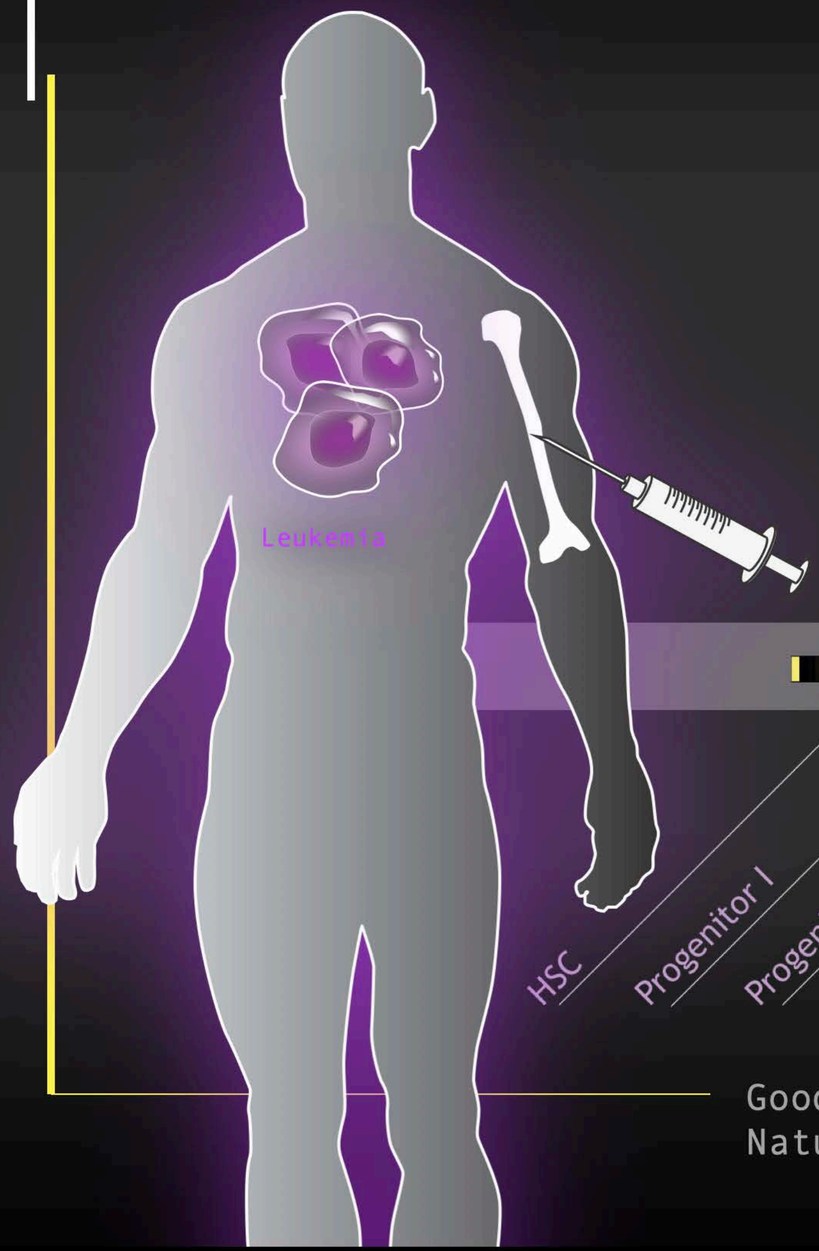
Assess MRD

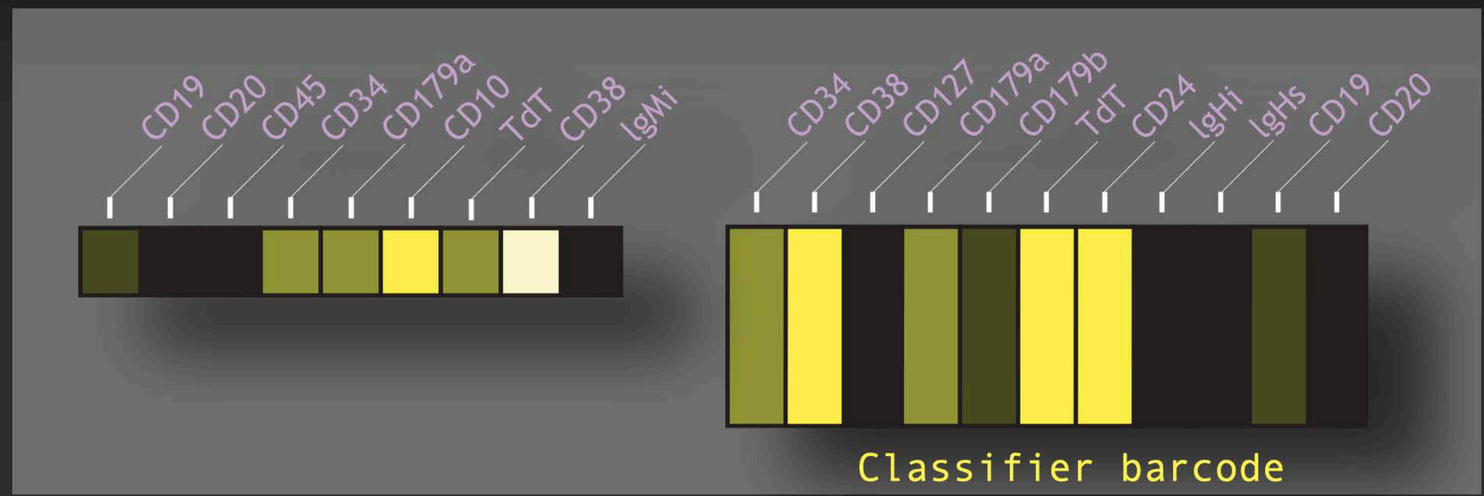
80% Cure

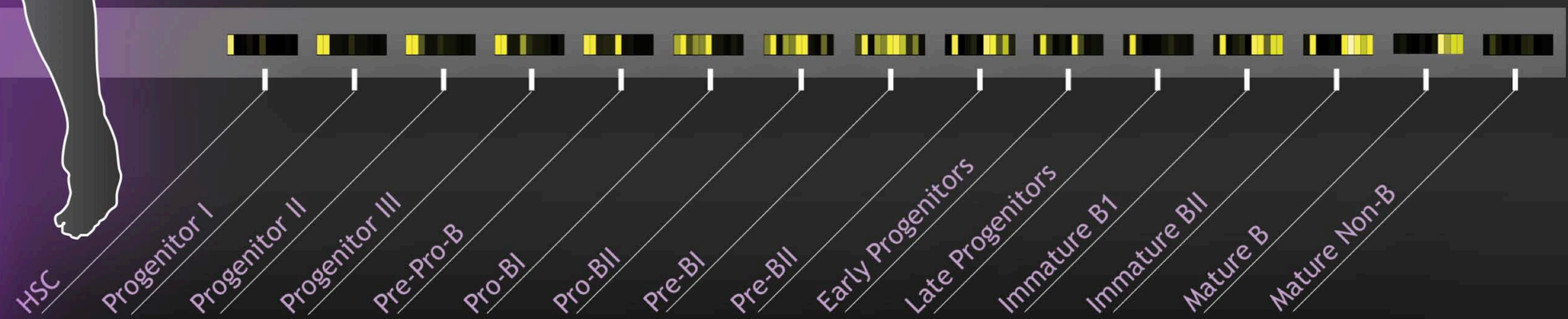
20% Relapse

MAPPING LEUKEMIA CELLS •

Closest Normal Stage in B Lymphopoiesis







Good et al,
Nature Medicine, 2018

MAPPING LEUKEMIA CELLS •

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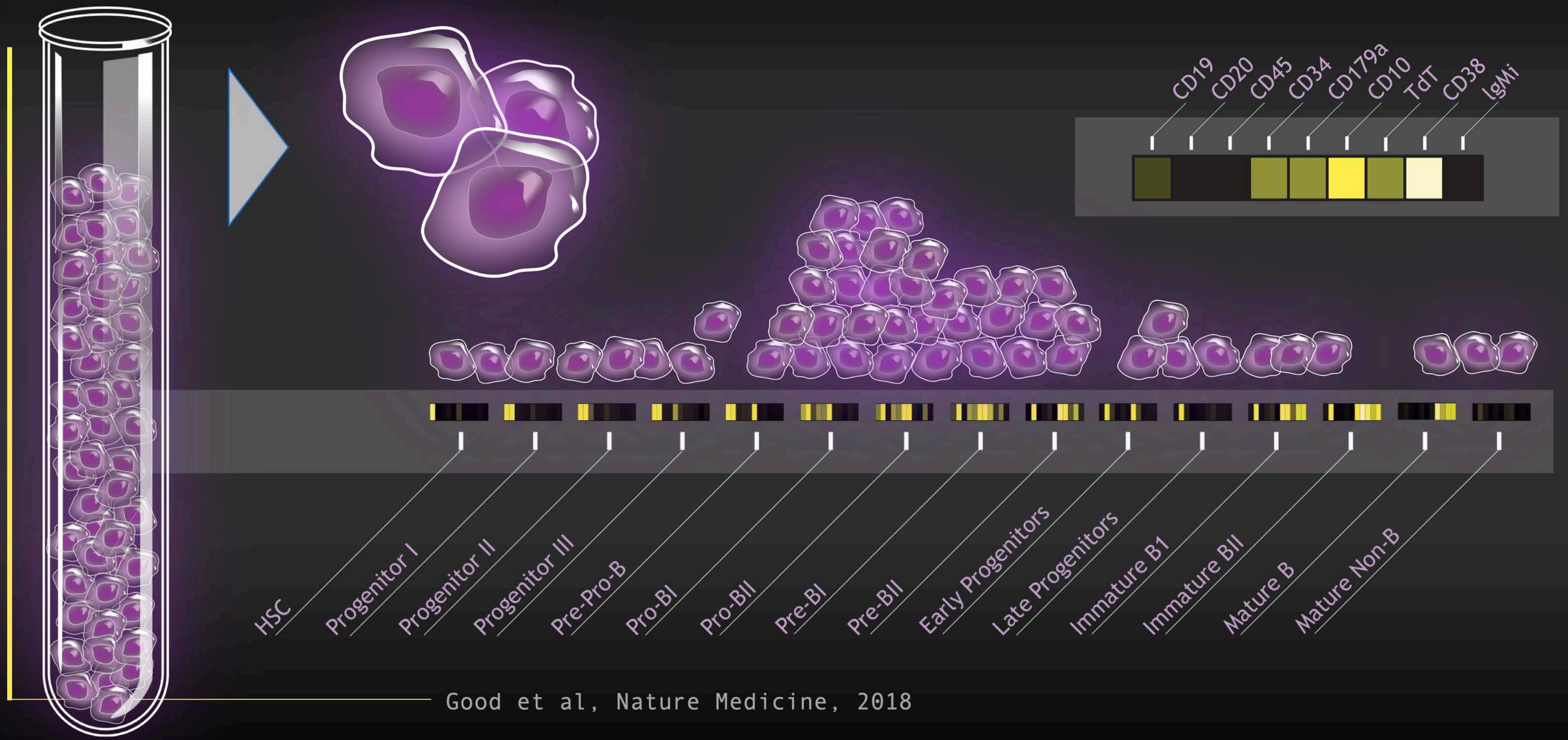
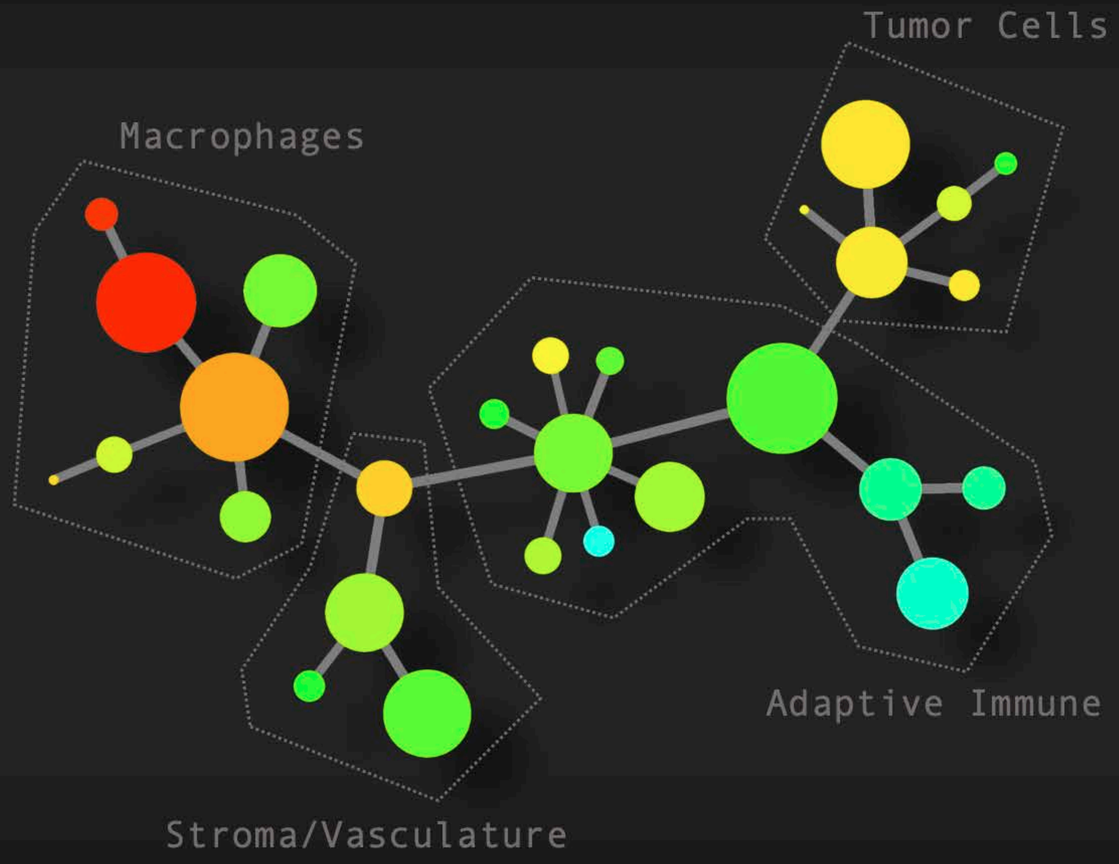
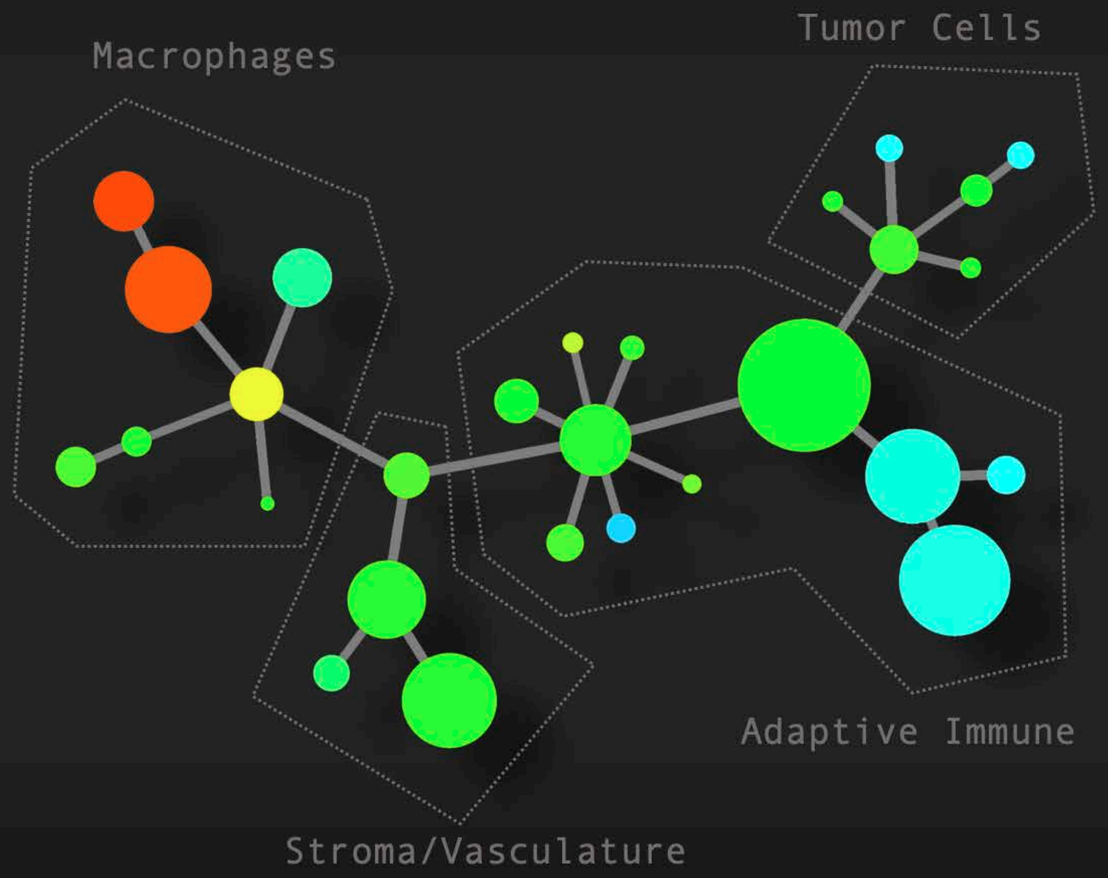


Good et al, Nature Medicine, 2018

CELL CLUSTERING • Brentuximab @CD30, response in CTCL



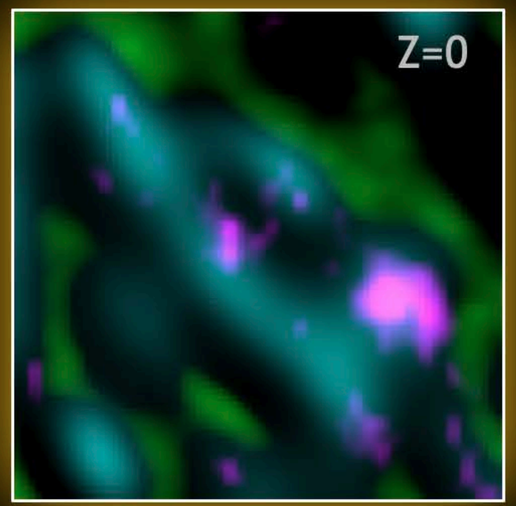
Pre-treatment

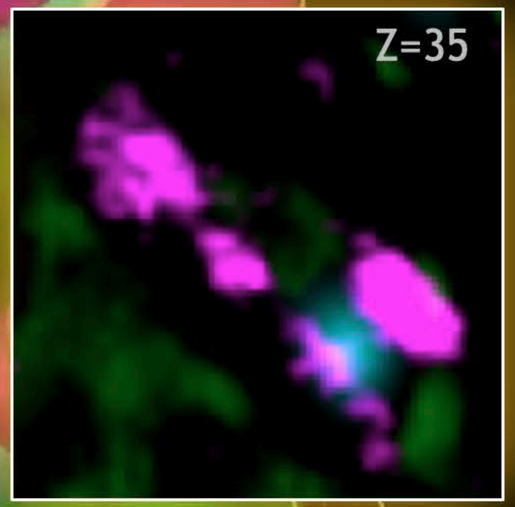


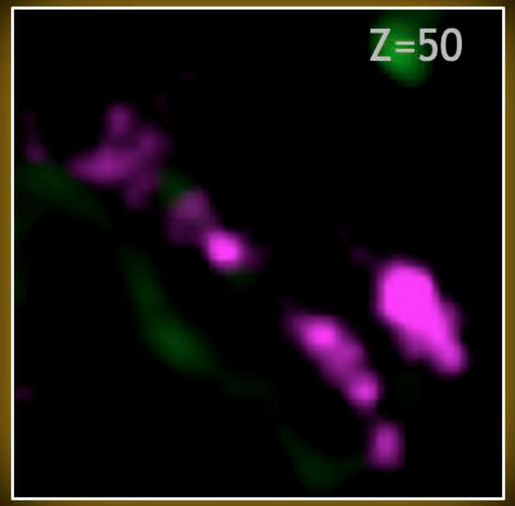
Post-treatment (responder)

3D NANO-IMAGING • Mitochondria & Protons

DR Congo
1995 (Kikwit)







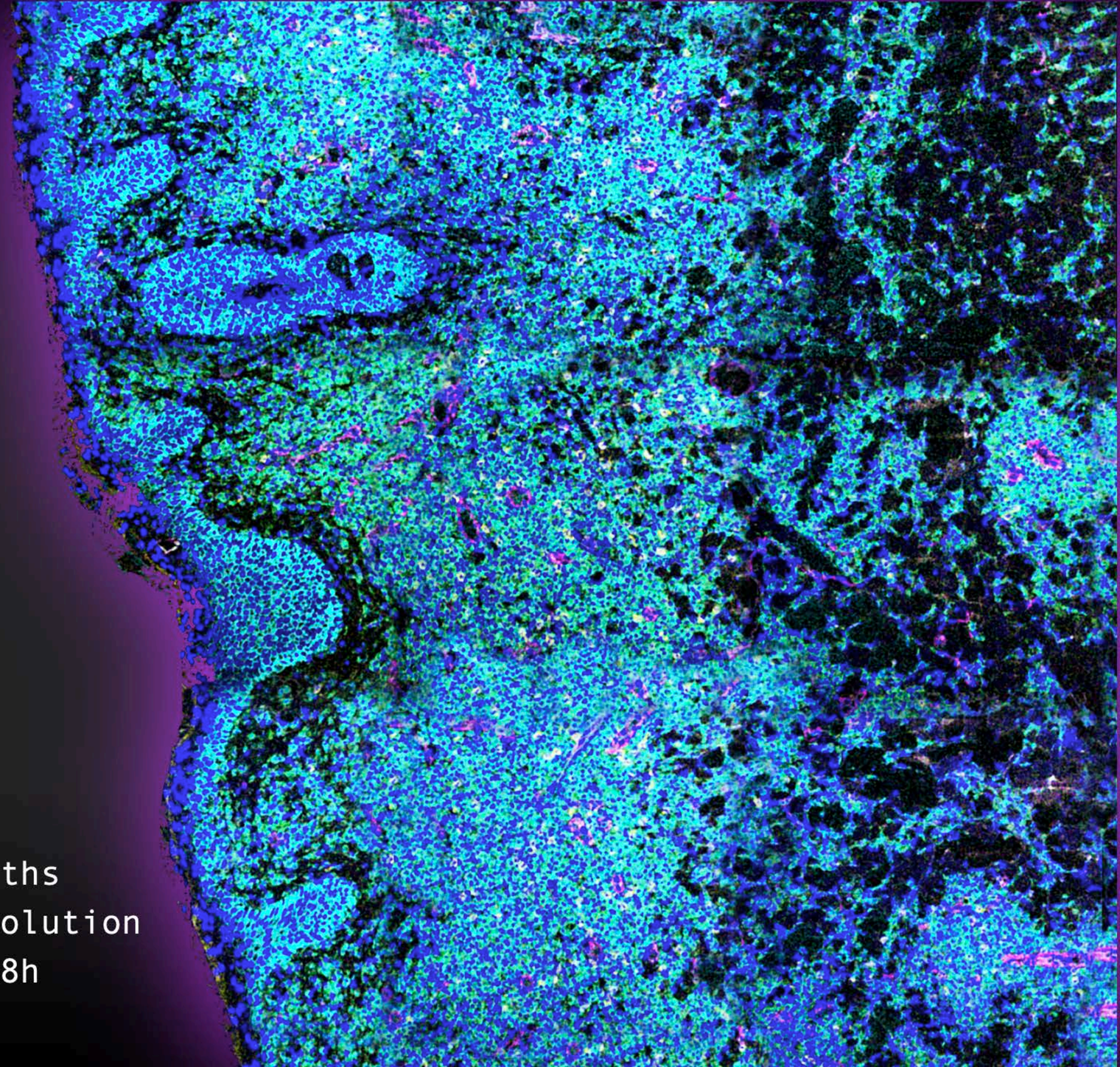
Single Mitochondria

Nitrogen $^{12}C^{14}N$ Tom20 ^{197}Au Proton $^{12}C^{1}H$

CTCL Plaque •

- Pan Keratin
- Vimentin
- HLA-DR
- dsDNA
- CD8

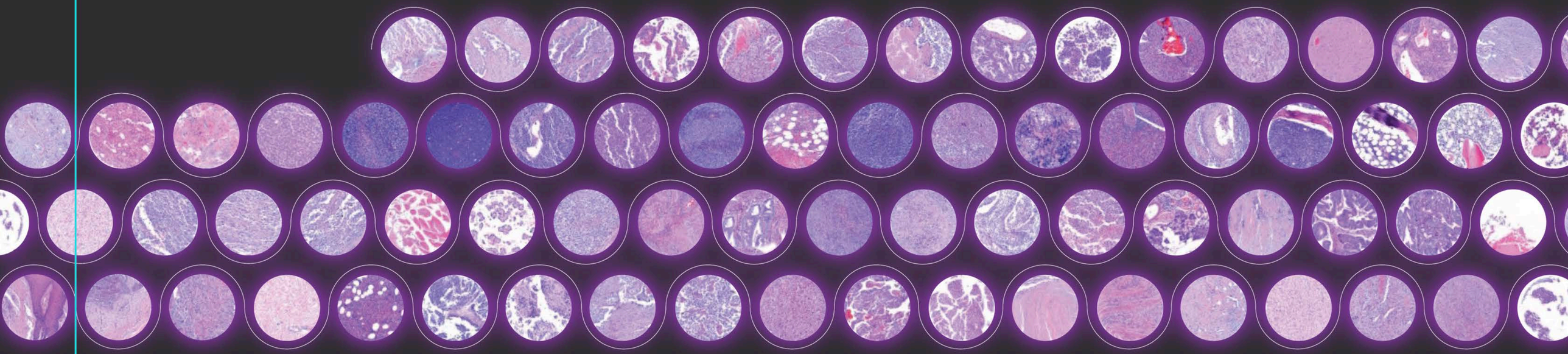
- 1.5mm * 1.5mm
- 42 Channels
- 3 x 3 FOVs, 3 depths
- 300-500nm X-Y resolution
- Time to acquire: 8h



EXPANDING CODEX •

Christian Schuerch
Darci Philipps

TISSUE ATLAS



FFPE

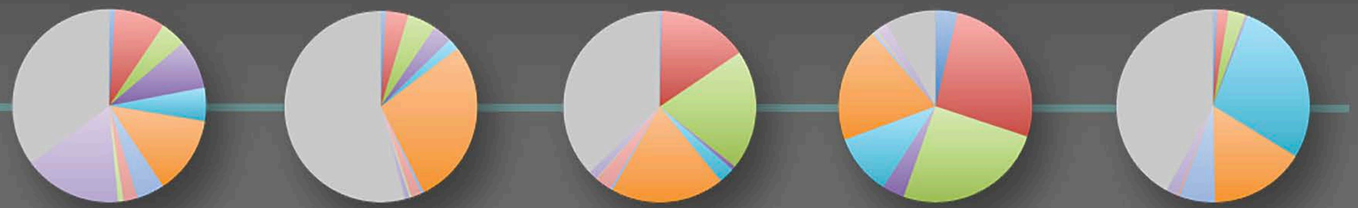
’Studying Malignancies, One Cell at a Time’

- ADVANCED PROTEOMIC TECHNOLOGIES -

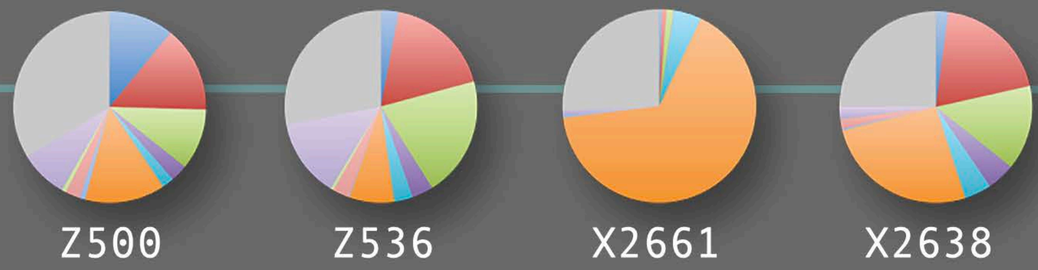
VARIATION • Frequencies of Immune Cell Subsets

- B Cells
- CD4 T Cells
- CD8 T Cells
- NK Cells
- Myeloid DC
- Macrophages
- Monocytes
- Non Classical Monocytes
- Basophils
- Granulocytes
- Unassigned Cells

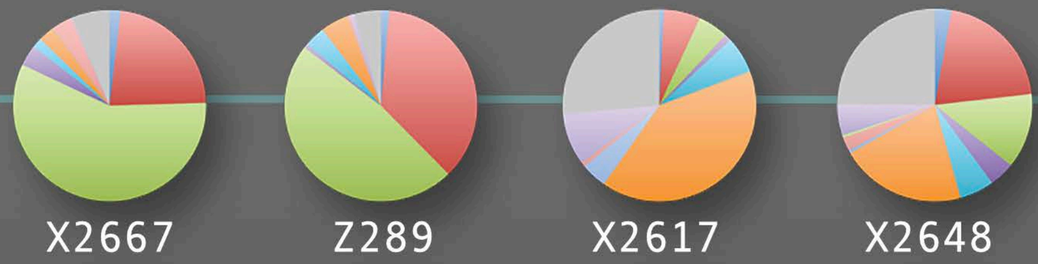
CD45+ 3-18%



CD45+ 20-60%



CD45+ 60-75%



CD45+ 80-95%

High Parameter

IMMUNE PROFILING • Host Responses to Infection

From Blood to Tissues

David McIlwain, PhD

NIH | Center for Human Immunology
National Institute of Allergy & Infectious Disease

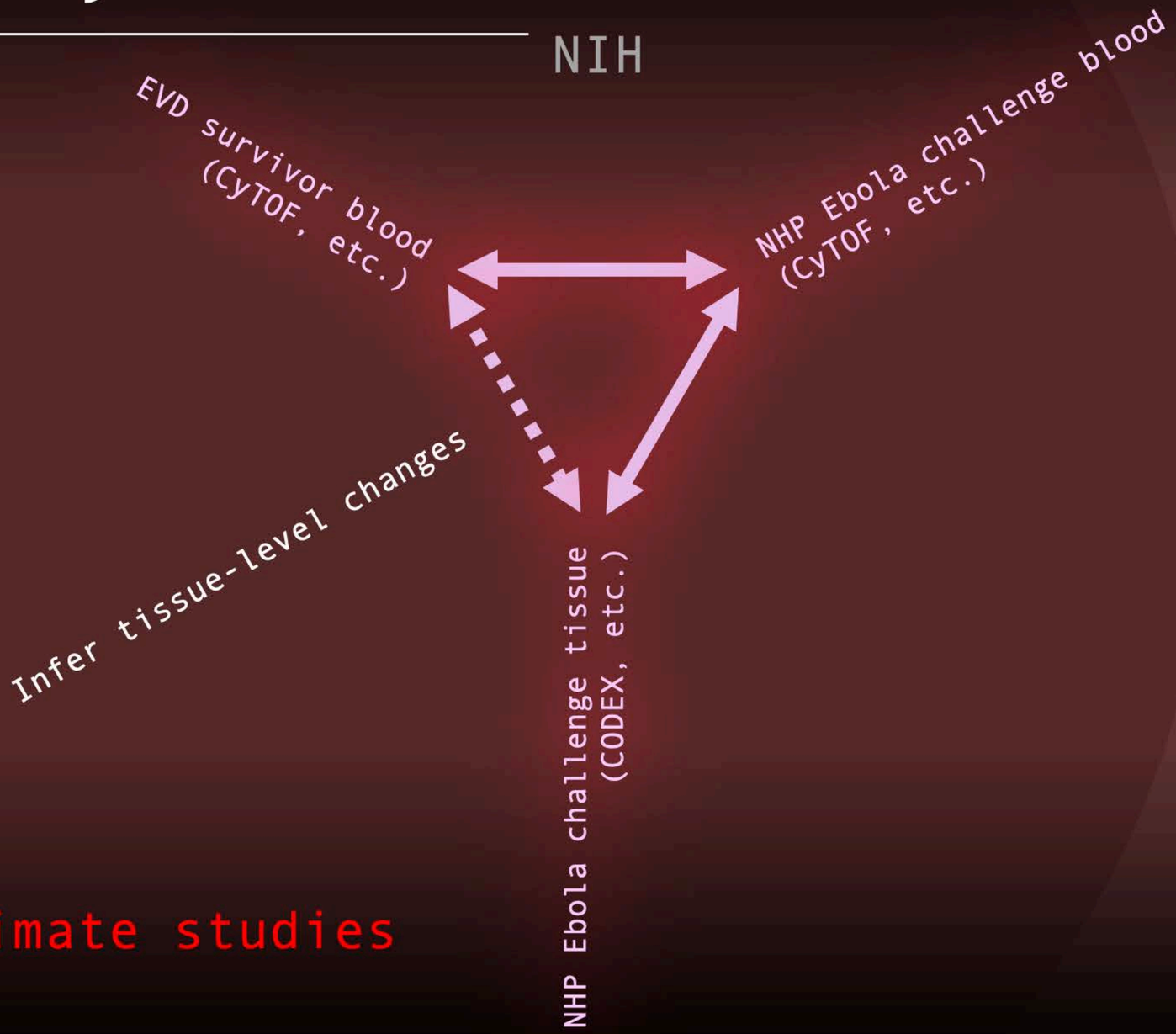
STANFORD MEDICINE

Influenza

- 5-15% of the world’s population affected annually
- 250,000-500,000 deaths/yr globally
- Economic costs $100 billion/yr for U.S. alone
- Pandemic potential: 50 million deaths in 1918

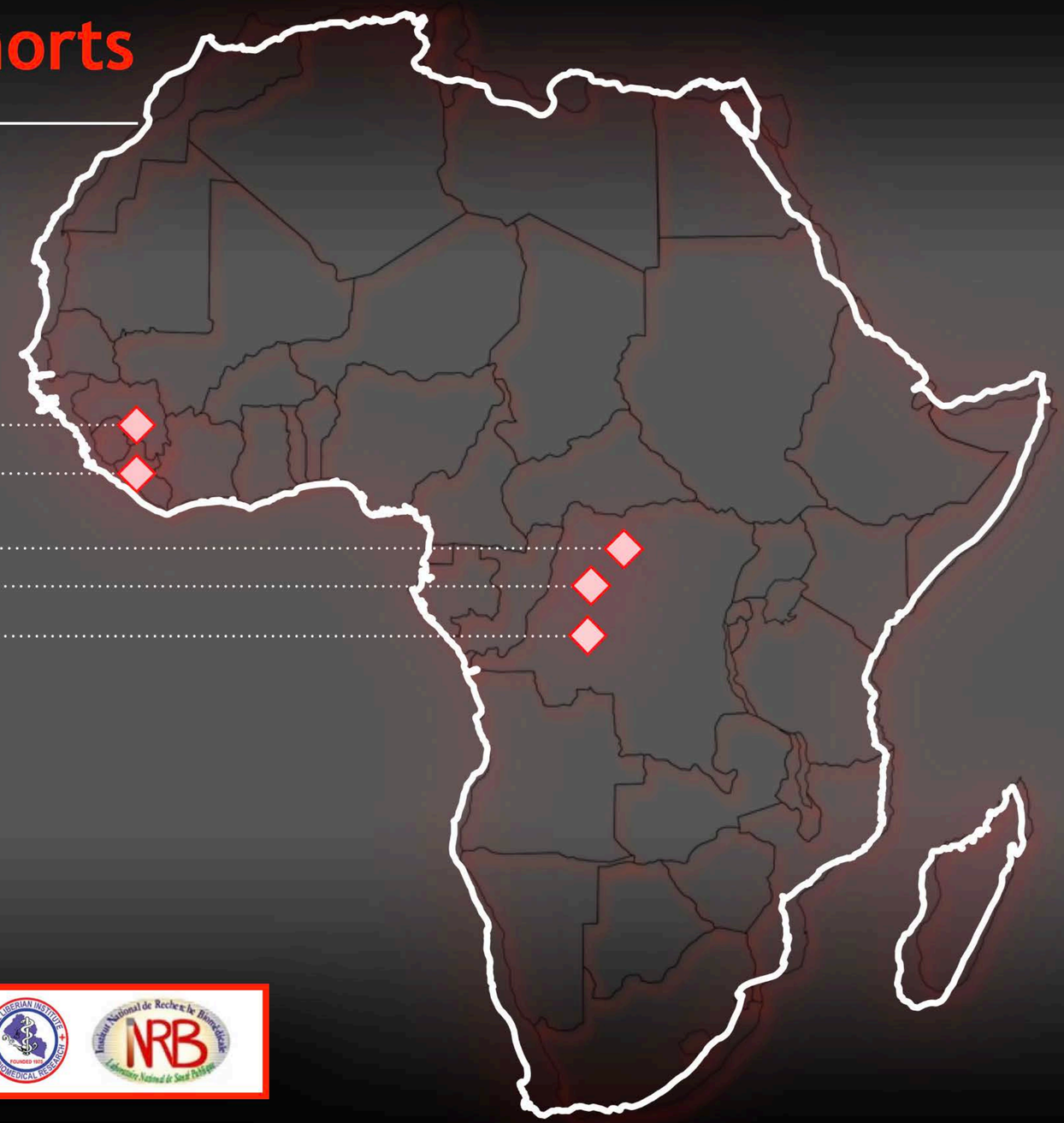
STUDY • Cross Species CyTOF Panel

NIH



Connecting to primate studies

STUDY • EVD Survivor Cohorts

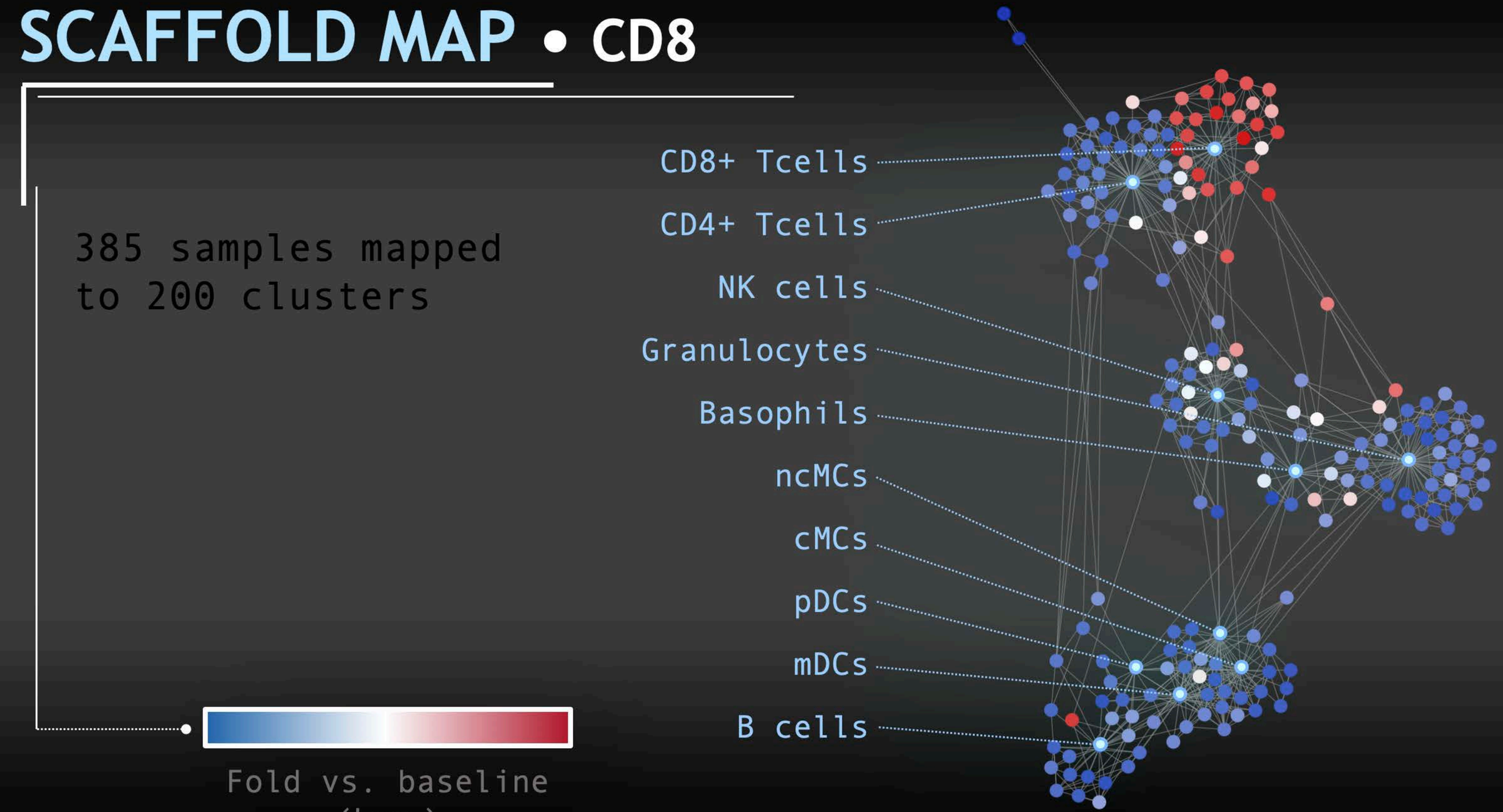


Collaborators:

UCLA FIELDING SCHOOL OF PUBLIC HEALTH · EMLab · Public Health England · NIH National Institute of Allergy and Infectious Diseases · The Liberian Institute for Biomedical Research · Institut National de Recherche Biomédicale NRB

SCAFFOLD MAP • CD8

385 samples mapped
to 200 clusters

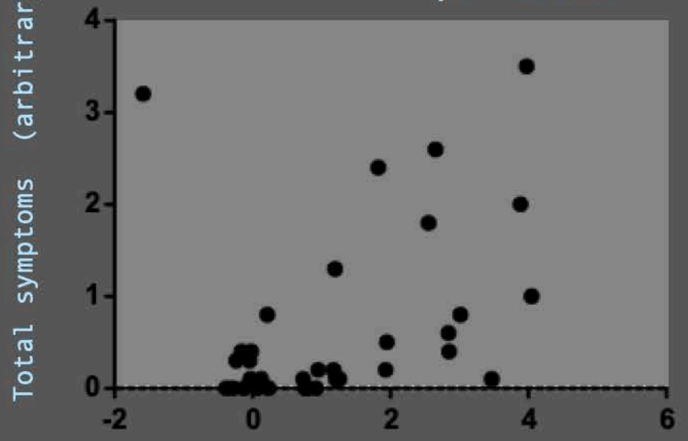


CORRELATIONS • H1N1

preliminary data

CD14+CD16+ Mono
(Day 6)/baseline (log_2)

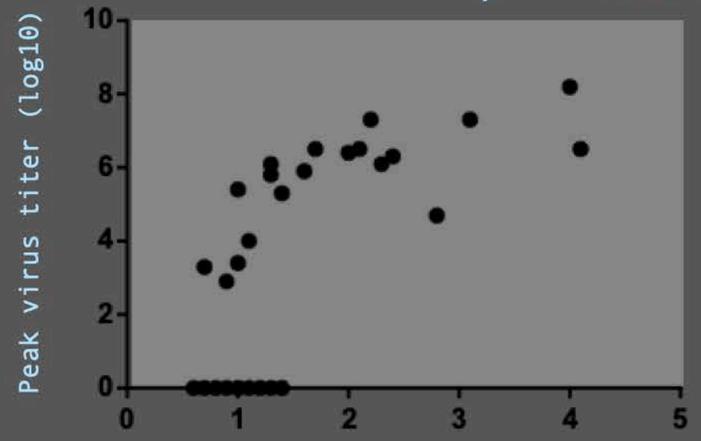
r = 0.37
p =0.03



Symptoms

CD16+CD14+ Mono
(Day 5)/baseline

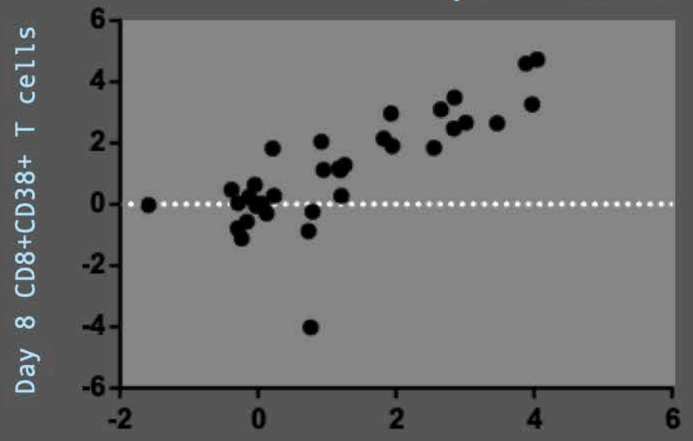
r = 0.72
p= <0.0001



Virus titer

CD14+CD16+ Mono
(Day 5)/baseline

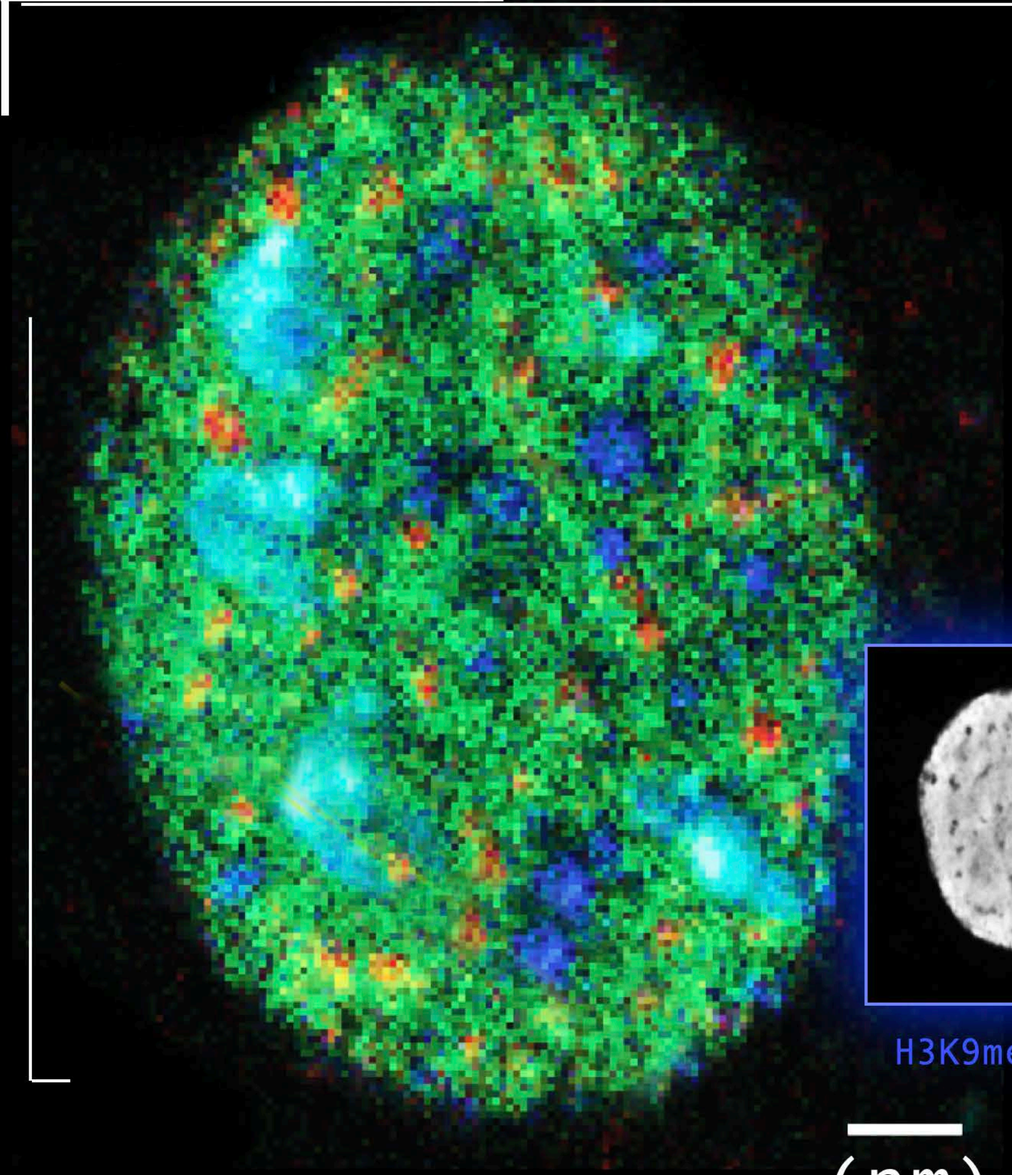
r = 0.79
p =<0.0001

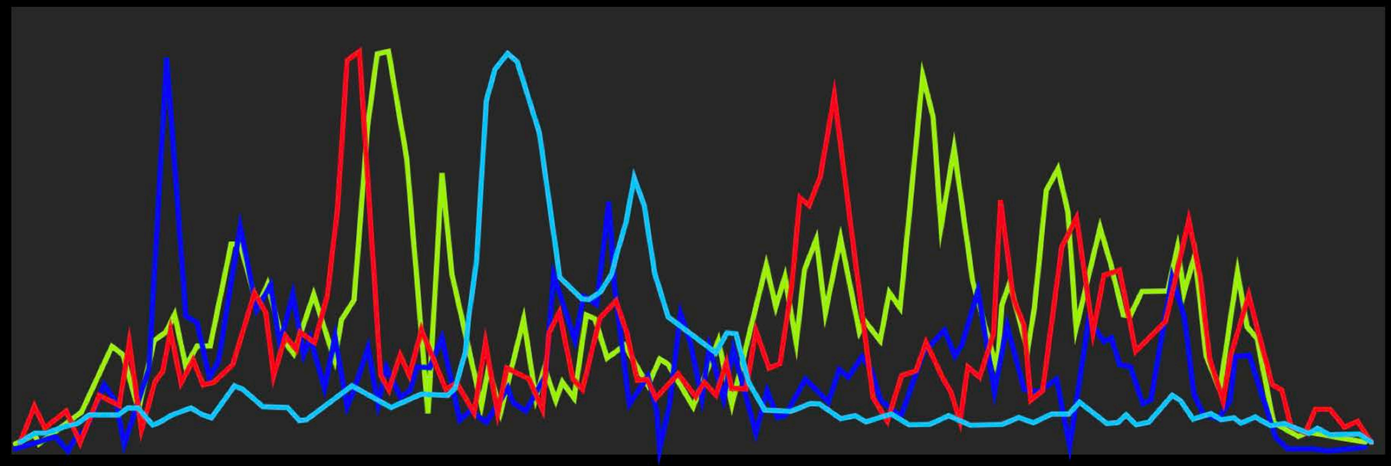


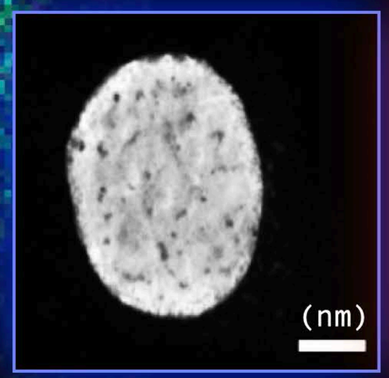
Activated T cells

- CD14+CD16+ Intermediate monocytes correlate with symptoms, virus titer, and T cell activation

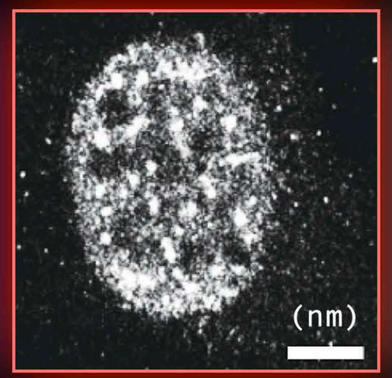
3D MIBI • Determining Molecular Distribution with Precision



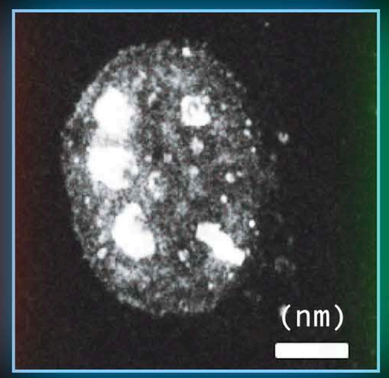




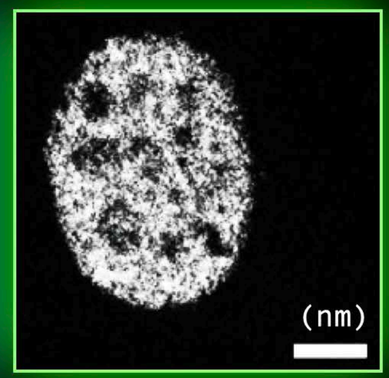
H3K9me3 (81Br)



SC35 (197Au)



Nucleolin (19F)



H3K27Ac(127I)

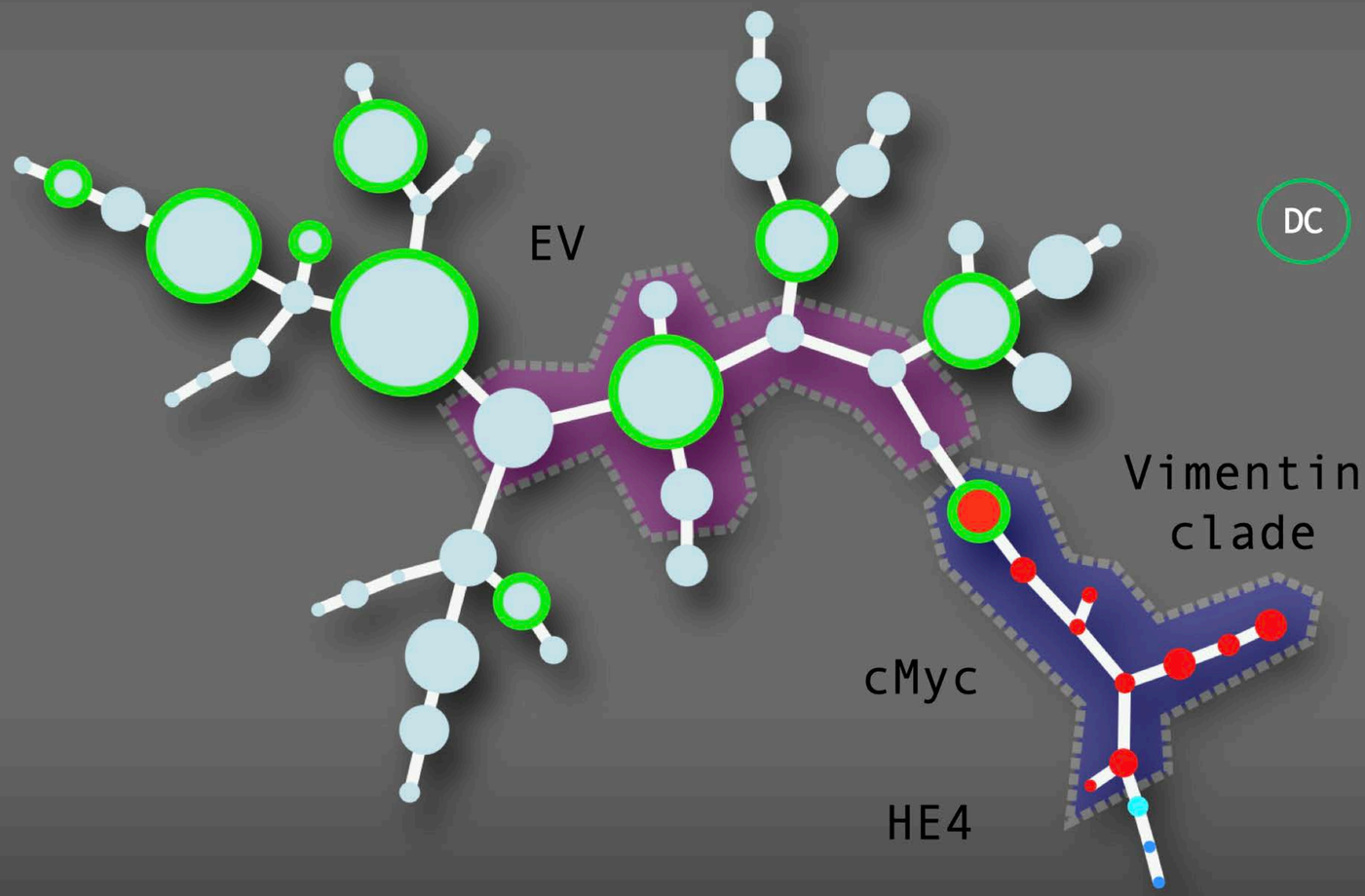
THERAPEUTIC OPPORTUNITIES

Three new tumor cell types recur across samples

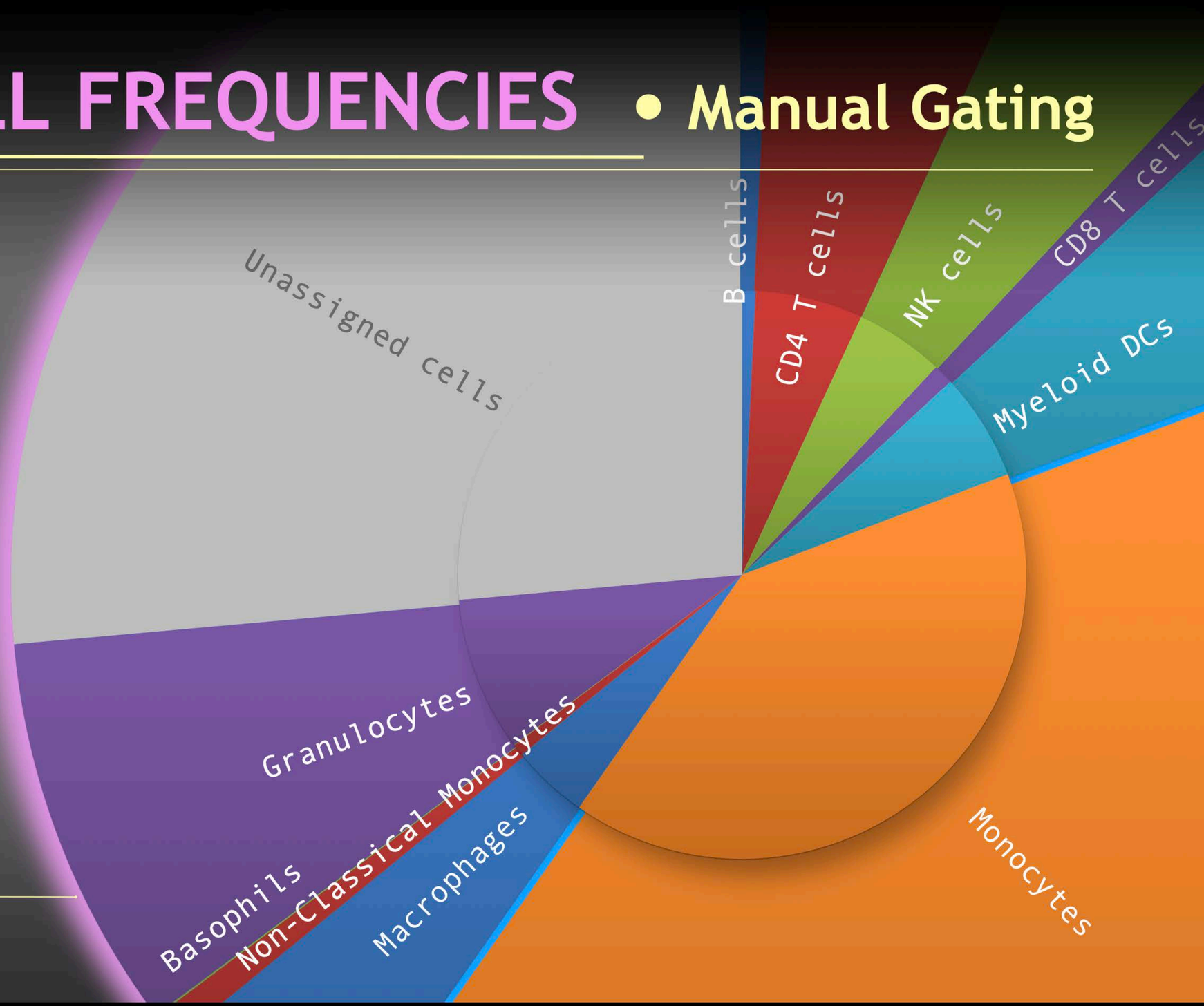
- EV co-expressing cells
- Dominant clusters
- V/cMyc/HE4 cell subsets

Clinical relevance

- New targets
- Diagnostic test
- Time to relapse
- Extent of surgical debulking



IMMUNE CELL FREQUENCIES • Manual Gating



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Dept. of Microbiology & Immunology
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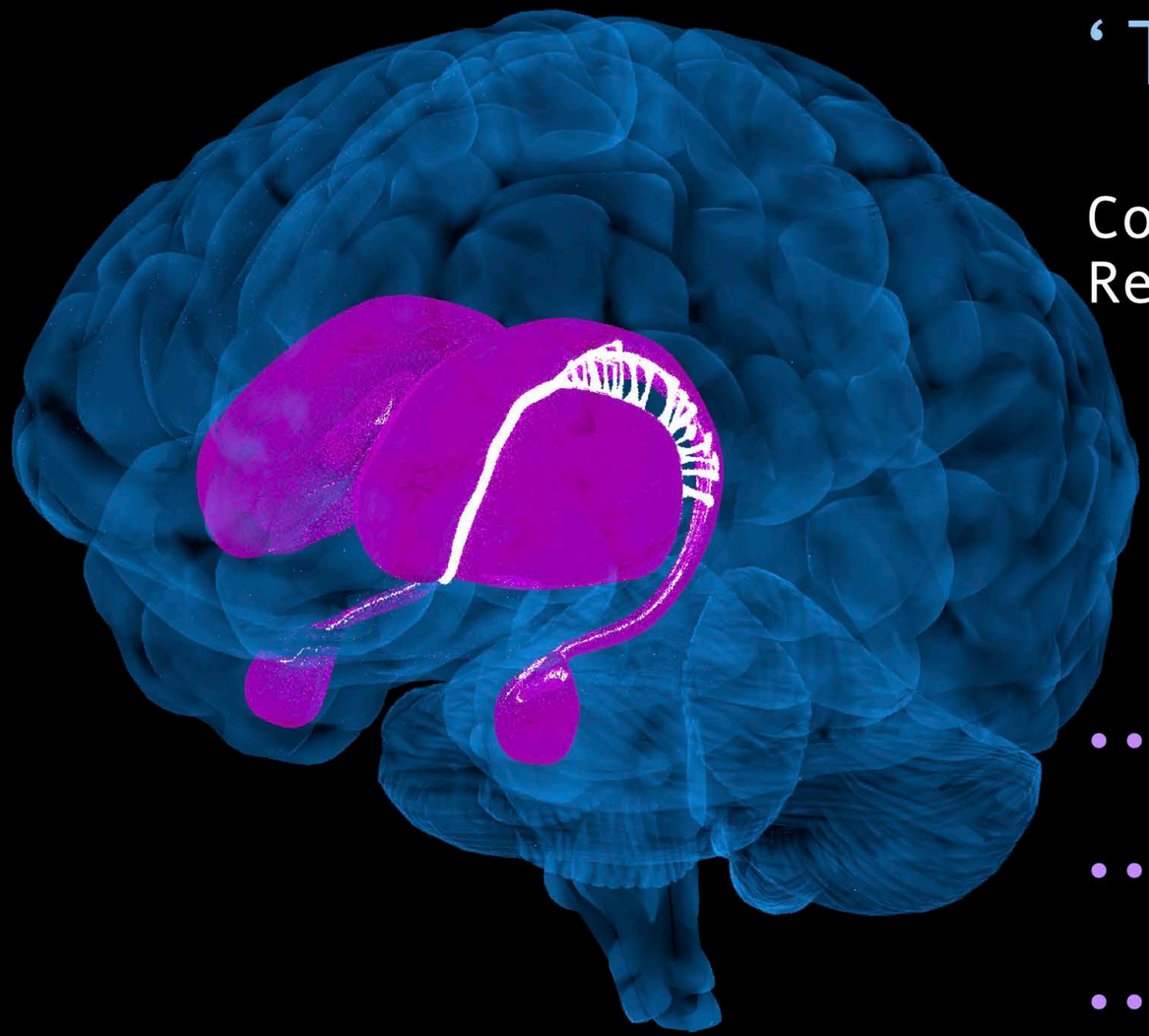
Baxter Lab for Stem Cell Biology

X

Christopher Green
MD, PhD

Forensic Neurology
Dept. of Diagnostic Radiology
Detroit Med. Cntr &
Wayne State School of Med.
Med:For, Inc. Chairman

CAUDATE ←→ PUTAMEN • An Introduction



Together -

‘THE DORSAL STRIATUM

Cognitive, Executive, Decision-Making
Relay-Center for High-Functioning

- ’The Oldest’
- ’The Most Evolutionarily Developed’
- ’The Most Advanced’

HYPOTHESIS • Caudate Overdevelopment

CASE CRITERIA

Pick first 8 [of 24] in random series of
IntFxSndx Diagnosed Experiencers

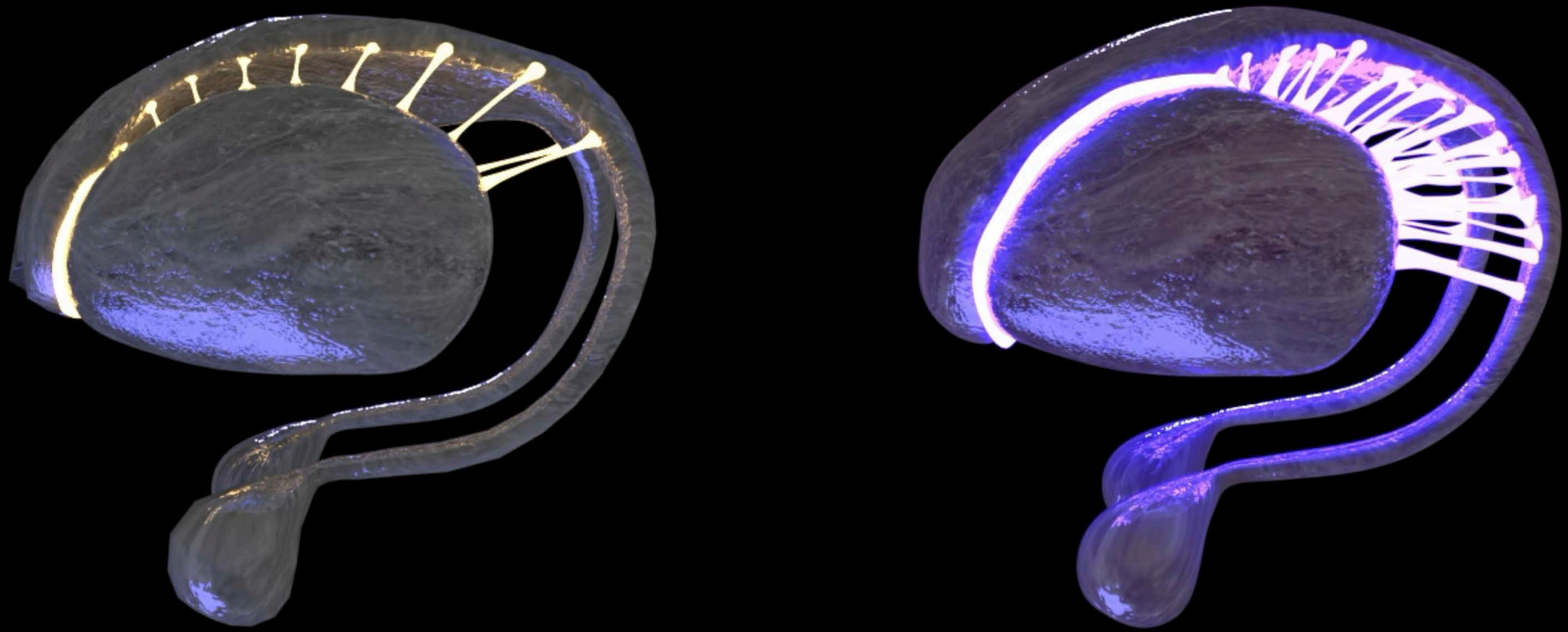
Common Syndrome Sub-Set Features

- Orbs
- High Strangeness events
- Fear
- Family witnessed / followed
- FUO
- Multiple witnesses

NOT Required (but may have)

- Marks
- Endocrinopathy
- Neurological findings w/ MRI positives

CONNECTIVITY • ‘Normal’ vs. ‘Hyper’



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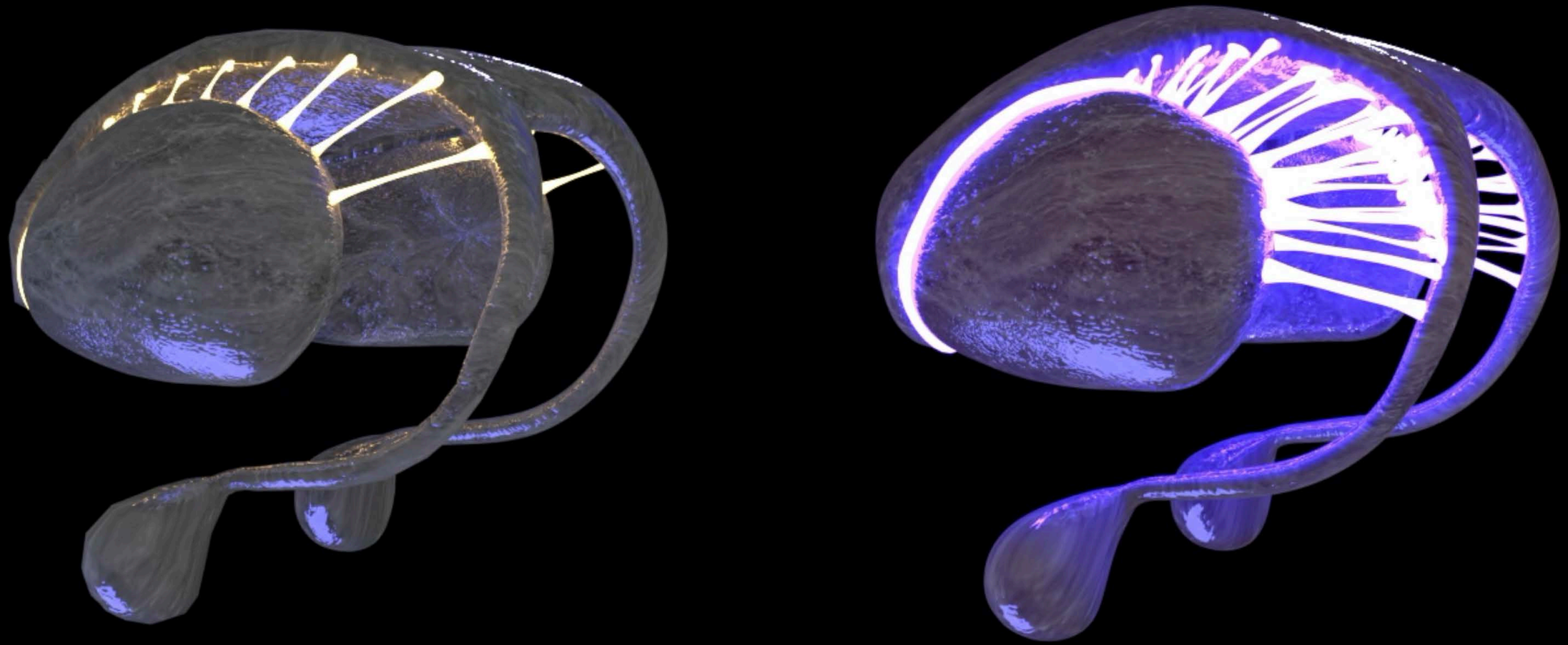

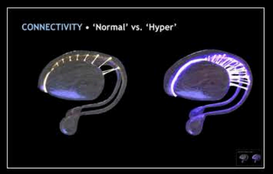

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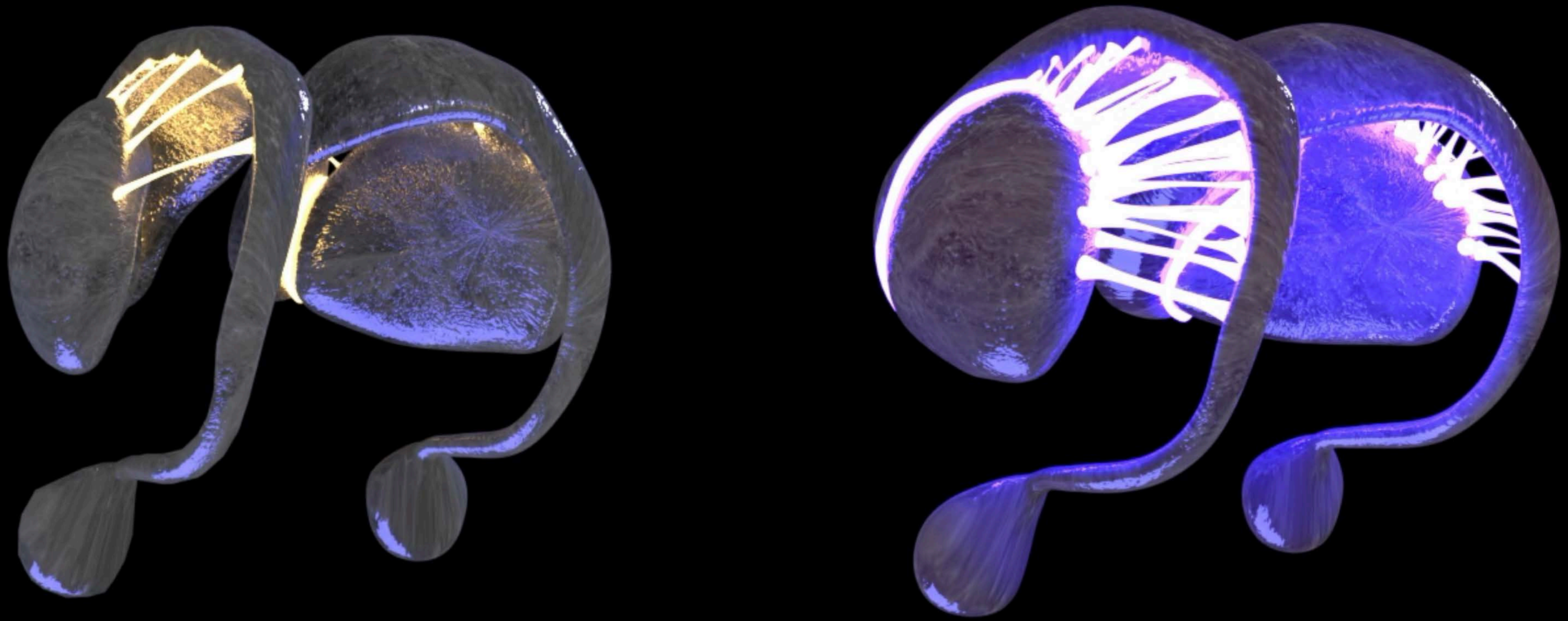

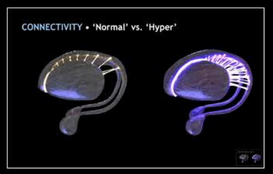

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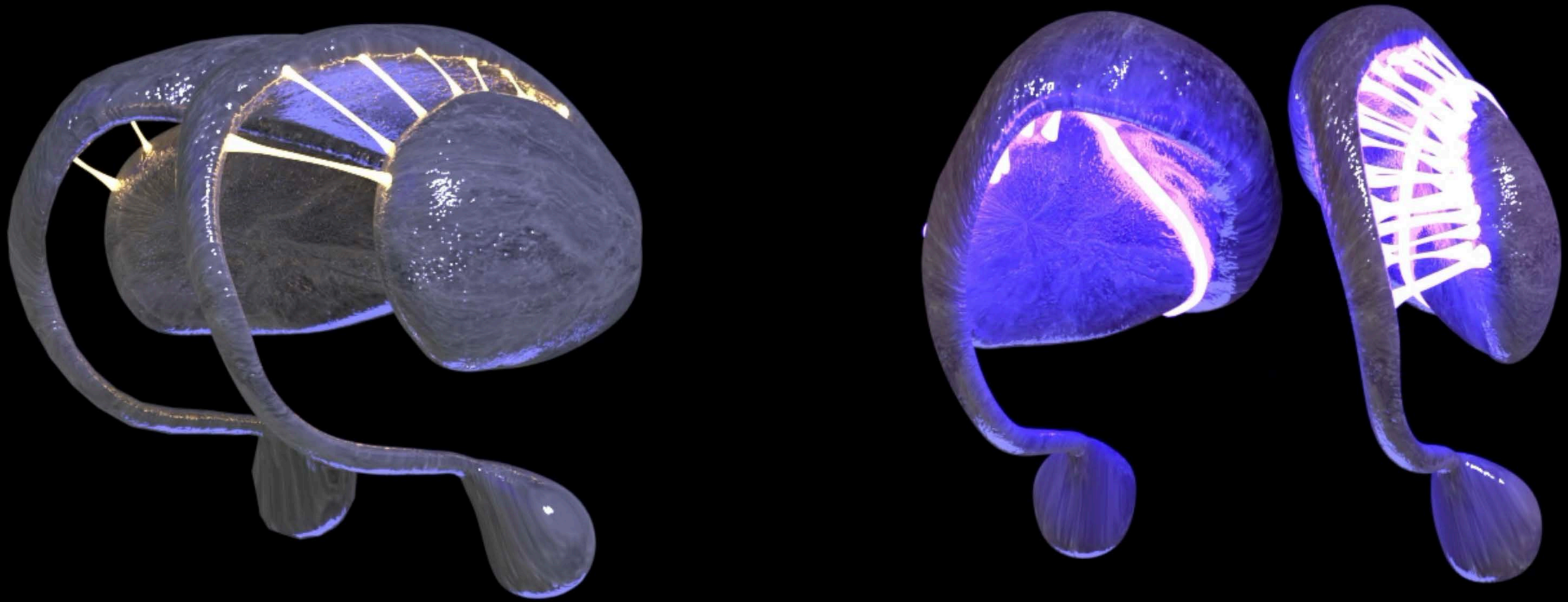

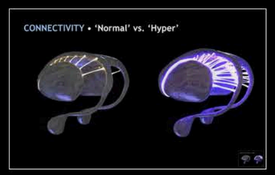

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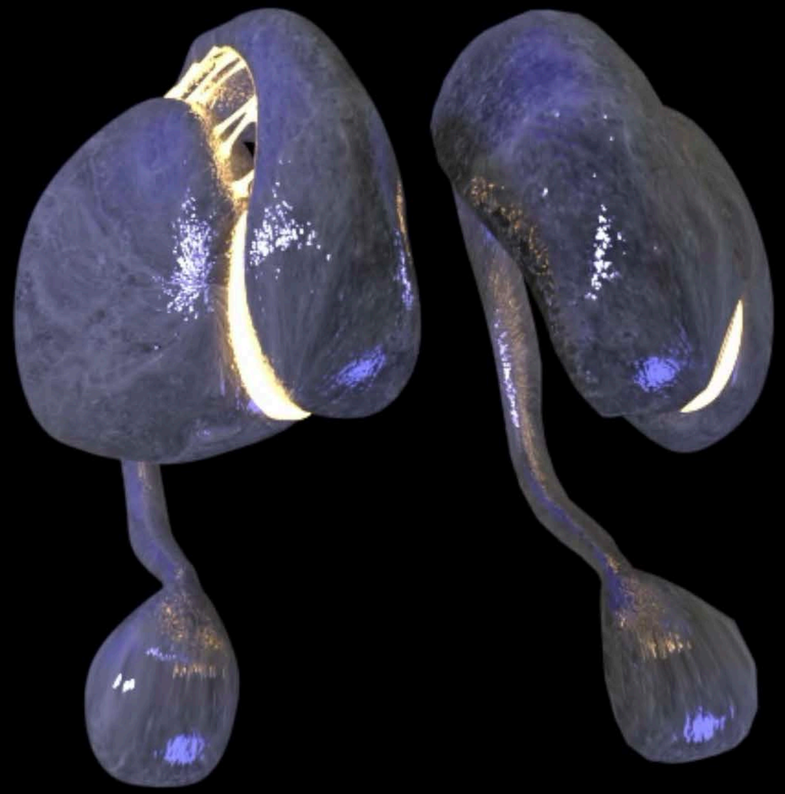

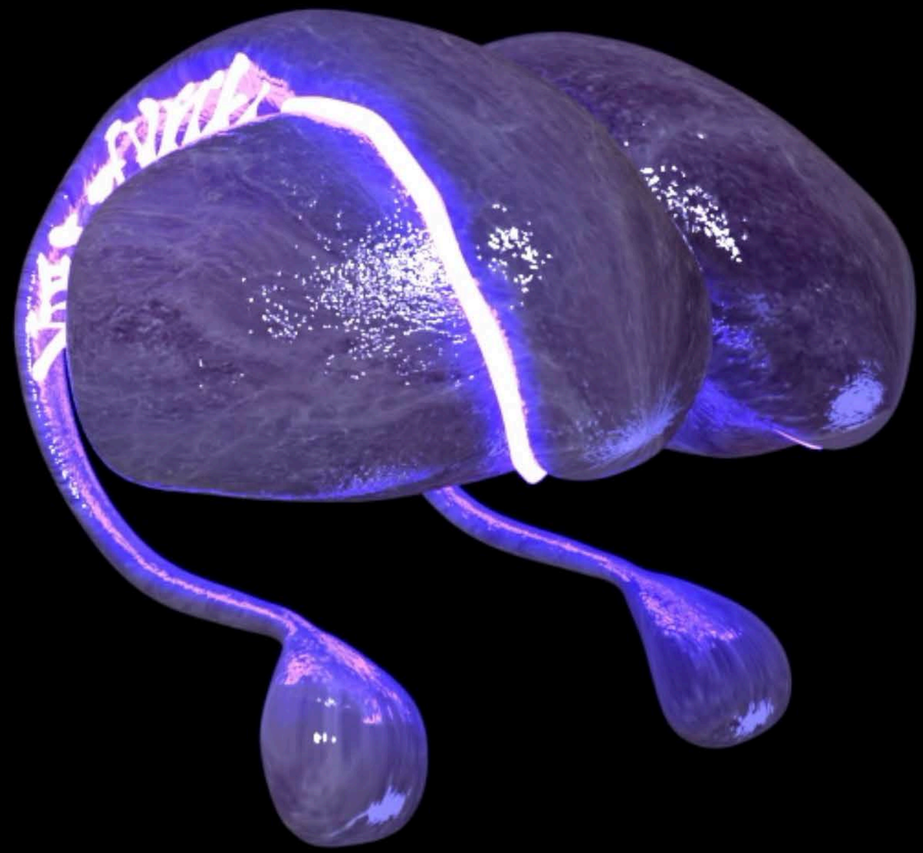

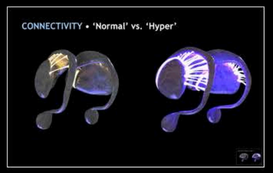

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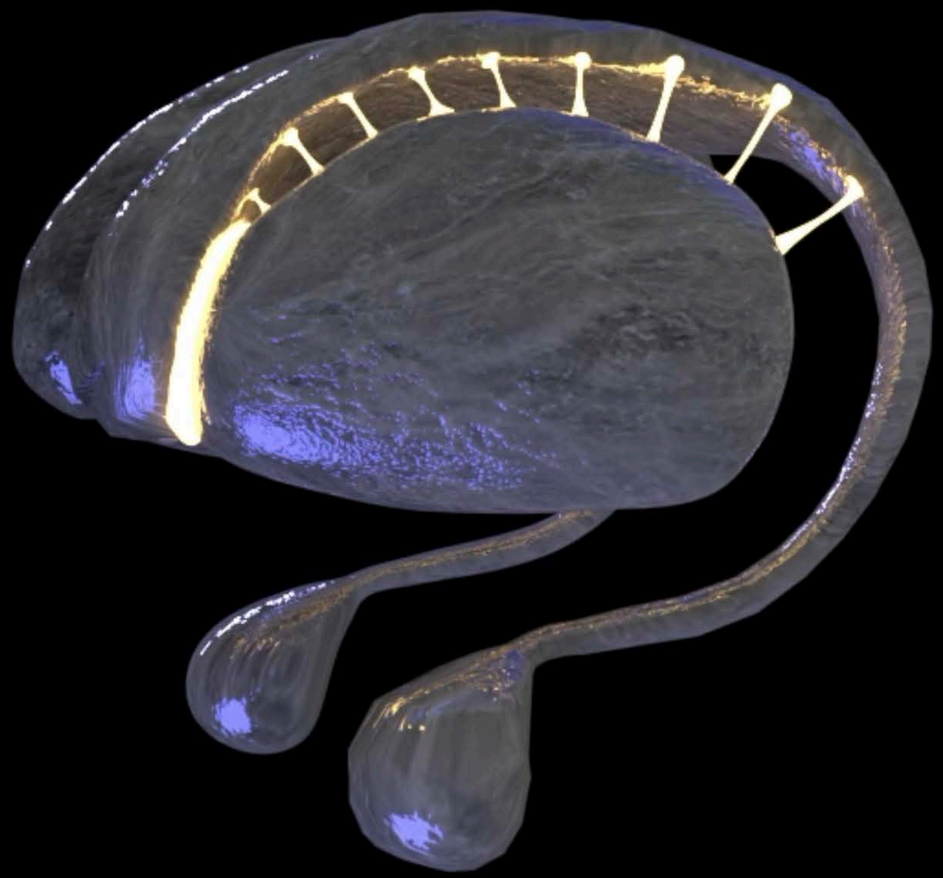
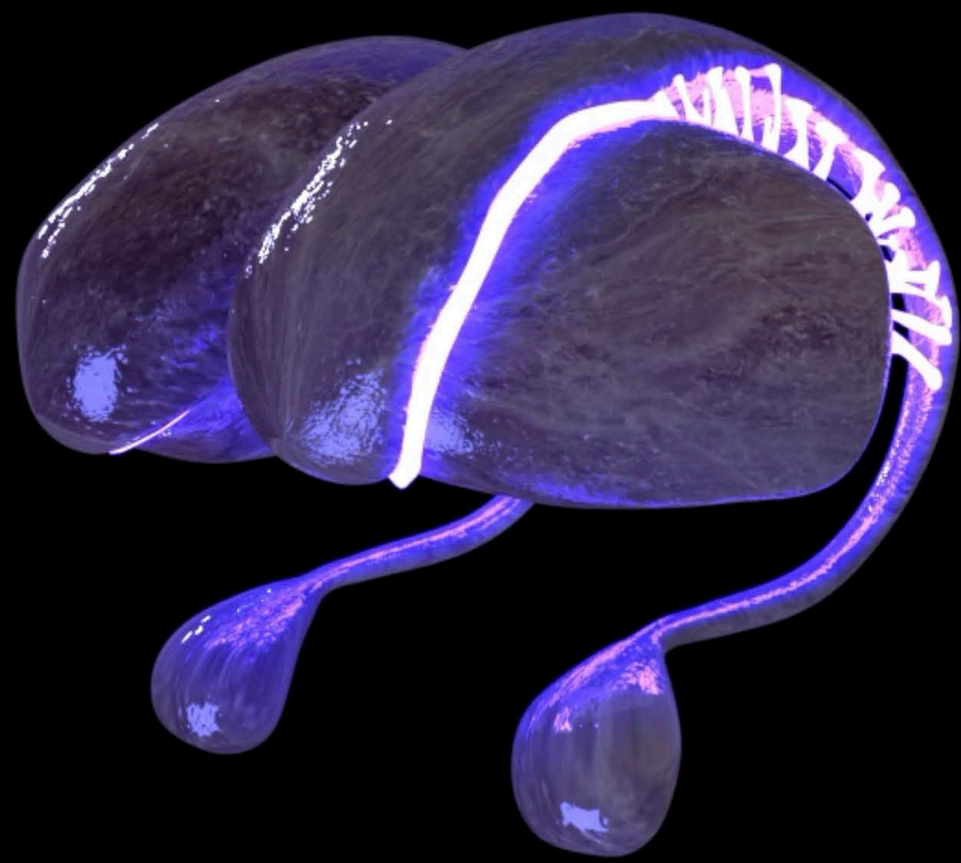
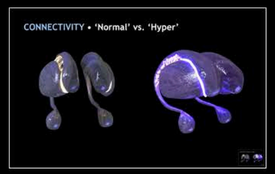






ÅTOMNAUT

A.M.P.

Operating Principles

AMP combines 3 microscope technologies:

FIM field-ion microscopy

••• structure: surface imaging using gas ions

APT atom probe tomography

••• chemistry/color: element identification via TOF-MS

FIB focussed ion beam

••• in-situ sample prep: mills needle-shaped specimens

Patented laser-decontamination silences interference produced by the technology combination; e.g. FIM requires an imaging gas, APT requires gas-free, a difference in vacuum pressure requirements of 1,000,000x.

AMP solves the two biggest problems with predecessor techniques:

1. Specimen preparation: 50x faster. Months into days, makes AMP ‘routine’.
2. Complete atomic resolution: 100x improvement. Every atom, 3D position & identity.

Markets

Pharma

$ 1,100,000,000,000

Trillion

Protein Crystalography

$ 1,300,000,000

Billion

7% CAGR

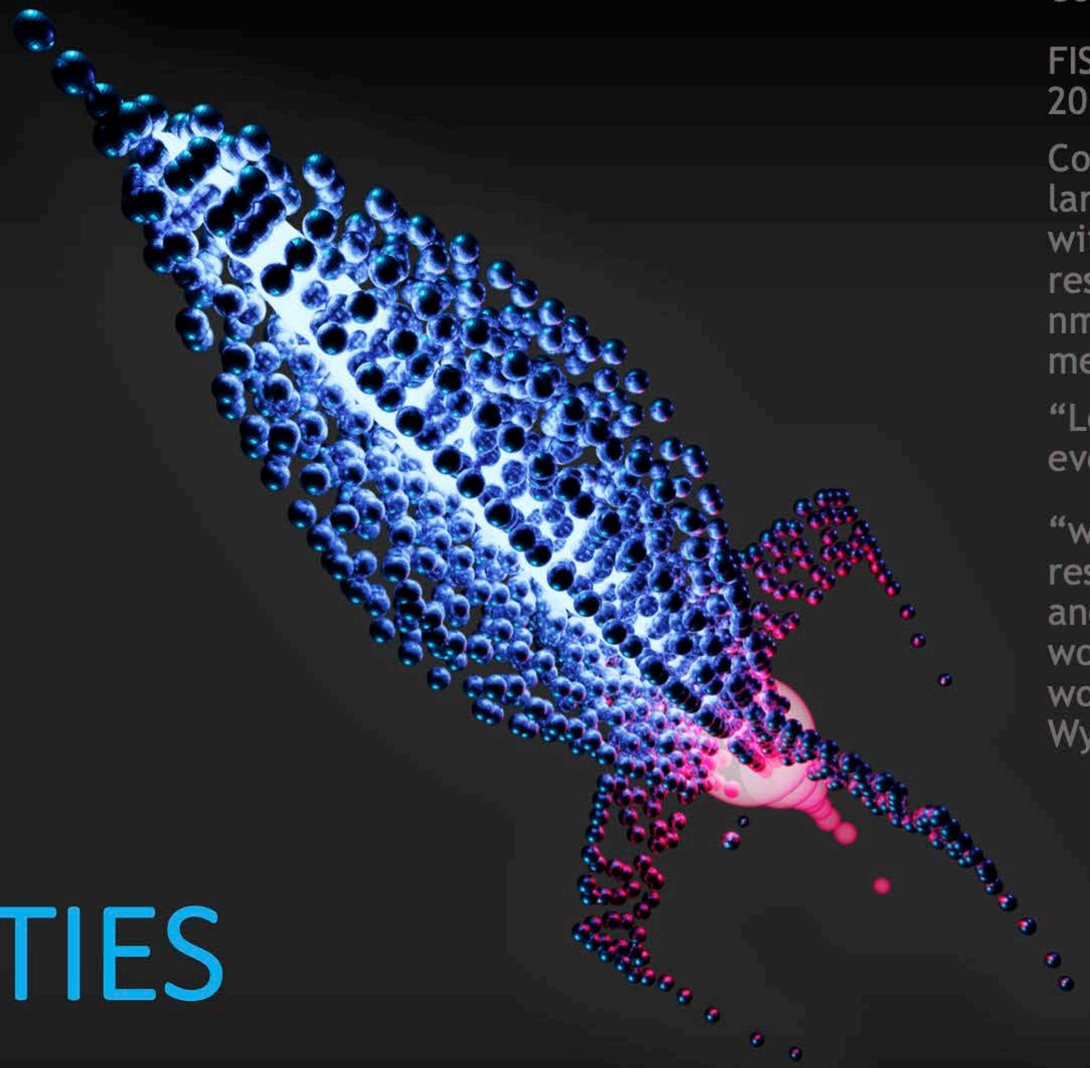
R&D

$ 182,000,000,000

Billion

From DNA strands to extended chromatin, AMP can provide full field-of-view 3D atom maps.

Not as a competitor with high-speed DNA sequencing, but as a unique 3D spatial & chemical mapping tool of DNA, AMP will reveal previously hidden structure-function relationships.



Comparative technology

FISSEQ by ReadCoor
2016 Series A, $25M

Compared to AMP, FISSEQ views a larger region of data (µm scale) but with poorer spatial and chemical resolution – AMPs opportunity is in nm-scale spatial sequencing measurements.

“Locating the products of genes is everything”

“will give neurologists, cancer researchers, clinicians, geneticists, and others the 3D coordinates of working genes that will inform their work in new ways.” (Terry, 2016, Wyss Institute)

OPPORTUNITIES

DNA / RNA

Atomic Scale Spatial Sequencing