Next-Generation Connectomics (NGC) via optical in-situ multiplexing

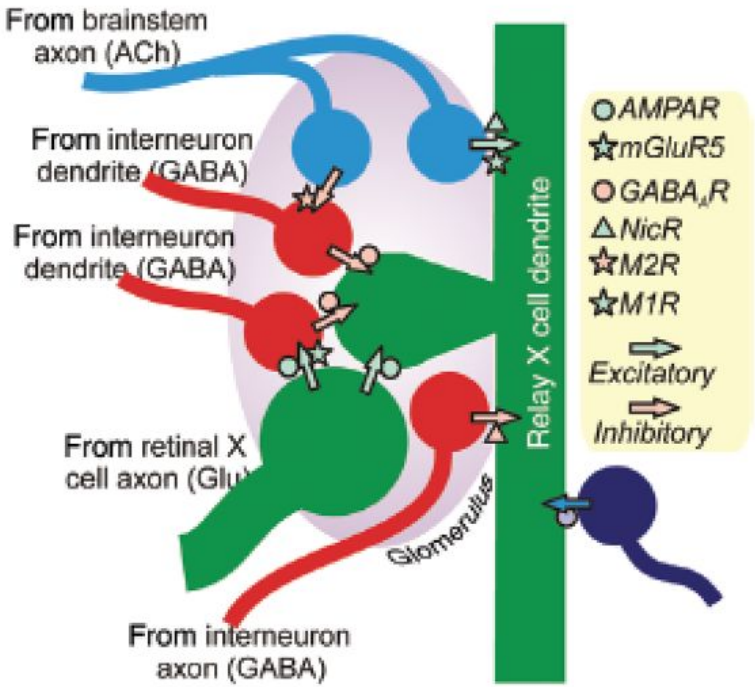
Adam Marblestone, PhD

DOE-NIH workshop: Experimental modalities for whole-brain connectivity mapping (March 5th 2021)
Session 2: Projectome to Connectome Imaging
Synapto-Projectomes: Toward connectome relationships in target fields
Strategies to bridge spatial and temporal gaps

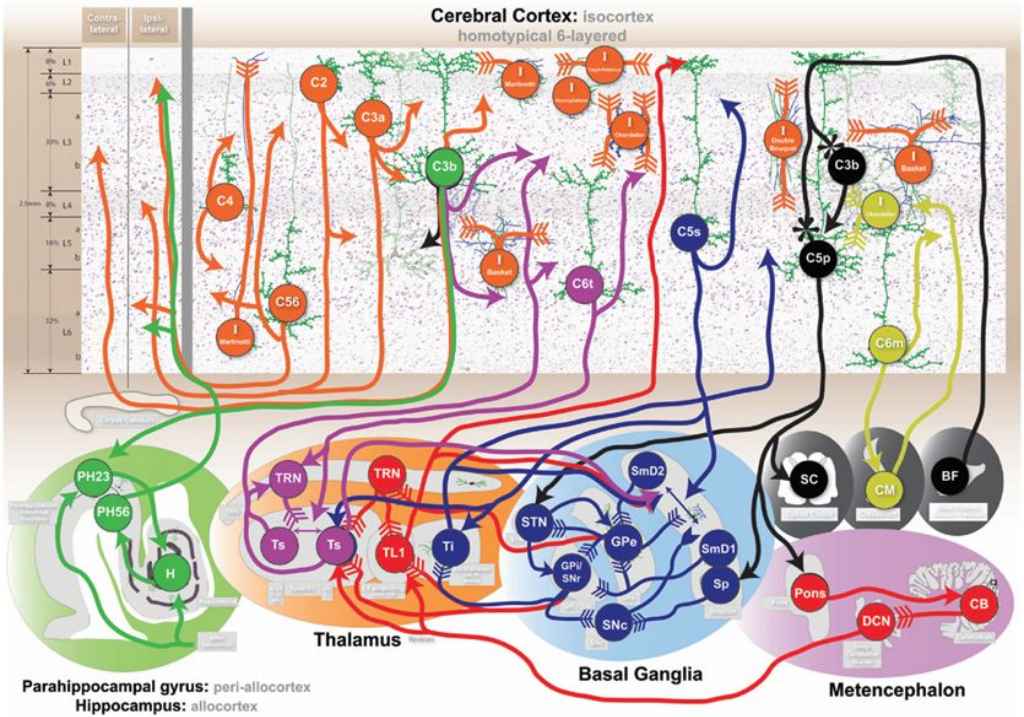
w/ Ed Boyden, George Church, Tony Zador, Richie Kohman, Sam Rodriques, Andrew Payne, Bobae An, Dawen Cai, Fred Shen, Fei Chen, Dan Goodwin, Andrew Xue, Ruihan Zhang, Shahar Alon, Oz Wassie, Anu Sinha, Kathleen Leeper, Evan Daugharthy, Sam Inverso, Grace Huynh, Ian Peikon, Justus Kebschull, Xiaoyin Chen, Reza Kalhor, Alex Vaughan, David Markowitz, Jacob Vogelstein, Manos Karagiannis, Luis Kang, Tay Shin, Amauche Emenari, Bobby Kasthuri, Chris Rowlands, et al

The challenge of connectomics is the brain's vast dynamic range of linked structure

From *synapses* with particular *molecular* features, terminating on particular *dendritic compartments* of particular *cell types*....

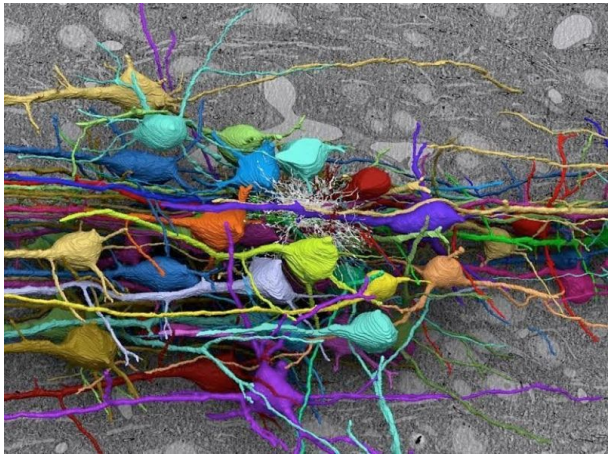


...to *circuits* spanning across several brain *areas* located centimeters apart, <u>yet which still retain this molecular, dendritic and cell-type specificity at the synaptic level</u>



Murray Sherman; Soren Solari

Electron microscopy (EM) approach



All structure is derived bottom-up, from tracing nanoscale membrane morphology

Each pixel answers only: "**Am I membrane, or not membrane?**"

This means that very high spatial resolution and fine sample handling is needed

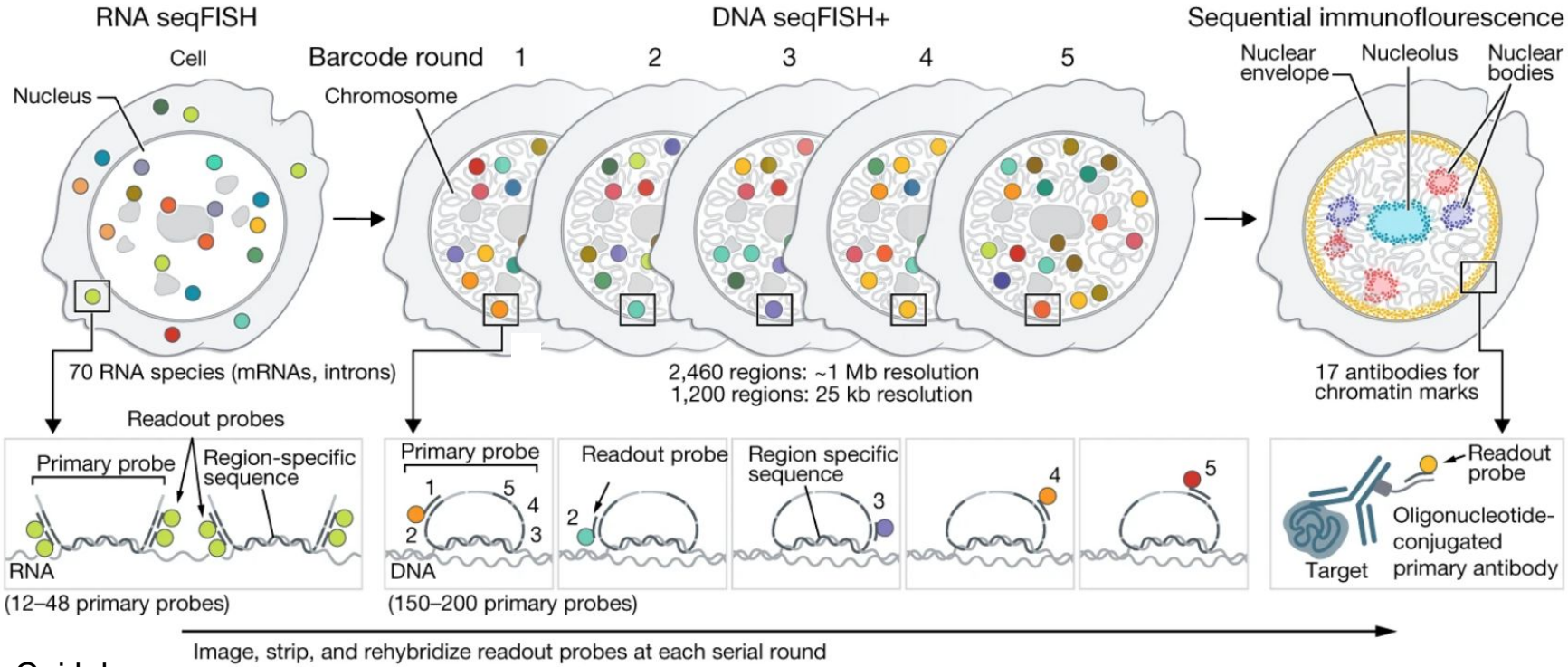
Only get one "cycle" of imaging each pixel

Even if we find a good way to encode "colors" in EM, it will be a small, fixed set of colors

Combinatorial "barcoding" via *sequences* of colors is not admitted, since electrons are destructive

Optical approaches read out combinatorial color-codes by assaying each pixel many times in sequence

Example:
Multiplexed, in-situ optical readout of DNA, RNA & protein via *cyclic* fluorescent staining and imaging



Long Cai lab
(*Nature*, 2021)

From grayscale, to billions of effective colors

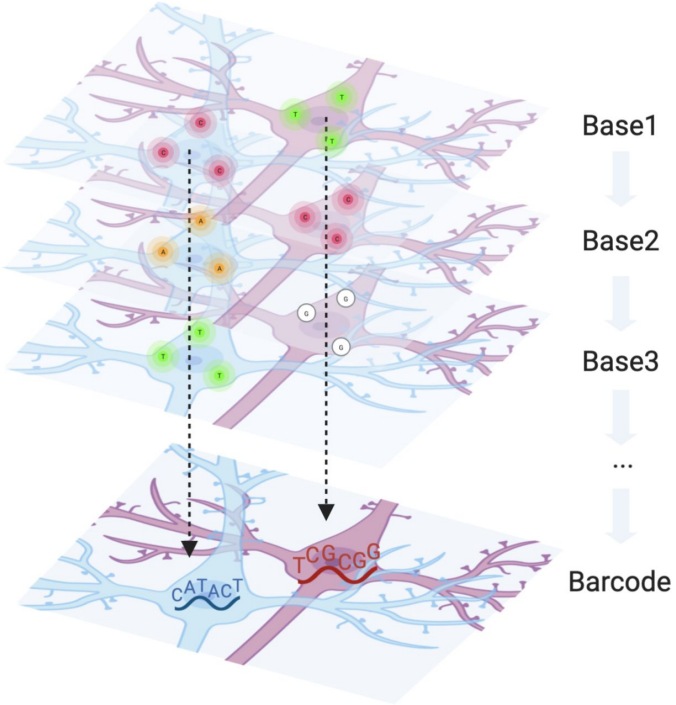
K colors → K^N effective colors

K ~ 4 colors, N > 15 cycles

Billions of effective colors

Optical approaches read out combinatorial color-codes by assaying each pixel many times in sequence

With appropriate “barcoding” of cells (Zador, 2012), each pixel can directly answer: “**Which cell am I part of?**”

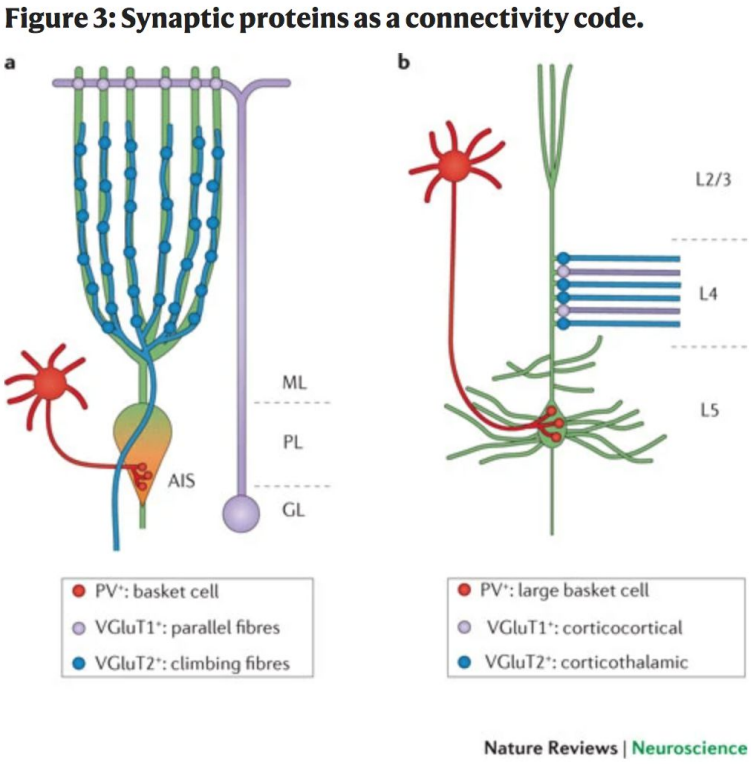


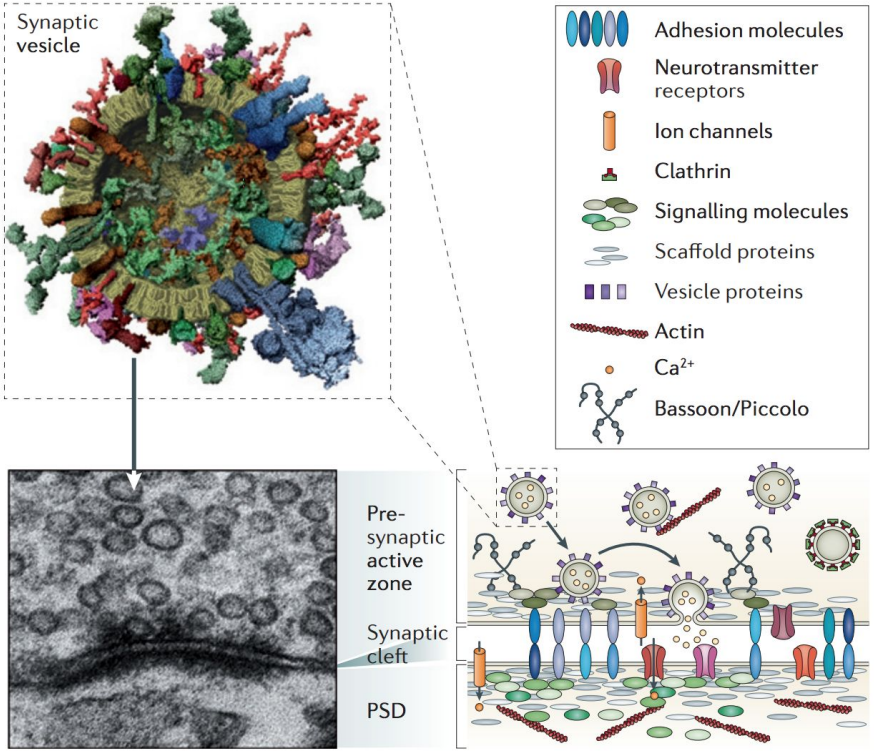
Filling spatial gaps:
Connectivity determined even if one doesn’t physically trace the membrane from synapse back to parent somas

“**wiring diagrams**” vs. “**volume reconstructions**”

Optical approaches read out combinatorial color-codes by assaying each pixel many times in sequence

Likewise, each pixel can directly answer: "Which molecule am I?"





O'Rourke, Weiler, Micheva, Smith (2012)

Next-Gen Connectomics (NGC) exploiting barcoding and optical in-situ multiplexing

Core near-term value proposition (my opinion):

"*Synapto-projectome*" or "*sparse connectome*", bridging spatial gaps

Cell-type-resolved, layer-resolved, dendritic-compartment-resolved

Reduced resolution requirements, easier sample handling/slicing, simpler computation, and lower cost

Molecular annotation of cells, synapses, and other subcellular features (e.g., gap junctions)

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Use cases:

Connectivity of neurons between distal areas, *without* tracing in between

Molecular composition of synapses, linked to molecular composition (and/or perturbations) of their parent cells

High-throughput sparse connectomics: synaptic-level analysis of brain-wide circuits, across many brains/variants

"Whole brain inputs to a single neuron" (and other such specialized use cases)

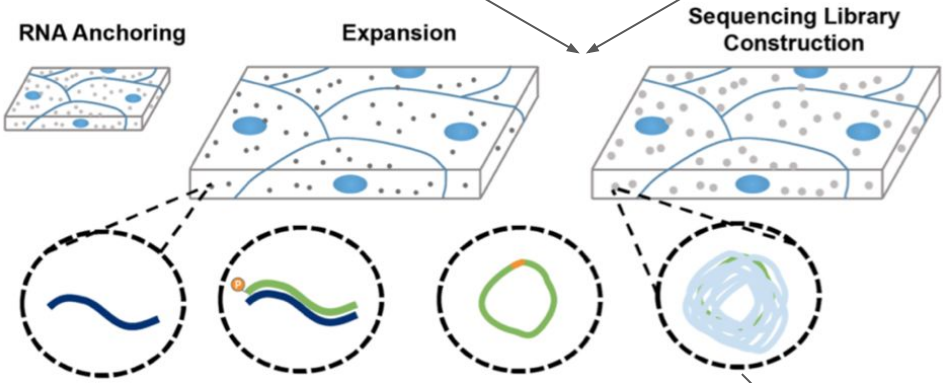
(Longer term: *approaching* dense connectomics via 20x expansion microscopy, lipid staining + other tools)

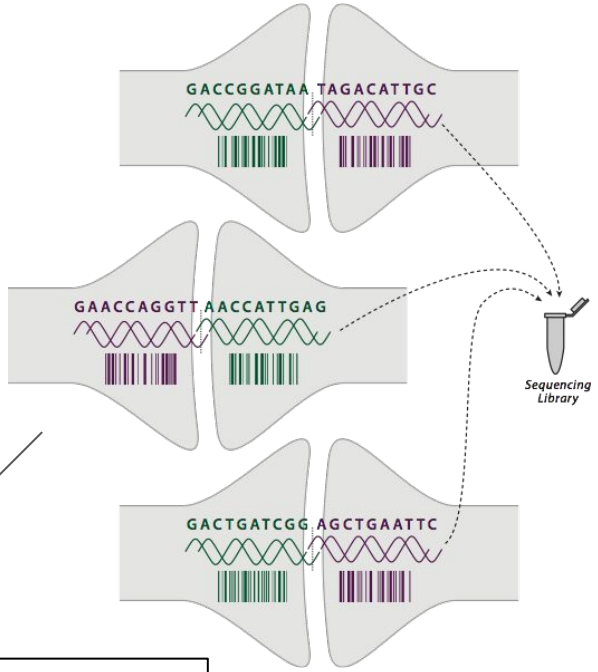
Next-Gen Connectomics (NGC) calls for *integration* of existing molecular & optical technologies

Expansion Microscopy (ExM)

In-Situ Fluorescent "Multiplexing"

Cellular Barcoding





Automation

<u>Next-Generation Connectomics (NGC)</u>

Fast, cheap, scalable

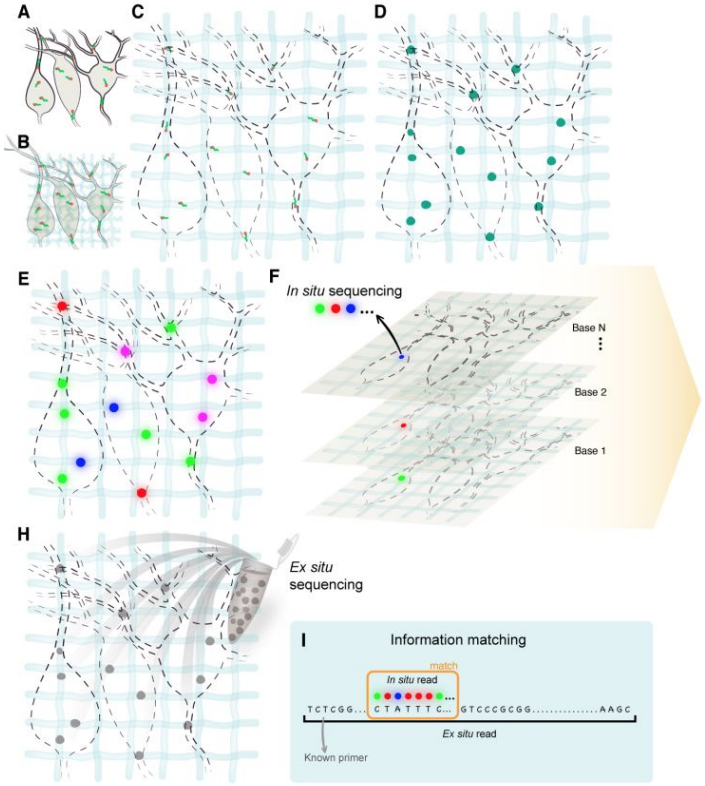
"Molecularly annotated"

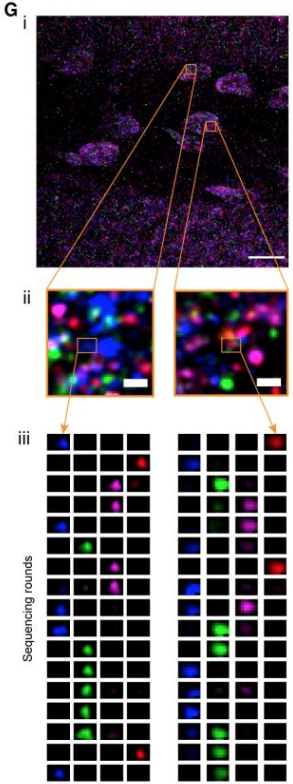
Robust, long-range (fills spatial gaps)

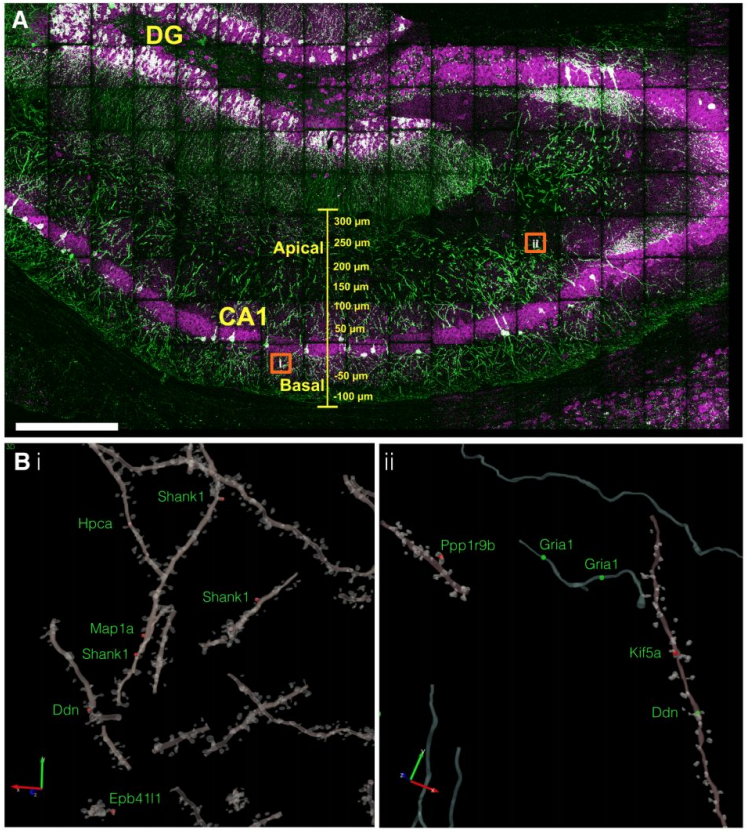
We have demonstrated that such integration is possible, first with ExM + in-situ multiplexing (ExSeq)...

Expansion Microscopy (ExM) ***In-Situ* "Multiplexing"**

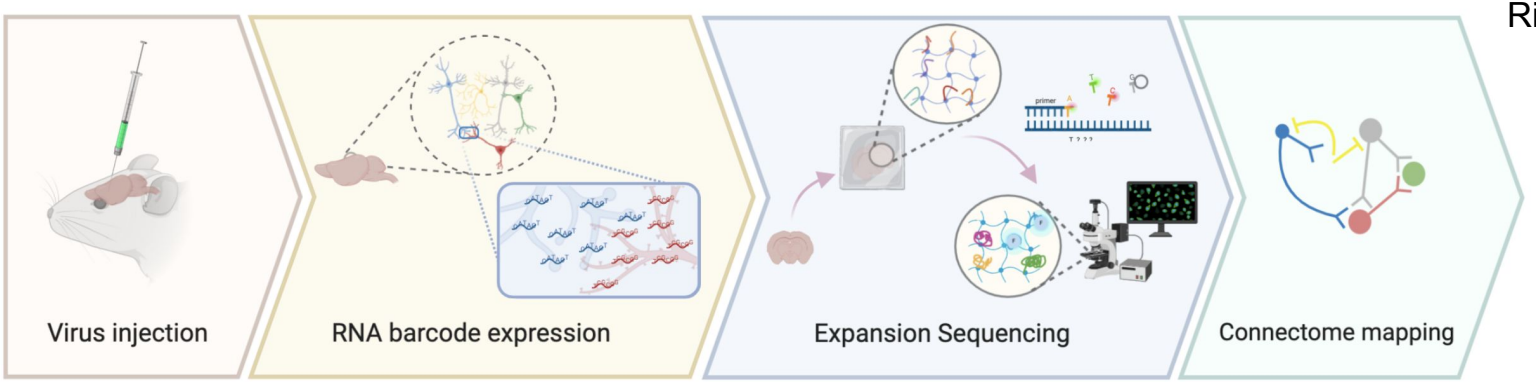
Expansion Sequencing (ExSeq, *Science*, 2021)
Sequencing RNA transcripts inside dendritic spines...





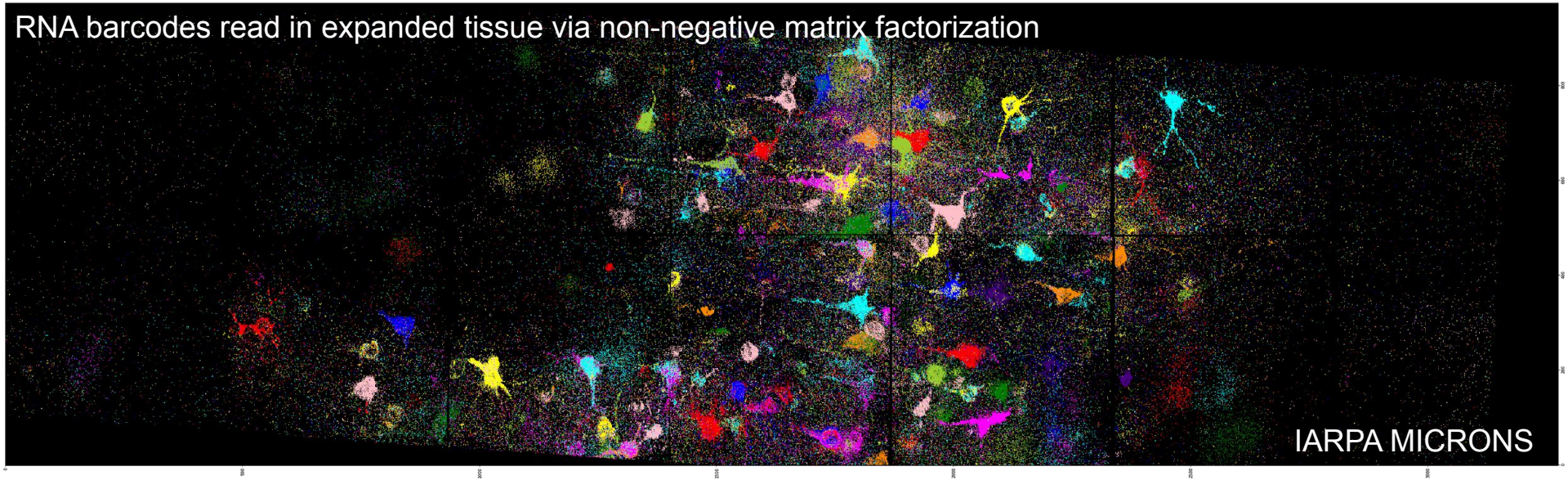


…and now reading Zador's neuron-specific RNA barcodes in mouse cortex with ExSeq



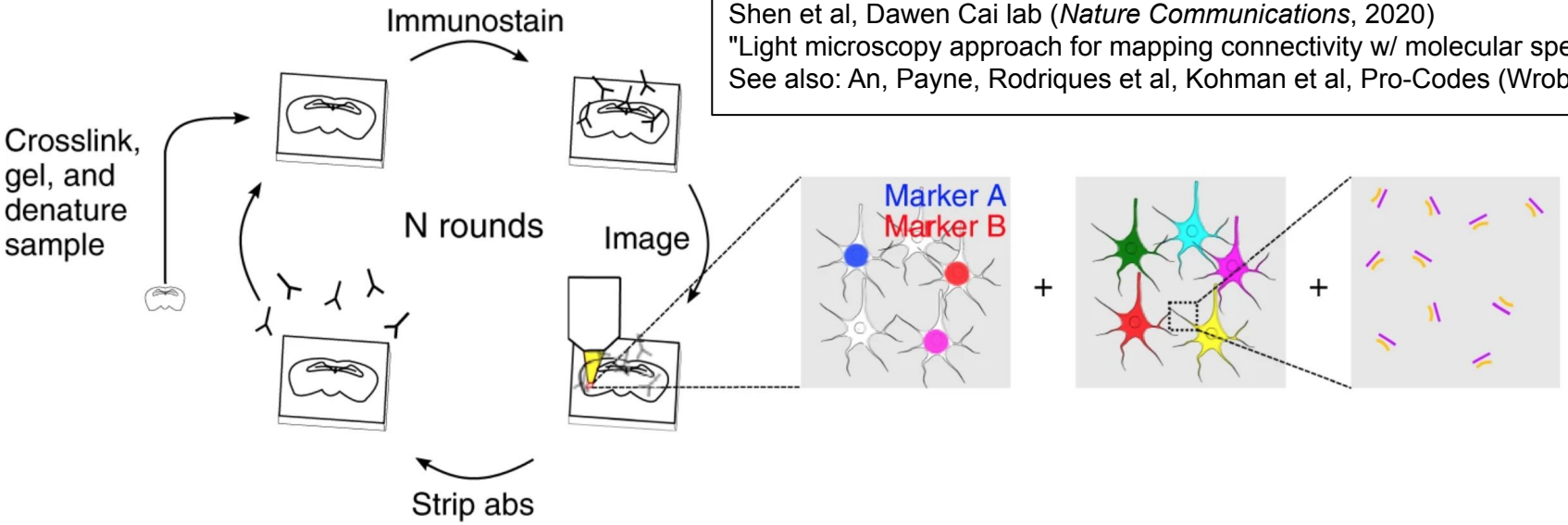
Richie Kohman (Wyss)
[preliminary data]

RNA barcodes read in expanded tissue via non-negative matrix factorization



Reading *protein* barcodes with ExM & cyclic immunofluorescence

Shen et al, Dawen Cai lab (*Nature Communications*, 2020)
"Light microscopy approach for mapping connectivity w/ molecular specificity"
See also: An, Payne, Rodriques et al, Kohman et al, Pro-Codes (Wroblewska, 2018)



Protein barcodes:

much higher copy #s, and we know they can be trafficked efficiently throughout cells (cf., GFP)

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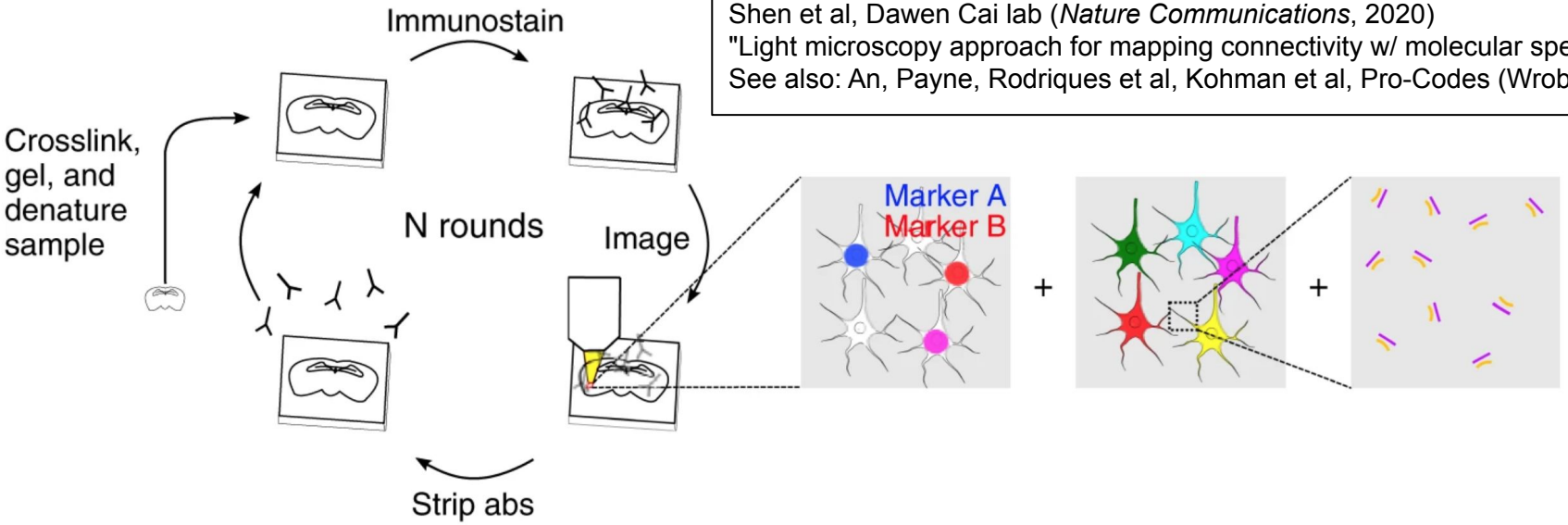

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Natural extensions:

Convert to a nucleic acid multiplexing problem using DNA-conjugated (1ary) antibodies

Post-expansion immunostaining (“expansion revealing”, ExR, 2020) to increase tag density

ExM-compatible chemical lipids stains for even denser staining (e.g., Shin, Karagiannis, Kang, 2019, +2021)

Cell-identifying barcodes could also be used as training data for lipid tracing algorithms, and so on

1) What resolution is required to attain meaningful wiring models?

50 nm in XY&Z (with cell identity of each voxel via multi-cycle readout of fluorescent barcodes)

Achievable with ~8x expansion microscopy (ExM, TREx), assuming one of:

- Optical microscope w/ isotropic <400 nm point-spread function (e.g., diSPIM, Shroff 2013)
- *Post-ExM sectioning* in Z to < 400 nm, plus simple widefield fluorescence microscope (think “smartphone camera”)
    - ExM also allows local signal amplification to make cell barcodes or molecular tags *bright* enough for this

2) Which imaging technologies could be driven to scale and most easily disseminated?

Optical ‘scopes + fluid cycling, **driven by commercialization** of FISSEQ / MERFISH / CODEX & similar.

3) Compare and contrast single large-scale efforts to democratizing data collection.

Both! Need **centralized, focused, medium-scale, supra-academic** effort(s) for robust methods integration + optimization.

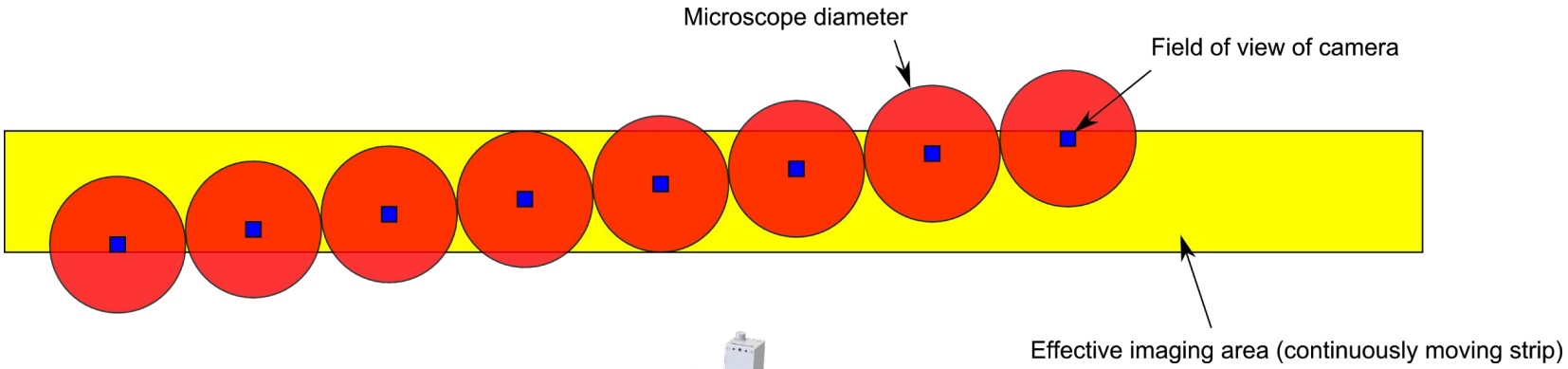
(Illumina democratized sequencing; *building* Illumina required tight-knit, well-funded startup. BGI is centralized, meanwhile.)

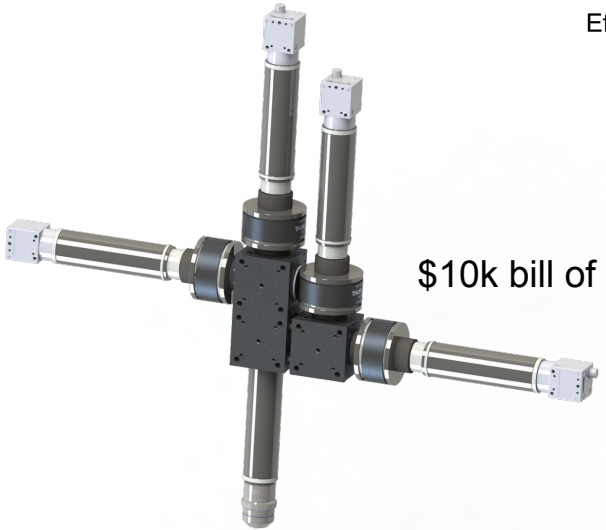
4) How do you encode dynamics in static brain maps (adding the time domain)?

Co-registration with calcium/voltage imaging at the cellular level, plus new methods like “signaling reporter islands” (**SiRIs**)

In-situ multiplexing for readout of cell *lineage* (via CRISPR **recorders**), “RNA velocity”, RNA timestamps, plus cell barcodes

Example long-term embodiment for ultra-fast/cheap optical imaging system via widefield conveyor-tape





$10k bill of materials per camera field of view

Post-expansion sectioning resolution required: 50 nm x (expansion factor) ~ 400 nm versus <20 nm for EM