What (kind of computer) is the brain?

New Destinations in Artificial Intelligence
MIT Media Lab
Taught by Joscha Bach in Fall 2015

Presenter: Adam Marblestone, PhD
MIT Synthetic Neurobiology Group

Experimental Projects with: Boyden, Church, Zador and Cai labs
Theoretical Projects with: Marcus, Kording, Hayworth, Dean & others
Outline

• Musings about the brain’s high-level architecture and how it relates to modern AI
  • Early clues and the birth of neural networks research
  • Deep learning as a sub-module
  • Who is the trainer?
  • Basal ganglia gating of information flow between working memory buffers
  • The problem of variable binding and potential solutions

• How might we move towards a “complete” circuit diagram of the brain
  • Where we stand: Blue Brain etc.
  • Levels of description
  • “Assumption-proof” brain mapping principles
  • Towards Rosetta brain maps and models
Musings about brain architecture and AI
multi-layer feedforward networks trained by back-propagation
neural computation as hierarchical feature extraction?

Hubel and Weisel 1950s: tuned simple and complex cells in visual cortex
today’s “deep learning”: gradient descent of a cost function

Supervision signals define cost function
Large amount of labeled input data needed

Relatively unstructured network

Trained relatively unstructured network
today’s “deep learning”: gradient descent of a cost function

Supervision signals define cost function
Large amount of labeled input data needed

**back-propagation of errors**

\[
W_{46}' = w_{46} + \eta \delta \frac{df_k(e)}{de} - y_4
\]

\[
W_{56}' = w_{56} + \eta \delta \frac{df_k(e)}{de} - y_5
\]

Relatively unstructured network

Trained relatively unstructured network
“This is, I think, the recurring lesson of neural networks. Neural networks are shapeless, useless things unless they are driven into optimal configurations... The big, big lesson from neural networks is that there exist computational systems (artificial neural networks) for which function only weakly relates to structure. A neural network trained to recognize digits from MNIST can look structurally indistinguishable from a neural network that is untrained. More or less the same neural network architectures are currently used for speech recognition as for language translation... I really believe that we (/ the field of neuroscience) should defocus our discussion from "the computations" that the brain performs. Marr himself retired from neuroscience because he had concluded that neural networks could be very, very powerful computationally; he despaired that he would not be able to find enough constraints on brain function by examining their structure.... I would take heed from what has been learned by neural network researchers over the past 30 years: a neural network needs a cost function and an optimization procedure to be fully described; and an optimized neural network's computation is more predictable from this cost function than from the dynamics or connectivity of the neurons themselves.”
Recurrent networks: liquid state machines

Figure 1: A. Traditional gradient-descent-based RNN training methods adapt all connection weights (bold arrows), including input-to-RNN, RNN-internal, and RNN-to-output weights. B. In Reservoir Computing, only the RNN-to-output weights are adapted.

random recurrent network, only adjust the output weights
Recurrent networks: LSTMs

“back-propagation through time” based on error signal
How does this relate to the brain?

One popular direction: (e.g., Hawkins)

- View neural computation primarily as hierarchical feature extraction
- Assume there is one such algorithm common across all of cortex (canonical cortical microcircuit)
- Assume that the cortex is actually doing un-supervised learning (since supervised back-propagation is assumed to be biologically implausible)
- Seek to understand the universal learning algorithm of cortex

---

**Table 1. Belief propagation equations for an HTM node.**

1. Calculate likelihood over coincidence patterns.
   \[ P(x_{(i)}=1|e_{(i)}(t)) = \prod_{j=1}^{M} P(c_{(i,j)}(t)) \]  
   where coincidence pattern \( c_{(i,j)} \) is the co-occurrence of \( i \)-th Markov chain from child 1, \( i \)-th Markov chain from child 2, ..., and \( i \)-th Markov chain from child \( M \).

2. Calculate the feed-forward likelihood of Markov chains using dynamic programming
   \[ P(x_{(i)}=1|e_{(i)}(t)) = \sum_{c_{(i,j)}} P(c_{(i,j)}(t)) \sum_{t=1}^{T_{(i,j)}} P_{(i,j)}(t-1|t) \sum_{z_{(i,j)}} P(z_{(i,j)}(t)) \]  
   where \( P_{(i,j)}(t-1|t) \) is the transition probability from state \( t-1 \) to state \( t \) in the \( i \)-th chain.

3. Calculate the belief distribution over coincidence patterns
   \[ Bel(c_{(i,j)}) = \sum_{x_{(i,j)}} P(x_{(i,j)}|c_{(i,j)}) \]  
   where \( Bel(c_{(i,j)}) \) is the belief in the coincidence pattern \( c_{(i,j)} \).

4. Calculate the messages to be sent to child nodes.
   \[ m_{(i,j)}(t) = \sum_{c_{(i,j)}} P(c_{(i,j)}|x_{(i,j)}) \]  
   where
   \[ m_{(i,j)}(t) = \begin{cases} 1, & \text{if } x_{(i,j)} \text{ is a component of } c_{(i,j)} \\ 0, & \text{otherwise} \end{cases} \]

---

Figure 8. Mapping between neocortex hierarchy and HTM hierarchy. (A) Schematic of neocortex inside the skull. The neocortex is a thin sheet of several layers of neurons. Different areas of the neocortical sheet process different information. Three successive areas of the visual hierarchy – V1, V2 and V4 – are marked on this sheet. The connections between the areas are reciprocal. The feed-forward connections are represented using green arrows and the feedback connections are represented using red arrows. (B) A slice of the neocortical sheet, showing its six layers and columnar organization. The cortical layers are numbered 1 to 6 layer 1 is closest to the skull, and layer 6 is the inner layer, closest to the white matter. (C) Areas in the neocortex are connected in a hierarchical manner. This diagram shows the logical hierarchical arrangement of the areas which are physically organized as shown in (A). (D) An HTM network that corresponds to the logical cortical hierarchy shown in (C). The number of nodes shown at each level in the HTM hierarchy is greatly reduced for clarity. Also, in real HTM networks the receptive fields of the nodes overlap. Here they are shown non-overlapping for clarity.

doi:10.1371/journal.pcbi.1000532.g008

doi:10.1371/journal.pcbi.1000532.t001
Hierarchical pattern recognition is just a small part of the picture

<table>
<thead>
<tr>
<th>Computation</th>
<th>Potential algorithmic/ representational realization(s)</th>
<th>Potential neural implementation(s)</th>
<th>Putative brain location(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid perceptual classification</td>
<td>Receptive fields, pooling and local contrast normalization</td>
<td>Hierarchies of simple and complex cells (Anselmi et al., 2013)</td>
<td>Visual system</td>
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<tr>
<td>Complex spatiotemporal pattern recognition</td>
<td>Bayesian belief propagation (Lee &amp; Mumford, 2003)</td>
<td>Feedforward and feedback pathways in cortical hierarchy (George &amp; Hawkins, 2009)</td>
<td>Sensory hierarchies</td>
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<td>Learning efficient coding of inputs</td>
<td>Sparse coding</td>
<td>Thresholding and local competition (Rozell, Johnson, Baraniuk, &amp; Olshausen, 2008) or other mechanisms</td>
<td>Sensory and other systems</td>
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<tr>
<td>Working memory</td>
<td>Continuous or discrete attractor states in networks</td>
<td>Persistent activity in recurrent networks (X.-J. Wang, 2012)</td>
<td>Prefrontal cortex</td>
</tr>
<tr>
<td>Decision making</td>
<td>Reinforcement learning of action-selection policies in PFC/BG system</td>
<td>Reward-modulated plasticity in recurrent cortical networks coupled with winner-take-all action selection in the basal ganglia (Rajesh P N Rao, 2010)</td>
<td>Prefrontal cortex and basal ganglia</td>
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<tr>
<td>Routing of information flow</td>
<td>Context-dependent tuning of activity in recurrent network dynamics</td>
<td>Recurrent networks implementing line attractors and selection vectors (Mante, Sussillo, Shenoy, &amp; Newsome, 2013)</td>
<td>Common across many cortical areas</td>
</tr>
</tbody>
</table>

Frequently Asked Questions for: The Atoms of Neural Computation

Gary F. Marcus, Adam H. Marblestone, Thomas L. Dean

(Submitted on 31 Oct 2014)
Hierarchical pattern recognition is just a small part of the picture

<table>
<thead>
<tr>
<th>Shifter circuits</th>
<th>Divergent excitatory relays and input-selective shunting inhibition in dendrites (Olshausen, Anderson, &amp; Van Essen, 1993)</th>
<th>Common across many cortical areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oscillatory coupling (Colgin et al., 2009; Ketz, Jensen, &amp; O’Reilly, 2014) and phase synchronization (Salinas &amp; Sejnowski, 2001)</td>
<td>Frequency filtering via feedforward inhibition (Akam &amp; Kullmann, 2010) or selective effects on spike coherence in excitatory/inhibitory networks with multiple classes of interneurons (Börgers, Epstein, &amp; Kopell, 2008)</td>
<td>Common across many cortical areas</td>
</tr>
<tr>
<td>Modulating excitation/inhibition balance during signal propagation</td>
<td>Selective modulation of inhibitory neurons in balanced networks, e.g., via cholinergic modulation (Vogels &amp; Abbott, 2009)</td>
<td>Common across many cortical areas</td>
</tr>
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<td>Gain control</td>
<td>Divisive normalization</td>
<td>Common across many cortical areas</td>
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<td>Sequencing of events over time</td>
<td>Feed-forward cascades</td>
<td>Language and motor areas</td>
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<td>Serial working memories</td>
<td>Prefrontal cortex</td>
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<td>Ordinal serial encoding through variable binding (Choo and Eliasmith, 2010)</td>
<td>Prefrontal cortex / basal ganglia loops</td>
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<td></td>
<td>Population coding (Georgopoulos, Schwartz, &amp; Kettner, 1988), probabilistic population coding (Ma, Beck, Latham, &amp; Pouget, 2008), or many variants</td>
<td>Motor cortex and higher cortical areas</td>
</tr>
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<td></td>
<td>Time-varying firing rates of neurons representing dot products with basis vectors, nonlinear (Shamir &amp; Sompolinsky, 2004) or probabilistic variants (Ma et al., 2006), and generalizations to high-dimensional vectors (Eliasmith et al., 2012)</td>
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<td>Indirection (Kriele, Noelle, Cohen, &amp; O’Reilly, 2013)</td>
<td>Patterns of cortical neural activity in an area encode “pointers”, and gate the activity of other pointer-specific areas via projections to and from the basal ganglia</td>
</tr>
<tr>
<td>Variable binding</td>
<td>Dynamically partitionable autoassociative networks (Hayworth, 2012) Holographic reduced representations (Eliasmith et al., 2012)</td>
<td>Selective gating of subsets of large-scale attractor networks linking many cortical regions</td>
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<td>Circular convolution applied to population coded vector representations in cortical working memory buffers, gated by thalamic signals</td>
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Table 1: Preliminary, illustrative example of a proposal for a partial taxonomy of elementary, reusable operations (left column) that could underlie the diversity of cortical computation, with possible neural implementations (middle two columns) and associated brain structures (right column), based on a survey of mechanisms from the existing computational neuroscience literature. Key sources influencing the overall breakdown include (Eliasmith, 2013; O’Reilly, 2006; Rajash P.N. Rao, Olshausen, & Lewicki, 2002). The

Frequently Asked Questions for: The Atoms of Neural Computation

Gary F. Marcus, Adam H. Marblestone, Thomas L. Dean

(Submitted on 31 Oct 2014)
Problems with the “canonical learning rule of cortex” direction:
- Ignores the power of genetically-defined circuit structures
- Ignores the diversity of possible computations
- Obsessed with unstructured networks
- Obsessed with pattern recognition
- Obsessed with sensory/motor cortex
  (as opposed to prefrontal cortex and hippocampus)
- Not a good model of “higher level cognition”

I suggest a different focus:
- Supervised back-propagation is biologically plausible
- View trainable deep-nets as modules that the brain can use
- Key questions:
  - Who is the trainer and what is the training data?
  - How are these networks organized in a larger architecture?
  - How does this larger architecture bootstrap cognition?
  - How can genetics encode heuristics to bootstrap learning?
Biologically plausible back-propagation: many possible mechanisms

Biologically Plausible Error-driven Learning using Local Activation Differences: The Generalized Recirculation Algorithm

Randall C. O'Reilly

Random feedback weights support learning in deep neural networks

Timothy P. Lillicrap¹, Daniel Cownden², Douglas B. Tweed³, Colin J. Akerman¹

Towards Biologically Plausible Deep Learning

Yoshua Bengio, Dong-Hyun Lee, Jorg Bornschein, Zhouhan Lin

(Submitted on 14 Feb 2015)
Integrated biological cognitive architectures: LEABRA and SPAUN

Figure 10: The PBWM (prefrontal cortex basal ganglia working memory) component of the Leabra architecture, capturing the dynamic gating of prefrontal cortex active maintenance by the basal ganglia, which is in turn modulated by phasic dopamine signals to learn what is important to maintain. The PVLV (primary value, learned value) system provides a biologically-based model of the dopaminergic system.

The Leabra Cognitive Architecture:
How to Play 20 Principles with Nature and Win!

Randall C. O’Reilly, Thomas E. Hazy, and Seth A. Herd
Department of Psychology and Neuroscience
University of Colorado Boulder
345 UCB
Boulder, CO 80309
randy.oreilly@colorado.edu
Integrated biological cognitive architectures: LEABRA and SPAUN

A Large-Scale Model of the Functioning Brain
Chris Eliasmith et al.
Science 338, 1202 (2012);
DOI: 10.1126/science.1225266
The central role of the basal ganglia: 
the cortex evolved in the context a basal ganglia 
action selection system

Figure 7. Comparison of the Mammalian and Lamprey Basal Ganglia

Evolutionary Conservation of the Basal Ganglia as a Common Vertebrate Mechanism for Action Selection

Marcus Stephenson-Jones,† Ebba Samuelsson,† 
Jesper Ericsson,† Brita Robertson,† and Sten Grillner†,†
†The Nobel Institute for Neurophysiology, Department of Neuroscience, Karolinska Institutet, SE-171 77 Stockholm, Sweden
thalamic gating of “copy and paste” operations between cortical working memory buffers, executing a sequence of steps controlled by the basal ganglia.
CHAPTER 9

Thalamic relays and cortical functioning

S. Murray Sherman*

Department of Neurobiology, Pharmacology & Physiology,
University of Chicago, Chicago, IL 60637, USA
sensation → stored sensory patterns → basal ganglia action selection → stored motor patterns → action

reinforcement learning

value fn
sensation → CORTEX
  more abstract stored sensory patterns
  → basalganglia action selection
  → CORTEX
  more abstract stored motor patterns
  → working memory buffers
  → associative memory
  → PFC → HIPPOCAMPUS

value fxn
reinforcement learning

action
Abstract stored motor patterns

sensation

reinforcement learning

CORTEX

more abstract stored sensory patterns

basal ganglia action selection

CORTEX

more abstract stored motor patterns

value fxn

sensation

working memory buffers

PFC

associate memory

HIPPOCAMPUS

action

Tuesday, October 27, 15
What is the brain’s “value function”? 

Figure 8: The (inverted) pyramid of goals, anchored at the base in the most basic of primary values (PVs), divided into positive and negative cases, handled by different areas e.g., positive = LH (lateral hypothalamus), negative = PBN (parabrachial nucleus) and VMH (ventromedial hypothalamus). At the next level up are learned value (LV) areas that can associate perceptual stimuli with PVs, to provide a prospective, anticipatory estimate of probability of goal attainment, e.g., CeM (medial central nucleus of the amygdala) and CeC, CeL (capsular and lateral central amygdala). The next level adds the basolateral amygdala (BLA), which has a much more extensive set of PV representations, and it supports LV sensory learning as well. BLA feeds into the ventral striatum, where some neurons learn to expect the timing, magnitude, and probability of PVs, contributing to dopamine responses reflecting the discrepancy from these expectations. At the highest level are the limbic frontal areas, including insula which represents highly differentiated primary values (e.g., different food tastes), OFC (orbital frontal cortex) which represents many different goals, continuing up the medial wall of mPFC (medial PPC) and anterior cingulate cortex (ACC).

O’Reilly: goal-driven cognition in the brain
What is variable binding?

the transitory or permanent tying together of two bits of information:

- a variable (such as an $X$ or $Y$ in algebra, or a placeholder like subject or verb in a sentence)

and

- an arbitrary instantiation of that variable (say, a single number, symbol, vector, or word).

Such processes appear to be outside the scope of uniform pattern recognition systems, yet are likely to be central both in language (e.g., in interpreting sentences that combine words in novel ways) and deductive reasoning. Variables likely figure prominently in other domains, as well, such as navigation, motor control, and higher-level vision (2, 10, 11).

unstructured neural nets trained by gradient descent don’t naturally produce full variable binding: need a dedicated mechanism.
Deep learning recently realized that it needs a dedicated mechanism for variable binding as well...

---

**Neural Turing Machines**

- Alex Graves  
  gravesa@google.com
- Greg Wayne  
  gregwayne@google.com
- Ivo Danihelka  
  danihelka@google.com
Indirection and symbol-like processing in the prefrontal cortex and basal ganglia

Trenton Kriete\(^a,1\), David C. Noelle\(^b\), Jonathan D. Cohen\(^c\), and Randall C. O’Reilly\(^a,1\)
<table>
<thead>
<tr>
<th>$x_1$</th>
<th>$x_2$</th>
<th>$x_3$</th>
<th>$x_4$</th>
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Symbol in slot $x_2$ has been successfully ‘transferred’ to slot $x_5$.

Block of synaptic connections projecting from neurons of $x_2$ to neurons of $x_5$.

**HYPOTHESIS & THEORY ARTICLE**


**Dynamically partitionable autoassociative networks as a solution to the neural binding problem**

Kenneth J. Hayworth*

Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA, USA
Hayworth DPANN
more abstract stored sensory patterns

sensation

CORTEX

more abstract stored motor patterns

action selection

basal ganglia

working memory buffers

working memory

PFC

associative memory

HIPPOCAMPUS

Enables variable binding?

value fxn

reinforcement learning

Tuesday, October 27, 15
Can this system “bootstrap” cognition by orchestrating the supervised training of cortex?

What genetically-programmed “clues” are needed?

cf., Poggio’s “implicit supervision” of learning

PFC

HIPPOCAMPUS

basal ganglia

action selection

value fxn

reinforcement learning

more abstract stored motor patterns

more abstract stored sensory patterns

working memory buffers

associative memory

sensation

to action

CORTEX

CORTEX

Tuesday, October 27, 15
How can we actually characterize the actual brain
Neural circuits are macroscopic in extent but nanoscopic in functional organization

long-range connections

dense 3D circuitry:
>1 synapse (connection) per um$^3$

[Gong et al, 2013]

[Mischenko, 2010]
Specialized molecular machinery at each synapse and in each neuron:
1000s of types of circuit elements & connections

(artists renditions)

Not just “excitatory” vs. “inhibitory”:
many timescales, learning rules, modulatory inputs, local computations...
all defined by specific variants of molecular machinery
Isn’t everything below this line just an “implementation detail”?

Mapping every dopant atom in a CPU wouldn’t give insight into its architecture.
But: I would argue...

In a brain (though not in an Intel chip), the molecules encode the rules of wiring and learning.

The molecular level shapes the high-level circuit architecture.

Mapping molecularly heterogeneity might give clues to the high-level architecture.
38,016 isoforms of a single axon guidance protein

Alternative splicing of Drosophila Dscam generates axon guidance receptors that exhibit isoform-specific homophilic binding.

Wojtowicz WM, Flanagan JJ, Millard SS, Zipursky SL, Clemens JC.
Claims that connectivity is statistical and predicted by morphology alone are only telling part of the story.

Statistical connectivity provides a sufficient foundation for specific functional connectivity in neocortical neural microcircuits.

Sean L. Hill$^{a,1,2}$, Yun Wang$^{b,c,1}$, Imad Riachi$^{a,1}$, Felix Schürmann$^{a}$, and Henry Markram$^{a}$

Only predicts part of the variance of one particular statistical metric.

Only tested in one area and one species.

The exceptions could be the basis for Gary’s FPGA-like micro-circuit configurations.

were independently and randomly placed. We compared the positions of physical appositions resulting from the incidental overlap of axonal and dendritic arbors in the model (statistical structural connectivity) with the positions of putative functional synapses (functional synaptic connectivity) in 90 synaptic connections reconstructed from cortical slice preparations. Overall, we found that statistical connectivity predicted an average of 74±2.7% (mean ± SEM) synapse location distributions for nine types of cortical connections. This finding suggests that chemospecific attractive and repulsive mechanisms generally do not result in pairwise-specific connectivity. In some cases, however, the predicted distributions do not match precisely, indicating that chemospecific steering and aligning of the arbors may occur for some types of connections. This finding suggests that random alignment of axonal and dendritic arbors provides a sufficient foundation for specific functional connectivity to emerge in local neural microcircuits.
Homeostatic rules link the molecular contents of connected cells: can molecules enforce “conservation laws” of neural circuit excitability?

Figure 4  Paired PD neurons have strongly correlated abundances of Shal and IH, but not BK-KCa and Shab, mRNA. (a-d) Abundances of each mRNA were plotted for electrically coupled pairs of PD neurons from each crab, with one PD neuron on the x-axis (PD1) and the other on the y-axis (PD2). (e) Absolute abundances of Shal versus IH mRNA in each individual PD neuron. (f) Ratio of Shal to IH mRNA for pairs of PD neurons.

Published online: 29 January 2006 | doi:10.1038/nn1639

Variable channel expression in identified single and electrically coupled neurons in different animals

David J Schulz1,2, Jean-Marc Goailllard1 & Eve Marder1
Trans-synaptic communication of cell-type-specific information

Presynaptic Neurexin-3 Alternative Splicing Trans-Synaptically Controls Postsynaptic AMPA-Receptor Trafficking

Jason Aoto, David C. Martinelli, Robert C. Malenka, Katsuhiko Tabuchi, and Thomas C. Südhof
Why should theorists demand molecularly detailed maps of entire circuits?
Why should theorists demand molecularly detailed maps of entire circuits?

Key computations are not local to an area...
“Rosetta Brain”

**Activity history**

**Behavior**

**Connectivity** (circuit diagram)

**Development** (cell lineage tree)

**Expression** (epigenetic cell types, + single-synapse proteomes)

**Need:** a technology to cheaply / rapidly measure all these variables in a single brain.

**Need:** (sub)cellular resolution + whole brain scope.

see essay by Church, Marblestone & Kalhor
Fine-grained mapping, today...

Current practice: widely believed that electron microscopy (EM) is the only viable method for cellular-resolution connectomics

Problems:
- Manual human segmentation, taking minutes per cubic micron (Kasthuri, 2015)
- Does not provide molecular information
Requires tracing greyscale images through thousands of successive slices: a deeply challenging problem.

Current Opinion in Neurobiology

Semi-automated reconstruction of neural circuits using electron microscopy
Dmitri B Chklovskii, Shiv Vitaladevuni and Louis K Scheffer
302 neurons: 50 person-years for C. elegans

$\text{\$\text{\$\$\$\$}}$ for mouse w/ no molecular info
$4^N$ possible DNA sequences of length N “letters”

Zador, Cepko, Tabin, Walsh, Church et al:
can give every neuron a uniquely-identifiable DNA “barcode”
Random viral-mediated delivery to neurons then leads to every neuron expressing a unique RNA barcode string:

\[ P(j, n) = n! \times \text{Binomial}(4^j, n)/(4^j)^n \]

where \( n \) is the size of the cell population and \( j \) is the DNA barcode length in nucleotides [61].

For \( n = 7.5 \times 10^7 \) neurons and \( j = 31 \) base-long barcodes, the probability of a duplication \( 1 - P(j, n) < 0.001 \) (the per-neuron probability of duplication is then roughly \( 10^{-11} \)). This corresponds to a total barcode population size of \( 4^{31} \approx 5 \times 10^{18} \).
**Zador:** Pair barcodes of connected cells, then sequence potential for extremely low cost due to cheap sequencing

extracts connectivity

but

1) scrambles the precise positions of cells + synapses

2) nontrivial to integrate w/ molecular “annotations” (e.g., gene expression)
Zador: Pair barcodes of connected cells, then sequence.

potential for extremely low cost due to cheap sequencing.
Barcoding would allow long-range as well as short-range connectomics.
Problem: we really need the spatial information

“...we found evidence that one BC type prefers to wire with a SAC dendrite near the SAC soma, while another BC type prefers to wire far from the soma. The near type is known to lag the far type in time of visual response”

Space-time wiring specificity supports direction selectivity in the retina

Solution:

read the DNA barcodes *right where they are*

- At synapses

- On their way: in axons/dendrites

*without* grinding up the tissue
digital $4^N$ color microscopy

$=\quad ... N$ cycles

(Read one letter of DNA at a time)
Fluorescent In-Situ DNA Sequencing (FISSEQ):

A

Microscope

Fluorescence In Situ Sequencing

Image 1

Image 2

Image 3

Image 4

Image n

B

Image Analysis

Sequencing-by-ligation in the nucleus

Highly Multiplexed Subcellular RNA Sequencing in Situ

Je Hyuk Lee,1,2,† Evan R. Daugharty,1,2,4* Jonathan Scheiman,1,2 Reza Kalhor,2 Joyce L. Yang,2 Thomas C. Ferrante,2 Richard Terry,1 Sauveur S. F. Jeanty,3 Chao Li,1 Ryoji Amamoto,3 Derek T. Peters,3 Brian M. Turczyk,3 Adam H. Marblestone,1,2 Samuel A. Inverso,1 Amy Bernard,9 Prashant Mali,2 Xavier Rios,2 John Aach,5 George M. Church1,2,*
Fluorescent In-Situ DNA Sequencing (FISSEQ):

**Digital $4^N$ Color Microscopy**

A

**Fluorescence In Situ Sequencing**

Image 1

Image 2

Image 3

Image 4

Image n

B

**Image Analysis**

Highly Multiplexed Subcellular RNA Sequencing in Situ

digital $4^N$color microscopy for barcode connectomics

with Zador, Church, Boyden, Peikon, Kebshull, Daugharthy et al

Tuesday, October 27, 15
digital 4<sup>n</sup>color microscopy for barcode connectomics

with Zador, Church, Boyden, Peikon, Kebshull, Daugharthy et al
digital \(4^n\) color microscopy for barcode connectomics

Key issue: optical resolvability of synapses in 3D space

with Zador, Church, Boyden, Peikon, Kebshull, Daugharthy et al
An easier problem than electron microscopy?

Resolvability of synapses by strict criterion

Loss fraction vs. Isotropic resolution (nm)

Dataset: 1.85 synapses / um^3 in EM-sectioned mouse hippocampal neuropil (data analysis by Yuriy Mishchenko)

Marblestone et al 2013; with Zador, Church, Boyden, Mishchenko, Daugharty, Lee, Peikon, Kalhor, Kebschull et al
Expansion Microscopy (ExM):
Single-Synapse Resolution at the Voxel Acquisition Rates of a Conventional Microscope + Full Sample Transparency

Physical magnification: a new principle of microscopy

Sodium polyacrylate: polymer that swells in water (found in baby diapers)

Embed brain in permeating polyacrylate network, then add water

Expansion Microscopy (ExM):
Single-Synapse Resolution at the Voxel Acquisition Rates of a Conventional Microscope + Full Sample Transparency

Chen, Tillberg, Boyden, 2014
Barcoding and Readout of Protein Identities and Locations

DNA barcode anchored at target location

expanding hydrogel polymer strands

Target Protein/Structure

Tag
The “Rosetta Brain” integration project

Connectivity → In-vivo-generated cell barcodes (update 1x per division) → FISSEQ (Lee et al 2014) + ExM (Chen et al 2014)

Development

Expression

RNA Transcripts + DNA-barcoded antibodies