Animation: Julia Kuhl
visual input

1: flight
0: approach
Background 10 cd/m²
Spot 60 cd/m²

Position 5° S 10° A
in visual field

Spot size

Unit 3-26

Barlow, Hill, and Levick, 1964
"Santiago Ramon y Cajal - arguably the most accomplished anatomist in the history of neuroscience - became recognized as such not only because of his incredible anatomical skills and his indefatigable working habits, but also because of his uncanny sense of the functional implications of his work, a sense that made him a true genius in the field of biology."


http://www.geocities.com/ResearchTriangle/Lab/6722/quadiicirb.html
Component properties are essential

Hudspeth and Lewis 1988
THE STRUCTURE OF THE NERVOUS SYSTEM OF THE NEMATODE *CAENORHABDITIS ELEGANS*

BY J. G. WHITE, E. SOUTHGATE, J. N. THOMSON
AND S. BRENNER, F.R.S.

Laboratory of Molecular Biology, Medical Research Council Centre, Hills Road, Cambridge CB2 2QH, U.K.

(Received 9 August 1984 - Revised 12 November 1984)

1979

THREE-DIMENSIONAL COMPUTER RECONSTRUCTION OF NEURONS AND NEURONAL ASSEMBLIES

E. R. Macagno, C. Levinthal, and I. Sobel
Department of Biological Sciences, Columbia University, New York, New York

1984

Three-Dimensional Reconstruction from Serial Sections

RANDLE W. WARE
California Institute of Technology
Pasadena, California

AND

VINCENT LoPRESTI
Columbia University, New York, New York

1975

Three-Dimensional Reconstruction from Serial Sections

RANDLE W. WARE
California Institute of Technology
Pasadena, California

AND

VINCENT LoPRESTI
Columbia University, New York, New York

1972

Three Dimensional Reconstruction from Serial Sections

CYRUS LEVINTHAL & RANDLE WARE®
Department of Biological Sciences, Columbia University, New York, NY 10027

1972
Animation: Julia Kuhl

Denk and Horstmann 2004, Leighton 1981
EM contrast is provided by highly charged (heavy) nuclei.

Tissue

<table>
<thead>
<tr>
<th>Period</th>
<th>1st period</th>
<th>2nd period</th>
<th>3rd period</th>
<th>4th period</th>
<th>5th period</th>
<th>6th period</th>
<th>7th period</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H (Hydrogen)</td>
<td>Li (Lithium)</td>
<td>Be (Beryllium)</td>
<td>B (Boron)</td>
<td>C (Carbon)</td>
<td>N (Nitrogen)</td>
<td>O (Oxygen)</td>
</tr>
<tr>
<td>2</td>
<td>He (Helium)</td>
<td>Be (Beryllium)</td>
<td>Mg (Magnesium)</td>
<td>Al (Aluminium)</td>
<td>Si (Silicon)</td>
<td>P (Phosphorus)</td>
<td>S (Sulphur)</td>
</tr>
<tr>
<td>3</td>
<td>B (Boron)</td>
<td>C (Carbon)</td>
<td>N (Nitrogen)</td>
<td>O (Oxygen)</td>
<td>F (Fluorine)</td>
<td>Ne (Neon)</td>
<td>Na (Sodium)</td>
</tr>
<tr>
<td>4</td>
<td>Si (Silicon)</td>
<td>P (Phosphorus)</td>
<td>S (Sulphur)</td>
<td>Cl (Chlorine)</td>
<td>Ar (Argon)</td>
<td>K (Potassium)</td>
<td>Ca (Calcium)</td>
</tr>
<tr>
<td>5</td>
<td>Al (Aluminium)</td>
<td>Si (Silicon)</td>
<td>P (Phosphorus)</td>
<td>S (Sulphur)</td>
<td>Cl (Chlorine)</td>
<td>Ar (Argon)</td>
<td>K (Potassium)</td>
</tr>
<tr>
<td>6</td>
<td>C (Carbon)</td>
<td>N (Nitrogen)</td>
<td>O (Oxygen)</td>
<td>F (Fluorine)</td>
<td>Ne (Neon)</td>
<td>Na (Sodium)</td>
<td>Mg (Magnesium)</td>
</tr>
<tr>
<td>7</td>
<td>O (Oxygen)</td>
<td>F (Fluorine)</td>
<td>Ne (Neon)</td>
<td>Na (Sodium)</td>
<td>Mg (Magnesium)</td>
<td>Al (Aluminium)</td>
<td>Si (Silicon)</td>
</tr>
</tbody>
</table>

* Lanthanide series
  - La (Lanthanum) 57
  - Ce (Cerium) 58
  - Pr (Praseodymium) 59
  - Nd (Neodymium) 60
  - Pm (Promethium) 61
  - Sm (Samarium) 62
  - Eu (Euridy) 63
  - Gd (Gadolinium) 64
  - Tb (Terbium) 65
  - Dy (Dysprosium) 66
  - Ho (Holmium) 67
  - Er (Erbium) 68
  - Tm (Thulium) 69
  - Yb (Ytterbium) 70
  - Lu (Lutetium) 71

* * Actinide series
  - Ac (Americium) 89
  - Th (Thorium) 90
  - Pa (Protactinium) 91
  - U (Uranium) 92
  - Np (Neptunium) 93
  - Pu (Plutonium) 94
  - Am (Americium) 95
  - Cm (Curium) 96
  - Bk (Berkelium) 97
  - Cf (Californium) 98
  - Es (Einsteinium) 99
  - Fm (Fermium) 100
  - Md (Mendelevium) 101
  - No (Nobelium) 102

Slide by Kevin Briggman
3D-EM Techniques

ssTEMCA

SBEM

AT(L)UM

FIB-SEM

A) ssTEMCA: mouse visual cortex at 4 x 4 x 45 nm$^3$. B) ATUM-SEM: Mouse cortex at 3 x 3 x 29 nm$^3$. C) SBEM: mouse retina at 12 x 12 x 25 nm$^3$. D) FIB-SEM: cortex at 5 x 5 x 5 nm$^3$. Images by: D. Bock (A), K. Hayworth, J. Lichtman (B), K. Briggman (C), and G. Knott (D).
Monte-Carlo simulations yield point-spread functions for block-face imaging

Hennig and Denk, 2007
BACKSCATTERING COEFFICIENTS FOR LOW ENERGY ELECTRONS

A.M.D. Assa’d and M.M. El Gomati

The Department of Electronics, University of York, York Y01 5DD, U.K.

(Received for publication August 6, 1996 and in revised form December 9, 1996)

Joy&Joy, 1996

Figure 4. The backscattered electron coefficient as a function of the atomic number for different electron beam energies (a) for Ar ion cleaned surfaces, (b) as inserted from Bongeler et al. (1995).
epoxy
~100 μm
Background 10 cd/m²
Spot 60 cd/m²

Position 5° S 10° A in visual field

Spot size

Unit 3-26

Barlow, Hill, and Levick, 1964
Briggman et al. 2011
Briggman et al. 2011
'synapses'

all contacts

Briggman et al. 2011
“Connectomics”...
3 Hidden Layers
all nodes shown

Input

x edge

y edge

z edge

Srini Turaga
Viren Jain
...
Sebastian Seung
MIT
Joergen Kornfeld

Hahnloser et al., 2002: Sparse time code

Long et al., 2010: Rapid depolarization underlies the burst
11x11x29 nm, ROTO stain combined with HRP-DAB protocol for labeling, ECS preservation

Kornfeld et al. (under review)
Quantified for 9 axons, 504 postsynaptic dendrites

HVC\textsubscript{(RA)} axons with synapses colored by postsynaptic type

Kornfeld et al. 2017
Similar network architecture proposed by Binas et al., 2014 for cortical sequence generation (coupled winner-take-all)

Kornfeld et al. 2017
Januszewski, Kornfeld, Li, Pope, Blakely, Maitin-Shepard, Tyka, Denk, Jain. arXiv 2017
Michal Januszewski (Google), Jeremy Maitin-Shepard (Google), Peter Li (Google), Joergen Kornfeld (MPINB), Viren Jain (Google)
Completed reconstruction through targeted tracing with KNOSSOS
Total workload for small data set: ~900 hours
Automated synaptic connectivity inference for volume electron microscopy

Sven Dorkenwald, Philipp J Schubert, Marius F Killinger, Gregor Urban, Shawn Mikula, Fabian Svara & Joergen Kornfeld
Songbird basal ganglia datasets

450 somata 11,000 somata
The three ingredients for reinforcement learning

1 Behavioral context (relative song time)

2 Trial efference copy (random song change)

3 Reward signal (dopaminergic reward prediction error)

Goldberg and Fee. J Neuroscience 2011
Fee. Current Opinion in Neurobiology 2014
Links between plasticity and spines e.g. Yuste and Bonhoefer. Ann. Rev. Neuroscience 2001
Preliminary analysis with manually identified cells
11-fold higher synaptic area of LMAN shaft synapses in comparison to HVC synapses
Optokinetic response (OKR) (Eye movements)

Optomotor response (OMR) (Swimming)

Rotation (clockwise)

Translation (forward)

Fumi Kubo

Fabian Svara
Direction-selective cells

*Accessory optic system (AOS) / Nucleus of the optic tract (NOT) in mammals

All RGC axons cross the midline in fish; binocular integration therefore requires additional crossings.

Modified from Masseck and Hoffmann (2009)
From functional imaging to electron microscopy (EM) reconstruction

Pretectal Ca$^{2+}$ imaging

Fixation and staining

Serial block-face EM of whole larval brain

HuC: GCaMP5G, 5 dpf

approx. 4-30 neurons per each type (approx. 200 neurons in total)

Data size: ~12 TB
Larval Zebrafish Whole-Brain EM

≈ 80'000 neurons in the brain

≈ 7 months, 28000 sections at 25 nm
(at 14 x 14 nm resolution, with standard SBEM setup)

Fish drawing: Julia Kuhl
X-ray microCT

Allows exact measurement of sample geometry after embedding, even in opaque epoxy.

microCT illustration adapted from documentation by SCANCO medical AG
microCT

Linescanning + dynamic mosaic

50 µm
Reconstruction of functionally characterized pretectal cells: Current status

195 cells traced (partially consolidated)
Consensus path length: 49 cm

Tracing: ariadne-service GmbH
C. elegans

Fly brain

Mouse cortical column

Mouse brain

1 mm

$10^{-3}$ mm$^3$

$10^2$ neurons

$10^{-1}$ mm$^3$

$10^5$ neurons

$10^{-2}$ mm$^3$

$10^4$ neurons

$10^3$ mm$^3$

$10^8$ neurons

slide by Kevin Briggman
Zeiss Team:
- Pascal Anger
- Thomas Kemen
- Mario Mützel
- Stefan Schubert
- Dirk Zeidler

Specifications:
- 61 parallel beams
- Acq. rate: 1.22 Gpixel/s
- Resolution: 4-10 nm

Sample at 30 kV

SE  BSE
back-scattered electron

secondary electrons
Sample: Shawn Mikula
Coating: Benjamin Titze
Imaging: Tomasz Garbowski & Dirk Zeidler (Carl Zeiss Microscopy GmbH)
mSEM prototype system in development

Landing energy: 1.5 kV
Beam deceleration: 30 kV
Pixel size: 6 nm
Dwell time: 50 nS
Acquisition rate: 1.22 GHz
Serial Thick Section Gas Cluster Ion Beam Scanning Electron Microscopy

Kenneth J. Hayworth¹, David Peale¹, Zhiyuan Lu², C. Shan Xu¹ and Harald F. Hess¹

¹ Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, United States.
² Department of Psychology and Neuroscience, Dalhousie University, Halifax, Canada.

Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) is used to volume image heavy metal-stained, plastic-embedded biological samples with resolutions below 10 x 10 x 10nm, an ability that is especially important in connectomics [1]. FIB-SEM samples are typically restricted to be <50μm in the direction of the FIB beam because glancing angle milling results in artifacts over longer distances [1]. Removal rate is also restricted due to a current/spot size tradeoff. These limitations are especially problematic when one contemplates combining FIB with the increased speed offered by multibeam SEMs like the 91 beam Zeiss MultiSEM [2]. The MultiSEM’s minimum field of view is ~180μm, and its imaging rate is approximately two orders of magnitude faster than FIB’s milling rate. These considerations appear to preclude the integration of traditional FIB milling with MultiSEM imaging.

To overcome these limitations we chose to develop a broad ion beam milling approach using Gas Cluster Ion Beams (GCIB). GCIB delivers low-energy atoms to a surface and therefore does not require the use of a glancing angle. GCIB has been used for semiconductor polishing and for profiling in mass spectroscopy [3]. We attached a GCIB-10s gun from Ionoptika to a Zeiss Ultra SEM. Using a 10kV beam of Ar2000 (clusters of 2000 argon atoms), we verified that smooth, sub-10nm removal was possible from the surface of 100nm thick tissue sections. In order to obtain surfaces sufficiently smooth to produce quality secondary electron (SE) images (using 1.2kV landing energy and InLens detection
Figure 1. GCIB-SEM imaging. (A) SE image after multiple rounds of GCIB milling. (B) Cross section through dataset of three consecutive 1 μm thick sections prior to computational flattening.

Figure 2. Final GCIB-SEM dataset after computationally flattening and volume-stitching the three consecutive 1 μm thick sections together.
Illustration: Yamada et al., 2001
Stumbling Blocks, Pitfalls, Showstoppers, etc.:

Synapses (chemical), strength and other parameters

Synapses (electrical), existence etc.

Synapses (modulatory), etc.

Channel distributions

Variation between individuals

Isn’t all that’s interesting encoded in the genome?