Brain Connectivity Mapping

IJCNN Tutorial

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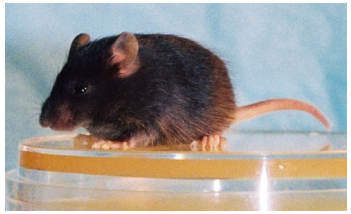
Introduction and Overview

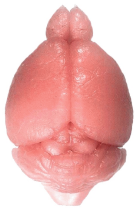
Mapping Brain Connectivity



Organism

C57BL/6 mouse

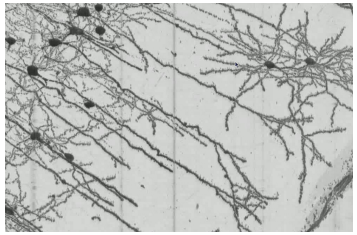
http://mouseatlas.org



Brain

Mouse brain

http://nervenet.org



Connectivity

Brain circuits (Mouse cortex)

- First step toward Understanding brain function: from structure to function.
- Approach: Omics

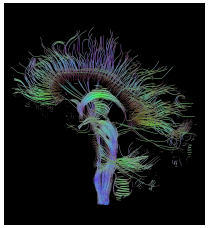
Age of the "Omics"

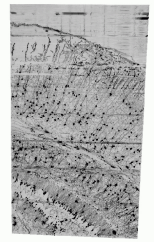
Biology has entered the age of "Omics".

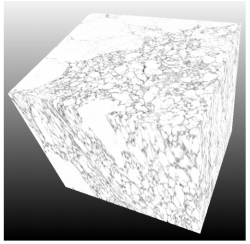
- "X-ome" means a complete collection of X
  - Derived from -$\omega\mu\alpha$ (-oma) in Greek.
  - "X-omics" means the study of "X-ome".
- Examples:
  - genome, proteome, metabolome, physiome, etc.
- Why study "omics"?
  - Can understand how the whole system works.

Connectomics

Connectome: Collection of all connections between all neurons in the brain (Sporns et al. 2005; Sporns 2012; Seung 2012).







Imaging:	Diffusion Tensor Imaging (DTI)	Light Microscopy	Electron Microscopy
Scale:	$\sim$ 10 cm cube	$\sim$ 1 cm cube	$\sim$ 100 µm cube
	Whole Human brain	Whole Mouse brain	Hundreds of neurons
Resolution:	$\sim$ 1 mm cube	$\sim$ 1 µm cube	$\sim$ 10 nm cube
Time:	hours	weeks	year
	See e.g.Hagmann et al. (2007)	Mayerich et al. (2008)	Denk and Horstmann (2004)

DTI image source: `http://en.wikipedia.org/wiki/File:DTI-sagittal-fibers.jpg`

Why Connectomics?

- Brain evolution is mostly evolution of the architecture (connectome), not the elements (neurons) (Swanson 2003)
- Current state of neuroscience is too specialized, local, and fragmented.
- Huge accumulation of (local) experimental (anatomical, physiological, genetic, behavioral) data.
- Need a framework to integrate the scattered data for a system-level understanding of the brain.

Current Status of Connectomics

- Nematode C. elegans: Only available connectome (White et al. 1986).
- Mostly focused on data acquisition (microscopy and imaging).
- Analysis framework leading behind.

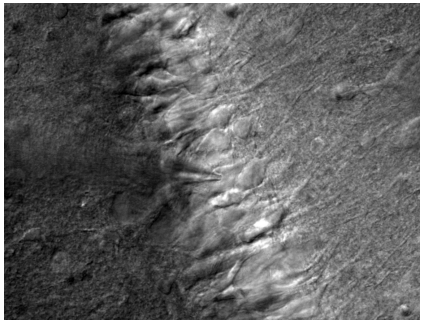
Overview

1. Staining and Labeling
2. Imaging
3. Data and Online Resources
4. Analysis
5. Wrap Up

Part I

Staining and Labeling

Staining and Labeling



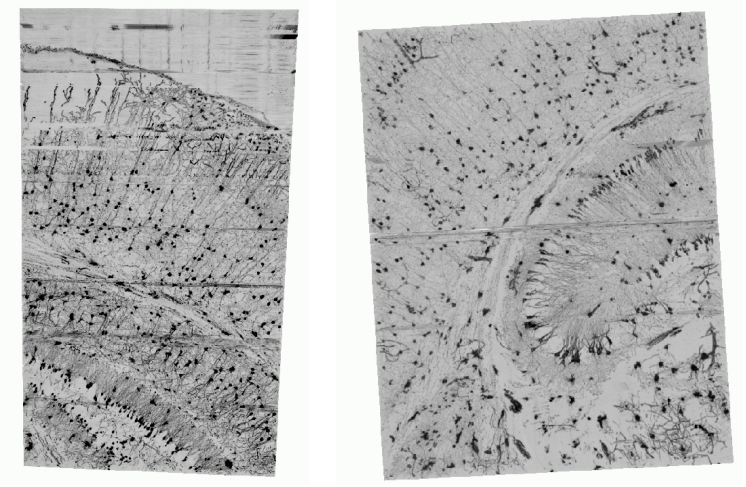
http://commons.wikimedia.org/wiki/File:WholeCellPatchClamp.jpg

- Need: Very low contrast between neurons and non-neuronal cells/tissue in the brain (see image above).
- Chemical stains and molecular labels are used to provide contrast.

Types of Stains/Labels

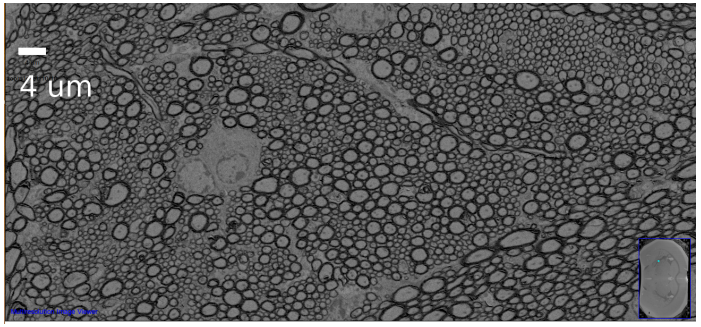
- Sparse (few neurons marked) vs. dense (all neurons marked)
- Random (random population marked) vs. targetted (specific cell types marked).
- For use with different imaging methods: light microscopy, electron microscopy, fluorescence microscopy, etc.

Golgi



- Stains whole neurons (axons unreliably stained): Sparse ($\sim$1% stained), Random, Whole brains can be stained.
  Ideal for light microscopy.

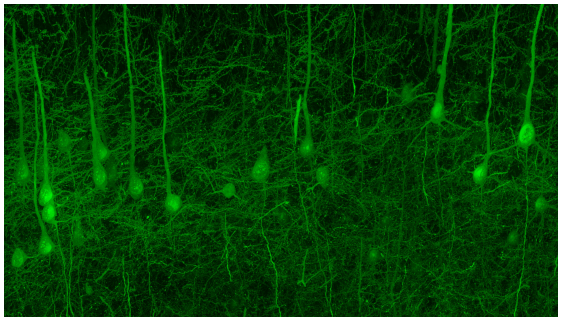
Osmium Tetroxide (OsO_4)



From `http://connectomes.org`. See Mikula et al. (2012). wbPATCO stain (OsO_4 variant)

- Stains lipid (all cell membranes): Dense, Unselective, Whole brains can be stained. Ideal for electron microscopy.

Immunofluorescence Labeling



`http://smithlab.stanford.edu/Smithlab/Array_Tomography.html`

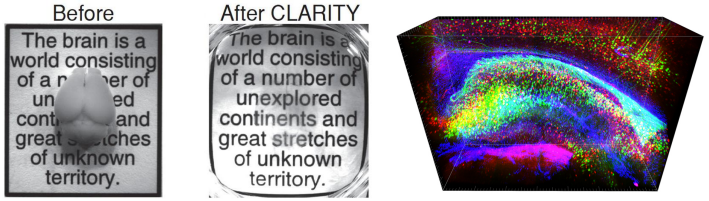
YFP expressed in whole neurons (false color added)

- Targets specific molecules (e.g. proteins): Sparse, Targetted
- Use antibody (to attach to antigen in the target) linked to fluorophore (directly or indirectly).

Tracer Injections

- Fills neurons near injection site (whole neurons): Sparse (local to injection site), Unselective, Can span long distances.
- Anterograde (soma toward axon terminal), Retrograde (axon terminal toward soma)
- Viral: anterograde or retrograde. Can cross synapses through infection to highlight higher-order connections (e.g., Pseudorabies virus)

Other Relevant Techniques



`http://directorsblog.nih.gov/the-brain-now-you-see-it-soon-you-wont/`

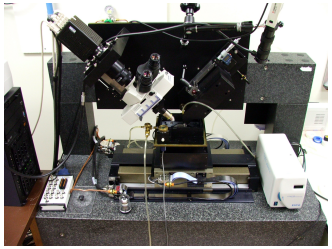
`http://clarityresourcecenter.org/` (Chung and Diesseroth 2013)

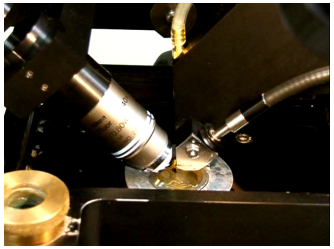
- Making brain tissue transparent: remove lipid, replacing with hydrogel for structural support.
- CLARITY: Allows imaging using multiple immunostains over large volumes of brain tissue.

Part II

Imaging

Knife-Edge Scanning Microscope

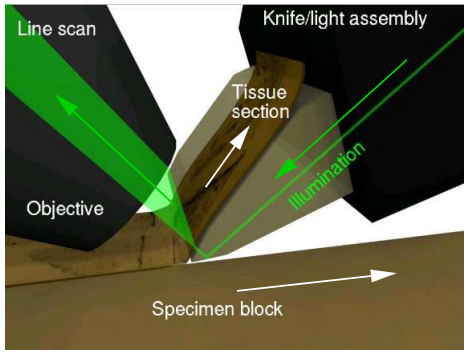




Mayerich et al. (2008); Chung et al. (2011)

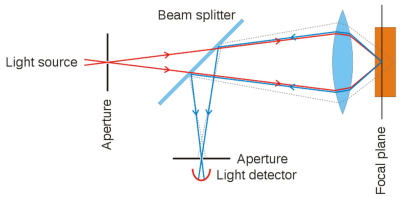
- Physical sectioning, as opposed to optical sectioning (e.g. confocal).
- Light microscopy, bright-field imaging (fluorescence in the works).
- Stains: Golgi (neuron morphology), Nissl (soma), India ink (vasculature). (Fluorescence imaging in the works.)
- 0.6 µm × 0.7 µm × 1 µm voxel resolution.
- Custom software for control, image capture (Kwon et al. 2008).
- Compare to MOST (based on KESM) (Li et al. 2010).

Imaging Principles of the KESM



- Image while cutting (line-scan at the tip of the knife).
- Transmission illumination through the diamond knife.
- Tissue thickness: 1 µm (or possibly less).

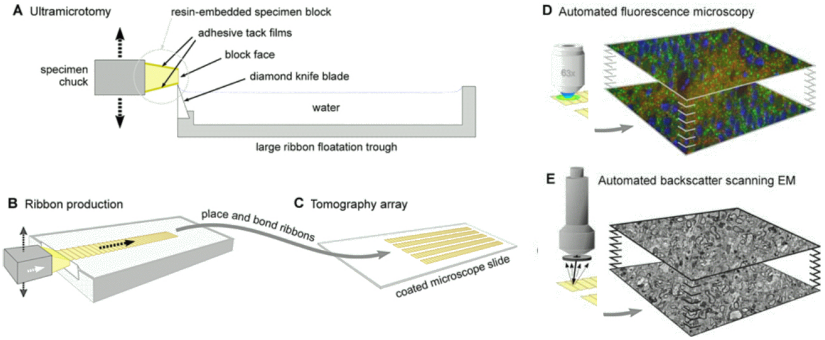
Confocal Microscopy



http://en.wikipedia.org/wiki/File:Confocalprinciple_in_English.svg

- Optical, not physical sectioning: Imaging at a specific focal depth. Scanning. Fluorescence imaging.
- Depth limit (max 1 mm) (Murray 2011).
- Also see two-photon (and multi-photon) imaging.

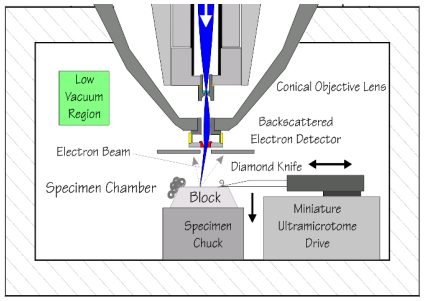
Array Tomography



Micheva and Smith (2007)

- Ultrathin sections transferred on glass slide.
- Repeated washing and staining allows perfectly registered volume data from multiple staining modalities.

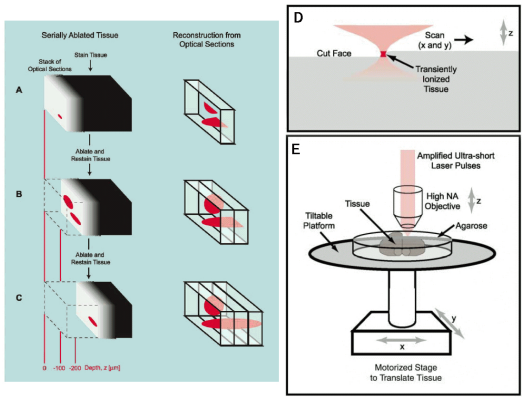
SBF-SEM (or SBEM)



Denk and Horstmann (2004)

- Microtome installed inside the vacuum chamber of an SEM.
- Commercially available from Gatan.

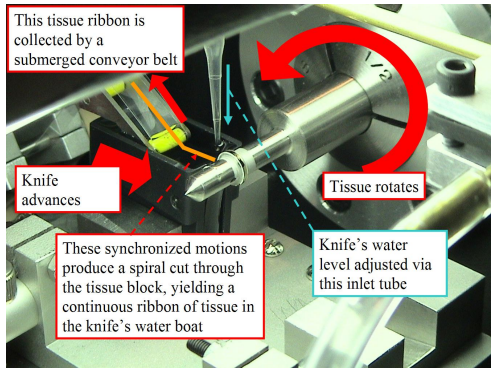
All-Optical Histology



Tsai et al. (2003)

- Hybrid of physical sectioning and optical sectioning (cf. Serial Two-Photon Tomography (Ragan et al. 2012)).
- Femtosecond laser pulses used to ablate $\sim$ 150 µm sections, followed by multiphoton imaging.

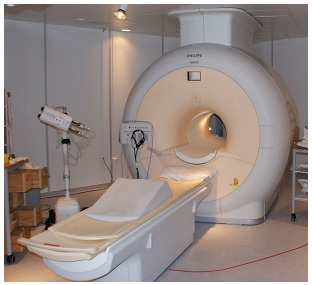
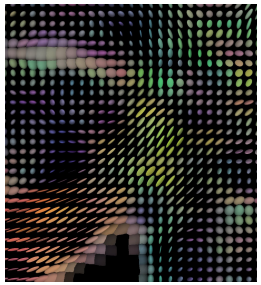
ATLUM



Hayworth et al. (2006)

- Continuous sectioning using a lathe.
- Sectioned tissue collected on adhesive tape.
- Post-staining and imaging of tape library with Transmission EM.

Diffusion Tensor Imaging (DTI)

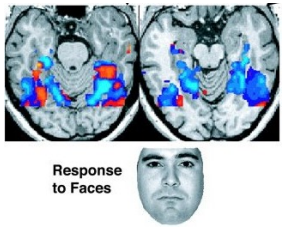



http://en.wikipedia.org/wiki/File:MRI-Philips.JPG

http://en.wikipedia.org/wiki/File:DTI-axial-ellipsoids.jpg

- Based on Magnetic Resonance Imaging (MRI). Low resolution ($\sim$100 µm).
- Detect anisotropic diffusion patterns of water molecules along figer tracts.

functional MRI (fMRI)



Haxby et al. (2001) (image cropped)

- Brain activity measured through BOLD (blood oxygen level dependent signal).
- Region-to-region connectivity can be inferred based on activity correlation or causality (dynamic causal model, Granger causal model): (Friston 2009).

Comparison

Table 1: **Summary Comparison.**

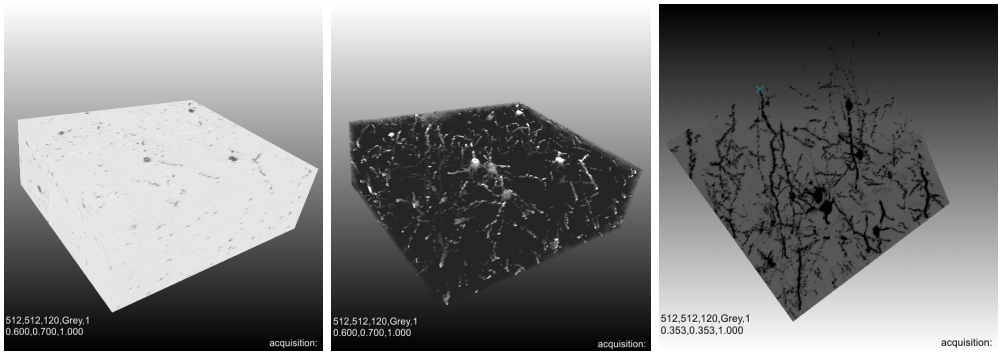
Method	nm-scale	µm-scale	High-Volume	High-Throughput
KESM (Mayerich et al. 2008) (cf. Li et al. 2010)	–	○	○	○
Confocal	–	○	–	–
All-Optical Hist. (Tsai et al. 2003)	–	○	○	–
Serial Two-Photon Tomography (Ragan et al. 2012)	–	○	○	–
Array Tomography (Micheva and Smith 2007)	○	○	–	–
SBF-SEM (Denk and Horstmann 2004)	○	–	–	–
ATLUM (Hayworth et al. 2006)	○	–	○	–
MRI/diffusion MRI (Jacobs et al. 1999; Hagmann et al. 2007)	–	–	○	○

nm-scale: $\sim$10 nm (thickness of cell membrane)
µm-scale: $\sim$1 µm (diameter of dendrites, axons, capillaries, etc.)
High-Volume: $>$ 1 cm^3 (approximate volume of mouse brain and other organs)
High-Throughput: $<$ 100 hours (for $\sim$50 scanned organs per year)

Part III

Data and Online Resources (with Demo)

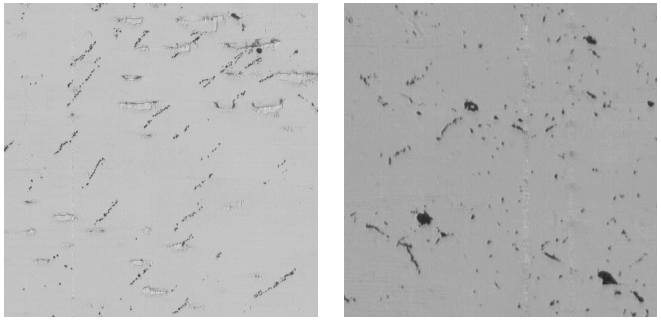
KESM Data



300 µm × 350 µm × 120 µm block

- Basically a huge 3D stack made up of 2D images.
- Details such as dendritic spines can be observed.

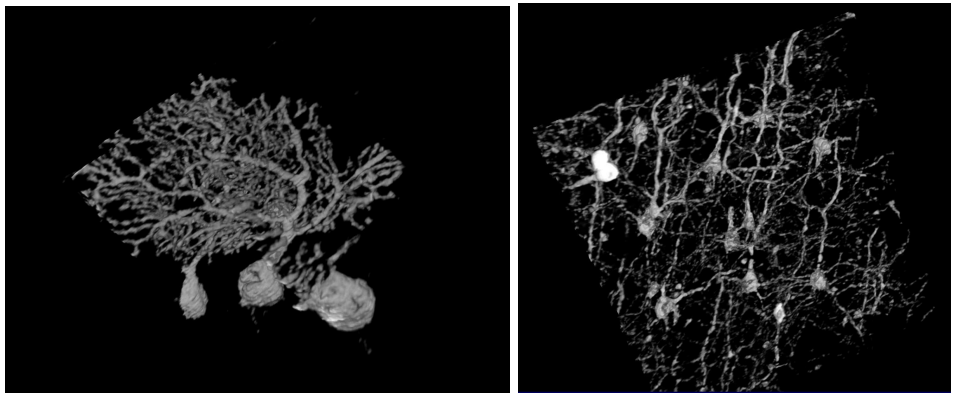
KESM Data (Image Stack)



Cerebellum (Golgi) Cortex (Golgi)

- Flythrough of 3D stack: Looks like a movie in 2D.
- Each frame = 1 µm-thin section.

KESM: Volume Visualization

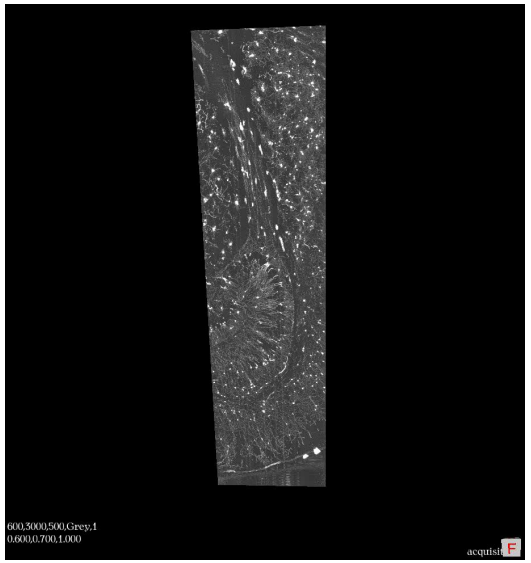


Golgi (Cerebelum) Golgi (Cortex)

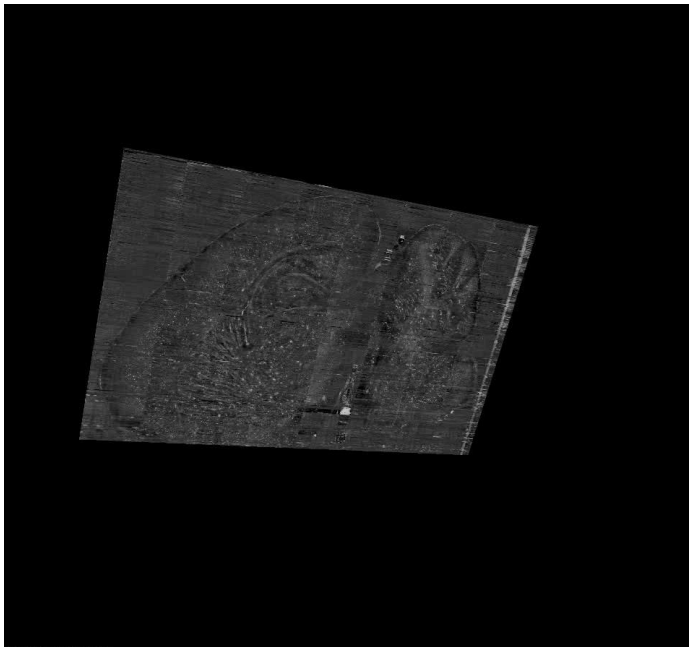
3D visualization of

- Purkinje cells and pyramidal cells.

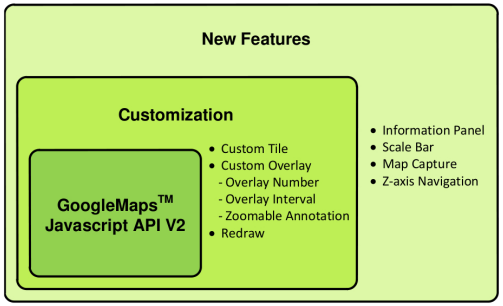
KESM: Local Circuits (Hippocampus)



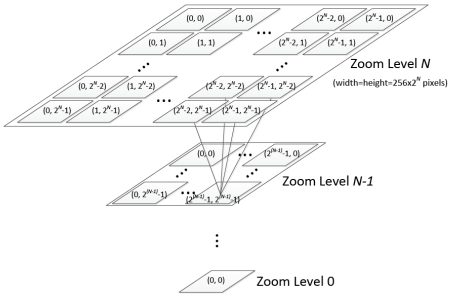
KESM Whole Brain: Neurons (Golgi)



KESM Brain Atlas



API layers

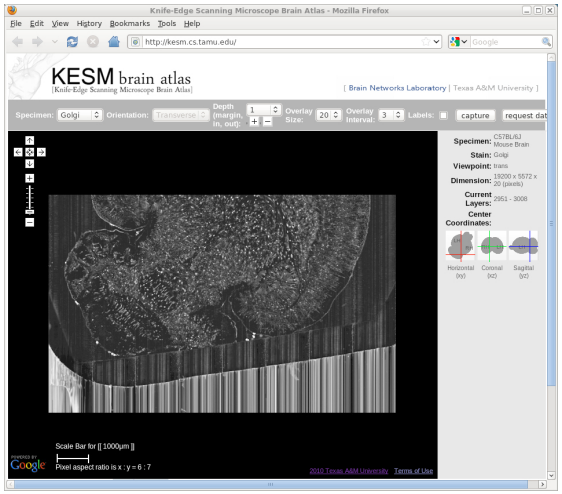


Tiling Scheme

- Multi-scale tiles.
- Semi-transparent images.
- Google Maps API (v2).

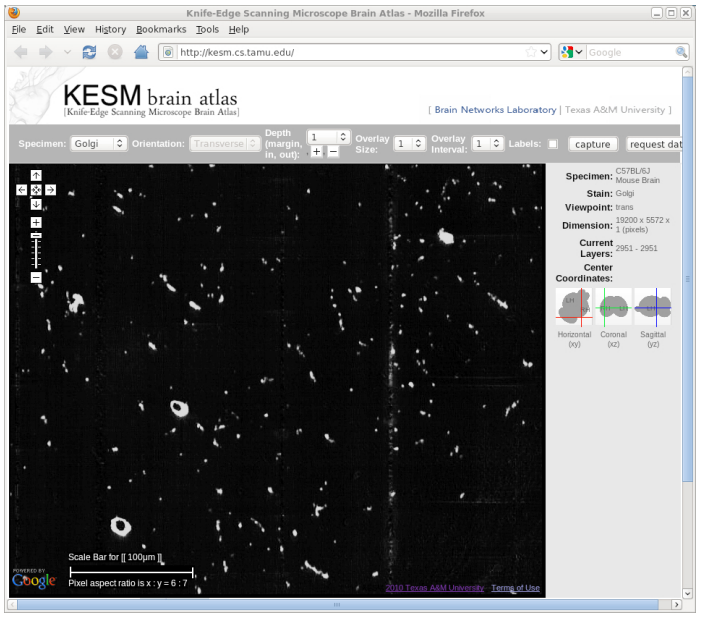
  $\longrightarrow$ KESM Brain Atlas

KESM Brain Atlas (KESMBA)

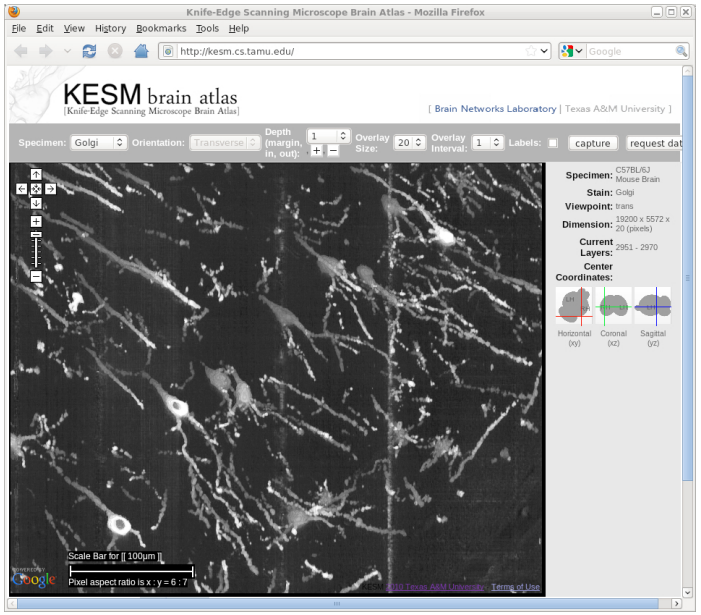


- `http://kesm.org` (Chung et al. 2011).
- Open to all! Even runs on smartphone browsers (can be slow).

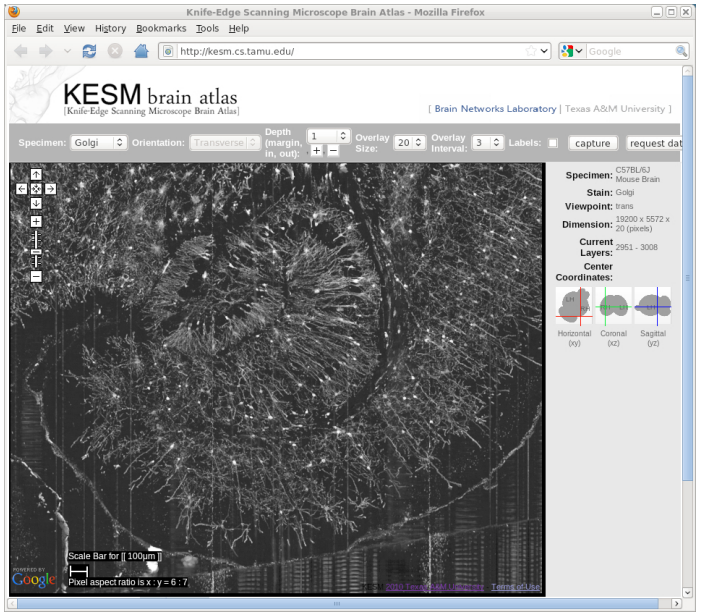
KESMBA: Single Overlay



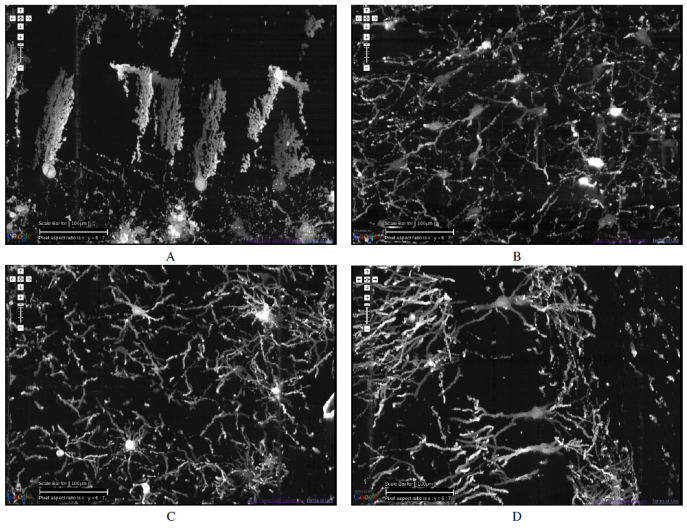
KESMBA: 20 Overlays



KESMBA: Zoomed Out

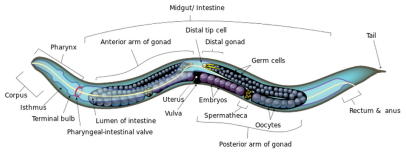


KESMBA: Some Samples



A: Cerebellum, B: Inferior colliculus, C: Thalamus, D: Hippocampus

OpenWorm Project

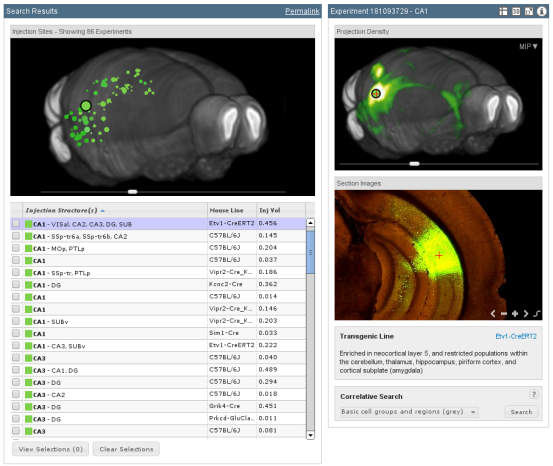


http://en.wikipedia.org/wiki/File:Caenorhabditis_elegans_hermaphrodite_adult-en.svg

http://www.openworm.org/

- C. elegans connectome, downloadable in XML (NeuroML), for multicompartment models.
- Ultimate goal of constructing a detailed simulation of the whole worm.

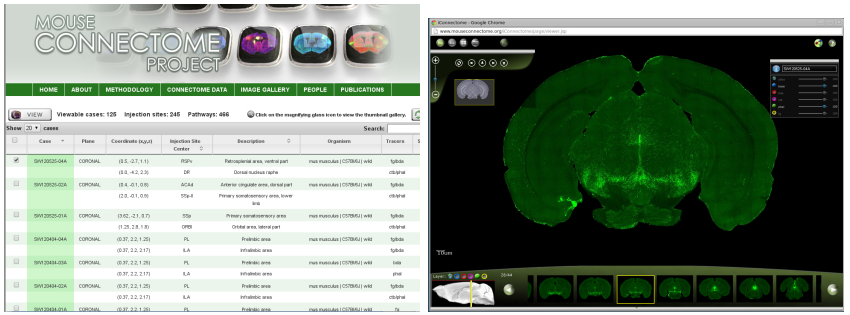
Allen Brain Atlas: Mouse Connectivity



`http://connectivity.brain-map.org`

- Tracer injection-based (1010 injection sites).
- Fluorescence microscopy.

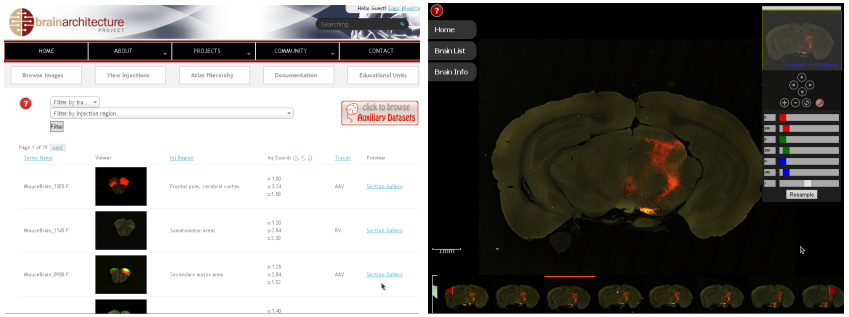
Mouse Connectome Project (UCLA)



`http://www.mouseconnectome.org/` (Hintiryan et al. 2012)

- Tracer injection-based (245 injection sites).
- Fluorescence microscopy.

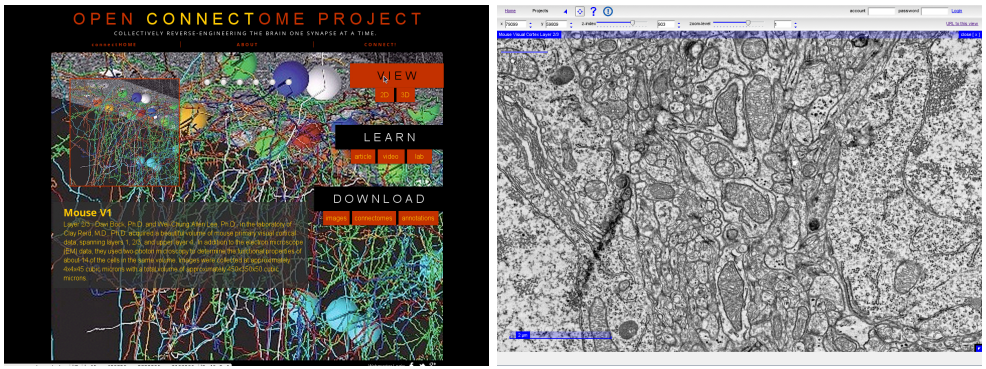
Brain Architecture Project (CSHL)



`http://brainarchitecture.org` (Mitra 2012)

- Tracer injection-based (235 injection sites, mouse).
- Fluorescence microscopy. Other species also available.

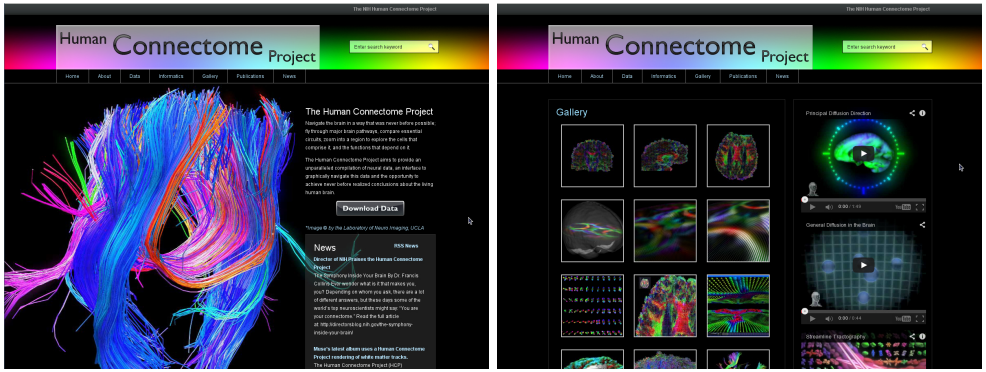
Open Connectome Project



`http://www.openconnectomeproject.org`

- EM data from mouse visual cortex (Bock et al. 2011).

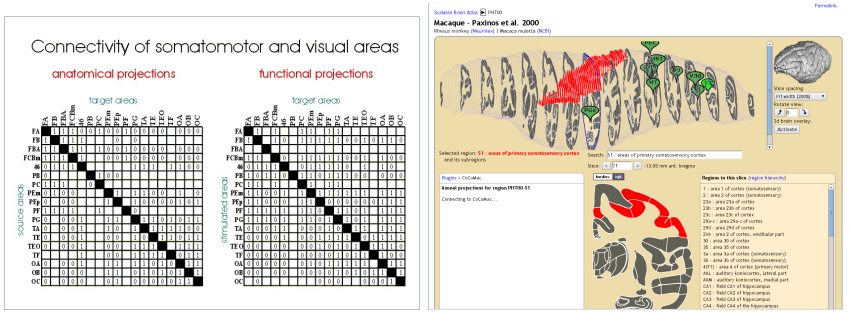
Human Connectome Project



http://www.humanconnectomeproject.org

- DTI data from human (Van Essen et al. 2012).
- Also see (Hagmann et al. 2007).

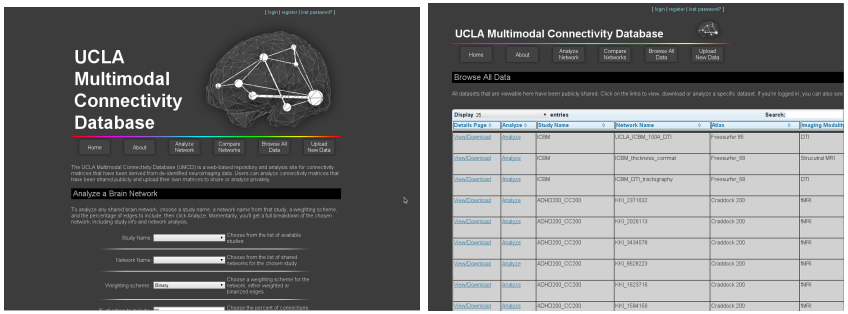
CoCoMac



http://cocomac.org http://scalablebrainatlas.incf.org

- Macaque brain connectivity (based on 2508 tracer injections, 39,748 connection details, collected from the literature).
- Second version under preparation: http://cocomac.g-node.org/

UCLA Multimodal Connectivity DB



http://umcd.humanconnectomeproject.org

- MRI-based connectivity database.

Part IV

Analysis

Geometric Reconstruction

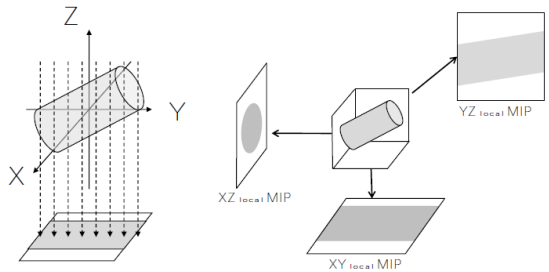
Raw data or volume visualization is not enough:

- We need to reconstruct the geometric structure of the objects in the data.
- Data can be huge (several TB): manual tracing is not an option.
- We need automated algorithms.

Reconstruction Approaches

- Segment-then-connect: the most common approach
- 3D convolutional network: Jain et al. (2010)
- Template-matching-based vector tracing: Al-Kofahi et al. (2002); Han et al. (2009b,a); Han (2009); Luisi et al. (2011)
- Semi-automated reconstruction: Yang and Choe (2011b)
- Topology-constrained reconstruction: Yang and Choe (2011a); Jain et al. (2007)
- Crowd sourcing: Eyewire.org (Seung and Burnes 2012).

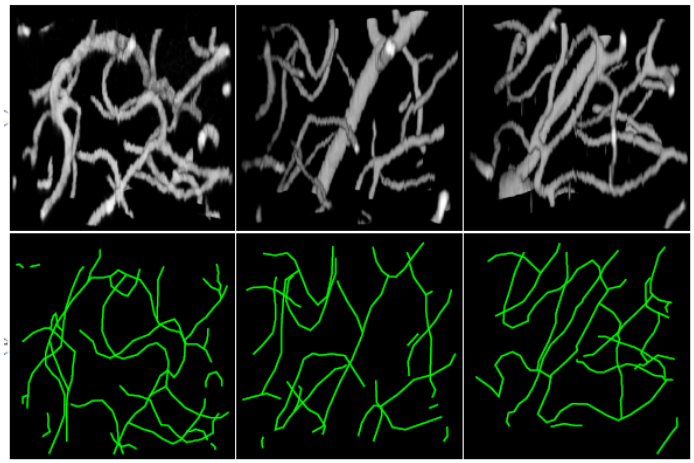
Tracing Example: MIP-Based Tracing



Han (2009)

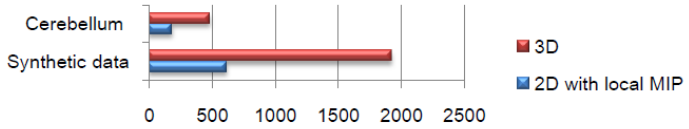
- Maximum-Intensity Projection (MIP).
- MIP-based tracing: Trace on projected 2D images.

MIP-Based Tracing Results



- KESM mouse vasculature data.

MIP-Based Tracing Performance



- MIP-based approach about 3× faster than 3D version.

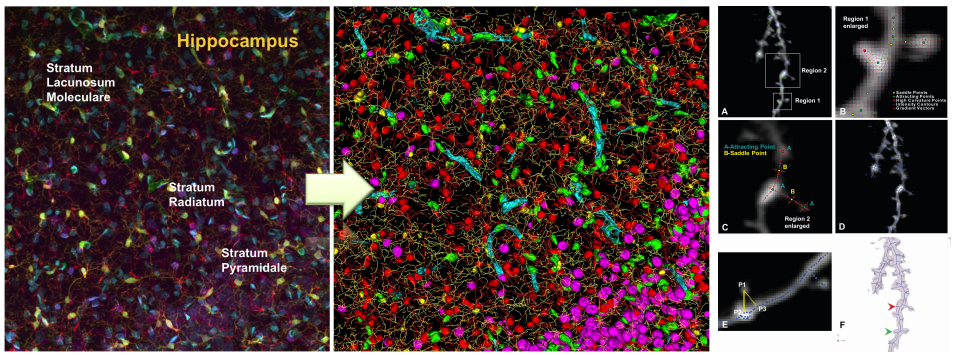
MIP-Based Tracing: Validation

	ϕ			φ		
	μ	σ	p-value	μ	σ	p-value
R1	0.1518	0.1762	0.4188	1.2131	0.3529	0.6853
R2	0.1325	0.1804		1.1294	0.3016	

Error

- Validation against small manual ground-truth (R1 and R2).
- ϕ = centerline deviation, φ = length difference.

FARSight Toolkit (U Houston)



`http://www.farsight-toolkit.org` (Luisi et al. 2011)

- 2D and 3D image analysis toolkit.

Other Reconstruction Tools

- KNOSSOS: 3D image data (mostly for EM) analysis tool (mostly manual). `http://www.knossostool.org/`
- EyeWire: crowd-sourcing EM reconstruction portal. `http://eyewire.org/`
- Reconstruct: EM reconstruction tool (manual). `http://synapses.clm.utexas.edu/tools/reconstruct/reconstruct.stm`
- Generic (yet powerful) tools:
  - ImageJ: `http://rsbweb.nih.gov/ij/`
  - ITK: `http://www.itk.org/`

Connectivity Analysis

- Graph-theory based analysis (Sporns 2002, 2011)
  - In-degree, out-degree, cluster index, power law
- Motif analysis (Milo et al. 2002).
  - Statistics of small sub-graph patterns.
- Dynamics (Thiel et al. 2003; Sporns and Tononi 2002)
- Large-scale simulation based on DTI (Izhikevich and Edelman 2008)
- Time is a crucial factor in connectivity analysis (Choe 2004).

Part V

Theoretical Insights

Wrap Up

Thinking Beyond Connectomics

Connections alone not enough:

- Sign: excitatory/inhibitory
- Weight: synaptic strength
- Delay: both conduction delay and integration time
- Molecular dynamics and gene expression
- Plasticity

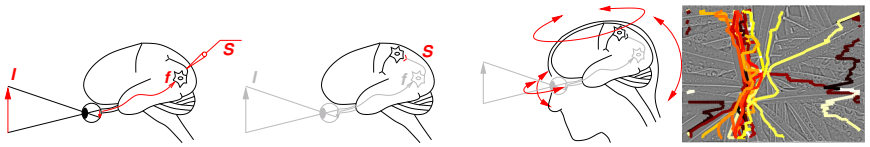
Is the Brain Enough? – Will Need the Body

- Brain is part of the body and a lot of function is performed by the spinal cord and the peripheral nervous system.
- To fully understand brain function, it must be understood in the context of the entire body.
- Imaging whole organisms may be necessary for a true understanding of brain function.

Risk of Doubling our Task?

- Without a proper theoretical framework for analysis, the resulting simulation can be as complex and hard to understand as the real brain.
- Such blind simulation could double our task.
- However, it has distinct merits:
  - Full read/write access and localized lesions.
  - Can investigate subjective phenomena such as consciousness (have the brain simulation study itself!).
  - Systematic, programmatic investigation becomes possible (automated science).

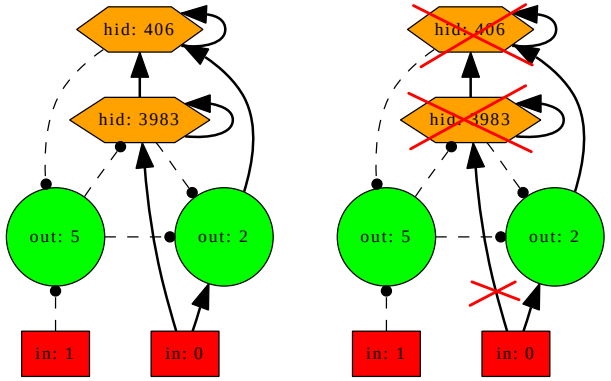
Conceptual Breakthroughs Needed



Choe and Smith (2006); Choe et al. (2007)

- Posing the right questions (Choe and Mann 2012): Internal perspective, problems faced by the brain itself.
- Sensorimotor perspective (Choe and Smith 2006; Choe et al. 2007).
- Developmental perspective.
- Evolutionary perspective (Chung and Choe 2011; Kwon and Choe 2008; Choe et al. 2012).
- Temporal perspective (Choe 2004; Lim and Choe 2008).

Inferring Function from a Brain Network: A Cautionary Tale



Analyze this!

- hid = hidden neuron, out = output neuron, in = input unit, arrow = excitatory connection, disc = inhibitory connection

Conclusion

- Understanding brain function requires a system-level investigation at a microscopic resolution.
- Innovative microscopy technologies are enabling a data-driven investigation linking the microstructure to the system level.
- A robust, accessible informatics platform is needed for knowledge discovery.
- Deep theoretical insights are needed to guide our investigation.

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