Long-Term Goal of the BNL

- Image and reconstruct the mouse connectome at a sub-micrometer resolution.
  - connectome = full connection matrix of the brain.
- Understand brain function: Structure $\rightarrow$ function.

Background: Connectomics

- Study of the connectome, the full connection matrix of the brain (Sporns et al. 2005).

Imaging: Diffusion Tensor Imaging

<table>
<thead>
<tr>
<th>Scale</th>
<th>Human brain</th>
<th>Mouse brain</th>
<th>Several neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>$\sim 1$ mm cube</td>
<td>$\sim 1$ µm cube</td>
<td>$\sim 10$ nm cube</td>
</tr>
<tr>
<td>Time</td>
<td>weeks</td>
<td>weeks</td>
<td>year</td>
</tr>
</tbody>
</table>

Motivation and Research Issues

Vasculature
Whole brain: 1 cm cube
∼50 µm cube

Neurons
Whole brain: 1 cm cube
∼200 µm cube

- Very large 3D volumes of biological data (TBs).
- Very high resolution.
- Details are too fine to be visible at the scale of the whole volume.

→ Innovative visualization methods are needed

Overview

1. Instrument: Knife-Edge Scanning Microscope
2. Data: Mouse brain data
3. Visualization

Part I
The Instrument: Knife-Edge Scanning Microscope

Mayerich et al. (2008); McCormick (2004)

The Instrument: Knife-Edge Scanning Microscope

- Physical sectioning, as opposed to optical sectioning.
- 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).
Operational Principles of the KESM

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

KESM Imaging

Brain specimen is embedded in plastic block.

Plastic block is moved toward the knife.

Thin tissue slides over knife and gets imaged.
Successive line scan constructs a long image.

One sweep results in a $\sim 4,000 \times 12,000$ image ($\sim 48$ MB).

One brain results in $\sim 25,000$ to $40,000$ images.

• Movies showing the KESM in action.
Related 3D Microscopy

Physical sectioning:

- Array Tomography (Micheva and Smith 2007)
- ATLUM (Hayworth 2008)
- SBF-SEM (Denk and Horstmann 2004)

Hybrid: Ablation + confocal

- All-Optical Histology (Tsai et al. 2003)

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**Part II**

**The Data**

Abbott (2008); Choe et al. (2009, 2010)

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**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 [Movies]**

- Flythrough of 3D stack: Looks like a movie in 2D.

Cerebellum (Golgi)  
Cortex (Golgi)  
Spinal cord (India ink)
**KESM: Volume Vis. [Movies]**

- Nissl (Cortex)
- Golgi (Cortex)
- Golgi (Cerebellum)
- India ink (Spinal cord)
- Golgi (Pyramidal cell)
- Golgi (Purkinje cell)

**KESM: Whole Brain [Movies]**

- Close-up
- Sagittal
- Coronal
- Vascular network in the mouse brain stained with India ink.
- Whole brain at 0.6 µm × 0.7 µm × 1.0 µm resolution.

---

**Part III**

**Visualization**

Eng and Choe (2008); Choe et al. (2011)

**Issues in Visualization**

- Very large volume (∼ 24,000 × 12,000 × 5,500 ≈ 2 TB)
- Fine detail (typical fibers ∼ 1 to 2 µm diameter).
- We want a global perspective, but preserve fine detail.
Two Approaches

1. Thin slab fly-through:
   - View the whole volume, but only show a thin slab.
   - Interactively move around the slab perpendicular to one sectioning plane.
   - More of a visualization know-how than an algorithm.

2. Web-based rendering using image overlays:
   - Google Map-like interface (multi-scale tiling).
   - Transparent image overlays for 3D.
   - Pseudo-stereo by offsetted overlays.

Getting Oriented: Golgi Brain

- Partial view of the whole-brain Golgi data set (horizontal section, seen from above).
- Data block width = 2.88 mm. Horizontal section.

Whole Block Reveals Little

- Looking at entire block is not informative.
- Nor is looking at a single layer.
• Flying through ∼100 µm-thick slabs reveals intricate detail.

Thin-Slab Visualization [1/2]

Part III.2
Visualization

Web-Based Rendering Using Image Overlays

• Again, single images convey little information.
• Looking at the images as a movie does not help either.
• Looking at the whole set at once does not either.
• Try that for a 2 TB image stack!

Thin-Slab Visualization [2/2]

Sagittal Horizontal

• Thin-slab visualization of new full-brain Golgi data.
Goals and Requirements

• Goal 1: Visualization in 3D
• Goal 2: Broad dissemination:
  – No high-end hardware.
  – No custom application.
  – Platform independence.
  – Runs in a standard web browser without plugins.

Approach: Overlay w/ Dist. Attenuation

Off the coast of Dubrovnik, Croatia MIP Distance Attenuation
(The inspiration)

How to visualize an image stack? (Eng and Choe 2008)

• We can overlay the images in HTML, using CSS.
• Simple overlay (MIP) is not good.
• We need distance attenuation (haze effect).

Approach: Pseudo-3D Rendering

• Generate stereo pair by shearing the image stack.
• Cross merge the above pair.

Approach: Pseudo-3D Rendering

• Generate stereo pair by shearing the image stack.
• Parallel merge the above pair.
Putting It Together: KESM Brain Atlas

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

→ KESM Brain Atlas

KESMBA: Live Demo

- Email choe@tamu.edu for username/password.
Stereo Pseudo-3D Rendering

Cross viewing.
Stereo Pseudo-3D Rendering

Parallel viewing.

Cross viewing.

Wrap Up
Conclusion

• High-throughput physical sectioning microscopes are enabling the acquisition of detailed neural circuitry data at the whole brain scale.

• New visual exploration techniques are needed.

• Web-based light-weight database interface allows quick, intuitive exploration of the data.

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References


In Memory of Bruce H. McCormick

Bruce H. McCormick (1928–2007)

• Designer of the Knife-Edge Scanning Microscope

• Co-Founder of Scientific Visualization (with Tom DeFanti and Maxine D. Brown)


