

PROJECT DESCRIPTION

A. Introduction

Advances in high-throughput, high-resolution, high-volume microscopy techniques in recent years have enabled the acquisition of extremely large volumes of neuroanatomical data (see [17] for a review, and §B.1). The *Knife-Edge Scanning Microscope* (KESM), developed and hosted in the project team's lab, is one of the first such instruments to generate whole-brain-scale data at submicrometer resolution [17, 21, 59, 63] (also see [50] based on the same principles).

We have successfully built a web-based 3D atlas, serving our whole-brain-scale mouse Golgi data set (neuronal morphology) and India ink data set (vascular network). In this **renewal application** (for our CRCNS data sharing project, our **only source of funding** for the KESM project at the moment [expires 12/2011]), we will extend both the data set and the web-based 3D atlas features and capabilities for broader access, increased usability, and accelerated scientific discovery.

The proposed extensions (compared to the prior grant) include:

- *New Data*: (1) Whole mouse brain, in Nissl; (2) Whole mouse brain, in Golgi (previous Golgi data set was 1/3 incomplete). (3) Small volumes of transgenic mouse brain expressing green fluorescent protein (GFP) in GABAergic neurons.
- *Enhanced atlas features*: (1) Open Layers API, instead of Google Maps API, with performance enhancements; (2) Registration to the rodent-standard Waxholm space; (3) Registration to the Allen Brain Atlas; (4) All three orientations supported (horizontal, coronal, and sagittal); (5) Extensive labeling with tracing of limited volumes; (6) Enhanced unit-volume viewer; and (7) A turn-key mirroring facility and Torrent seeding (for peer-to-peer data sharing of full data set download at maximum resolution).

Quality and scientific importance of the data: The KESM data sets are unique in their detail and extent compared to other currently available data sets. Submicrometer voxel size across entire small animal brains such as that of the mouse has only been possible through KESM (Nissl, India ink, Golgi)[1, 17, 20, 21, 63] or KESM-like instruments (Golgi) [50]. The new addition of the mouse Nissl data set [18] enables detailed studies of cortical and subcortical distribution of neuronal cell bodies, and the complete mouse Golgi data will provide rich local circuit data and allow morphological variability studies. Inclusion of small volumes of GFP data will enable reliable tracing of axons and open the door for multichannel data, e.g., from Array Tomography [72].

Anticipated range of uses for research and education: Please refer to the project summary and §D.9.

Scientific merit: Please refer to the project summary and the Research Plan (§D).

Broader impact: Please refer to the project summary and the Broader Impact Plan (§E).

B. Background: Relationship to Similar Resources

Our project will provide whole-brain scans at a level sufficient to reconstruct individual neurons, cell bodies, and microvascular structures. This fills a critical gap in existing 3D volume microscopy modalities, brain atlases, and 3D neuronal reconstructions, which we briefly review here.

B.1. High-throughput, High-resolution, 3D Microscopy

Critical to our proposed work are the massive biological data sets at a cellular level to which our computation will be applied. The technologies for producing such massive data are actively

being developed. Such methods can lead to the complete description of the *connectome*, the study of neural connectivity at the whole-brain scale, identified as a *grand challenge* in neuroscience [89]. Examples of such methods include our Knife-Edge Scanning Microscopy (KESM) [59, 66, 67, 68, 69] (also see [50] based on the same principles), All-Optical Histology [95], Array Tomography [73], Serial-Block-Face Scanning Electron Microscopy (SBF-SEM) [28], and Automatic Tape-Collecting Lathe Ultramicrotome (ATLUM) [38]. Table 1 shows a comparison chart of these microscopy technologies. Unlike Array Tomography and SBF-SEM, KESM can survey large volumes of biological tissue (whole small animal organs), and KESM is an order of magnitude faster than All-Optical Histology. SBF-SEM has the advantage of ultra high resolution, and Array Tomography is ideal for repeated imaging of a single specimen with multiple immuno stains and even with SEM.

Table 1: **Summary Comparison.**

Method	Resol. (x & y)	Resol. (z)	Volume	Modality	Time
All-Optical Hist. [95]	0.5 μm	1 μm	1 cm^3	Fluorescence	~900 hours
KESM [59] (cf. [50])	0.3–0.6 μm	0.5–1 μm	1 cm^3	Bright field, Fluorescence*	~100 hours
Array Tomography [72]	~0.2 μm	0.05–0.2 μm	$\sim 100^3 \mu\text{m}^3$	Fluorescence, EM	N/A See SBF-SEM
SBF-SEM [28]	~0.01 μm	~0.03 μm	$\sim 100^3 \mu\text{m}^3$	EM	296.3 days [†]
ATLUM [38]	~0.01 μm	0.05 μm	$\sim 2.15^3 \text{ mm}^3$	EM	See SBF-SEM

*Experimental scans initiated. [†] 200 μm cube at 10 nm \times 10 nm \times 50 nm resolution [28].

B.2. Brain Maps and Atlases

The 3D mouse brain atlas, at a typical macroscale spatial resolution of 10 μm , is an indisputable guide to navigation within the mouse brain [79, 80].

The High Resolution Mouse Brain Atlas [87], based on The Atlas of the Mouse Brain and Spinal Cord [86], uses C57BL/6J strain mice specimens, and has 572 coronal sections where each section is 20 μm thick. The odd-numbered sections are stained with the Nissl stain and the even-numbered sections are stained for myelin. The atlas has 10 μm *x*- and *y*-resolution, and interpolates three equidistant images to obtain a 10 μm resolution in the *z*-axis to yield 10 μm isomorphic voxels. For example, the resolution of slices in this atlas is as follows: 572 coronal sections at 20 μm thickness yielding an interpolated voxel size of 10 μm \times 10 μm \times 10 μm , which is too coarse to map out the morphology of individual neurons.

The Mouse Atlas Project (MAP) [53] is developing a probabilistic atlas of the adult and developing C57BL/6J mouse. MAP consists of not only data from Magnetic Resonance Microscopy (MRM) and histological atlases, but also a suite of tools for image processing, volume registration, volume browsing, and annotation. MAP will produce an imaging framework to house and correlate gene expression with anatomic and molecular information drawn from traditional and novel imaging technologies. This digital atlas of the C57BL/6J mouse brain is composed of volumes of data acquired from μ MRI, blockface imaging, histology, and immunohistochemistry. MAP technology provides the infrastructure for the development of the Allen Brain Atlas [54] (see below). Also see the related Mouse BIRN (Biomedical Informatics Research Network).

The Allen Brain Atlas [3, 49] contains detailed gene expression maps for ~20,000 genes in the C57BL/6J mouse. A semi-automated procedure was used to conduct *in situ* hybridization and data acquisition on 25 μm -thick sections (*z*-axis) of the mouse brain. The *x*-*y*-axis resolution of the images range from 0.95 μm to 8 μm . The Allen Brain Atlas is the first comprehensive gene expression map at the whole-brain level, and is currently accessed over 4 million times per month,

with over 250 scientists browsing the data on a daily basis (see [3], frequently asked questions in the "community page").

The Mouse Brain Library (MBL) [82] is developing methods to construct atlases from celloidin-embedded tissue to guide registration of MBL data into a standard coordinate system, by segmenting each brain in its collection into 1200 standard anatomical structures at a resolution of 36 μm . Algorithms are to be designed to segment each brain in the MBL into a set of standard anatomical structures like those defined in the rat atlas produced by [24], whose computerized 3D atlas [25] was built from stained sections for the mouse brain that reconstructs Nissl-stained sectional material, a 17.9 μm isotropic 3D data set, from a freshly frozen brain of an adult male C57BL/6J strain mouse.

NeuroMouse [78] is an interactive graphical database which provides structural, molecular, and genetic information on the adult murine nervous system and its relevance to human neurobiology. It is based on photographic serial sections in the coronal, sagittal, and horizontal planes with average plate distance of $\sim 300 \mu\text{m}$.

The Edinburgh Mouse Atlas Project (EMAP) [8, 31] is building a digital atlas of mouse embryonic development. The EMAP atlas is based on the books of mouse embryonic development by Theiler [92] and Kaufman [44]. It will consist of a series of interactive 3D computer models of mouse embryos at successive stages of development with defined anatomical domains linked to a stage-by-stage ontology of anatomical names.

The μMRI Atlas of Mouse Development [41], a 3D digital atlas of mouse development, allows querying cerebral structures of a 13.5 days post coitum (dpc) mouse embryo and viewing 3-D reconstructions of those structures as well as reconstructions of the entire embryo. It contains non-annotated μMRI sections at several stages of development. Gene expression patterns can be input into the atlas.

BrainMaps.org [10, 75] is an internet-enabled, high-resolution brain map. The map contains over 10 million mega pixels (35 terabytes) of scanned data, at a resolution of 0.46 $\mu\text{m}/\text{pixel}$ (in the x - y plane). The atlas provides an intuitive web-based interface for easy and band-width-efficient navigation, through the use of a series of subsampled (zoomed out) views of the data sets, similar to the Google Maps interface. Even though the x - y plane resolution is below 1 μm , the z -axis resolution is orders of magnitude lower (for example, one coronal brain set has 234 slides in it, corresponding to a sectional thickness of 25 μm).

Whole Brain Catalog (WBC) [96] is a 3D virtual environment for exploring multiple sources of brain data (including mouse brain data), e.g., Cell Centered Database (CCDB, see below), Neuroscience Information Framework (NIF), and the Allen Brain Atlas (see above). WBC has native support for registering to the Waxholm Space, a rodent standard atlas space [42]. Multiple functionalities including visualization, slicing, animations, and simulations are supported.

In summary, there are several mouse brain atlases available, with data from different imaging modalities, but their resolution is not high enough in one or more of the x , y , or z axes to show morphological detail of neurons or microvascular segments.

B.3. Databases of Three-Dimensional Reconstruction of Neurons

The low spatial resolution in existing whole-brain-level brain maps and atlases have been pointed out as a major limitation. Near-micron-level reconstructions of brain areas do exist, but only for a small volume. Part of the reason is that, in many cases, the geometric reconstructions were done manually, with the aid of interactive editing tools like NeuroLucida [35, 74], Reconstruct [33], or Neuron_Morpho [12, 26].

The Duke/Southampton Archive of Neuronal Morphology, an on-line archive of neuronal geometry [14, 15] includes full 3D representations of 124 neurons from the rat hippocampus, obtained following intracellular staining with biocytin and reconstruction using Neurolucida. The archive includes data both in the native format as supplied from the digitization software, and in a simpler, 3D standardized format (given the extension ‘SWC’ in the archive). The data for the SWC files are obtained by fitting cell segments in three dimensions with cylinders, directly confirming the location and size of these shapes using a computer-based tracing system.

NeuroMorpho.org, is a centrally curated collection of reconstructed neurons, currently containing 4508 cells from various species and brain regions [6]. The data are available for download in SWC format. *L-neuron* is a modeling and analysis project that is associated with this database, where statistical features of dendritic geometry and stochastic generation of (statistically) realistic neurons are studied [5, 7, 84].

The Cell Centered Database (CCDB) [16, 56] houses high-resolution 3D light and electron microscopic reconstructions spanning the dimensional range from 5 nm^3 to $50\text{ }\mu\text{m}^3$ produced at the National Center for Microscopy and Imaging Research (NCMIR) [76]. The current CCDB has over 80 tables containing descriptive data that models the entire process of reconstruction, from specimen preparation to segmentation and analysis.

The SynapseWeb [34] is a portal into a dense network of synaptic connections and supporting structures in the gray matter of the brain that can be fully visualized only through 3D electron microscopy. It provides an interface for examining volumes of brain tissue at nanometer resolutions which have been reconstructed from serial section electron microscopy. Currently, the SynapseWeb houses three brain volumes ranging from $62\text{ }\mu\text{m}^3$ to $108\text{ }\mu\text{m}^3$ from the CA1 regions of rat hippocampus.

In summary, there are several excellent neuronal morphology databases that serve the neuroscience community, but they are limited to a small number of neurons from limited volumes.

B.4. Atlasing and Neuronal Morphology Description Standards

A rapid increase in web-based resources serving neuronal morphology and atlas-scale data sets gave rise to the need for data representation standards.

The Waxholm Space [42] is a new standard atlasing space for rodents. The effort to build this standard space was motivated by multiple non-standard, yet widely used coordinate spaces such as those in the Allen Brain Atlas [3, 49] or Paxinos and Franklin’s atlas [79].

As for neuronal morphology, NeuroML [77] has become the de facto standard (XML). BrainML [11], on the other hand, provides an XML framework for the exchange of general neuroscience data at the whole brain scale.

C. Prior Work

We have completed the sectioning and imaging of two additional whole mouse brains, resulting in 2 TB of data per brain, at a submicrometer resolution. We have also developed a prototype data dissemination framework for web-based delivery, and visualization and tracing algorithms for small volumes of data.

C.1. Imaging with the Knife-Edge Scanning Microscope

The Knife-Edge Scanning Microscope (KESM, US patent #6,744,572) [17, 48, 64, 66, 67, 69] has been designed at Texas A&M University (TAMU) in recent years with support from the National Science Foundation (MRI award #0079874; McCormick, PI), the Texas Higher Education

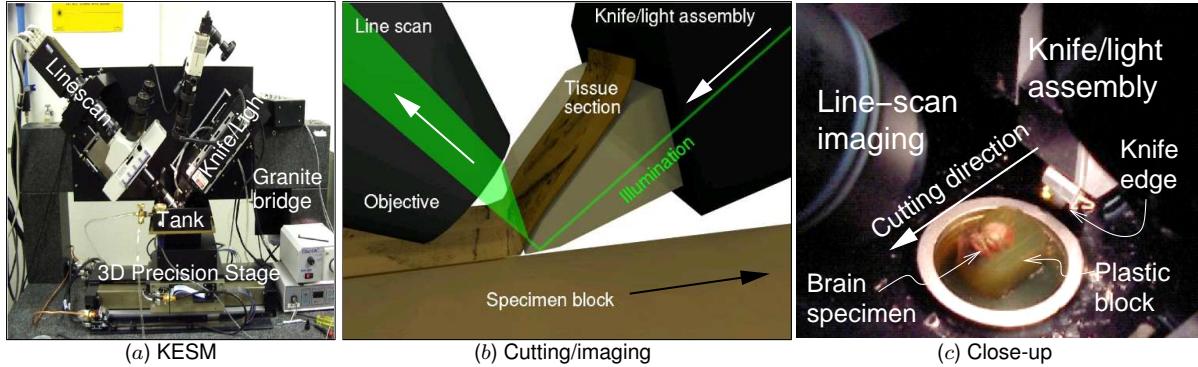


Figure 1: The Knife-Edge Scanning Microscope. (a) Photo of the KESM instrument showing line-scan/microscope, knife/light assembly, granite bridge, and 3D precision stage. (b) Specimen undergoing sectioning by knife-edge scanner (thickness of section is not drawn to scale). (c) Close-up photo of the line-scan/microscope assembly and the knife/illumination.

Coordinating Board (ATP award #000512-0146-2001; Keyser, PI), and the National Institute of Neurological Disorders and Stroke (Award #1R01-NS54252; Choe, PI). The instrument, shown in Fig. 1a, is capable of scanning a complete mouse brain ($\sim 310 \text{ mm}^3$) at 300 nm sampling resolution within 100 hours when scanning in full production mode. The instrument comprises four major subsystems: (1) precision positioning stage, (2) microscope/knife assembly, (3) image capture system, and (4) cluster computer. The specimen, a whole mouse brain, is embedded in a plastic block and mounted atop a three-axis precision positioning stage. A custom diamond knife, rigidly mounted to a massive granite bridge overhanging the three-axis stage, cuts consecutive thin serial sections from the block. Unlike block face scanning, the KESM concurrently both cuts and images (under water) the tissue ribbon as it advances over the leading edge of the diamond knife. A white light source illuminates the rear of the diamond knife, and in turn illuminates the brain tissue at the leading edge of the diamond knife with a strip of intense illumination reflected from the beveled knife-edge, as illustrated in Fig. 1b. The microscope objective, aligned perpendicular to the top facet of the knife, images the transmitted light. A high-sensitivity line-scan camera repeatedly samples the newly cut thin section, imaging a stripe 20 μm wide across the tissue ribbon and just beyond the knife-edge, prior to subsequent deformation of the tissue ribbon after imaging. Finally, the digital video signal is passed through image acquisition boards and stored in a dedicated cluster computing system. A custom software developed in-house at Texas A&M is used to control the stage movement and imaging process in a fully automated manner. See Fig. 2 for imaging results.

C.2. Rapid tracing of fibrous matter

In order to turn the raw data into a geometric description of the objects of interest (i.e., reconstruction), we are currently developing rapid tracing algorithms for fibrous matter such as neuronal processes and neurovasculature [65], which is broadly classified as a vector tracing method [2, 13]. Our method has been used successfully on KESM data. See [60].

C.3. Web-based light-weight 3D data stack viewer

We have taken initial steps in making available the massive volume image stack from KESM over the internet using standard web browsers without any add-on or plug-in. The basic idea is to use image overlays with distance attenuation and offsetting (shearing) to generate a stereo pair for a vivid 3D viewing experience. See [32] for details.

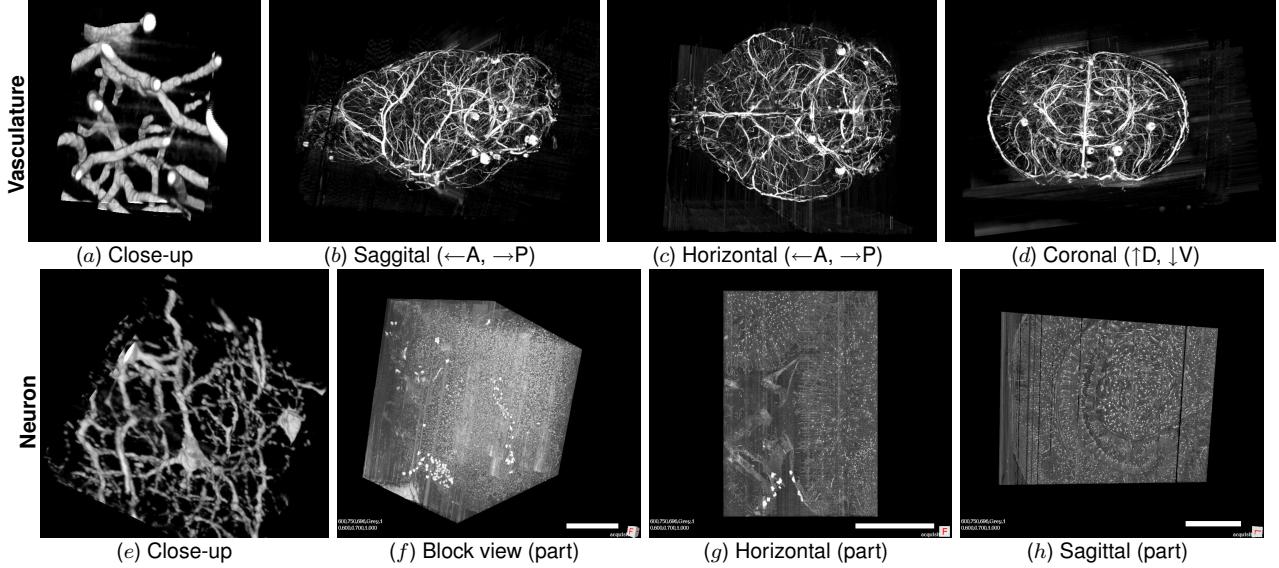


Figure 2: KESM Data. Volume visualizations of KESM data stacks are shown for the vascular data set (top row, India ink stain) and the neuronal data set (bottom row, Golgi stain). (a) Close-up of the vascular data. Width $\sim 100 \mu\text{m}$. (b–d) Three standard views of the whole mouse brain vasculature (subsampled from high-resolution data). Width $\sim 10\text{mm}$. (e) Pyramidal cells from the visual cortex. Width $\sim 100 \mu\text{m}$ (f) A large sub-volume from the Golgi data set. Fine details are washed out. (scalebar = 1.44 mm) (g) A thin slab from (f) reveals intricate circuits (horizontal section). (scalebar = 1.44 mm) (h) A thin slab from (f) reveals intricate circuits (sagittal section). (scalebar = 1.44 mm)

C.4. Results from prior NSF support

For results from the NSF MRI award (#0079874, PI: McCormick), see §C.1–§C.2. The NSF-supported MRI project received two subsequent funding from the Texas Higher Education Coordinating Board (ATP#000512-0146-2001; Keyser, PI) and the National Institute of Neurological Disorders and Stroke (#1R01-NS54252; Choe, PI), producing over 30 publications since 2001. Co-PI Keyser’s research (#0220047; Keyser, PI) on accurate and robust operations on curved geometry resulted in 12 publications (see CV). The prior support by NSF most immediately relevant to this proposal is NSF CRCNS data sharing grant #0905041 (PI: Choe). The main, direct outcome of this grant is the KESM Brain Atlas, a web-based atlas containing whole-brain-scale Golgi and India ink data sets from the KESM (<http://kesm.org>; see Fig. 3 and 4). Publications made possible by this NSF grant are as follows: 3 journals [22, 23, 63], 4 conferences (full paper) [47, 62, 97, 98], 4 abstracts [18, 19, 90, 91], and 4 demos/exhibits at conferences [21, 90, 91] and other scientific meetings.

D. Research Plan

Here, we will discuss how we plan to organize and deploy our data sets, and what kind of supportive framework we will develop for effective and efficient dissemination.

D.1. Prepare Standardized Data Sets

We have already acquired full data sets for two additional mouse brains (C57BL/6J mouse), one stained in Golgi (neuronal morphology) and one in Nissl (cell bodies). Fig. 5 shows the new Golgi data set and Fig. 6 the new Nissl data set. The old Golgi data set was missing about 1/3 of the anterior pole, so the new complete Golgi data set fills the gap in the data. The Nissl data set is

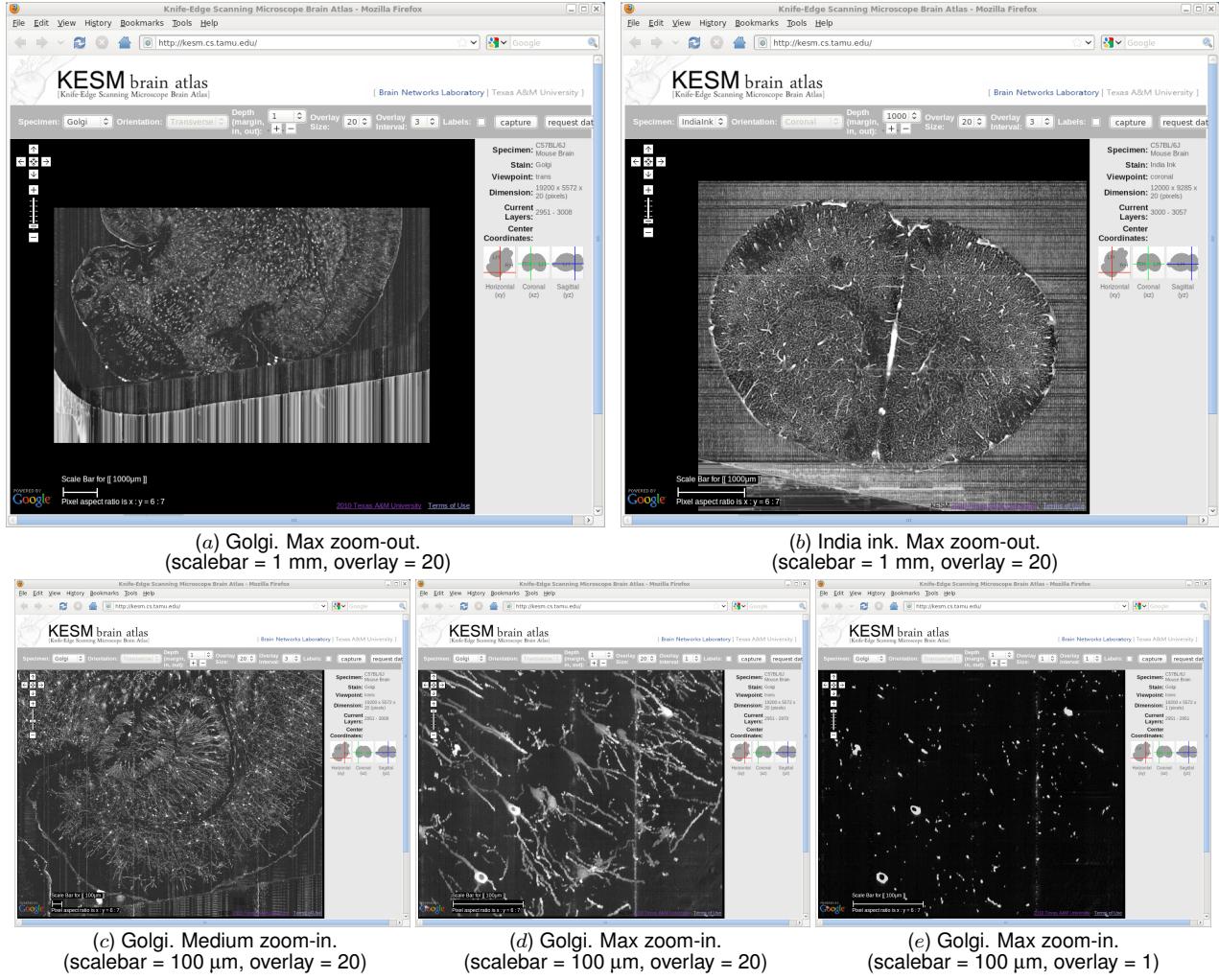


Figure 3: KESM Brian Atlas Prototype. Screenshots from the prototype KESM Brain Atlas are shown. (a) Golgi data set at max zoom-out. Overlay of 20 sections ($1 \mu\text{m}$ thick/section) at an interval of 3 (total visualized thickness of $60 \mu\text{m}$). Horizontal section. (b) India ink data set at max zoom out. Overlay method was the same as (a). Coronal section. (c) Golgi data set at medium zoom-in. Overlay of 20 sections at an interval of 3 (thickness = $60 \mu\text{m}$). The spiral-like Dentate gyrus and CA1 in the hippocampus are prominently visible. (d) Golgi data set at max zoom-in. Overlay of 20 sections at an interval of 1 (thickness = $20 \mu\text{m}$). Details of the hippocampus is shown. (e) Golgi data set at max zoom-in (same region as (d)). A single section (thickness = $1 \mu\text{m}$). Viewing individual sections like these does not give much insight. These results are a direct outcome of our on-going NSF CRCNS data sharing grant. **The atlas is now publicly available at <http://kesm.org>.**

a new modality, complementing the existing modalities of Golgi and India Ink. These data sets will augment our previous Golgi data set and India ink data set (vascular network) featured in our on-going CRCNS data sharing project (Fig. 2 and 3). We will also conduct additional imaging as needed, as part of our other on-going project (AUP 2009-197, approval date 10/13/2009, expiration date 10/12/2012). Most notably, we will section and image a small volume of commercially available GFP GABAergic mouse, using laser illumination (25mW, 470nm). Our initial experiment with fluorescent micro-beads embedded in nitrocellulose gave promising results (Fig. 7). We will also conduct Golgi and Nissl imaging to improve image quality. We expect this additional imaging to be completed by year 1, before the expiration of our AUP, but will extend it as needed.

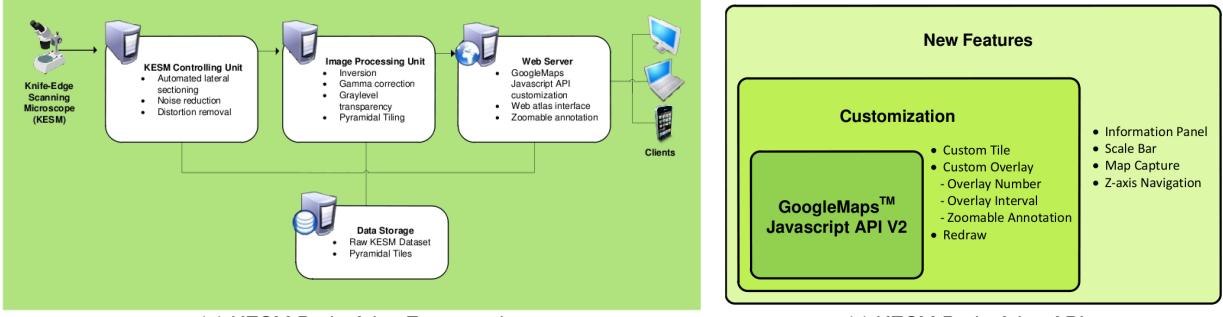


Figure 4: **KESM Brain Atlas Framework and API.** The overall system framework and the API organization of the prototype KESM Brain Atlas are shown. We will transition from Google Maps API to OpenLayer API.

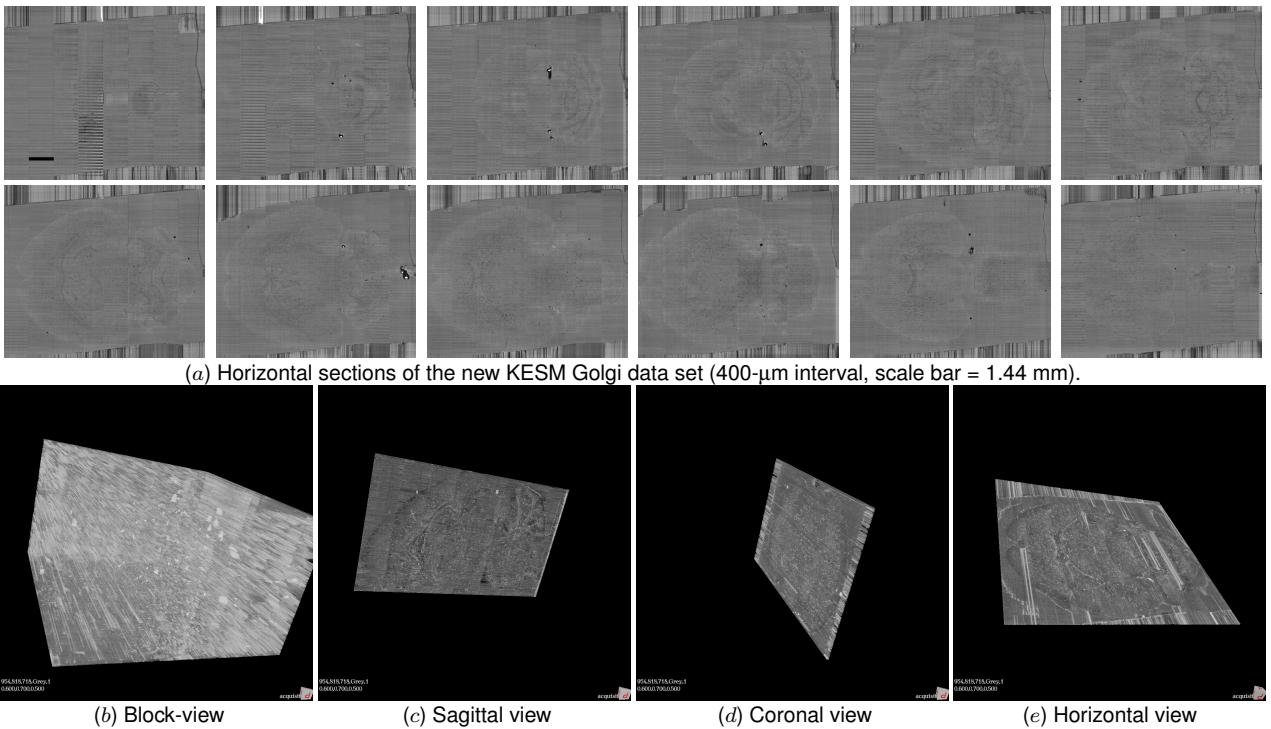


Figure 5: **KESM Whole-Brain Golgi Data (New).** The new KESM Golgi data set is shown. (a) 1 micron-thin coronal sections are shown at a 400- μ m interval. Scale bar (in first frame) = 1.44 mm. (b) A block view of the full data set. Fine circuit features are washed out and not visible at this level. (c) A thin sagittal slab of the block shows intricate circuits. (d) A thin coronal slab. (e) A thin horizontal slab.

We will extend our existing data scheme and develop algorithms to convert the raw image stack data into a multi-scale, standardized format. Data sets need to be prepared in two forms, one for web-based dissemination (multi-scale image stack, in JPG and/or PNG) and one for local viewing (unit-volume files, in DICOM, Analyze, and other standard data formats).

D.1.1. Imaging artifact removal and image registration

Knife-edge scanning microscopy has its own sources of noise and artifacts. The primary sources of error include: misalignment of the knife edge (causing a uniform gradient to appear in the image), defects in the knife blade (causing “streaks” in the y -axis of the image), oscillations in the light source due to power fluctuation (causing a regular pattern of light changes along the y -axis),

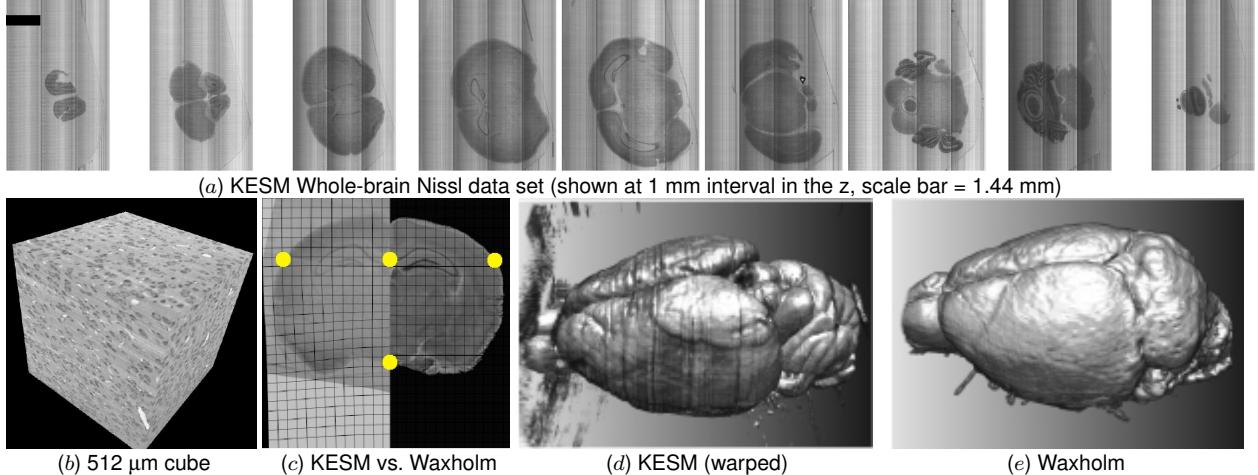


Figure 6: KESM Whole-Brain Nissl Data Set (New). Whole-brain Nissl data set from the KESM is shown. (a) Coronal sections spanning the entire brain, from anterior to posterior, at an interval of 1 mm, are shown. Voxel resolution = $0.6 \mu\text{m} \times 0.7 \mu\text{m} \times 1.0 \mu\text{m}$. Adapted from [18]. (b) A $512 \mu\text{m}$ cube is shown. This figure shows the 3D-nature of the KESM data set at its full resolution. (c) Registering the KESM Nissl data set (left) to the Waxholm space (right). The grids show the local warping applied and yellow discs the manually selected fiducial markers (not drawn to scale). (d) 3D visualization of the KESM Nissl data volume registered to the Waxholm space. Fine details such as the folds in the cerebellum can be seen (such details are not visible in (e)). (e) 3D visualization of the Waxholm reference atlas.

and knife chatter (causing horizontal lines and loss of most data at those pixels) in the image. Image registration issues are minimal due to the way KESM is designed and built. However, stopping and resuming can introduce slight misalignments between neighboring stacks of images. We have already developed methods for automatically cleaning this data [64]. These methods involve basic image processing techniques such as using a baseline scan for lighting, stretching contrast and normalizing intensities, and a variation on a local windowing approach. We have also developed preliminary methods for registration [48], and will extend these methods in this project. The proposed application will use proven standard image processing and GUI libraries. Furthermore, we will parallelize the image processing and image registration algorithms, since the current versions take a long time to go through the data sets. For this task, we will utilize the IBM p5 575+ grid (40-node, 640-processor, 1.9GHz, 32GB/node, 20TB disk storage) acquired by our department through the NSF Computing Resource Infrastructure grant (#0551685, PI: Valerie Taylor). This will allow us to experiment with the image processing parameters to produce an optimal normalized data set.

D.1.2. Prepare data for multi-scale viewing

We will continue to prepare a multi-scale data set from the original data sets. Efficient navigation will require multiple resolutions of the data to be communicated between the client and server. In order to reduce communication bandwidth, data sets at different zoom levels will be pre-generated and stored in our source database as scaled images (for web-based browsing) and also as cubical unit volumes (for download and local visualization, typically less than 0.5 GB in size). We will also prepare pre-overlaid data sets to make the viewing speed up to 20 times faster (see Fig. 11 for the performance of the current system). The storage overhead is expected to be minimal for the multi-scale format: for a 10-fold zoom out, 10 TB of data reduces to 10 GB, and so forth (i.e., 1,000-fold reduction in each step). When the user is looking at larger portions of the data, a lower resolution data set will be transmitted. As the user navigates in to look at specific features, higher resolution data will be sent, only for that local region.

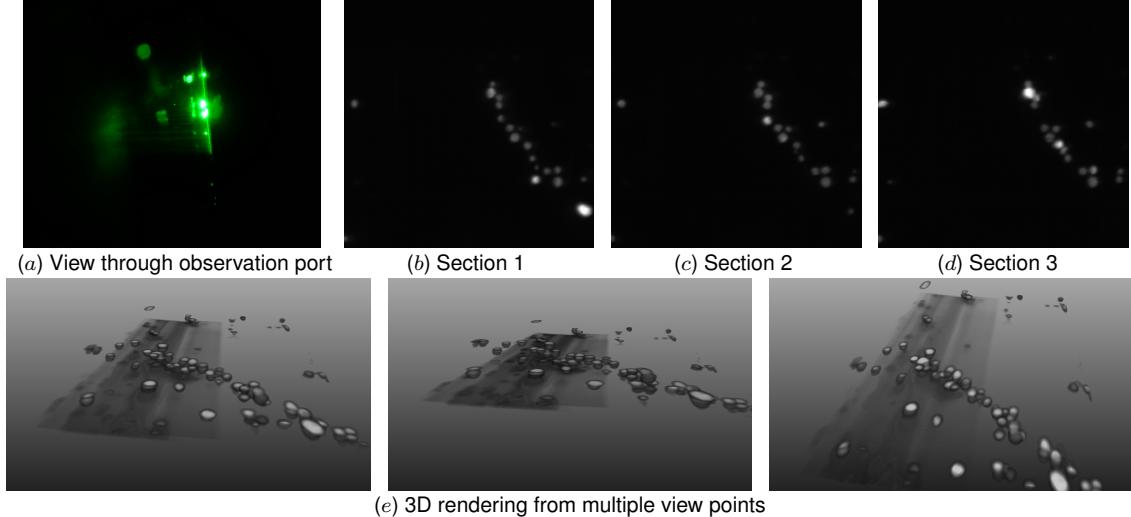


Figure 7: Fluorescence Pilot Results using KESM. Initial fluorescence imaging results using KESM are shown. Latex beads ($10\text{ }\mu\text{m}$ diameter) embedded in nitrocellulose were imaged, using 473 nm, 25 mW diode laser line generator (excitation) and Chroma D535/40M 34320 512 nm–552 nm emission filter. (a) Fluorescence observed through observation port. Scattered latex bead debris can be seen on the knife edge (long vertical bright region in the middle). (b)–(d) Fluorescence scanned with KESM. The images appear as grayscale since we are using a monochrome linescan camera. (e)–(f) 3D rendering of a small volume including (b)–(d). Note: the beads are $10\text{ }\mu\text{m}$ in diameter.

D.2. Transform KESM Data to Support All Three Standard Views

The current KESM brain atlas only supports the native orientation of the data sets. The Golgi data sets were obtained in horizontal sections, while the India ink and Nissl data sets were in coronal sections. However, as can be seen in Fig. 8, restricting to a single orientation hampers exploration and understanding of the data. We would like to minimize the need to download unit volumes for full 3D inspection, so a good alternative is to provide all three standard orientations: horizontal, coronal, and sagittal. The updated web atlas will have a pull-down menu to choose between these three standard orientations.

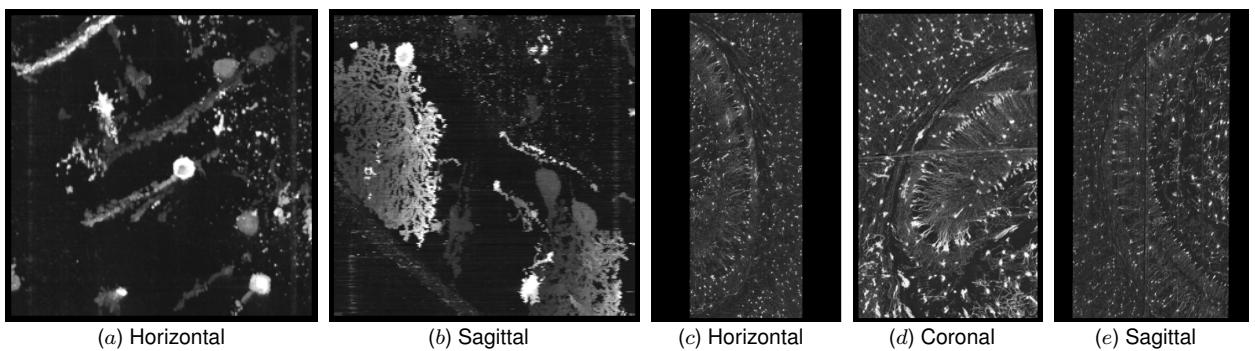


Figure 8: Comparison of Different Orientations. Change in the orientation reveals more intuitive details. (a)–(b) Cerebellar Purkinje cells. (a) In this standard view (horizontal), it is hard to see the familiar planar dendritic arbor of the Purkinje cell. (b) In this sagittal view, we can clearly see the familiar shape of the Purkinje cell. (c)–(e) The hippocampus, seen from multiple orientations.

D.3. Register KESM Data to the Waxholm Space

To enhance interoperability between the KESM mouse brain atlas and other mouse brain atlases, we will transform our KESM data sets into the Waxholm space, a new standard coordinate space for rodents [42]. The benefit of this step is mutual. We can pull detailed annotations at no cost from existing resources (see §D.6 for details) which include target volumes and a total of 55 3D data sets in the Waxholm space [42]. In turn, our high-resolution KESM data can provide rich anatomical data to other atlases. We have already begun work on registering KESM data to the Waxholm space, in collaboration with Dr. Jyl Boline at the International Neuroinformatics Coordinating Facility (INCF). The algorithm to the right outlines the steps. We employ a landmark-based 3D registration framework and use a stable 2D rigid deformation method for fine registration. This method, introduced by [83], is called “rigid transformations using Moving Least Squares (MLS)”. Figs. 6c–e show our initial results (Nissl data). We will apply this algorithm to all KESM data sets.

Algorithm 3D Registration Framework

- 1: $KIs \Leftarrow$ Prepare 2D images in the coronal plane from KESM.
 - 2: $WIs \Leftarrow$ Prepare 2D images in the coronal plane from the WHS atlas.
 - 3: $SI_s \Leftarrow$ Downscale KIs with the similar WIs size.
 - 4: $RIs \Leftarrow$ Roughly transform SI_s into WIs .
 - 5: $CPs \Leftarrow$ Identify corresponding anatomical landmarks between RIs and WIs .
 - 6: $Ps \Leftarrow$ Set deformation parameters based on CPs .
 - 7: $DI_s \Leftarrow$ Deform RIs using 2D rigid MLS deformations with Ps .
 - 8: $V \Leftarrow$ Reconstruct 3D volume from DI_s .
 - 9: Prepare 2D images in the horizontal plane from V and the WHS atlas, and follow the coronal plane steps.
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D.4. Register KESM Data to the Allen Brain Atlas

We will also register our KESM mouse brain atlas to the Allen Brain Atlas. The Allen Brain Atlas uses a custom coordinate space, and access to the atlas is facilitated using the Allen Brain Atlas API (ABA-API). There is active on-going work to register the Allen Brain Atlas into the Waxholm space, so in the long run, registering KESM data to Waxholm space will effectively have the data registered to the Allen Brain Atlas as well. However, we cannot wait for the completion of that transition, so we will use the same registration framework outlined above in §D.3 to register the KESM data sets to the Allen Brain Atlas. Once we have generated a transformed KESM data set, we will utilize ABA-API to overlay gene expression data onto the KESM brain atlas. The use of the gene expression data can help link physiological parameters to the purely anatomical data in the KESM brain atlas (see e.g. [94] on linking gene expression data and physiological parameters).

D.5. Transition from Google Maps API to OpenLayers API

In the current on-going CRCNS data sharing project, we adopted the Google Maps API and extended it with atlasing features such as minimap, scale bars, location indicator, and most importantly the transparent overlay for pseudo-3D rendering (Fig. 4). Some of these tasks were pretty straight-forward, but we experienced multiple difficulties when trying to extend the Google Maps API. Furthermore, the Google Maps API is strictly controlled by a commercial entity, and requires a license key, so an open-ended customization and deployment may become troublesome.

In order to overcome these limitations, we propose to transition our API to the OpenLayers API. The OpenLayers API is a free mapping and layering API supporting similar functionality as the Google Maps API, while maintaining a BSD-style license (clear BSD). OpenLayers is actively developed and maintained, unlike some other open source projects that have gone dormant. We will port our current Google Maps-based system to the OpenLayers API. Just like Google Maps API, OpenLayers API is also based on Javascript, so we expect the transition to be smooth. Both Google Maps and OpenLayers fundamentally catered toward 2D data, so we will have to make necessary adjustments to enable 3D rendering. Dealing with 3D data imposes exciting challenges. Here, we propose to continue with our light-weight approach to 3D rendering, only using

standard HTML, Javascript, and Cascading Styling Sheets (CSS) to achieve a quick, effective, and resource-efficient web-based interface. The open API based on OpenLayers will allow efficient layering of different types of annotations on the source volume database (e.g., see what Wikimapia, <http://www.wikimapia.org>, has done with the Google Maps data and API). Such annotations are recognized as an integral part of biological databases and will facilitate an increased rate of scientific discovery through the development and use of automated informatics tools [57].

D.6. Extensive Labeling by Annotation Import and Tracing

While navigation provides the ability to explore the KESM data, annotation is necessary to create and store interpretations of the data. Annotation features rely on the ability to specify views of the data and their subcomponents. Such a specification must include the visible portion of the data, the particular visualization of the data, and any overlays or data associated with the view. Annotations can be any form of user-specified content (textual, graphical) attached to a region in such a display, such as anatomical names, physiological characteristics, related literature citations, etc. Geometric primitives will be implemented so that regions of interest can be marked by pointing, clicking, and dragging the mouse on the zoomable web-based display. Depending on the primitive (point, line, oval, rectangle, polygon, or free-form closed contour), control points will be recorded and stored in an XML file associated with each annotation. This XML file will also include textual descriptions (see discussion on ontologies below), and linkage to neighboring annotations in case several object boundaries together form a single macro object. See, e.g., Wikimapia (<http://www.wikimapia.org>) in relation to Google Maps. Annotations can also be used to identify important or interesting areas in data [85].

Another important issue is that of the ontology. With the increase in the number of biological databases, one major concern is about interoperability of data, and thus standardization is becoming an important issue. A common ontology to express domain knowledge is thus important. There are existing efforts in this direction such as the Subcellular Anatomy Ontology (SAO) by the Cell-Centered Database (CCDB) project [57], NeuroML [77], and BrainML [11]. We will adopt these standards in the annotation system. Metadata conforming to these ontologies will be stored in XML files, which can be easily parsed and used in Javascript.

With the annotation framework in place, we will utilize existing annotations in the Waxholm Space data volumes and in the Allen Brain Atlas data volumes. As the KESM data will be registered with these two standard atlas space as part of the proposed project (§D.3 and §D.4), we expect this step to be straight-forward. A main research issue is to reciprocate this back to the Waxholm Space atlases and to the Allen Brain Atlas. We will develop compatible API calls so that those using the Waxholm Space atlases and Allen Brain Atlas can import anatomical data from our KESM Brain Atlas.

Finally, we will perform automated tracing and semi-automated validation in small sample volumes from major regions of the cortex and other structures such as the thalamus, basal ganglia, hippocampus, and cerebellum. Full tracing is far beyond the scope of this project, and we are seeking alternative funding sources for that (NSF ABI proposal). For tracing and validation, we will use our own algorithms [37, 58, 61, 71, 97, 98] and the DIADEM-award-winning [51] FARSIGHT toolkit (by Badrinath Roysam's group at University of Houston) [52].

D.7. Develop an Enhanced Unit-Volume Viewer

We have developed a prototype unit-volume viewer for use with downloaded unit volumes from the KESM brain atlas (Fig. 9). Our unit volumes are also viewable using existing visualization packages such as Amira (commercial) and MeVisLab (free for noncommercial usage). Our prototype unit-volume viewer replicates a subset of functionality of Amira and MeVisLab. However, extended

usage of our unit-volume viewer and MeVisLab has allowed us to discover some non-obvious needs that could greatly enhance user experience and rate of discovery. These needs include: (1) scale bars, (2) thin-slab sweep (see Fig. 10), (3) ruler, and (4) movie clip generator (from item 2). Some of these features are available in Amira and MeVisLab, but it requires a lot of effort to use these features. We will incorporate these features in our enhanced unit-volume viewer and provide an easy-to-use interface.

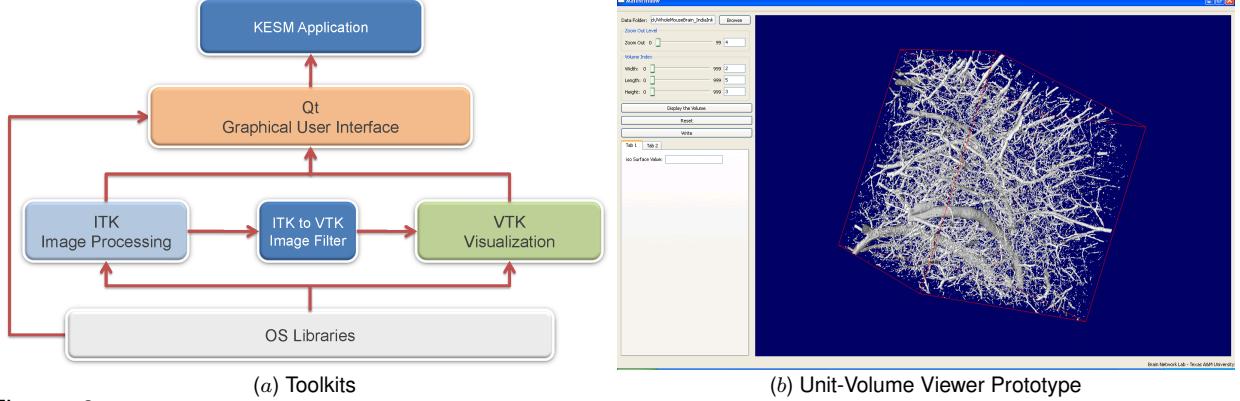


Figure 9: Toolkits and Prototype Unit-Volume Viewer. (a) The different toolkits and libraries used in our prototype unit-volume viewer are shown. (b) A screenshot of our unit-volume viewer displaying a small vascular network data is shown.

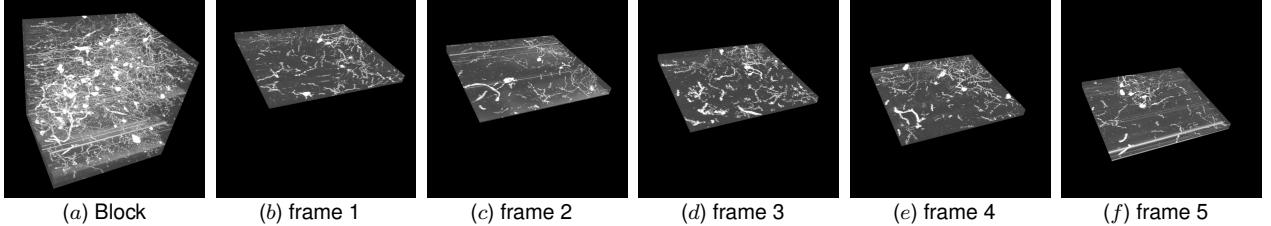


Figure 10: Sweeping Through the Volume with a Thin Slab. (a) When thick volumes are visualized, it is hard to appreciate the detailed circuit inside the block (block size = $360 \mu\text{m}^3$ cube). (b)–(f) Sweeping through the volume with a thin slab can reveal local circuits and their change over the depth of the block.

D.8. Develop a Turn-Key Mirroring Facility and Initiate Torrent Seeds

Our 2 years of experience with the prototype KESM Brain Atlas gave us a strong need to set up mirror sites to facilitate broader dissemination of our data and our neuroinformatics framework. First of all, we would like to note that the download time to view a single snapshot in the KESM web atlas (see Fig. 3) at any moment is strictly bounded by the browser window size. Although the full data can range up to 2 GB, due to the multi-scale representation and the tiling scheme, only $2 \times 4 \times n \times s$ bytes need to be downloaded at any moment, where 2×4 is the tile rows and columns (each tile is a 256×256 pixel image), n is the number of overlays, and s is the average size of each image tile. As we can see from our initial performance study (Fig. 11), download time for a small number of overlays ($n \leq 10$) scales well. However, for larger values of n , the download/rendering time diverges depending on the locality of

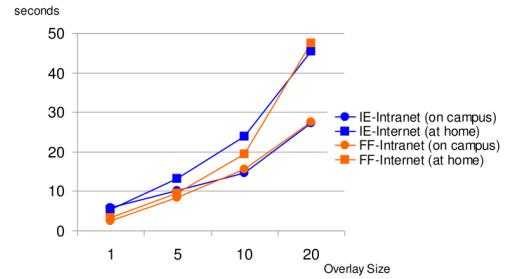


Figure 11: KESM Atlas Access Time. x-axis: number of overlays. y-axis: download time (seconds).

the data (e.g., campus network vs. through third-party ISP could show a factor of 2 difference). To minimize download delay and to increase user experience, we are planning to deploy mirror sites on all major continents, in collaboration with colleagues and with international entities such as the INCF and OpenConnectomics.org. To facilitate this, we will develop a turn-key mirroring facility so that we can quickly deploy our KESM brain atlas on third-party mirror sites. The mirroring facility will consist of an external hard drive or a network attached storage (NAS) that will be populated with our data (full multiscale tiling). The facility will be self-contained, with full atlasing source code. Simply mounting the external drive (or NAS) below the mirror site's web directory will instantly create a mirror site, with minimum effort on the part of the mirror site maintainers. We will modularize the atlasing code so that as much functionality as possible is pulled from our main atlas code base. We expect this scheme to minimize maintenance in the long-run, and reduce the need for periodic updates of the mirror site code base.

Finally, we acknowledge that some users would need the full, maximum resolution data sets. For this, we will set up a Torrent seed for rapid, distributed, peer-to-peer download. Once a sizable number of users have downloaded full data sets and start seeding them, general accessibility of the full raw data will dramatically increase.

D.9. Anticipated Usage of the Atlas in Research

Dynamic analysis: Certain dynamic parameters such as conduction delay can be estimated based on axon length and diameter. Simply calculating the delay distribution can already provide great insights into brain function. For example, [93] showed that the complexity of network dynamics critically depends on the delay distribution. Also see [88] on the relationship between neuroanatomy and brain dynamics.

Connectivity estimation: Data based on LM typically show only a fraction ($\sim 1\%$ for Golgi) of the entire population of neurons. That is, the data is sparse. In this case, we need to estimate connectivity. Methods like those proposed by [43] can be used for this purpose. Also, a systematic simulation study can be conducted with a full synthetic circuit, by dropping a certain proportion of connections and observing the resulting change in behavior. The degree of redundancy in the connections (both for real and synthetic circuits) will play an important role here.

Linking with gene expression data: The connectome is fundamentally a static structure. How can the physiological properties be inferred from just the structure? [94] shows a possibly powerful solution to this: Use gene expression data. They found that gene expression and electrophysiological properties are closely correlated. The availability of very large gene expression atlases such as the Allen Brain Atlas [49] (22,000 genes), and imaging modalities such as Array Tomography that support molecular as well as EM imaging [72] are great resources for this kind of approach (see, e.g., [55]).

Inter- and intra-specimen variability estimation: Simply measuring the morphological variability among the same class of neurons can provide valuable insights into how redundant or specialized the functions are (Gerald Edelman, personal communication, 2009; see [6] for an existing morphological database). Even when connectivity is not known, just examining the dendritic trees can give deep insights into neural computation [70, 81].

Brute-force parameter search and simulation: Of course a straight-forward yet potentially valuable approach is to start with computational simulation based on detailed neuronal morphology (cf. the Blue Brain Project [55]). The reconstructed geometry can be used to construct multi-compartment models (see e.g. [27]). Appropriate parameters such as channel conductance, capacitance, etc. need to be figured out. Tools like NEURON, GENESIS, neuroConstruct, and NeuGEN can be used for multi-compartment simulation and parametrized synthetic circuit gener-

ation/simulation/analysis [4, 9, 30, 36, 39, 45, 46]. Data from the KESM can help narrow down on the range of various parameters for these simulations (see e.g. [29]).

Investigate the effect of link fidelity: A great matter of debate in connectomics is whether individual connections matter (detailed EM info needed), or whether they can be averaged (diffusion MRI is enough). Some results suggest that dropping even a single spike in the initial condition can have a global effect on the entire cortex within 0.5 second (see [40]’s large-scale simulation study of the thalamocortical system based on Diffusion Tensor Imaging data). However, considering that the brain in a normal operating environment is always anchored to the present input stimulus, constantly resetting the initial condition, this may not be a serious issue. Issues like these can be studied based on circuit data estimated from the KESM data sets.

E. Management and Broader Impact

Management Plan: See the budget justification for PI responsibilities and project timeline.

Plan for Preparation and Deployment: See §D.1 for the data preparation process. We have acquired a dedicated domain name to set up a web portal (<http://kesm.org>). First, the source database with the mouse brain data from KESM will be made available on the internet as a web service (year 1). Next, the open API for access and annotation of the database will also be made available online, with continued maintenance by the project team (year 2). The current atlasing code is already available at <http://sourceforge.net/p/kesmba>. We will actively advertise this new resource through various channels: mailing lists, news briefs in scientific publications, personal contact, and demos and exhibitions at scientific meetings. We will also organize short courses and workshops to expand and support the user community. For the above, the PI (Choe) will depend on his extensive experience in most of the above activities.

Software Development and Sustainability: The PI/Co-PIs will design the main software architecture and do partial implementation, and a graduate student will assist in the design process and carry out the implementation. In order to ensure continued support of the informatics platform developed through this project, we will design and implement a protocol for software development, documentation, and education. We will make extensive use of the collaborative software development platform SourceForge. All members of the development team (both internal and external) will be trained to abide by this protocol. Involvement of external developers as well as department-funded graduate students (typically two students who carry out a dual duty as teaching assistants) will ensure continued support beyond the funding period. We expect maintenance to require less effort once the initial platform is implemented during the project period. We will also seek continued funding from follow-up grants and from collaborations made through the web platform (there are continued software development and maintenance programs at the National Institutes of Health).

Education and Outreach Plan: We will train a computer science graduate student so that he/she can become an expert in neuroinformatics. We have trained undergraduate students in the past through the NSF Research Experience for Undergraduates program, and will continue to utilize this mechanism. The neuronal data from the whole mouse is expected to serve as a rich resource for educational use. The multi-scale data scheme, together with custom annotations, will allow us to turn the web-based interface into a major educational resource for all levels of education (K-12 to graduate). We will set up a scaled-down version of the KESM Brain Atlas on <http://kesm.org/kids> so that K-12 students and teachers can have a centralized access. The K-12 education portal will include (1) interactive tutorials highlighting the history of neuroscience and the key role played by neuroanatomy and (2) an Easter-egg hunt game using the web-based atlas, to allow students to search for key anatomical features they learned in the tutorial.

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