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Three-Dimensional Imaging Using Knife-Edge Scanning Microscopy

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A prototype Knife-Edge Scanning Microscope (KESM) has been designed at Texas A&M University in recent years [1]. The goal of the KESM is to allow the scanning of high-resolution data sets of tissue, allowing for the reconstruction of complete cellular systems such as a whole mouse brain. The instrument, shown in Fig. 1(a), is capable of volume digitizing a complete mouse brain (~1cm^3) at 300nm sampling resolution within 100 hours. The instrument comprises four major subsystems: (1) precision positioning stage, (2) microscope/knife assembly, (3) imaging system, and (4) cluster computer. The brain specimen 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 serial sections from the block. A white light source illuminates the brain tissue at the diamond knife tip with a strip of intense illumination. A microscope objective, aligned perpendicular to the top surface of the knife, images the light reflection from the knife-edge, as illustrated in Fig. 1(b). Thus, the diamond knife serves a dual use: as an optical prism and for physical sectioning. A high-sensitivity line-scan camera captures the newly-cut thin section just beyond the knife-edge, prior to subsequent deformation of the tissue ribbon. Finally, the digital video signal is passed through image acquisition boards and stored for subsequent analysis in a cluster computing system. The cluster consists of 5 servers, each dual processor (1.1-1.5 GHz), 2GB of memory, a combined 1 TB hard drive capacity, and linked by a Cisco gigabit/s switch.

Knife-edge scanning, introduced in the KESM instrument, not only preserves image registration throughout the depth of the specimen block but also isolates the tissue above the knife from that below to eliminate undesirable events (back-scattering of light and bleaching of fluorescent-stained tissue below the knife). Knife-edge scanning supports all known forms of microscopy: absorption imaging using transmitted light, and reflected light imaging using bright-field, dark-field, DIC, and fluorescence. Methods have been developed for reducing both noise and required knife sharpening. Several new database techniques, developed by Koh [2], are used for data storage and retrieval.

Fig. 2 shows a section of Nisssl-stained mouse brain. Several consecutive KESM sections can be taken and processed, allowing the extraction of isosurfaces representing cell bodies in the original tissue, as seen in Fig. 4(a) and Fig. 4(b). Fig. 3 shows Golgi-stained mouse brain tissue.. After sectioning and processing, we are able to extract the morphology of neuron cell bodies and their processes, as illustrated in Fig. 4(c, d, e) [3].

[1] B.H. McCormick, Inventor, *System & Method for Imaging an Object*, U.S. Patent Application #09/948,469, U.S. PTO (for Knife-Edge Scanning).

[2] W. Koh and B.H. McCormick, *Specifications for Volume Data Acquisition in Three-Dimensional Light Microscopy*, Technical Report TR2003-7-5, Department of Computer Science, Texas A&M University, College Station, TX, 2003.

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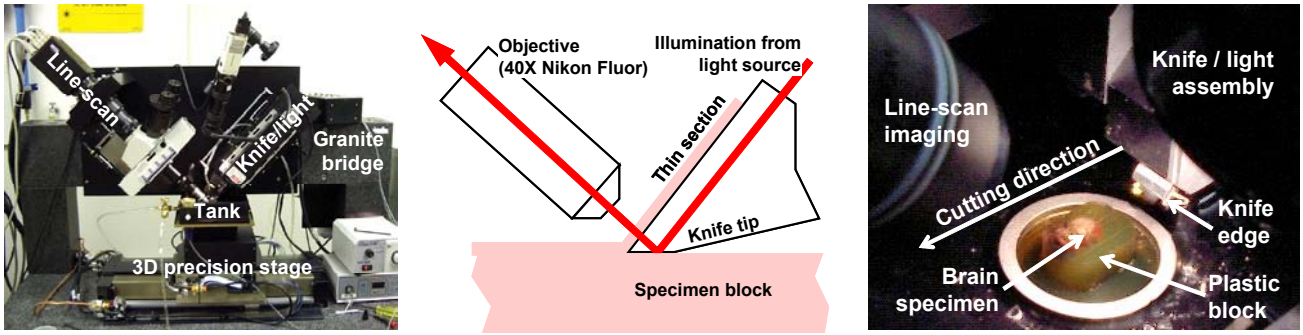


Fig. 1. (a, left) Photo of the KESM. (b, center) Diagram showing light refraction and imaging of knife edge. (c, right) Close-up photo of the line-scan/microscope assembly and the knife/light assembly.

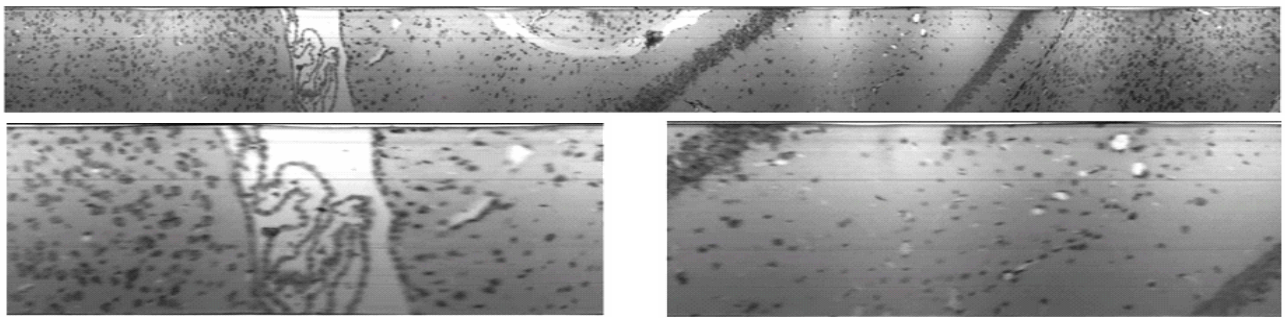
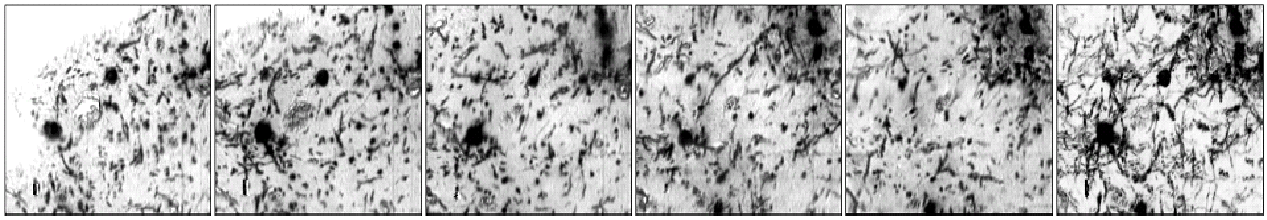
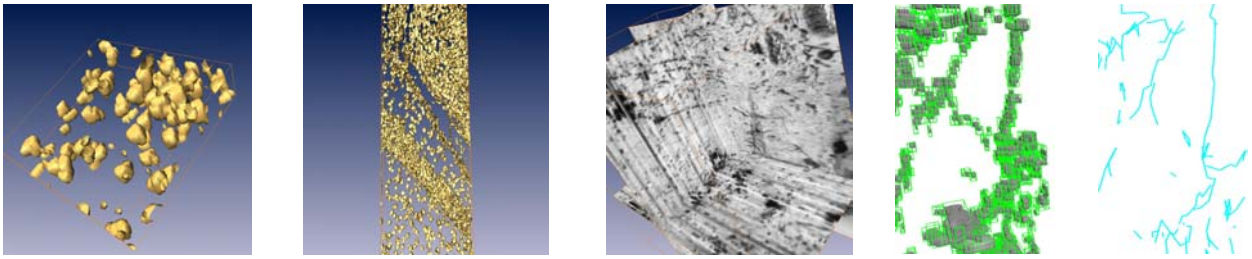


Fig. 2. Nissl-stained section of a mouse brain at 10X magnification (coronal section) with close-up images of the lateral ventricle (left) and hippocampus (right).



(a) Slice 0 (b) Slice 5 (c) Slice 10 (d) Slice 15 (e)Slice 20 (f) Overlay

Fig. 3. Sequence of Golgi-stained tissues at 10X. (a-e) Every fifth slice is shown from slice 1 to slice 20. (f) An overlay of all 20 slices is shown. Neurons and their processes are clearly visible.



(a) (b) (c) (d) (e)

Fig. 4. (a,b) Reconstruction of cell bodies from 10x scan of Nissl-stained hippocampus. (c) a view of three cross sections through a volume of scanned Golgi data. (d) an L-block covering of a portion of the set. (e) the initial threads reconstructed.