NANOMACHINING FOR HIGH-RESOLUTION SCANNING OF MAMMALIAN BRAIN MICROSTRUCTURE

B.H. McCormick[1], D.M. Mayerich[1] and M Wiercigroch[2]
[1]Department of Computing Science, Texas A&M University, College Station, TX, 77843-3112 USA
[2]Department of Engineering, University of Aberdeen, Aberdeen AB24 3UE, UK

ABSTRACT

The paper discusses how ultra precision nanomachining can provide a breakthrough in reconstructing the neuronal networks of mammalian brains. To explore this unknown territory a new instrument developed at Texas A&M, the *Knife-Edge Scanning Microscope (KESM)*, will be presented. The instrument comprises four major subsystems: precision positioning stage, microscope/knife assembly, imaging system, and cluster computer. The specimen (a whole mouse brain) is embedded in a plastic block and mounted atop a three-axis precision positioning stage. The instrument uses diamond knife sectioning of the plastic-embedded brain, with layers typically a few hundred *nm* thick. Sectioning at 300*nm* resolution allows to create an aligned volume data set of ~12 terabytes representing the tissue microstructure of entire brain. Preliminary cutting tests have shown that the major obstacle to obtain robust data are self-excited oscillations (chatter) generated during the cutting process, when sequential layers are being removed from the sample. It is clear now, that the regeneration effect and free oscillations caused by the sudden nature of the tool engagement into the sample, play paramount roles. Slight alterations of the cutting velocity for each pass have partially suppressed the chatter, however, not to extend that KESM can be used effectively unmodified. The main modifications to the current design are aimed to produce a chatter free operation. This can be achieved by employing an ultra-precision three-axis CNC lathe designed for single point diamond turning, and featuring the angular and axial motion accuracy of 8*nm* and and subnanometer slide feedback resolution.

1 INTRODUCTION

The neuronal connectivity of human and other mammalian brains is largely uncharted. Anatomically correct network models of the brain do not exist at present for the mammalian brain of any species; there is simply not enough three-dimensional (3D) neuroanatomical data available concerning mammalian brain microstructure and, specifically, its distribution of neurons and their connectivity. To explore this unknown territory, we aim to image and reconstruct the whole mouse brain in three dimensions at a neuronal level, exhibiting the cell bodies and dendritic and axonal arborization of the neurons. Then 3D geometric modelling will be undertaken in order to construct anatomically correct models of mouse brain networks. This knowledge is a fundamental precursor to detailed functional modelling of mammalian brain networks. To facilitate this development new large-scale 3D microscopes can be designed, capable of processing teravoxels of image data per day, to open the internal connectivity of brains of all species to measurement and modelling of brain architecture at a neuronal level of detail. Below we propose a first such instrument designed to exploit recent advances in nanomachining.

We propose to characterise the mouse brain by discovering its basic circuits, which model the topology and geometry of a significant part of the entire brain network. Basic circuits in the mammalian brain are characterised by (1) the morphology and neurotransmitter type of their participating neurons (typically ~5 types/nucleus or cortical area) and (2) their characteristic interconnection patterns. Fig. 1. shows one such basic circuit, first described by Ramon y Cajal ca. 1905 [1], now believed to be replicated at least 100,000 times in the mouse cerebral cortex. Shepherd [2] pioneered this mode of description of mammalian brain networks in his classic *Synaptic Organization of the Brain*. Shepherd's text exhibits ~60 known basic circuits with their variants. We propose to extend this first catalogue of basic circuits by analysing those we discover empirically from our model reconstruction of networks in the mouse brain. Furthermore, the

spatial distribution of these basic circuits can be determined, their lattice structure where applicable, and more generally, their mutual interconnection.

Basic circuits will be treated as *stochastic generators* whose *instances* serve to wire a portion of the mouse brain. Very much in the same manner as genes generate proteins by providing templates for their construction, we view the catalogue of basic circuits as providing templates for wiring up the 30M neurons of the mouse brain. This effort will initiate a new search for this compendium of basic circuits, although at first we will probably extract only a sizeable fraction of candidate basic circuits in the *Mouse Brain Web*.

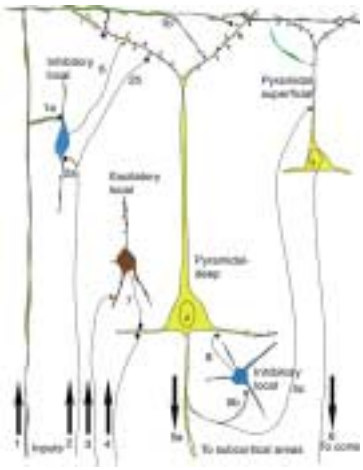


Fig. 1. Dominant basic circuit of cerebral cortical networks [42]

Our imaging and reconstruction of the whole mouse brain will differ from earlier efforts in several notable aspects. First, the proposed research will use our 3D KESM to scan the mouse brain at 300nm resolution and create an aligned volume data set of ~12 terabytes representing the tissue microstructure of each brain. Second, this 3D microscope, whose data-acquisition rates exceed 100 MB/s, can scan a whole mouse brain within 100 hours. Third, our group will develop new tissue preparation techniques to allow *en bloc* staining and embedding, with Nissl, Golgi-Cox, and transgenically induced protein stains such as GFP. Fourth, this research project will develop new 3D parallel image-processing algorithms, including our *polymerization algorithm*, to reconstruct and visualise the brain microstructure geometrically. Lastly, an I/O-intensive cluster computer with attached storage area network (SAN) will be designed to support the endeavour.

2 PROTOTYPE KESM AND PRELIMINARY RESULTS

A unique instrument, the *Knife-Edge Scanning Microscope (KESM)* [3] has been designed at Texas A&M University in recent years. The instrument, shown in Fig. 2(a), is capable of volume digitizing a complete mouse brain (~310mm^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 specimen (that is, 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 serial thin sections from the block. A light source illuminates the brain tissue at the diamond knife tip with a strip of intense illumination reflection from the beveled knife-edge, as illustrated in Fig. 2(b). Thus, the diamond knife performs two distinct functions: as an optical prism in the collimation system of the microscope and as the tool for physical sectioning. A microscope objective, aligned perpendicular to the top surface of the knife, images the illuminated narrow band of tissue in the newly cut section just beyond the knife-edge, prior to subsequent deformation of the tissue ribbon.. This image is digitally encoded by a high-sensitivity line-scan camera. Finally, the digital video signal is passed through image acquisition boards and stored for subsequent analysis in a cluster computing system. The current cluster consists of 5 servers, each dual processor (1.1-1.5 GHz), 2GB of memory, a combined 1 TB hard-drive capacity, all linked by a Cisco gigabit/s switch.

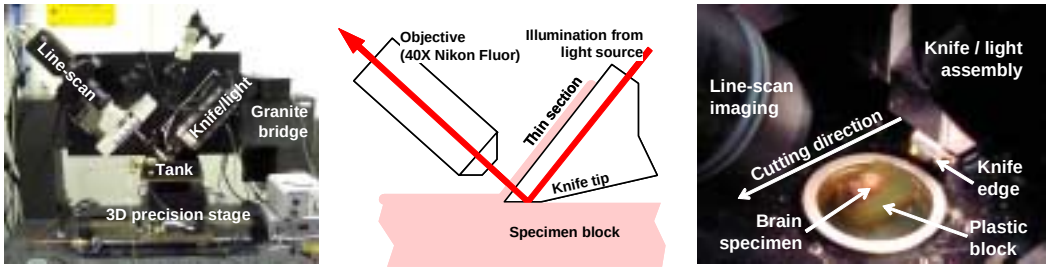


Fig. 2. (a, left) Photo of the KESM showing line-scan/microscope, knife/light assembly, granite bridge, and 3D precision stage. (b, center) Specimen undergoing sectioning by knife-edge scanner (thickness of section is exaggerated). (c, right) Close-up photo of the line-scan/microscope assembly and the knife/light assembly.

The prototype KESM has been validated on Golgi- and Nissl-stained mouse brain specimens, and is currently producing high-quality 2D and 3D data. *Nissl staining* targets the RNA in the cytoplasm of all neurons, as well as the DNA in all cell bodies. As a result, all cell bodies are visible, but the dendritic arbors and axons remain unstained. Thus, Nissl staining allows us to reconstruct the distribution of all cell bodies in the mouse brain, and in particular their distribution within the six layers of the cerebral cortex. Fig.3(a) shows a coronal slice (10X magnification objective) of a Nissl-stained mouse brain containing the lateral ventricle, hippocampus and ventral part of the cortex. Different layers of the mouse brain are clearly visible as density changes. Enlarged views in Fig.3(b-c) show the lateral ventricle, running through the central area of the brain, and the hippocampus. With this objective (10X), the individual cells that outline the lateral ventricle can also be seen.

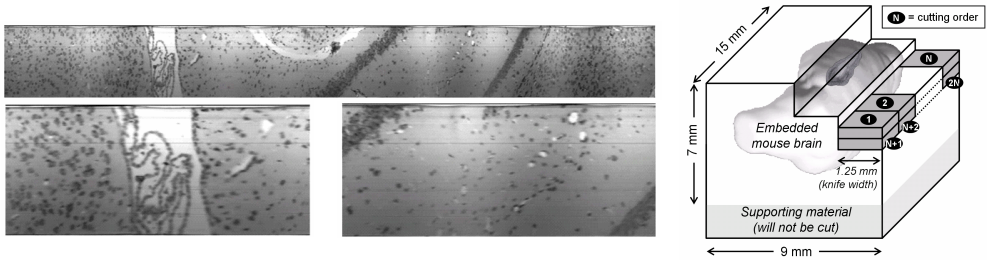


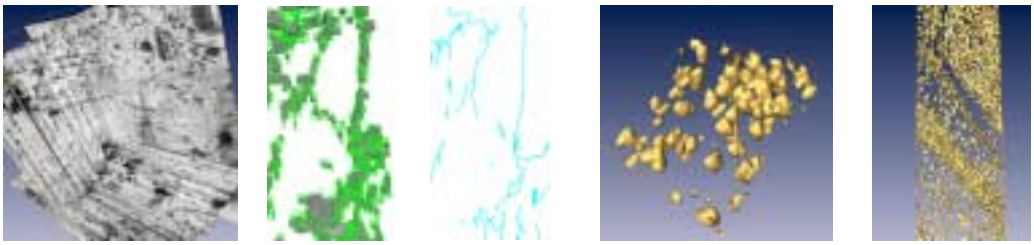
Fig. 3. (a, top) KESM scan of Nissl-stained ribbon of a mouse brain using a 10X objective (coronal section). (b, left) Close-up of the lateral ventricle. (c, middle) Close-up of the hippocampus. (d, right) Stair-case cutting of a specimen block into ribbons (section thickness not drawn to scale). A ribbon image is split into image stacks (denoted by circled numerals) and off-loaded to cluster nodes for processing and storage.

Our prototype KESM was tested on *GAT1-GFP mice* [4]. In our preliminary results fluorescence was suppressed by our embedding technique. However, it has been recently reported that GFP fluorescence can be observed in cells embedded in the low-viscosity acrylic resin LR White [5]. This resin equals the "hardness" of other resins currently employed with the KESM (i.e., Epon) which, given that harder materials produce better results with ultramicrotomes, makes LR White an ideal choice for our GAT1-GFP studies.

Data compression and geometric reconstruction of the scanned data are vital to the success of our proposed work. We need a method that (1) will work locally (vs. evaluating over the whole volume at once), (2) that is well-suited for representing long, thin, branching structures (vs. the blobby structures commonly reconstructed from volume data), (3) that supports high data compression in sparse data situations, and (4) that works very quickly so it is able to process one slice before the next one arrives. In examining existing volumetric representations and reconstruction methods, we found all of them to be inappropriate in at least one of these areas. We have introduced a new data structure, the L-block, which addresses the specific features of KESM-

scanned neuron data. Along with the L-block data structure we have begun work on a polymerisation strategy, by which locally identified neuron data is joined together automatically to form long dendritic "threads." The L-block and the polymerisation strategy are presented in [6], but will be improved during the project, as more data comes in.

Using these procedures, we have begun reconstruction of neuron processes from *Golgi-stained* volumetric data (Fig. 4(a)). Though this work is still in its preliminary stages, L-block coverings and initial thread reconstruction with the polymerisation strategy are demonstrated in Fig. 4(b,c), respectively. Reconstruction results of cell bodies from *Nissl-stained* specimens are shown in Fig. 4(d,e). These results were obtained with the Amira software for isosurface reconstruction; no processing was applied to the images beyond intensity equalisation, showing that even raw data is suitable for reconstruction.



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

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

3 CHATTER GENERATION ON KESM

From the machining point of view, the KESM can be classified as a small-scaleplaning machine, which uses a single point cutting tool. Preliminary cutting tests have shown that the major obstacle to obtain robust data is generation of self-excited oscillations (mechanical chatter) during the cutting process, when sequential layers are being removed from thespecimen. The exact explanation of this phenomenon has yet to be further studied, but it is clear now, that the regeneration effect and free oscillations caused by the sudden nature of the tool engagement into the specimen, play paramount roles. To grasp a sufficient understanding of main the problems involved, a brief discussion of the cutting mechanics based on [7] is given below.

Generally a cutting process results in dynamic interactions between the machine tool, the cutting tool and the workpiece and therefore its mathematical model should take into account its kinematics, dynamics, geometry of the chip formation and the mechanical and the thermodynamic properties of both the workpiece and the cutting tool. The mechanics of chip formation is recognised even more now than before as a key issue in a further development of machnining technologies. The physical complexity in describing and analysing a cutting process comes from the interwoven phenomena such as elasto-plastic deformations in the cutting zones, variable friction force acting on the cutting tool, heat generation and transfer, adhesion and diffusion, and material phase transformations, to name but a few. A schematic showing three main deformation zones and listing all important phenomena influencing the mechanics in the cutting process is given in Fig. 5(a). Understanding the relationships between these phenomena is critical for an adequate modelling of a specific cutting process. It is important to note that most of the phenomena (e.g. friction) are strongly nonlinear and interdependent. First studies on chatter date back to 1907, when the first significant work was published on metal cutting mechanics [8], however, the real breakthrough was achieve in mid-1940s when the first physical model of the chip formation was established [9]. Figure 5(b) shows this model, which is called the *orthogonal*

cutting model. Here, the uncut layer (initial depth of cut), h_0, of the workpiece in the form of a continuous chip is seen to be removed along the shear plane. Subsequently, the chip of thickness h flows along the face of the tool, where it encounters friction on the too-chip interface. The width of the chip remains unchanged, hence the stress field can be considered in two dimensions. The cutting force, F_c, and the thrust force, F_t, determine the vector R, which represents the resistance of the material being cut acting on the cutting tool. In stationary cutting conditions, this force is compensated by the resultant force generated in the shear stress field, and the friction on the rake surface.

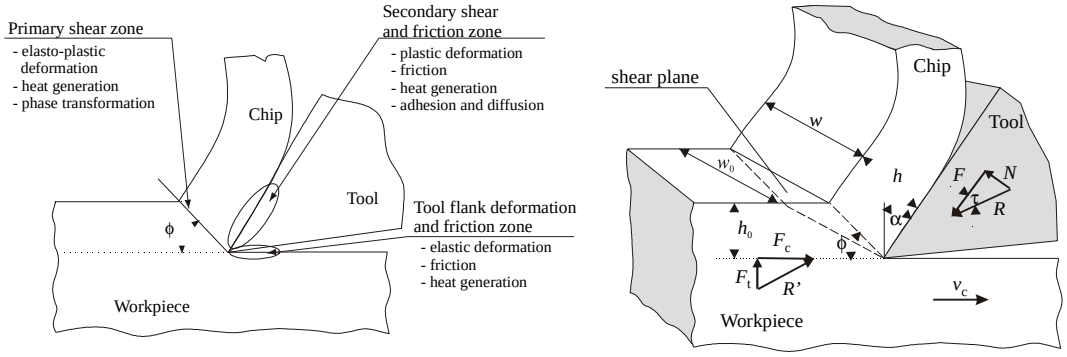


Fig.5. (a) Three main deformation zones with all important physical phenomena influencing the cutting process, (b) physical model of orthogonal cutting developed by Merchant [9]

The chip formation mechanism is controlled by instant cutting parameters such as feed, velocity and depth of cut. Any variation in these parameters almost instantaneously changes the force loading on the cutting tool and consequently the chip and the workpiece surface geometry. If these alterations of the chip geometry (e.g. thickness) start to be periodic, the appearance of chatter is imminent. The first attempts to describe chatter were made by Arnold [10]; however, a convincing mathematical model and analysis were given by Tobias and Fishwick [12]. In general, chatter can be classified as primary and secondary. Another classification distinguishes frictional, regenerative, mode-coupling, and thermo-mechanical chatter.

4 DESIGN OF NEW CHATTER FREE KESM

As can be seen from Section 3 some good data has been obtained on the original KESM [] as slight alterations of the cutting velocity for each pass have partially suppressed the chatter, however, not to extend that KESM can be used effectively unmodified. The main modification is to produce a chatter free operation by increasing the stiffness and producing a continuous chip. This can be achieved by employing an ultra-precision three-axis CNC lathe designed for single point diamond turning, where the angular and axial motion accuracy is around 8*nm* and the machine has subnanometer slide feedback resolution. Details of this new design are given in the paragraphs to follow.

A knife-edge scanning microscope (KESM) is comprised of three components: (1) a precision CNC machine, which uses a diamond knife to section a workpiece containing plastic-embedded tissue, such as small animal brains; (2) an image capture system, using a microscope objective, microscope optical train, and line-scan camera for imaging newly cut tissue as it passes over the edge of the knife; and (3) a cluster computer/storage area network, that provides data compression, segmentation, three-dimensional reconstruction, and visualization; and stores both the image and reconstructed data.

In this new design, a continuous section, or tape, of the newly sectioned tissue can be optionally extracted and stored. This tape can then be selectively processed and edited offline. For example, the tape can be counter-stained, then selectively scanned, and used to "paint" a 3D reconstruction of neurons and glial cells in the tissue with the supplementary information so gleamed. Alternatively, choosing a 6mm wide diamond knife, a tape of consecutive ultra-thin sections (~300nm thick) of mouse brains in transverse section can be extracted and, as above, processed and examined offline.

5 CONCLUSIONS

The paper discusses how ultra precision nanomachining can provide a breakthrough in reconstructing the neuronal networks of mammalian brains. To explore this unknown territory a new instrument developed at Texas A&M University, the *Knife-Edge Scanning Microscope (KESM)*, was presented and details of a new design combating shortcoming of the original KESM were given.

The instrument uses diamond knife sectioning of the plastic-embedded brain, with layers typically a few hundred *nm* thick. Sectioning at 300*nm* resolution allows to create an aligned volume data set of ~12 terabytes representing the tissue microstructure of entire brain. Preliminary cutting tests have shown that the major obstacle to obtain robust data are self-excited oscillations (chatter) generated during the cutting process, when sequential layers are being removed from the sample. The main modifications to the original design are aimed to produce a chatter free operation. This can be achieved by employing an ultra-precision three-axis CNC lathe designed for single point diamond turning, and featuring the angular and axial motion accuracy of 8*nm* and and subnanometer slide feedback resolution.

References

1. S. Ramon y Cajal, *Histology of the Nervous System of Man and Vertebrates (2 Volume Set)*, English translation by N. Swanson and L.M. Swanson, New York: Oxford University Press, 1995.
2. G. Shepherd, ed., *The Synaptic Organization of the Brain*, 5th ed., New York: Oxford University Press, 2003.
3. B.H. McCormick, Inventor, System & Method for Imaging an Object, U.S. Patent Application #09/948,469, U.S. PTO (for Knife-Edge Scanning). Examiner has allowed 13 claims and this case will issue shortly as a U.S. Patent.
4. C.-S. Chiu, K. Jensen, I. Sokolova, D. Wang, M. Li, P. Deshpande, N. Davidson, I. Mody, M. Quick, S. Quake and H.A. Lester, "Number, density, and surface/cytoplasmic distribution of GABA transporters at presynaptic structures of knock-in mice carrying GABA transporter subtype 1-green fluorescent protein fusions", *J. Neurosci*., vol. 22, pp. 10251–10266, 2002.
5. K. Luby-Phelps, G. Ning, J. Fogerty and J.C. Besharse, "Visualization of identified GFP-expressing cells by light and electron microscopy", *J. Histochem. Cytochem*., vol. 51, no. 3, pp. 271-274, 2003.
6. B.H. McCormick, B. Busse, P. Doddapaneni, Z. Melek and J. Keyser, "Compression, segmentation, and modeling of filamentary volume data", accepted by *ACM Solid Modeling* '04.
7. M. Wiercigroch and E. Budak, "Sources of nonlineariries, chatter generation and suppression in metal cutting", *Phil. Trans. R.Soc. Lond.* A, vol. 359, pp. 663-693, 2001.
8. F.W. Taylor, "On the art of cutting metals", *Trans. ASME*, vol. 28, pp. 31-248, 1907.
9. E.M. Merchant, "Mechanics of the metal cutting process. I. Orthogonal Cutting and a Type 2 Chip", *J. Appl. Phys.*, vol. 16, pp. 267-275, 1945.
10. R.N. Arnold, "Mechanism of tool vibration in cutting steel", *Proc. Inst. Mech. Engrs*, vol. 154, pp. 261-276, 1946.
11. S.A. Tobias and W. Fishwick, "The chatter of lathe tools under orthogonal cutting conditions", *Trans. ASME*, pp. 1079-1088, 1958.