AUTOMATED LATERAL SECTIONING FOR KNIFE-EDGE SCANNING MICROSCOPY

J. Kwon, D. Mayerich, Y. Choe, B. H. McCormick

Department of Computer Science
Texas A&M University
College Station, TX

ABSTRACT

Recent advances in high-throughput microscopy are used to acquire large-scale anatomical information at the microscopic level. One of these methods, known as Knife-Edge Scanning Microscopy (KESM), allows large volumes of tissue to be imaged using *physical sectioning*. This method has been limited, however, by constraints on the field of view of the objective and the need to prevent damage to tissue before it is imaged.

In this paper, we describe a simple sectioning algorithm we use to overcome these constraints on tissue size. By maintaining a height field of the tissue surface, we are able to cut *lateral sections* while minimizing damage to un-imaged tissue. Although lateral sectioning introduces some deformation and tissue damage at the interface of the sections, the damage is minimal and the deformations can be compensated for using affine transformations.

Index Terms— knife-edge scanning microscopy, KESM, microscopy, serial sectioning

1. INTRODUCTION

High-throughput microscopy is a rapidly developing field for both optical [1] and electron [2] microscopy. These new techniques allow the imaging of large-scale anatomy at the microscopic level. One of the optical technologies in this field is Knife-Edge Scanning Microscopy (KESM) [3].

KESM is a high-throughput technique for quickly imaging large volumes of tissue at sub-micron resolution. Thin sections are cut from the specimen and imaged using a high-speed line scan camera. Since physical sections are actually cut from the specimen, KESM is not constrained by specimen thickness, which is a major limitation in optical sectioning techniques such as confocal [4] and multi-photon [5] microscopy.

In KESM, imaging and sectioning are performed simultaneously. A high-speed line-scan camera is used to capture an image of the tissue section as it passes over the top surface of a diamond knife (Fig. 1a). Illumination is provided through the diamond knife using a high-intensity illuminator. KESM allows image data from the specimen to be captured at the maximum speed of the camera (currently 4096 pixel lines can be captured at 44kHz). Since the tissue is destroyed during the imaging process, any tissue outside the field of view (FOV) is permanently lost, thereby limiting the section width that can be imaged using KESM.

In this paper, we describe a technique for overcoming this constraint. We make two major contributions:

- We describe an automated sectioning algorithm that allows neighboring tissue, outside the FOV of the objective, to be imaged in later passes.
- We justify the use of simple affine transformations that compensate for deformations in the captured data set due to our sectioning technique.

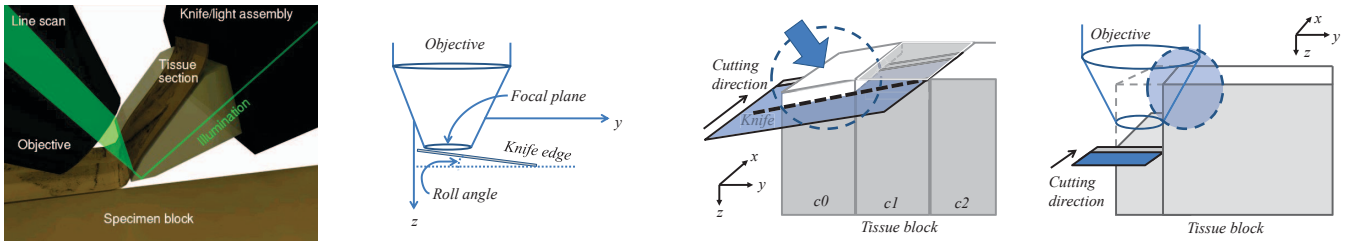
2. LATERAL SECTIONING

In order to image larger specimens, we perform *lateral sectioning* across the specimen surface. In traditional KESM, two motor stages are used during the cutting process. One vertical lift stage is used to adjust the level of the specimen relative to the knife. The second stage moves the specimen under the knife to perform cutting. Our lateral sectioning technique requires the use of an additional stage that moves orthogonal to both of these axes. In our current KESM setup, this stage is already present and used for accurate positioning of the specimen under the knife.

There are two ways to perform lateral sectioning, however care must be taken so that un-imaged tissue is not damaged.

- Lateral sections can be taken across the surface of the knife. Due to small misalignments in knife *roll*, this can cause damage to un-imaged tissue outside the FOV of the objective (Fig. 1b and c).
- This damage can be prevented by sectioning an entire *column* at a time. The distance between the knife edge and the objective limits the cutting depth because the side of the objective will come into contact with the uncut specimen block (Fig. 1d). In addition, as the cutting depth increases, there is more contact between the edge

The authors would like to thank Bernard Mesa at Microstar Technologies for his insight and assistance in KESM design.



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

Fig. 1. KESM lateral sectioning problems. (a) KESM performs imaging while cutting. (b) Small errors in the roll of the knife can (c) cause damage to un-imaged tissue. (d) Taking several consecutive sections stop tissue damage but may cause the objective to come into contact with the tissue.

of the knife and the neighboring tissue. This can cause tearing of the tissue and induce knife vibrations [6].

2.1. Stair-Step Sectioning

We use a simple cutting algorithm that avoids both of these problems by cutting small stacks of images in a stair-step fashion (Fig. 2). In order to avoid damage to the tissue or microscope, we must ensure that the cutting depth d is:

- large enough so that the overhanging part of the knife does not cause damage to un-imaged tissue
- small enough so that the microscope objective does not make contact with un-cut tissue
- small enough to keep knife vibrations and tissue tearing to a minimum

The first two constraints are easy to achieve because the focal distances of many optical objectives are 1mm to 2mm. We do, however, choose to minimize the column depth in order to minimize knife vibrations due to contact between the tissue and the knife.

The degree of knife misalignment is difficult to accurately measure. We can determine an upper bound for the knife misalignment based on the imaging constraints of the KESM. In order for the entire tissue section to be in focus, both points of the knife edge within the FOV of the objective must be within the focal depth. This means that the angle of the knife θ is constrained by:

$$\theta \leq \arctan\left(\frac{FD}{FOV}\right) \tag{1}$$

where FD is the focal depth of the objective and FOV is the field-of-view. If the roll angle of the knife is greater than θ, this can be detected by the user because part of the image will be out of focus. If θ complies with the above constraint, the angle of the knife cannot be detected using the imaging hardware available in KESM. Therefore, we compute a worst-case depth d based on the maximum possible un-detectible value of θ:

$$d = L_k \sin\theta_{max} - FD \tag{2}$$

where L_k is the length of the knife (Fig. 3) and $\theta_{max} = \arctan\left(\frac{FD}{FOV}\right)$.

2.2. Implementation

We implement stair-step sectioning by maintaining a height field of the tissue surface. We can then constrain cutting so that the height difference between two columns never exceeds the calculated value d from Equation 2. Changes to the cutting parameters (e.g. column thickness) can be handled robustly by simply resampling the height field in order to insure that there is no loss in data. The algorithm used to constrain the sectioning process is shown in Algorithm 1.

2.3. Tissue Damage

During the sectioning process, some tearing occurs at the interface of two neighboring columns (Fig. 4). Our sectioning experience tells us that the damage is less than $5\mu m$ in width in most cases. For our 10X (0.3 NA) objective, the horizontal pixel resolution is $0.6\mu m$ and the FOV is 2.5mm. This results in an average data loss of 0.2%.

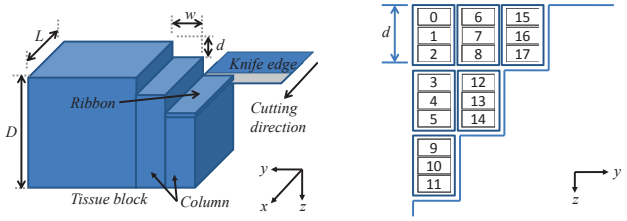


Fig. 2. Sections are cut in a stair-step fashion (left) and ordered so that neither the objective nor the knife comes into unwanted contact with the tissue (right).

Algorithm 1 Stair-step sectioning

$nNumOfCols \Leftarrow (int)(dTotalBlockWidth/dColWidth)$
$nCurCol \Leftarrow 0$
Initialize z positions in all columns
while $nCurColZ < nMaxBlockDepth$ **do**
  for $nIndexRibbon$ to $nPlankDepth$ **do**
    Section a tissue ribbon
  end for
  $nPlankThickness \Leftarrow nRibbonThickness \times PlankDepth$
  if $nCurColZ > (nNextColZ + nPlankThickness \times 2)$ **then**
    $nCurCol \Leftarrow nCurCol + 1$
  else
    $nCurCol \Leftarrow nCurCol - 1$
  end if
  Update $nCurColZ$
end while

2.4. Distortion

As mentioned in the previous sections, small misalignments in the knife orientation are difficult to detect and result in the knife contacting the specimen surface at a slight angle θ. Although the roll angle of the knife is the only misalignment that can cause unwanted tissue damage, note that it is also possible for there to be a slight yaw misalignment (Fig. 5a). Both of these knife angles cause each column to be imaged at a slight skew (Fig. 5b).

When the image stacks are placed next to each other, this results in misalignment at the interface between columns (Fig. 5c). We fix this misalignment by applying a translation to each column to compensate (Fig. 5d). The direction of translation parallel to the plane created by the column interface. Each component of the translation (x and z) is proportional to the angle of misalignment. We note two important properties of these offsets:

- The offsets are based on knife misalignment along two axes. These angles are too small to be measured with the KESM optics but can produce noticeable distortion in the data set.

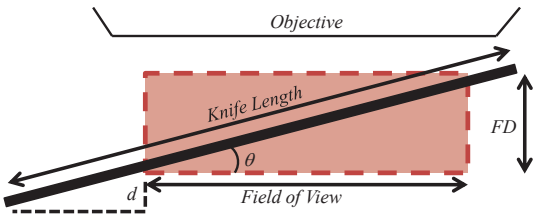


Fig. 3. Misalignment of the knife relative to the focal plane. θ represents the maximum undetectable misalignment. In practice, θ is quite small resulting in $d < 3\mu m$.

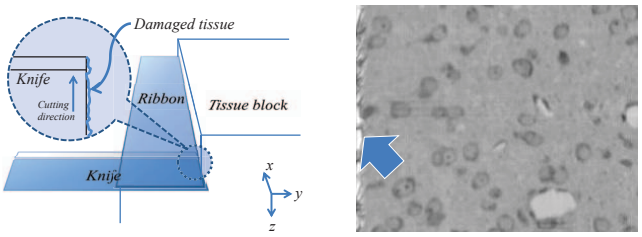


Fig. 4. Tissue damage due to lateral sectioning. (left) Damage occurs due to tearing at the interface of two columns. (right) This results in some data loss at the edge of the image (arrow).

- Since the offsets are based on the knife angle, they are constant throughout the entire data set.
- Practical constraints on the knife angle (discussed above) limit these offsets to very small values (1-4 pixels).

In our initial experiments, we attempted to determine these offsets automatically by aligning images representing the interface between the columns. The major difficulty with this approach is that tissue tearing, and therefore data loss, occur at this interface. Although it is possible to acquire image data slightly "inward" of the interface, this sampling was too coarse to allow effective alignment based on image data alone.

Since the knife misalignments are constant throughput a data set, we found that the offsets need to be determined manually only once. This was done by selecting a small volume of tissue at the interface of two neighboring columns. These volumes were aligned by using filament structures, such as vasculature and neuronal processes, as fiducials. Many of the larger filaments have trajectories that can be interpolated through missing data at the interface. After an initial estimated alignment, we can then explore several other samples along the interface to evaluate and refine the offsets. Since imaging can occur uninterrupted for several hours at a high data rate (approximately 30GB/hour), one-time manual alignment (requiring only a few minutes) was an efficient method for determining offsets between neighboring columns.

3. RESULTS AND CONCLUSION

We have used these sectioning and alignment techniques to increase the volume of tissue imaged using KESM. We have tested these techniques using two data sets:

- Mouse brain microvasculature stained using India-ink perfusion. This creates a high-contrast data set containing a dense network of blood vessels (Fig. 6).
- Rat somatosensory cortex stained with thionin (Nissl). This data set is lower contrast than the India-ink perfusion and contains significantly more data. In particular,

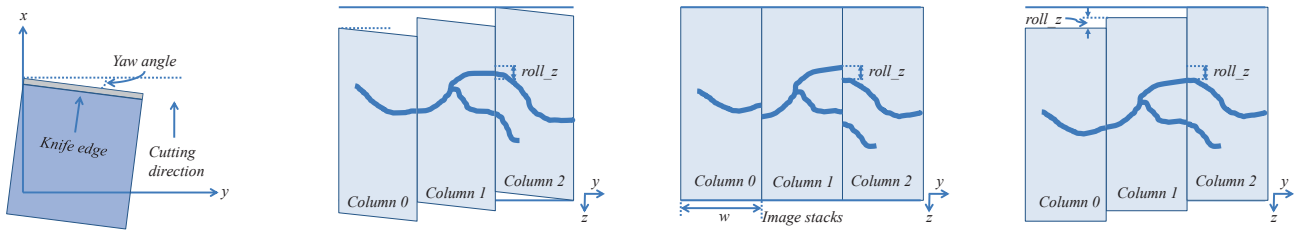


Fig. 5. Knife misalignments cause distortions. (a) Errors in knife yaw can also effect the data. (b) Both yaw and roll cause each column to be slightly skewed. (c) Aligning image data shows the misalignment between columns. (d) We can align neighboring columns by applying a translation along the interface plane.

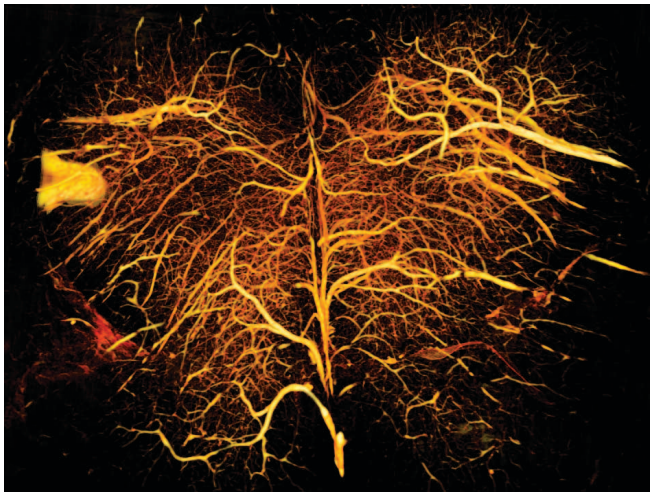


Fig. 6. Volume visualization of mouse spinal cord stained with India-ink. The tissue sample is approximately 2mm x 1mm in x and y. Several thousand sections were used to create the composite image (approximately 1mm deep).

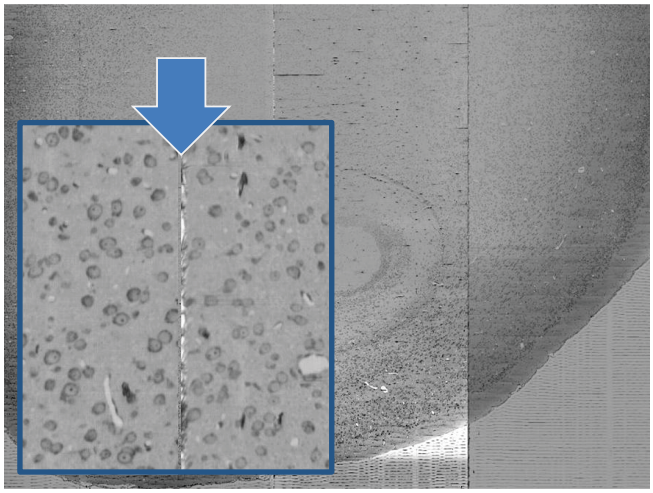


Fig. 7. Several columns of aligned rat brain. These sections include somatosensory cortex (left and right) and hippocampus (center). The inset shows a close-up of somatosensory cortex.

cell nuclei of all neuronal and glial cells are visible. In addition, vasculature is visible as unstained filaments which were used as fiducials for alignment.

These techniques have allowed us to image large volumes of mouse spinal cord and rat cortex, well beyond the capabilities of optical sectioning techniques and single-column KESM.

4. REFERENCES

[1] Kristina D. Micheva and Stephen J. Smith, "Array tomography: A new tool for imaging the molecular architecture and ultrastructure of neural circuits," *Neuron*, vol. 55, pp. 25–36, 2007.

[2] Winfried Denk and Heinz Horstmann, "Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure," *PLoS Biology*, vol. 2, pp. e329, 2004.

[3] Bruce H. McCormick and David M. Mayerich, "Three-dimensional imaging using knife-edge scanning microscopy," in *Microscopy and Micro-analysis*, Savannah, GA, 2004.

[4] J. B. Pauley, Ed., *Handbook of Biological Confocal Microscopy*, Plenum Press, New York, 1995.

[5] Winfried Denk, James H. Strickler, and Watt W. Webb, "Two-photon laser scanning fluorescence microscopy," *Science*, vol. 248, pp. 73–76, 1990.

[6] Marian Wiercigroch and Erhan Budak, "Sources of nonlinearities, chatter generation and suppression in metal cutting," *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, vol. 359, pp. 663–693, 2001.