

Specifications for Volume Data Acquisition in Three-Dimensional Light Microscopy

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Wonryull Koh

Bruce H. McCormick

Abstract—Knife-edge scanning microscopy facilitates automated data acquisition in the form of aligned image stacks from a thick tissue specimen. The effective field of view of the microscope objective determines the width of digital sections. The volume data sets involved are multi-terabyte in size, and require careful prescription of the volume data acquisition protocol. We define a protocol for the capture of aligned image stacks, or volume data set, from a workpiece using knife edge scanning microscopy. We describe the relevant terminology, workpiece specification, stage and workpiece coordinate systems, scanning conventions, microscope objectives specifications, camera specifications, scanning modes, and data storage and indexing scheme. We also show that the data acquisition protocol for knife edge scanning microscopy can be extended for the capture of registered image stacks from a fluorescently labeled thick tissue specimen undergoing Tsai et al's automated all-optical histology [3].

Keywords—three-dimensional light microscopy, knife-edge scanning microscopy, volume data acquisition, data storage and indexing

1 INTRODUCTION

Three-dimensional light microscopy promises to provide a system level description of brain and other organs at a cellular level of detail. New technology is now emerging to scan and reconstruct an entire mouse brain in three dimensions, exhibiting its cell bodies and forests of neuronal arbors. The acquisition of such volume data sets will enable detailed measurement and visualization of mammalian brain development and connectivity at a neuronal level of detail. Furthermore, whole brain data will allow us to construct an anatomically correct model of a significant portion of the mouse cerebral cortex, a precursor to large-scale functional simulation of the mouse brain cortex.

Currently, two techniques exist with a potential to scan and reconstruct an entire mouse brain in three dimensions—knife-edge scanning microscopy [1][2] and all-optical histology using ultrashort laser pulses [3]. Knife-edge scanning microscopy allows imaging the newly-cut tissue just beyond the knife edge as a thin section is cut away by an ultramicrotome, and supports all known forms of microscopy. The Brain Tissue Scanner [1][2], an instrument of local design that introduced knife-edge scanning microscopy, uses repeated knife-edge scanning to generate at 250nm sampling resolution a volume data set in the form of aligned image stacks. The all-optical histology technique developed by Tsai et al [3] makes use of successive iterations of imaging with two-photon laser scanning microscopy and tissue ablation with ultrashort pulses of infrared laser light to provide diffraction-limited volumetric data that is used to reconstruct the architectonics of labeled cells or microvasculature.

We present a method for the capture of an aligned image stack, or volume data set, from a workpiece using knife edge scanning microscopy. The volume data sets involved are multi-terabyte in size, and require careful prescription of the data acquisition protocol. Knife edge scanning microscopy as described throughout is the data acquisition process employed in the Brain Tissue Scanner [1][2], where the workpiece is assumed to be fixed rodent brain tissue embedded in plastic. However, other variants of knife edge scanning microscopy and workpiece specimens can be substituted without change of method. We first briefly summarize the knife edge scanning microscopy technique used in the Brain Tissue Scanner. We then describe the relevant terminology, workpiece specification, stage and workpiece coordinate systems, scanning conventions, microscope objectives specifications, camera specifications, scanning modes, and data storage and indexing scheme. Lastly, we show that the data acquisition protocol for knife edge scanning microscopy can be extended to Tsai et al's all-optical histology technique.

2 KNIFE EDGE SCANNING MICROSCOPY

Three subcomponents of the Brain Tissue Scanner [1][2] enable the capture of aligned image stacks from a workpiece using knife edge scanning microscopy: precision positioning system, tissue removal system, and image capture system.

Precision positioning system. The workpiece is mounted atop a three-axis precision stage. The encoder resolution for the X- and Y-axes is 20nm, and the encoder resolution for the Z-axis is 25nm. The three-axis precision stage maintains the physical location of workpiece and thus ensures registration of images from consecutive serial sections into an aligned image stack.

Tissue removal system. A custom diamond knife of local design serves as an ultramicrotome. The diamond knife tip not only serves as an ultramicrotome for physical sectioning but also as an optical element, i.e., prism in the microscope collimation optics.

Image capture system. A water-immersion microscope objective and a high-sensitivity line-scan camera are used. The microscope objective, aligned perpendicularly to the top surface of the diamond knife, images the edge of the knife. A line-scan camera images the newly-cut thin section just beyond the knife edge, prior to its subsequent deformation.

A workpiece undergoing knife edge scanning microscopy (Fig. 1) is concurrently cut with an ultramicrotome and scanned with a line-scan camera. Thus, each stroke of the knife scans and removes a thin section (typically $0.5\mu\text{m}$ thick) from the workpiece. The effective field of view of the microscope objective determines the width of thin sections. The Brain Tissue Scanner uses repeated knife-edge scanning to create a sequence of nested ascending (or descending) stairsteps in a workpiece. Fig. 2 shows a workpiece undergoing *stair-step cutting/scanning*. Each staircase corresponds to a plank, and is carved out by repeated strokes over the workpiece indexing down into the tissue before moving on to the next staircase. With stair-step cutting, a knife whose width is twice the size of the field of view of the microscope objective can be used—cutting the specimen first with the right-hand side of the knife blade, and then when that half dulls, cutting with the left-hand side, thereby minimizing the cost of knife sharpening.

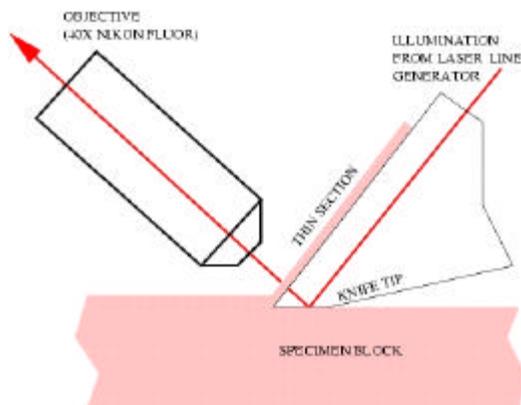


Fig. 1. Workpiece undergoing knife-edge scanning microscopy (thickness of sections exaggerated). Newly cut tissue is imaged with the microscope objective. Tissue is trans-illuminated using diamond knife as collimator for illumination from laser line generator [2].

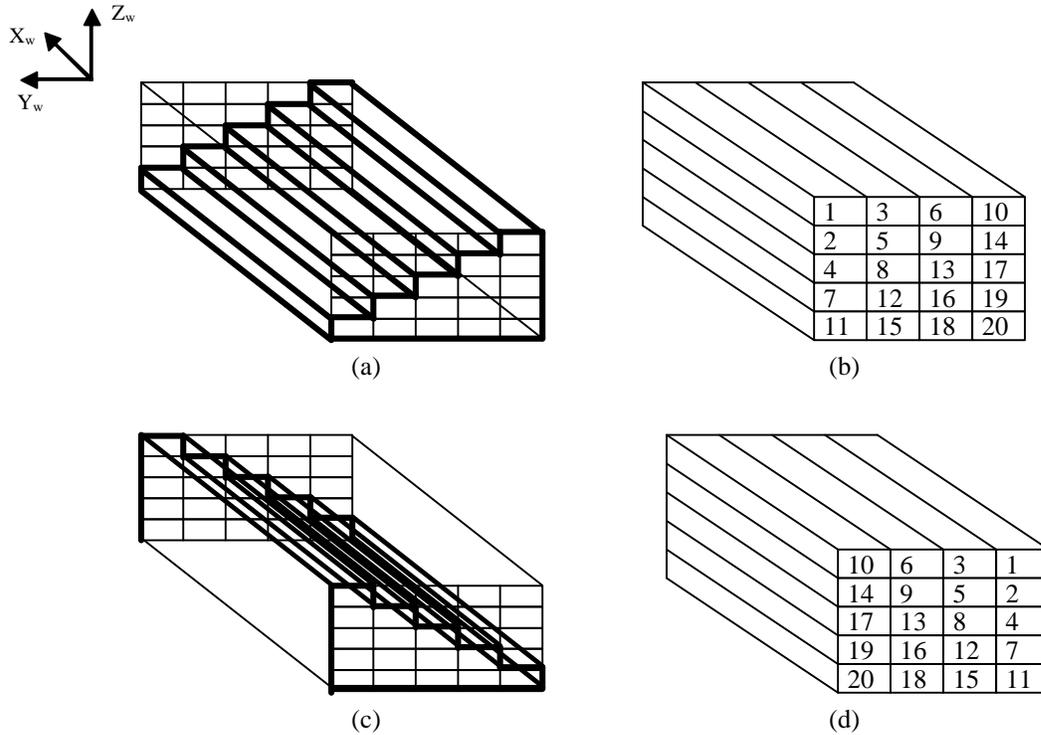


Fig. 2. (a) Ascending staircase constructed by removal of planks; (b) Sequence for plank cutting & scanning for ascending staircase; (c) Descending staircase constructed by removal of planks; (d) Sequence for plank cutting & scanning for descending staircase

3 TERMINOLOGY

3.1 Specimen

Specimen refers to a whole or part of a rodent brain embedded in plastic and molded on a tissue mount. The specimen, whose current size is limited to 15mm in length (X-axis), 12mm in width (Y-axis) and 9mm in height (Z-axis), fits within the well of the mold. The mold binds the specimen to a detachable specimen ring, which keys the mount to the Brain Tissue Scanner, allowing removal and replacement of the mount without loss of registration.

3.2 Plank, Plank Width, Plank Depth

Plank is a stack of images cut as one stair step in the stair-step cutting scheme (see Fig. 3). *Plank width* is determined by an effective field of view of the microscope objective. Currently, the plank width is nominally 0.625 mm for the 40X objective and 2.5mm for the 10X objective. For a specimen of 12mm width, it takes 20 planks of 0.625mm width laid side-by-side to cover the specimen.

Plank depth, physically, is computed from the number of consecutive sections cut in forming the plank. For the 40X objective, sections have a nominal $0.5\mu\text{m}$ thickness; for the 10X objective, sections have a nominal $1\mu\text{m}$ thickness.

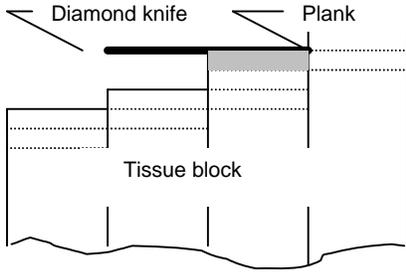


Fig. 3. Stair-step cutting, showing plank (gray) being cut. Knife width shown as 2X plank width

As data, a plank is considered as an image stack of long images that are w pixels wide. w is determined by the resolution of line-scan camera. For example, when line-scanning with a monochrome camera, a plank is a stack of long images that are 4096 pixels wide; with a color camera, a plank is a stack of long images that are 2048 pixels wide. All images of a plank start and stop at the same end points, and have equal length (the full length of the specimen). The plastic safely outside the range of the brain tissue is not imaged.

3.3 Image Stack

Image stack is a stack of square images created by uniformly dividing the length of each plank by the plank width. For example, a plank that is 15mm long and 0.625mm wide is partitioned into 24 image stacks.

3.4 Block, Big Voxel, Dark Voxel

Block refers to a repackaging of image stacks to make a nearly cubical data volume. A block is a *big voxel*, a $p_w \times p_w \times p_w$ volume element where p_w corresponds to the plank width. For example, the 15mm x 12mm x 9mm specimen block scanned by the 40X objective can be partitioned into 7,200 (24 x 20 x 15) big voxels. Each big voxel is a 0.625mm^3 cubical volume, and contains 15.7 GB of data (2048 x 2048 x 1250 RGB voxels). *Dark voxel* refers to a big voxel that lies outside the range of the rodent brain and thus does not contain any data.

4 WORKPIECE SPECIFICATION

A typical workpiece used in line-scanning is described in Table 1. The vertical lift stage indexes the sectioning thickness.

TABLE 1
Workpiece specifications

Material	Tissue embedded in plastic
Bounding Volume (Max.)	Cylinder (25mm dia. x 12.5mm depth)
Sectioning thickness (typically uniform for entire workpiece)	0.25-10 μm
Vertical repeatability (1 count)	25nm

5 STAGE AND WORKPIECE COORDINATE SYSTEMS

5.1 Stage Coordinate System

The stage conventions for home positions and directions of travel were set at the time of manufacture by Aerotech, Inc. The conventions (see Fig. 4) are:

- X_s -axis stage: X_s -axis homes to the left end of the stage and positive motion is to the right.
- Y_s -axis stage: Y_s -axis homes to the front and positive motion is toward the rear.
- Z_s -axis stage: Z_s -axis homes at the bottom and positive motion is upward.

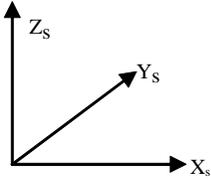


Fig. 4. Stage coordinates (a right-handed coordinate system)

5.2 Workpiece Coordinate System

Computer graphics conventions are used to set the world coordinates for the workpiece (see Fig. 5): the three-dimensional workpiece is viewed as if by a camera positioned above the workspace, looking downward along the stage Z_s -axis. The world coordinate system is right-handed and the object (workpiece) is considered to reside in the negative- Z half-space. We use X_w , Y_w , and Z_w for the coordinates of the volume data set generated from the workpiece:

- X_w -axis: home is at workpiece right, increasing to workpiece left
- Y_w -axis: home is at workpiece rear, increasing toward the workpiece front
- Z_w -axis: home is at workpiece top, increasing upward (workpiece is in negative Z half-space)

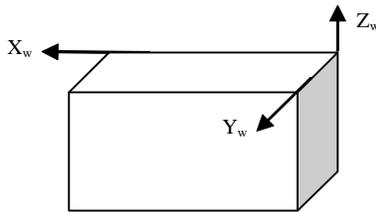


Fig. 5. Work piece coordinates (a right-handed coordinate system)

6 SCANNING CONVENTIONS

The workpiece is line-scanned along the positive X_w -axis as the air-bearing X_s -axis stage moves left-to-right. Scanning conventions for mapping stage coordinates to workpiece coordinates are:

- X-axis scanning: increment X_s -axis position of stage. Map X_s -coordinate to X_w by translation
- Y-axis scanning: increment Y_s -axis position of stage. Map Y_s -coordinate to Y_w by translation
- Z-axis scanning: increment Z_s -axis position of stage. Map Z_s -coordinate to Z_w by inversion and translation.

Fig. 6 and Fig. 7 show the mouse brain orientations for coronal and sagittal knife edge scanning microscopy.

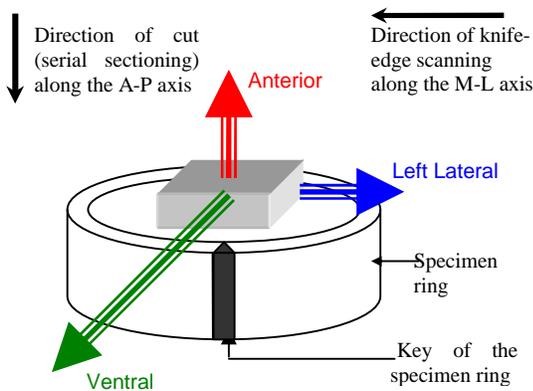


Fig. 6. Mouse brain orientation for coronal sectioning. Drawn as seen from standing in front of the BTS stage. Not drawn to scale.

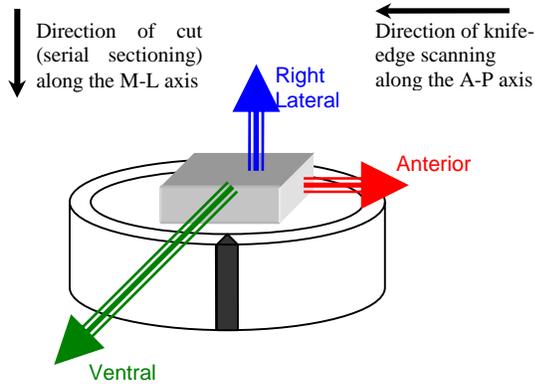


Fig. 7. Mouse brain orientation for sagittal sectioning. Drawn as seen from standing in front of the BTS stage. Not drawn to scale.

7 MICROSCOPE OBJECTIVE SPECIFICATION

Two water-immersion Nikon objectives, CFI60 Fluor 40X N.A. 0.8 and the CFI60 Fluor 10X N.A. 0.3, are currently used.

Table 2 gives the magnification (M), numerical aperture (N.A.), working distance (W.D.), and field of view (FoV) of these objectives. Table 2 also describes the resolving power (RP) and the number of resolvable pixels ($N_{\min}(I)$) for three wavelengths of light—blue ($\lambda=473\text{nm}$), green ($\lambda=532\text{nm}$), and red ($\lambda=670\text{nm}$). The resolving power (RP) defines the theoretical minimum resolvable distance between two points in the object plane. The Nyquist criterion, or its equivalent, Shannon's sampling theorem, states that the digitizing device must utilize a sampling interval that is no greater than one-half the size of the smallest resolvable feature of the optical image. Hence, $\frac{1}{2}\text{RP}$ is the sampling interval specified by the Nyquist sampling theorem. The number of pixels in the linear sensor array should be $2N_{\min}$, to avoid aliasing of the image data.

TABLE 2
NIKON CFI60 OBJECTIVES (COHERENT ILLUMINATION)

M	N.A.	W.D. (mm)	FoV (mm)	Blue (473nm)		Green (532nm)		Red (670nm)	
				$\frac{1}{2}$ RP (nm)	$2N_{\min}$	$\frac{1}{2}$ RP (nm)	$2N_{\min}$	$\frac{1}{2}$ RP (nm)	$2N_{\min}$
10X W	0.3	2	2.5	607	4,120	683	3,660	860	2,910
40X W	0.8	2	0.625	228	2,750	256	2,440	322	1,940

8 LINE SCAN CAMERA SPECIFICATIONS

Two line-scan cameras, Dalsa CT-F3 for monochrome imaging and Dalsa CL-T4 for color imaging, are used. Table 3 describes their specifications.

TABLE 3
SPECIFICATION FOR THE LINE-SCAN CAMERAS

	Dalsa CT-F3 (monochrome)	Dalsa CL-T5 (color)
Resolution	4906 x 96 TDI	2048 x 24/24/48 TDI (R/G/B)
Pixel Size	13 μm x 13 μm	14 μm x 14 μm
Aperture	53.3 x 1.3mm	28.7 x 1.6mm
Lens Mount	Custom	F-mount
Max. Line Rate	44 kHz	10.7 kHz
Data Rate	8 x 25 MHz	3 x 25 MHz
Data Format	8x8-bit LVDS	3x8-bit LVDS
Responsivity	180 DN/(nJ/cm ²)	380/260/220DN/(nJ/cm ²) (R/G/B)
Dynamic Range	610:1	R/G/B: 330/330/280:1
Nominal Gain Range	Fixed	6-17.5x

9 BRAIN TISSUE LINE-SCANNING MODES

High-resolution scanning (40X objectives) is used for the 3D reconstruction of stained neurons and fibers in the brain tissue. Low-resolution scanning (10X objective) is used for cell counts and 3D reconstruction of somata. Resolution in the Z-direction is determined by the thickness of the tissue sections, rather than by the optical resolution of the objective. Table 4 describes different line-scanning modes.

TABLE 4
BRAIN TISSUE LINE-SCANNING MODES

	High-resolution		Low-resolution	
	Monochrome	Color	Monochrome	Color
Sample size	250nm	300nm	600nm	1200nm
Objective	40X	40X	10X	
Effective FoV	0.625mm	0.625mm	2.5mm	
½ R.P. (green laser)	256nm	256nm	683nm	
Thickness of thin section	500nm	500nm	600nm	1200nm
Effective pixels	2440	2048	3660	2048
Tissue scanning speed (max)	11mm/s	3.21mm/s	26.4mm/s	12.8mm/s

10 DATA STORAGE AND INDEXING

10.1 Data Storage

Volume data obtained by three-dimensional microscopy with knife edge scanning is stored in four types of tables in a relational database: mold (see Fig. 8), specimen (see Fig. 9), block (see Fig. 10) and image (see Fig. 11)

Field	Type	Null	Key	Default	Extra
mold_id	int(11)		PRI	NULL	auto_increment
x_length	float			0	
y_width	float			0	
z_depth	float			0	
orientation	char(20)				

Fig. 8. Mold table. It describes the orientation and dimensions of the mold used

Field	Type	Null	Key	Default	Extra
specimen_id	int(11)		PRI	NULL	auto_increment
description	char(20)				
mold_id	int(11)		MUL	0	
stair-step mode	char(10)				
objective	char(4)				
plank_width	float			0	
plank_pixel_width	int(11)			0	
plank_depth	int(11)	YES		NULL	
section_thickness	float			0	
block_index_xmax	int(11)	YES		NULL	
block_index_ymax	int(11)	YES		NULL	
block_index_zmax	int(11)	YES		NULL	
num_blocks	int(11)	YES		NULL	

Fig. 9. Specimen table. It contains information pertaining to a specimen and the BTS configuration parameters used to scan the specimen.

Field	Type	Null	Key	Default	Extra
block_id	int(11)		PRI	NULL	auto_increment
specimen_id	int(11)		MUL	0	
x_index	int(11)			0	
y_index	int(11)			0	
z_index	int(11)			0	
host_name	varchar(20)				
dir_name	varchar(80)				

Fig. 10. Block table. It stores block's x-, y- and z- indices within the specimen mold. It also stores the names of the host machine and the directory where the block's image data resides.

Field	Type	Null	Key	Default	Extra
image_id	int(11)		PRI	NULL	auto_increment
block_id	int(11)		MUL	0	
z_position	int(11)			0	
file_name	varchar(255)				

Fig. 11. Image table. It stores an image's z-position within the block and its file name.

10.2 Data Indexing

Our current specimen (15mm x 12mm x 9mm) is partitioned into 24 x 20 x 15 data blocks, for a total of 7,200 blocks, where each block is a 0.625mm^3 cubical volume. Blocks are addressed as a 3-dimensional array indexed by their starting X, Y and Z positions in the workpiece: $B_{(x,y,z)}$ where $0 \leq x \leq 23$, $0 \leq y \leq 19$ and $0 \leq z \leq 14$. The X-index increases from right to left along the positive X_w -axis, and the Z-index increases from top to bottom along the negative Z_w -axis. The Y-index increases from front to back along the negative Y_w -axis for ascending stair-step cutting (see Fig. 12(a)), and from back to front along the positive Y_w -axis for descending stair-step cutting (see Fig. 12(b)).

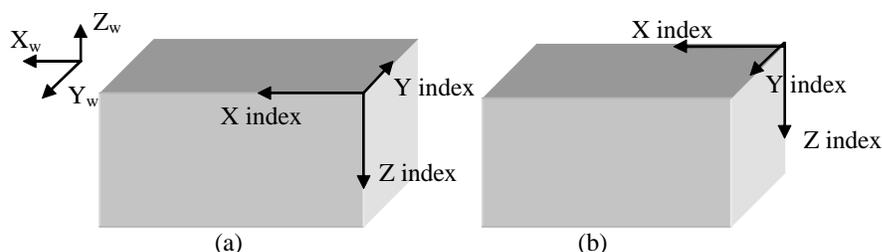


Fig. 12. Indexing directions for a specimen mold. (a) for ascending stair-step cutting; (b) for descending stair-step cutting

Blocks are organized in a storage area network as separate directories. Each block is matched to a directory named $N-P-Q-R$ where

- N is an alphanumeric string of length 8; N refers to the specimen from which the block is indexed.
- P, Q and R are numeric strings of length 4; P, Q and R are the X-, Y- and Z-indices of the block within the specimen mold, respectively.

Digital sections forming a block are stored in the directory named after the block, and each digital image file is named $N-P-Q-R-S.tif$ where

- N-P-Q-R corresponds to the directory name associated with the block as defined above.
- S is a numeric string of length 4; S is the image's Z-sequence number within the block.

11 EXTENSION TO THE ALL-OPTICAL HISTOLOGY TECHNIQUE

Tsai et al's all-optical histology technique [3] makes use of successive iterations of imaging with two-photon laser scanning microscopy (TPLSM) and tissue ablation with ultrashort pulses of infrared laser light to provide diffraction-limited volumetric data. We briefly summarize the all-optical histology technique [3], and describe an extended set of terminology, coordinate systems, scanning conventions, microscope objective specification, and data storage and indexing scheme for data acquisition protocol using all-optical histology.

11.1 All-optical histology

In all-optical histology, a single apparatus encompasses the ablation and TPLSM imaging optics. The tissue to be processed is positioned on an automated X-Y translation table that can be moved in a raster pattern. The surface layers of the tissue are imaged using TPLSM, and the region of the tissue that has been imaged is subsequently removed by laser ablation with amplified ultrashort laser pulses. The objective is lowered relative to the tissue sample, and the newly exposed surface is then imaged and ablated. The sequence repeats serially until the desired volume of tissue has been analyzed. This leads to a digitized block of optical sections from the labeled tissue [3].

As in knife edge scanning microscopy, all-optical histology technique uses a motorized stage to maintain the physical location of the workpiece, and thus obviates the need to register cut sections. However, three aspects of all-optical histology technique differ from the knife edge scanning microscopy: specimen, camera scanning mode, and tissue removal.

Specimen. Specimen in the all-optical histology refers to fluorescently labeled tissue that is not embedded in plastic. The use of TPLSM as imaging optics calls for specimen expressing fluorescent proteins (e.g., YFP, CFP, etc.). As embedding plastic tends to quench the fluorescence, the tissue may be fixed but not embedded in plastic.

Camera scanning mode. Area-scanning is used with TPLSM. A series of optical sections are imaged at increasing depth along the z-axis of the microscope. Each optical section is typically $200\ \mu\text{m} \times 200\ \mu\text{m}$, and the axial z-step is typically $1\ \mu\text{m}$ [3]. A total depth of approximately $110\ \mu\text{m}$ [3] of tissue is imaged per z-series.

Tissue removal. The finest, reproducible cut that can be made by laser ablation is reported to be approximately $2\ \mu\text{m} \times 2\ \mu\text{m} \times 6\ \mu\text{m}$ [3]. Typically, a $60\ \mu\text{m}$ thick layer of tissue is ablated between iterations of optical imaging. At each iteration, a total depth of approximately $110\ \mu\text{m}$ of tissue is imaged, of which $60\ \mu\text{m}$ represents new information and $50\ \mu\text{m}$ represents overlap with prior images as a means to cross-check alignment [3]. Optical sectioning is used to produce digital thin sections at $1\ \mu\text{m}$ interval, and laser ablation is used to remove tissue (a $60\ \mu\text{m}$ thick layer) that has been imaged. In contrast, in knife edge scanning microscopy, each thin section ($\sim 0.5\ \mu\text{m}$ with 40X objective) is removed by physical sectioning by the ultramicrotome as it is scanned by a line-scan camera.

11.2 Terminology

Specimen refers to a whole or part of a fluorescently labeled rodent brain tissue that may be fixed but not embedded in plastic. The motorized X-Y stage and the translation of the objective in Z-direction allow the iterative processing of tissue while maintaining absolute tissue coordinates.

Layer is a tissue section that is imaged and ablated from the specimen in one iteration of the optical imaging and laser ablation sequence.

Layer width and *layer length* correspond to the whole width and length of specimen, respectively.

Layer depth, or *layer thickness*, is typically $60\ \mu\text{m}$ for ablation, and $110\ \mu\text{m}$ for imaging.

Image stack is a stack of optical sections obtained as a z-series. For example, a z-series typically contains 110 successive focal planes ($200\ \mu\text{m} \times 200\ \mu\text{m}$) at $1\ \mu\text{m}$ interval, and thus an image stack comprises 110 digital images that correspond to the $200\ \mu\text{m} \times 200\ \mu\text{m} \times 110\ \mu\text{m}$ volume.

11.3 Workpiece Specification

A typical workpiece used in all-optical histology is described in Table 5. Optical sectioning indexes the sectioning thickness.

TABLE 5
WORKPIECE SPECIFICATIONS

Material	Fluorescently labeled tissue not embedded in plastic
Bounding Volume (Max.)	Cylinder (25mm dia. x 12.5mm depth)
Sectioning thickness (typically uniform for entire workpiece)	1-10 μm

11.4 Stage Coordinate System

The conventions for the X-Y stage coordinate system (see Fig. 13) are:

- X_s -axis stage: X_s -axis homes to the left end of the stage and positive motion is to the right.
- Y_s -axis stage: Y_s -axis homes to the front and positive motion is toward the rear.
- Z_{MO} -axis microscope objective: Z_{MO} -axis homes at the top of the stage and positive motion is upward.

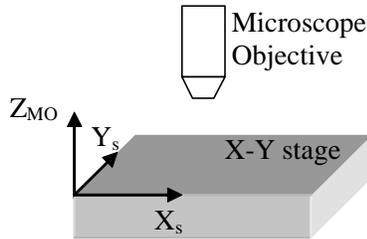


Fig. 13. Stage coordinates (a right-handed coordinate system)

11.5 Workpiece Coordinate System

Computer graphics conventions are used to set the world coordinates for the workpiece (see Fig. 14): the three-dimensional workpiece is viewed as if by a camera positioned above the workspace, looking downward along the Z_{MO} -axis. The world coordinate system is right-handed and the object (workpiece) is considered to reside in the negative-Z half-space. We use X_w , Y_w , and Z_w for the coordinates of the volume data set generated from the workpiece:

- X_w -axis: home is at workpiece right, increasing to workpiece left
- Y_w -axis: home is at workpiece rear, increasing toward the workpiece front
- Z_w -axis: home is at workpiece top, increasing upward (workpiece is in negative Z half-space)

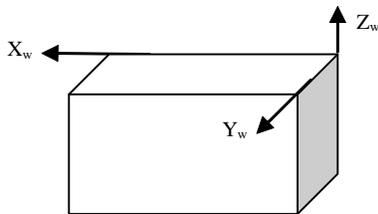


Fig. 14. Workpiece coordinates (a right-handed coordinate system)

11.6 Scanning Conventions

The workpiece is scanned by optical sectioning to yield successive z-series starting at the workpiece origin. Scanning conventions for mapping stage coordinates to workpiece coordinates are:

- X-axis scanning: increment X_s -axis position of stage. Map X_s -coordinate to X_w by translation
- Y-axis scanning: increment Y_s -axis position of stage. Map Y_s -coordinate to Y_w by translation
- Z-axis scanning: lower the microscope objective. Map Z_{MO} -coordinate to Z_w by inversion and translation.

Fig. 15 shows the scanning sequence for each optical imaging step.

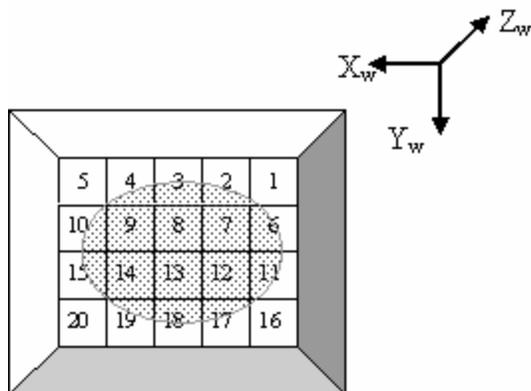


Fig. 15. Scanning sequence for acquiring successive z-series image stacks from a layer. Drawn as looking down on the workpiece positioned on the X-Y stage. Not drawn to scale.

11.7 Microscope Objective Specification

A water-immersion objective with 20X magnification and 0.5 N.A. is used. Table 7 describes the magnification (M), numerical aperture (N.A.), and the resolving power (RP) [4] of this objective for infrared light ($\lambda=920\text{nm}$). 920nm is the wavelength of light Tsai et al [3] used for iterative processing of a block of neocortex of a YFP labeled transgenic mouse.

TABLE 6
WATER-IMMERSION 0.5 N.A. OBJECTIVE

M	N.A.	$\frac{1}{2}$ RP at $\lambda=920\text{nm}$
20X W	0.5	552 nm

11.8 Data Storage

Volume data obtained by three-dimensional microscopy with all-optical histology can be stored in three types of tables in a relational database: specimen (see Table 7), imageStack (see Table 8), and image (see Table 9). The specimen table would contain information pertaining to a specimen, and imaging and ablation parameters used to scan and remove tissue, respectively. The imageStack table would store an image stack's x-, y- and z- indices within the specimen, and the names of the host machine and directory in which the image stack's image data resides. The image table would store an image's z-position within the block and its file name.

TABLE 7
SPECIMEN

Field	Type	Key
specimen_id	int	PRIMARY
description	char	
objective	char	
width	float	
pixel_width	int	
length	float	
pixel_length	int	
depth	float	
imaging_layer_depth	float	
ablation_layer_depth	float	
image_stack_xmax	int	
image_stack_ymax	int	
image_stack_zmax	int	
num_image_stacks	int	

TABLE 8
IMAGESTACK

Field	Type	Key
image_stack_id	int	PRIMARY
specimen_id	char	
x_index	char	
y_index	float	
z_index	int	
optical_section_thickness	float	
stack_size	int	
host_name	float	
dir_name	int	

TABLE 9
IMAGE

Field	Type	Key
image_id	int	PRIMARY
image_stack_id	char	
z_position	int	
file_name	char	

11.9 Data Indexing

A specimen can be partitioned into $p \times q \times r$ image stacks where each image stack is limited to a $200 \mu\text{m} \times 200 \mu\text{m} \times 110 \mu\text{m}$ volume, p is $(\text{specimen length})/(200\mu\text{m}) + 1$, q is $(\text{specimen width})/(200\mu\text{m}) + 1$, and r is $(\text{specimen depth})/(110\mu\text{m}) + 1$. Image stacks can be addressed as a 3-dimensional array indexed by their starting X, Y and Z positions in the workpiece: $B_{(x,y,z)}$ where $0 \leq x < p$, $0 \leq y < q$ and $0 \leq z < r$. The X- and Y-indices increase in the counter-clockwise radial direction starting at the specimen center (Fig. 15), and the Z-index increases from top to bottom along the negative Z_w -axis.

Image stacks can be organized in a storage area network as separate directories. Each image stack can be matched to a directory named $N\text{-}P\text{-}Q\text{-}R$ where

- N is an alphanumeric string of length 8; N refers to the specimen from which the block is indexed.
- P, Q and R are numeric strings of length 4; P, Q and R are the X-, Y- and Z-indices of the block within the specimen, respectively.

Digital sections forming an image stack are stored in the directory named after the block, and each digital image file is named $N\text{-}P\text{-}Q\text{-}R\text{-}S\text{-}t\text{if}$ where

- N-P-Q-R corresponds to the directory name associated with the block as defined above.
- S is a numeric string of length 4; S is the image's Z-sequence number within the block.

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