

Tomographic Modeling of Casted Blood Vascular Systems

*Roger C. Wagner
& Fred E. Hossler*

The three dimensional architecture of blood vascular systems is intimately associated with their function of irrigating the tissues they serve. These blood vessels, down to the smallest capillaries, can be injected with a plastic polymer which rapidly polymerizes into a cast of the blood space within them. After corrosion of the surrounding tissue, a hardened replica is left which accurately represents the essential geometry of the vessel system. Examination of these casts with a scanning electron microscope reveals images of striking clarity and depth. However, they cannot be viewed from every angle and interior vessels are often hidden. Also due to parallax when tilting, accurate measurement of the casts is difficult. The casting material has properties which permit tomographic acquisition of three dimensional data sets by laser confocal microscopy and Micro-CT (CAT Scans). These data sets can be surface rendered to produce 3D models which can be rotated and examined from any perspective. Interior vessels of compact capillary systems can be visualized by skeletonization of the model which is an accurate representation of the entire network. Since three dimensional data is precisely quantifiable, important parameters such as blood space volume, internal vessel surface area, vessel length and number of vessel intercepts can be computed. These approaches hold promise for a more detailed appreciation of tissue vascularization as well as for analysis of abnormal circulation in diseased tissues.





Fig. 1. A corrosion cast of the vasculature of a rabbit's ovary as viewed with a dissecting microscope. The capillary systems in the interior surround developing follicles which contain ova (eggs). A portion of the oviduct vasculature is visible at the lower right. The depth of focus is limited and portions of the cast are out of focus. Scale Bar = 1 cm

All animal tissues, except cartilage, are irrigated by a closed system of blood vessels. These form intricate, arborizing networks specifically designed to serve the function of organs and tissues. The three dimensional geometry of these vascular systems is difficult to visualize from serial sections and dissection reveals only the associations of macroscopic vessels.

Corrosion casts of whole animals or specific organs permit examination of blood vascular systems down to the level of capillaries, the smallest vessels. These are prepared by injection of a rapidly-hardening polymer (Mercox) followed by corrosion of the surrounding tissue with alkali. The remaining plastic cast faithfully represents the blood compartment within the vessels and thus the geometry of the vascular system itself.

Light microscopic examination of casts is limited by a shallow depth of focus and lack of sharpness throughout their depth (Fig. 1).

When examined with a scanning electron microscope (SEM), images of corrosion casts appear sharp and in focus throughout their depth (Fig. 2a). An anaglyph created from stereopairs (images taken from two tilt angles about 7 degrees apart) when viewed with red-cyan glasses, provides a very effective 3D representation (Fig. 2b). Tilting and rotating the specimen within the SEM reveals branching and cast features hidden in other views (Fig. 3).

Tilting, however, changes the relative dimensions and relationships of the vessels in a non-linear manner due to parallax. Consequently, quantitative

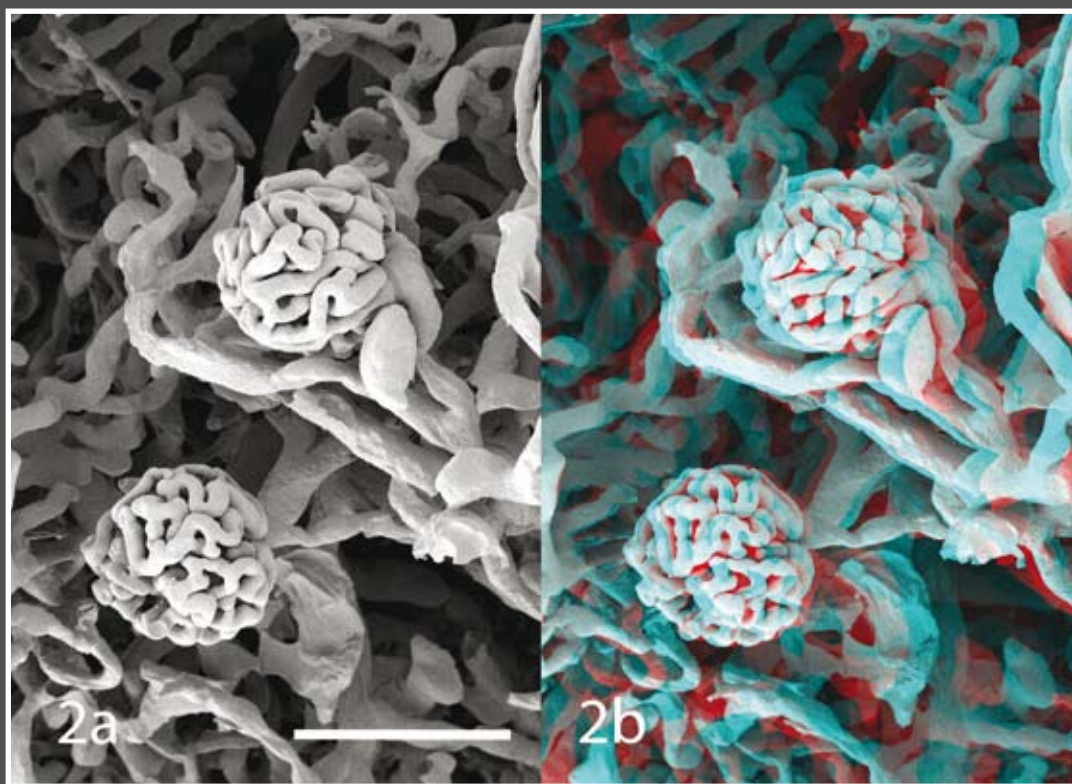


Fig. 2a. A scanning electron micrograph exhibiting two corrosion casted glomeruli in the kidney of a mouse. The sharpness of the image and depth of focus is greatly improved. Scale Bar = 100 μ m

Fig. 2b. An anaglyph representation of a stereopair of Fig. 2a taken at a 7 degree tilt angle difference. Using red-cyan glasses (red on left eye) a remarkable amount of three dimensionality can be perceived.

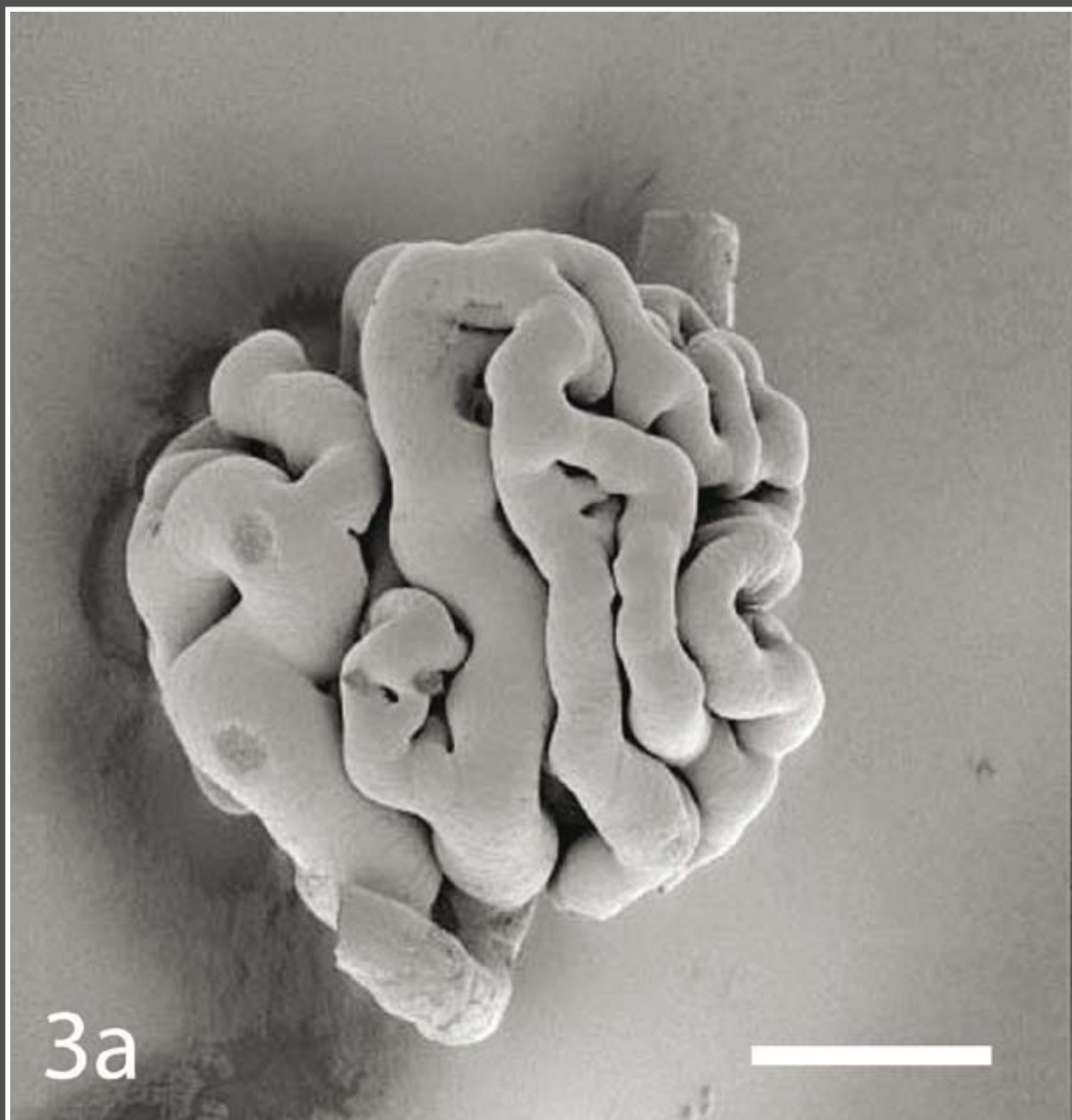
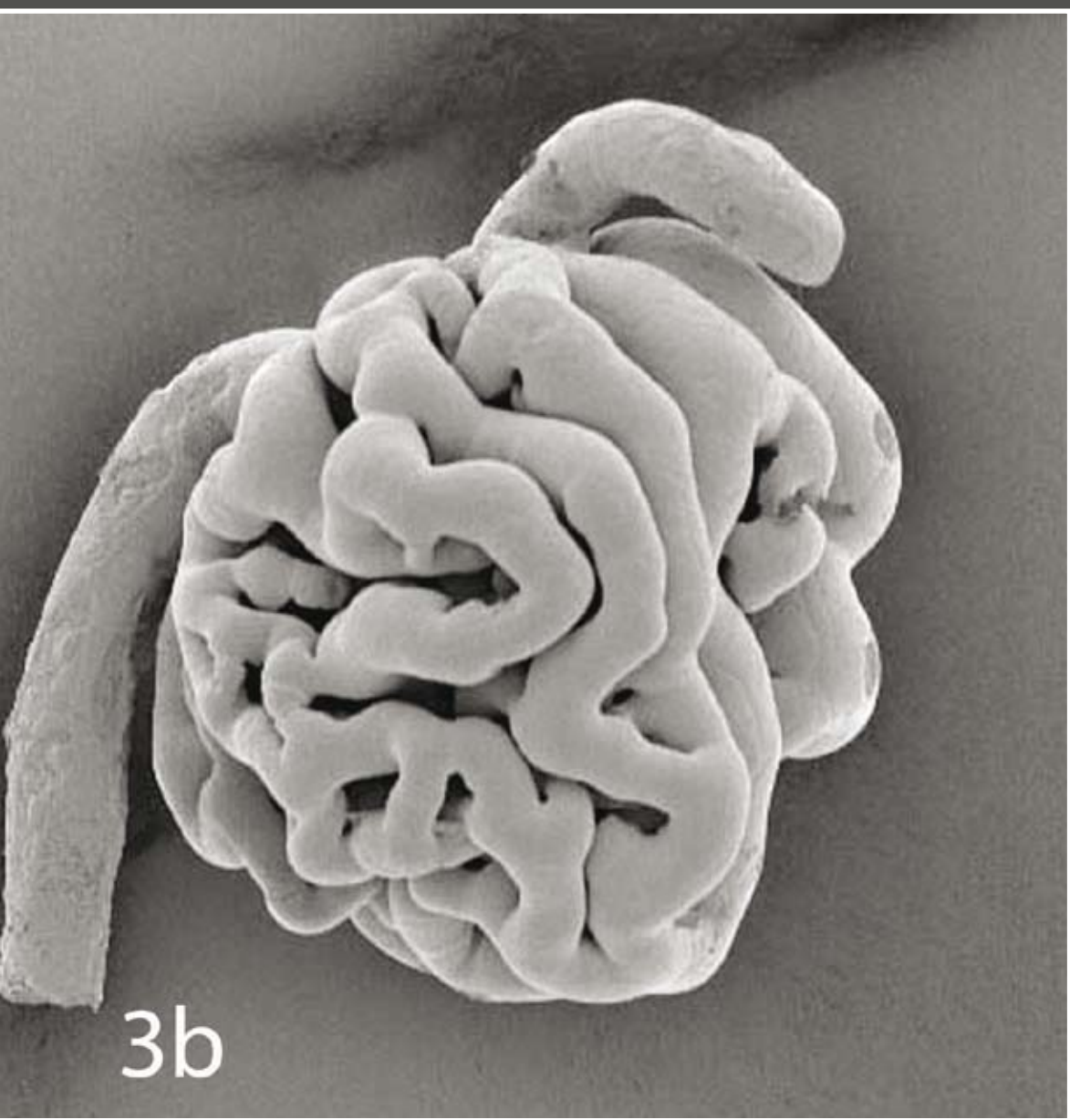


Fig. 3a & b. Images of a glomerular corrosion cast taken at a tilt difference of 30 degrees. Although portions of the cast not originally seen are evident, the dimensions and interrelationships of the vessels change in a non-linear fashion due to parallax. Precise measurements are difficult to obtain under these circumstances. Scale Bar = 40 μ m



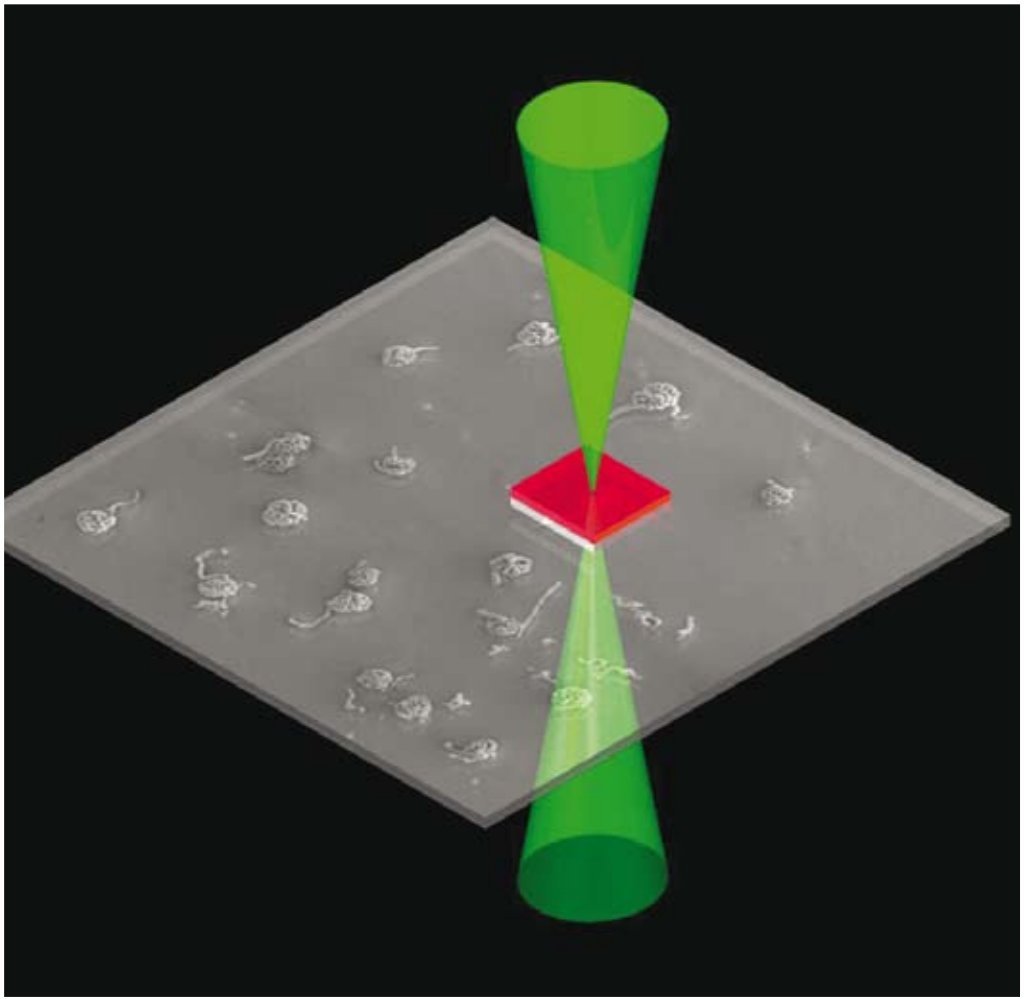


Fig. 4. An illustration of the manner in which laser confocal microscopy captures a fluorescent image. Only one plane of focus (red) is imaged by scanning a laser across the specimen in a raster. Fluorescence from above or below the plane of focus is eliminated resulting in a sharply defined image.

and morphometric data is difficult to obtain with the SEM. Also the interior organization of compact capillary systems is not revealed by imaging their surface (Fig. 3).

A solution to these limitations is the acquisition of tomographic three dimensional data sets by confocal microscopy and computer-assisted tomography (CAT scans). The casting plastic contains a dye which fluoresces and provides a fluorescent signal for confocal microscopy. With the addition of a heavy metal (lead), the casting plastic is also very X-ray dense providing a means for imaging with a CAT scan device.

In either case, acquired signals from the casting material must be intense and provide high contrast. When the 3D digital information in the form of Voxels (cubic pixels) is thresholded, a surface can be generated which closely approximates the true surface of the cast.

Modeling of Capillary Systems with Confocal Microscopy

In confocal microscopy, a laser beam excites fluorescing substances by scanning across a specimen in a square pattern called a raster. The resulting fluorescence signal is collected from only a single focal plane. Sharp, high contrast fluorescent images result from elimination of fluorescence from above and below the focal plane, a procedure known as optical sectioning (Fig. 4). Successive optical sections are acquired by moving the focus in increments through the specimen. Numerous optical sections through the specimen are called a z-stack and result in a three dimensional data set which is stored digitally as voxels (Fig. 5).

These three dimensional data sets can be processed and analyzed by software such as that provided by confocal microscope vendors or a powerful program called Amira (Mercury Computer

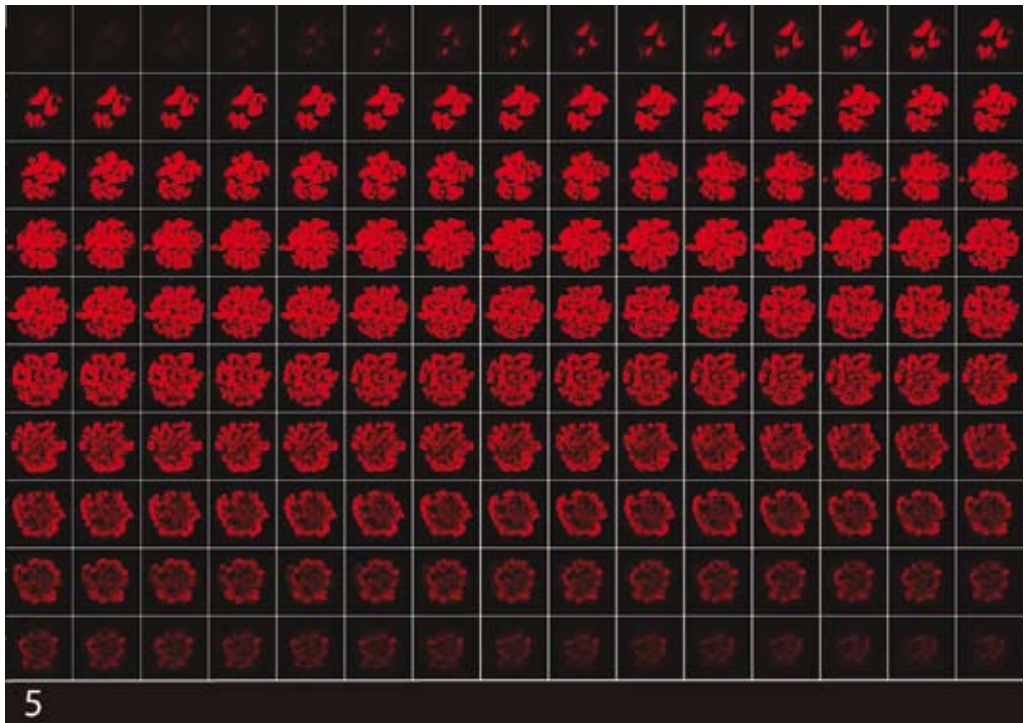


Fig. 5. Successive optical sections (160) acquired by moving the focus in small increments constitute a Z-stack, each image in perfect register with the others. This constitutes a three dimensional data set which is stored digitally as voxels.

Fig. 6a & b.
A scanning electron micrograph
of a kidney glomerulus and a
surface-rendered model of a similar
glomerulus appear remarkably
similar but the rendered model
can be rotated 360 degrees through
any angle for examination from any
perspective. Scale Bar = 50 μ m
Fig. 6c. An anaglyph representation
of a surface-rendered glomerular
model constructed from stereopairs at a 7
degree tilt differential. When examined
with red-cyan glasses (red on left
eye) considerable depth and three
dimensionality is perceived.

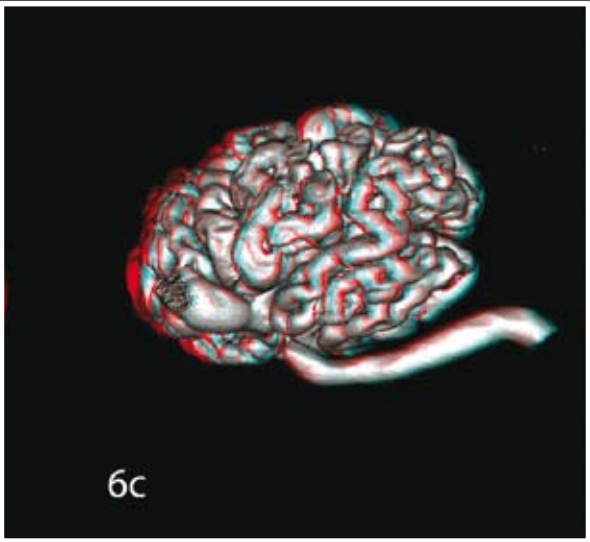
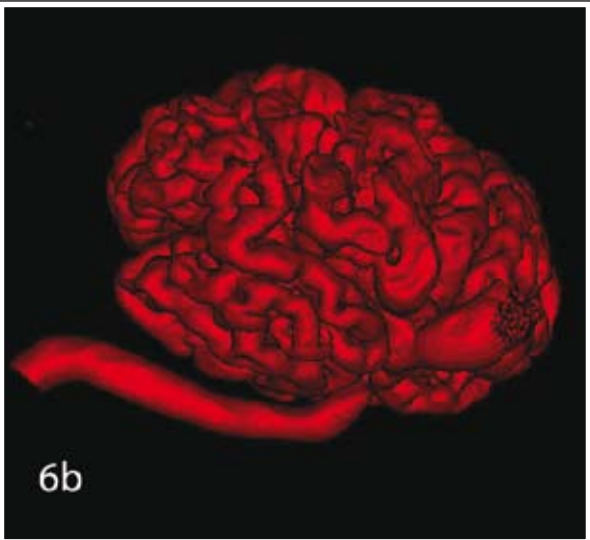
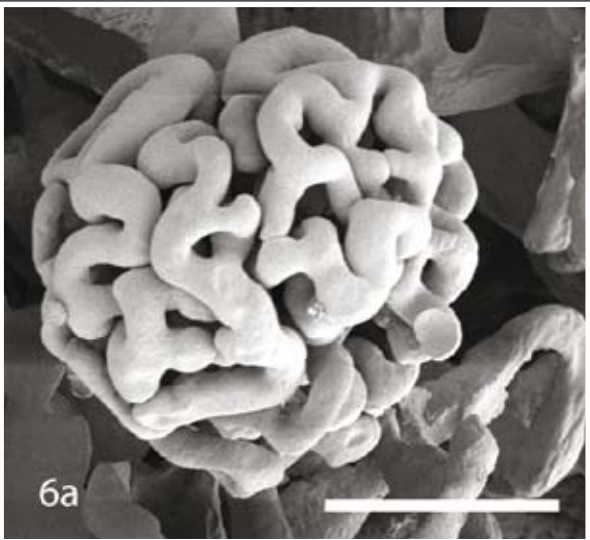


Fig. 7a. Skeletonization thins a model by eroding away all but a central axis of voxels. The resulting one-voxel-thick "skeleton" represents a three-dimensional network of all the vessels in the model and the interior vessels and their interconnections become visible. Scale Bar = 40 μ m

Fig. 7b. A transparent rendering of the original model with the skeleton at its interior helps to verify the accuracy with which the skeleton represents the geometry of the capillary network. The color coding of the skeleton indicates the relative diameters of the vessels from which the skeleton was derived.

Fig. 7c. Branch points or intercepts of vessels are marked with white dots. These can be computed from any skeleton as well as the total length of the capillaries and capillary segments between branch points.

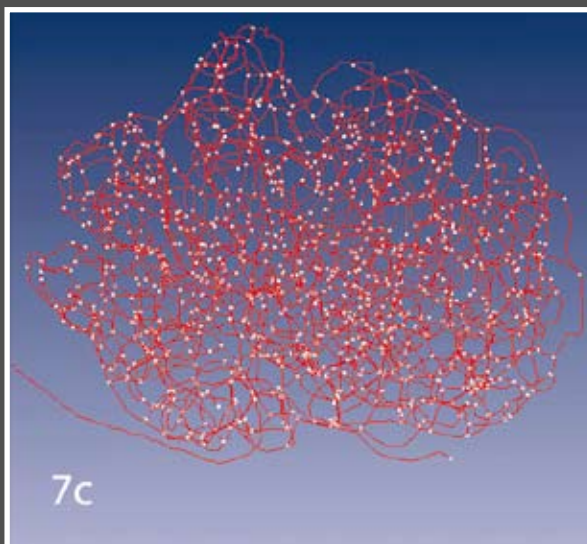
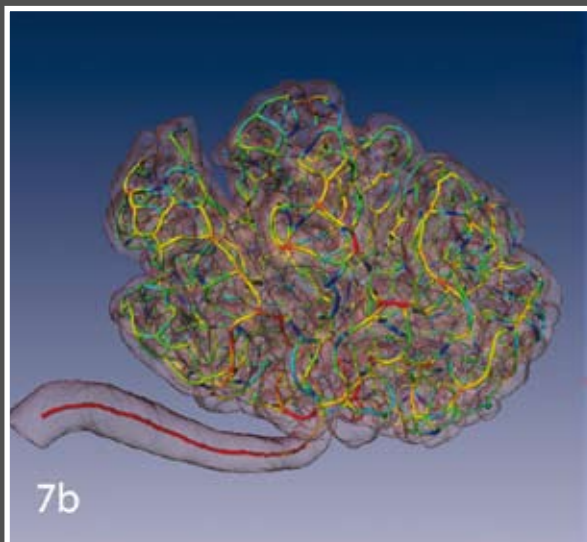
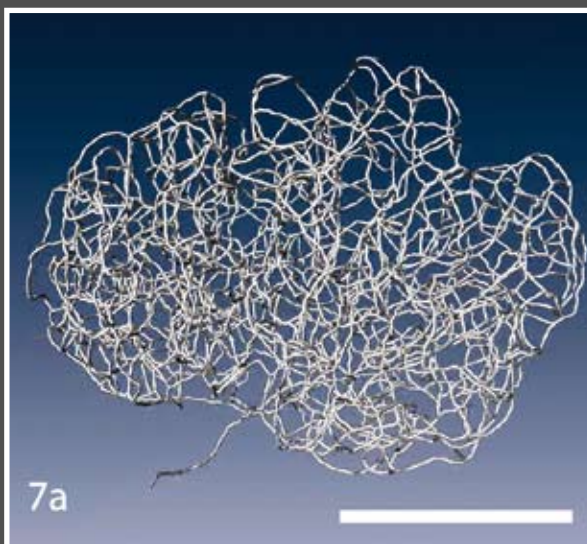


Fig. 8a. A scanning electron micrograph of a corrosion cast of the choroid layer in the back of the frog eye (choriocapillaris). The large orifice is the point of exit of the optic nerve from the eyeball. Scale Bar = 2 mm
Fig. 8b. A rendered model of the choriocapillaris looking remarkably like the SEM image.
Fig. 8c. A transparent rendering of the surface with the skeleton of the model visible in the interior. It is evident that the skeleton accurately represents geometry of the original vessel system along with branching and intercepts.

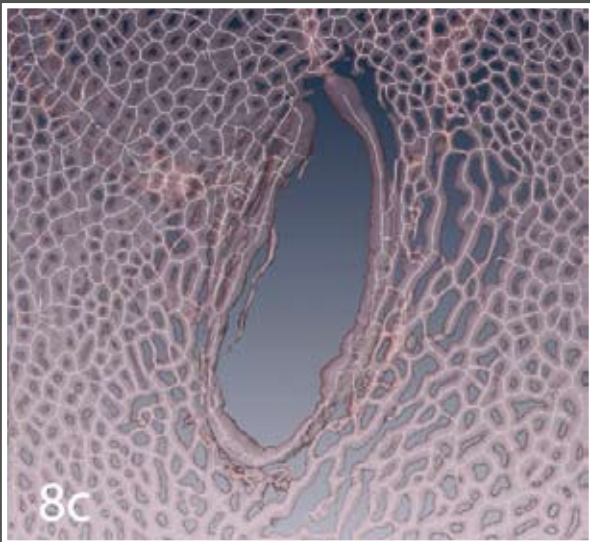
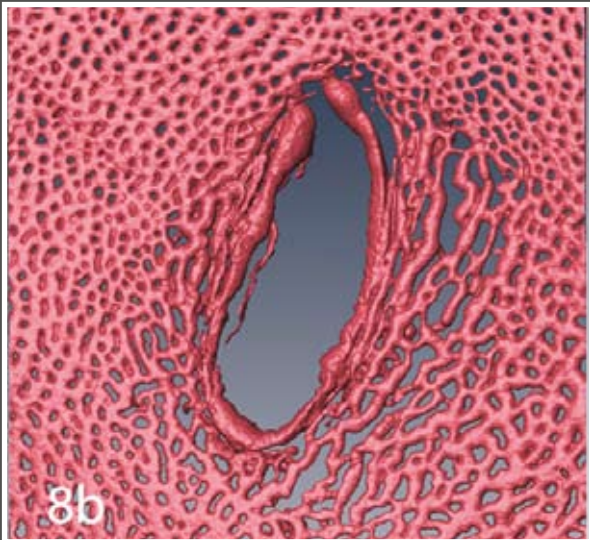
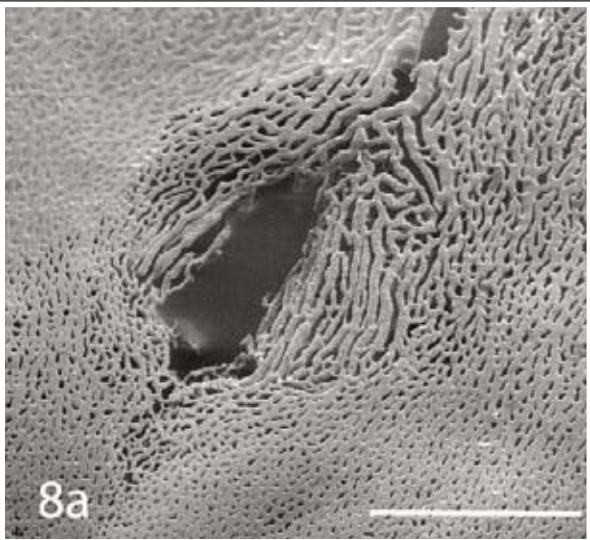
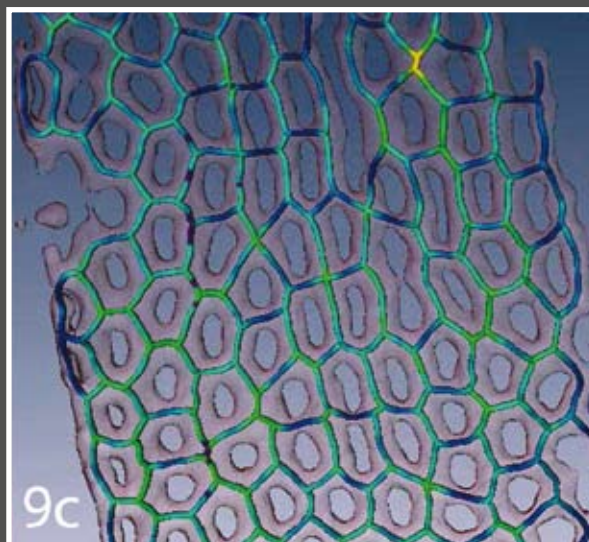
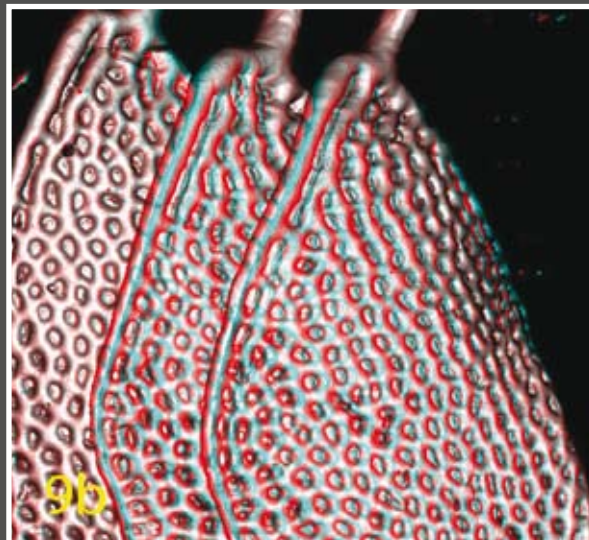
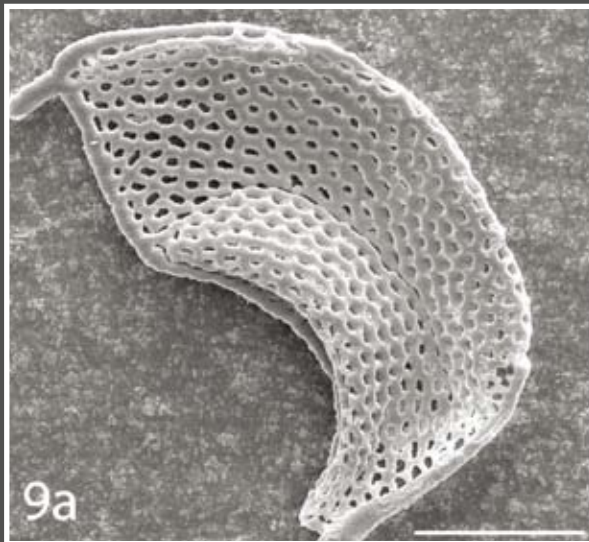


Fig. 9a. A scanning EM of a corrosion cast of a single gill filament from a striped bass.

Fig. 9b. An anaglyph presentation of three modeled gill filaments. When viewed with red cyan glasses (red of the left) a marked amount of depth and three dimensionality is perceived.

Fig. 9c. A transparent rendering of a fill capillary corrosion cast with the skeleton derived from the model in its interior.



Systems). A 3D data set consists of voxels of a wide range of signal intensities. Surface rendering a model requires thresholding or setting the voxel intensity where the surface is to be rendered. This is a somewhat arbitrary setting, however, the more intense the fluorescent signal the greater its contrast against background noise and the closer the threshold value will approximate the true surface of the cast.

Surface rendering results in strikingly clear models, which appear identical to SEM of the original cast (Figs. 6a & b). Unlike SEM images however, the model can be rotated 360 degrees through any angle for examination from any vantage point. Anaglyph stereo-images can also be generated using any two tilt angle differentials of about 7 degree (Fig. 6c). The voxel size for each data set is calibrated and from this important quantitative data such as volume (representing the size of the blood compartment within the vessels) and surface area (representing the inner surface of the blood vessels) can be easily computed.

Surface models of compact capillary systems such as the glomerulus of renal corpuscles of the kidney do not reveal interior vessels and their interconnections which are hidden from view. These can be visualized by a medial axis transform routine or "skeletonization". This thins the model by eroding away all but the central axis of voxels.

The result is a one voxel thick skeleton representing a three dimensional network of all the vessels in the model and the interior vessels and their interconnections become visible (Figs. 7a & b). From this skeleton, total vessel length as well as branch points or vessel intercepts can be computed (Fig. 7c).

The accuracy with which the skeleton represents the original model can be verified by viewing the skeleton simultaneously inside a transparent rendering of the original model (Fig. 7b). This can be more easily seen in flat capillary systems occurring in the same plane such as those in the

chorion of the eye (Fig. 8) or in the gill filaments of fish (Fig. 9).

An alternative way of viewing casted capillaries is to leave the tissue surrounding them intact. Casted kidneys can be left uncorroded, quick frozen and sectioned with a cryotome. A nuclear stain (Syto 13) is used to stain the sections and they are examined with a confocal microscope. These preparations result in images in which the casted vessels fluoresce as well as the nuclei and cytoplasm of the surrounding tissue cells (Fig. 10).

Confocal microscopes at low magnification can acquire data sets from casts of large regions of capillaries such as those in the alveoli of lung tissue (Fig. 11). However, larger vascular systems such as those serving whole organ systems require tomographic methods designed for acquisition of larger volumes such as CAT scans.

Modeling of Whole Organ Vascular Systems with X-ray Tomography

Tomographic imaging using X-rays contrasts dense materials such as bone. These materials impede X-rays resulting in a negative image. We have used CAT scanning to acquire 3D data sets of corrosion casted whole organs. By adding a soluble lead compound (tetraethyl lead) to the casting polymer, its density to X-rays is greatly enhanced resulting in a very intense, contrasty image. Successive planes of images are acquired and stored in three dimensional data sets which can be modeled in a fashion similar to confocal data sets.

Whole mouse kidneys were injected with Mercor to which 0.5 % tetraethyl lead was added and the tissue was corroded producing a whole organ cast. The enhanced X-ray density of the casting material resulted in intense, high contrast image planes of the cast. Stacks of successive images were acquired and processed with Amira software. These were

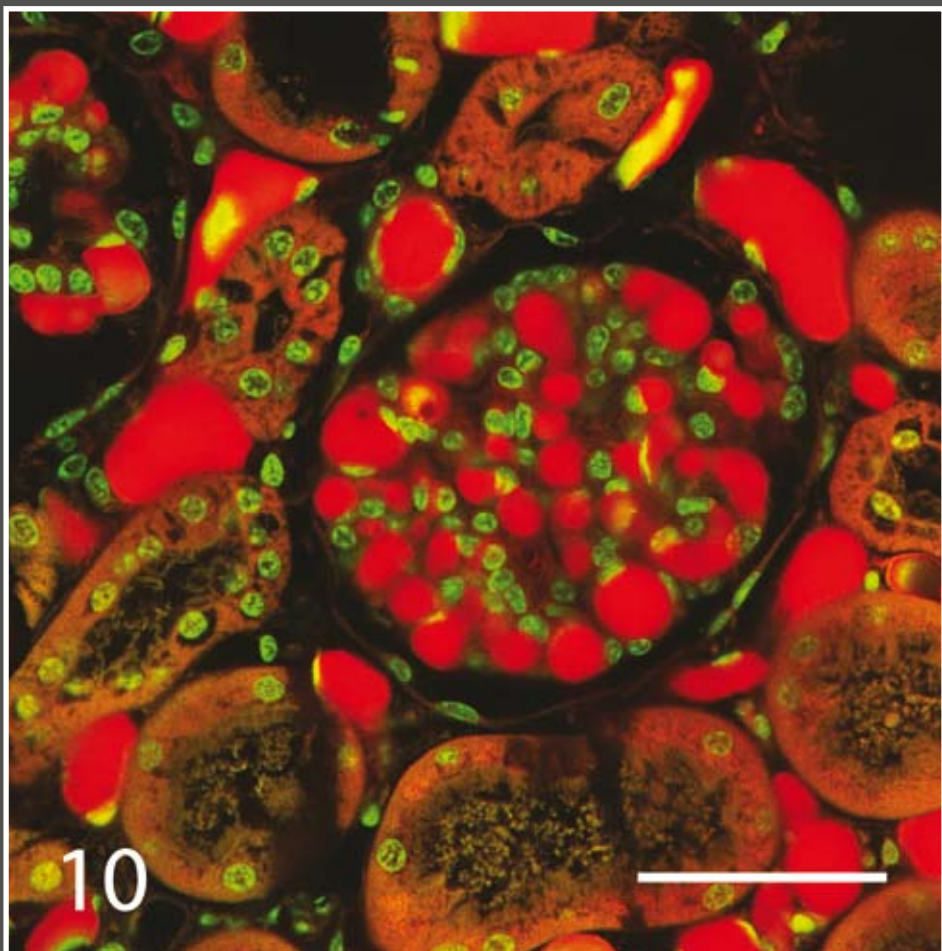


Fig. 10. A confocal image of a mouse kidney into which casting polymer had been injected but the surrounding tissue was left uncorroded. The specimen was rapidly frozen and sectioned on a cryomicrotome and the nuclear stain Syto 13 was applied to the section. The cast of the capillaries appears red and the nuclei of the endothelial cells of the capillaries and cells of the kidney tubules are green. The cytoplasm of the kidney tubule cells also fluoresces and appears reddish-brown. Scale Bar = 50 μ m

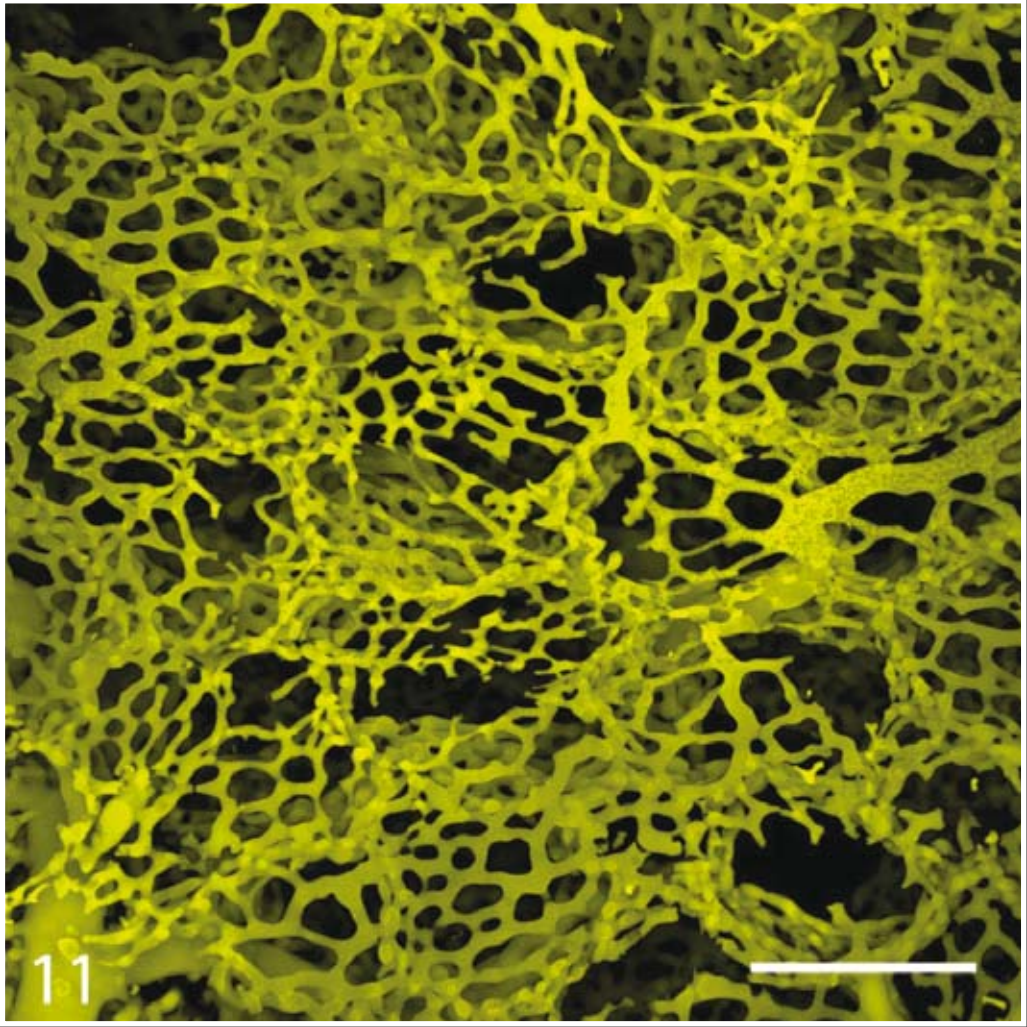


Fig. 11. A confocal fluorescence image of a series of optical sections (z-stack) through alveoli in the lung of a mouse. The prodigious supply of capillaries around each alveolus belies their function in exchange of blood gasses in internal respiration. Scale Bar = 250 μ m

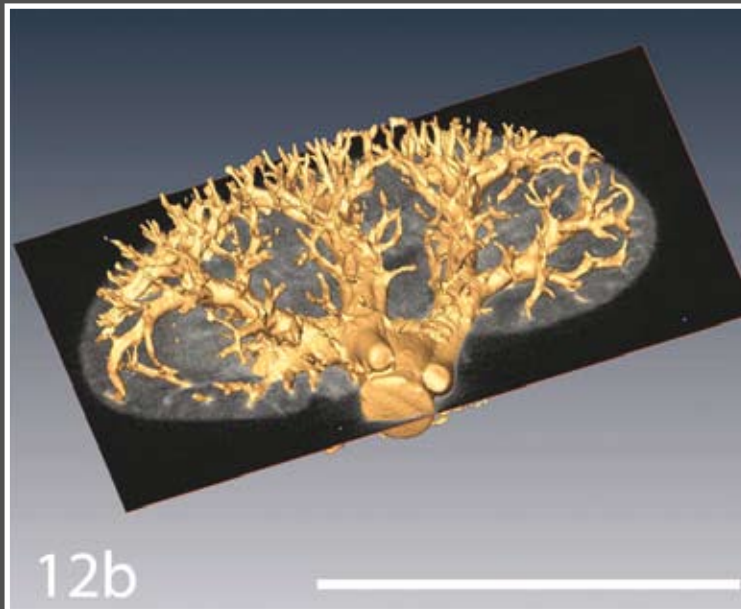
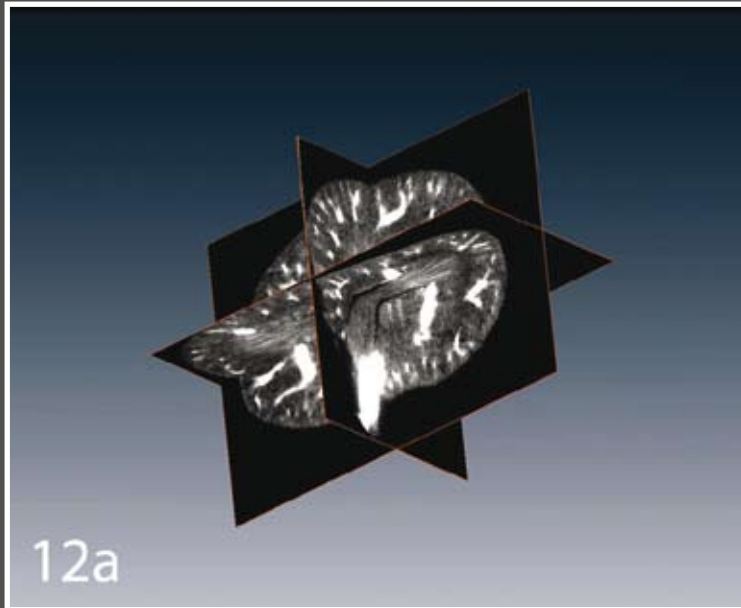


Fig. 12a. Three orthoslices (X,Y and Z) of thresholded X-ray signals of a mouse kidney corrosion cast take with a Micro-CT (CAT Scan).These planes can be made to sample any portion of the 3D data set.

Fig. 12b. A rendered model of the major vessels in a whole mouse kidney.A single orthoslice intersects the model showing regions containing smaller vessels, capillaries that cannot be resolved with Micro-CT. Scale Bar = 1 cm

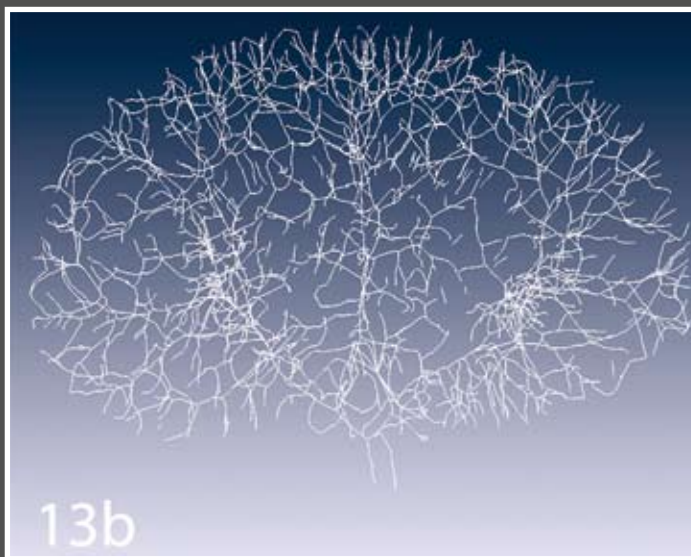
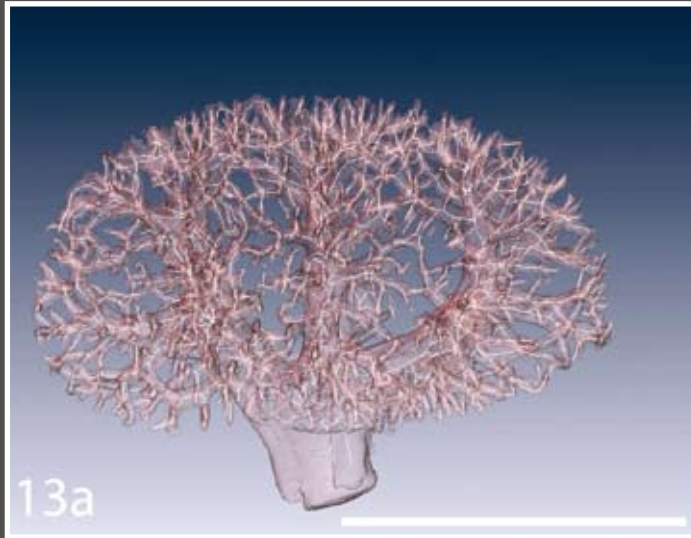


Fig. 13a & b. Skeletonization of these major vessel types represents their three dimensional geometry (a) and the skeleton (b) can be quantified to reveal vessel length, intercepts and number of segments. Scale Bar = 1 cm

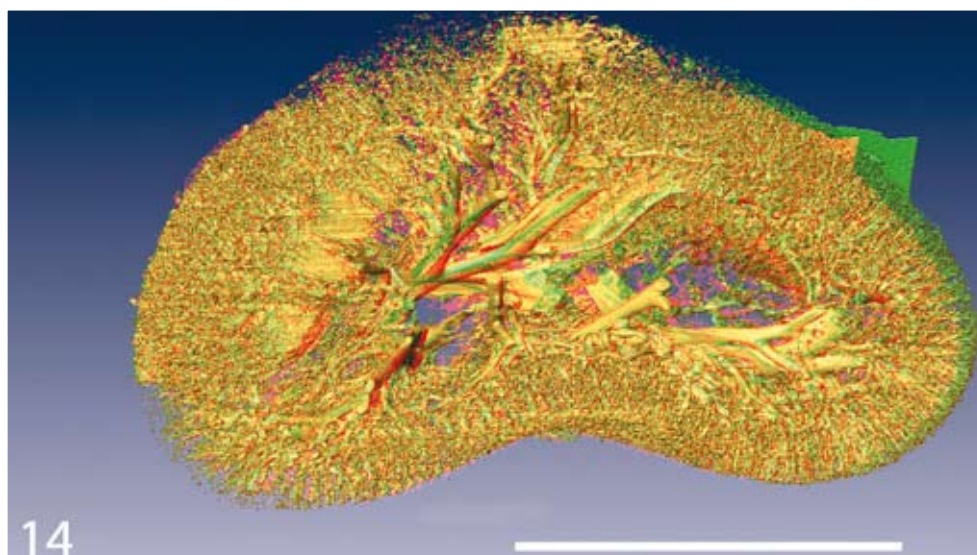


Fig. 14. An anaglyph representation of a modeled whole kidney corrosion cast permits appreciation of the three dimensional arrangement of major vessels in the kidney. Scale Marker = 1 cm

thresholded and could be visualized in either or all x, y and z planes (Fig. 12a). A surface rendered model reveals most of the major macroscopic vessels in the kidney (Fig. 12b). Unfortunately, the resolution provided by our Micro-CT scanner was not sufficient to reveal casts of vessels smaller than interlobular or arcuate arteries and veins (Fig. 12b).

Skeletonization of these whole organ models, however, faithfully represents the three dimensional geometry of the major vessel types (Fig. 13). The interrelationships, interconnections and branching patterns are evident in the skeletal structure. Anaglyph representations of these models permit visualization and appreciation of three dimensional arrangement of vessels in the kidney (Fig. 14).

Tomographic modeling of vascular corrosion casts provides accurate, quantifiable three dimensional information regarding blood vessel systems and hold great promise for understanding the blood supply to normal and diseased tissues.

We wish to express appreciation to Dr. Kirk Czymmek, Director of the Imaging Facility at the Delaware Biotechnology Institute, for acquiring confocal data of corrosion casts. We would like to thank Dr. Brian Andrews (Lab. of Neurobiology, NIH) for his suggestion of using tetraethyl lead as a contrasting agent for Mercox when using Micro-CT scans. We are also indebted to the Center for Translation Cancer Research at the University of Delaware for use of their Micro-CT.



Roger C. Wagner
Professor Emeritus of
Biological Sciences
University of Delaware
rag@UDel.Edu



Fred E. Hossler
Professor of Anatomy
and Cell Biology
East Tennessee State
University College of
Medicine
Hossler@etsu.edu

Dr. Roger Wagner received his Ph.D. in Cell Biology from the University of Minnesota. He was a post doctoral fellow at the Yale Medical School and has been on the faculty in Biological Sciences at the University of Delaware since 1973. He retired in 2005 and is presently Professor Emeritus of Biological Sciences. Dr. Fred Hossler received his Ph.D. in Pathobiology from the University of Colorado. He was a postdoctoral fellow along with Dr. Wagner at Yale. He was on the faculty of the LSU Medical Center and has been Professor of Anatomy and Cell Biology at the East Tennessee State University College of Medicine since 1981. Dr. Wagner and Dr. Hossler have been collaborators in research and the closest of friends for over 35 years. They share a fascination with biological structure and the beauty of living things. They also share a zany sense of humor not much appreciated by others.