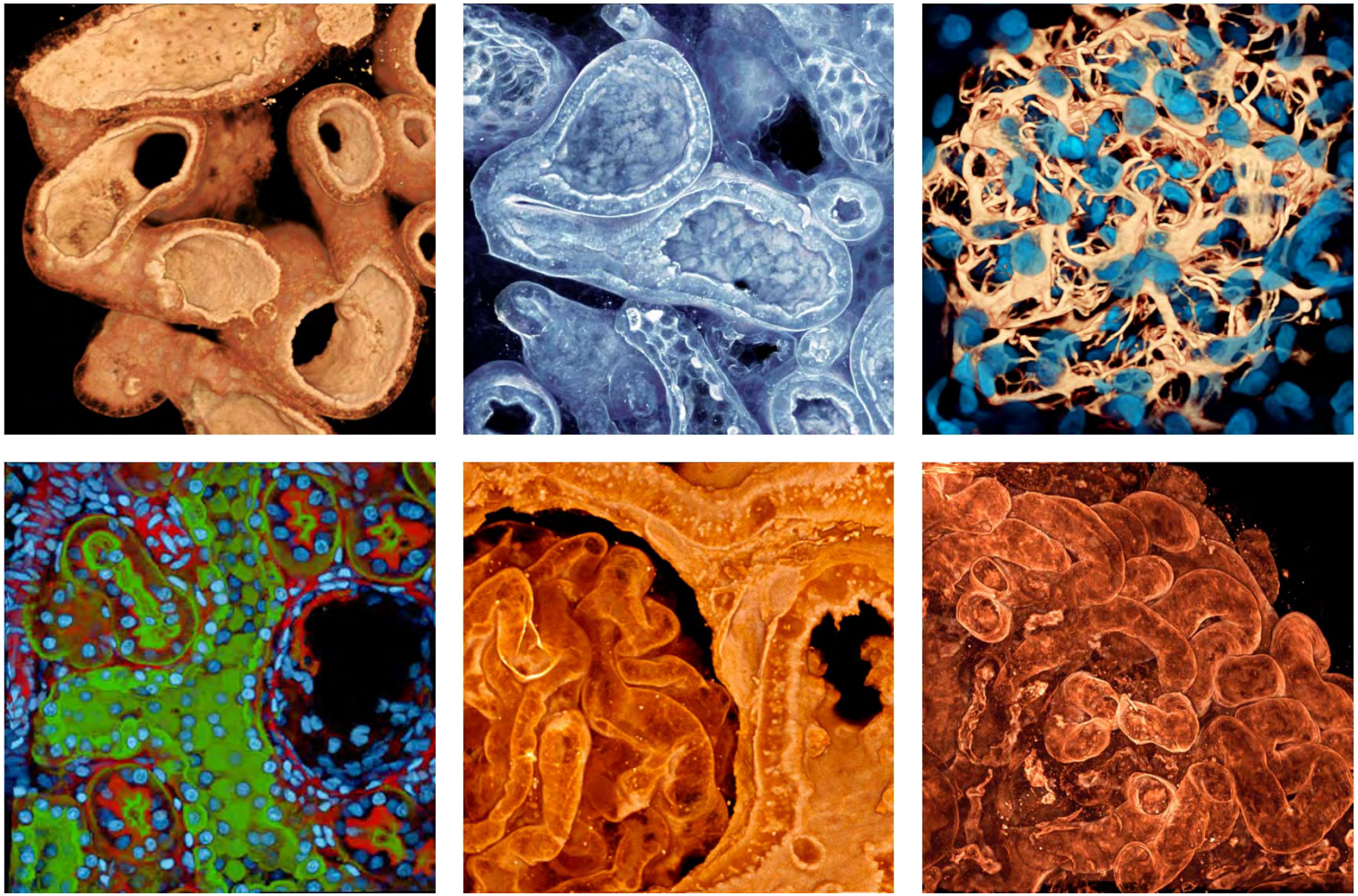
3D Visualization and Analysis

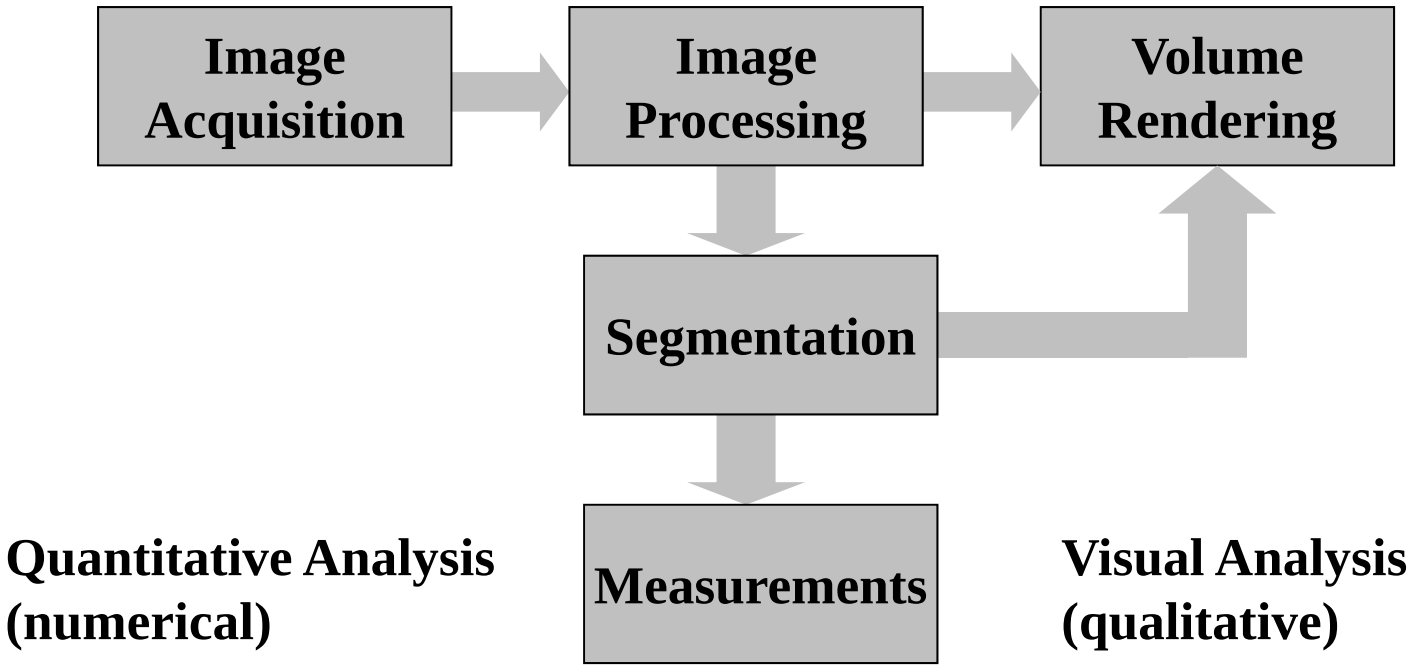
J. L. Clendenon

Aeon Imaging LLC



Data flow through the 3D imaging pipeline

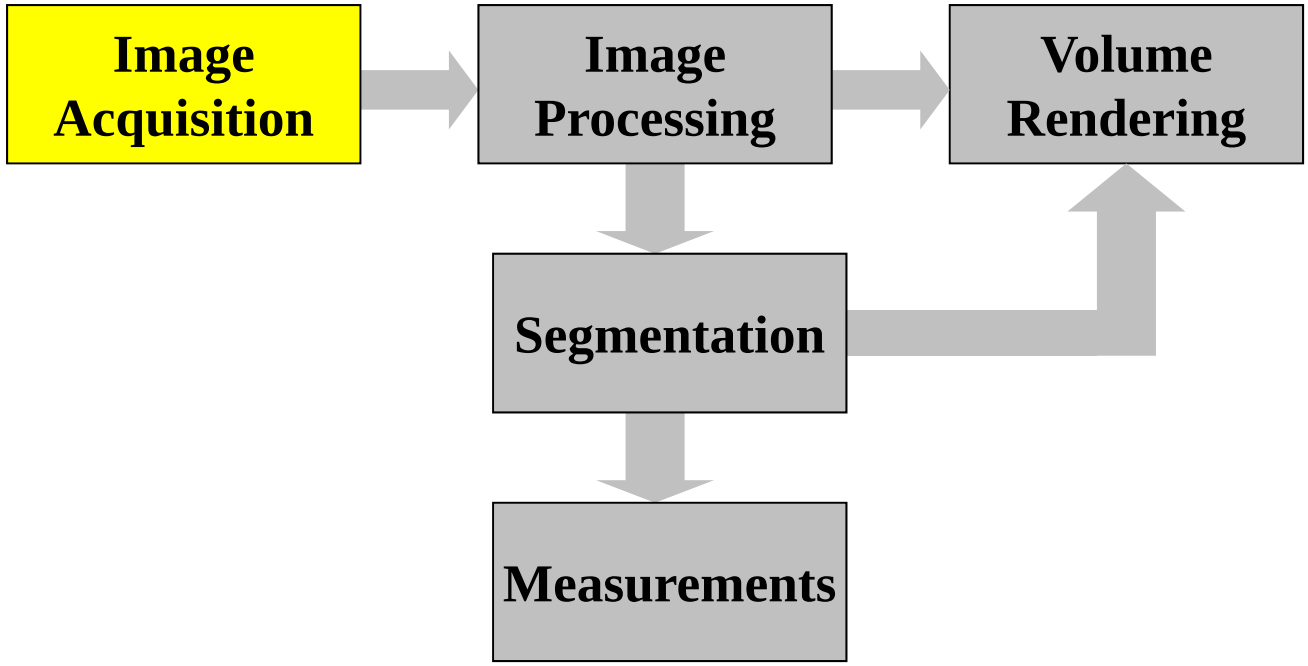
People involved in image-based research must perform several tasks: image acquisition, processing, rendering, segmentation, and measurement.



3D imaging software includes modules to perform one or more of these tasks

Image Acquisition

The starting point for 3D image processing and analysis is usually some form of cross-sectional image acquisition system.

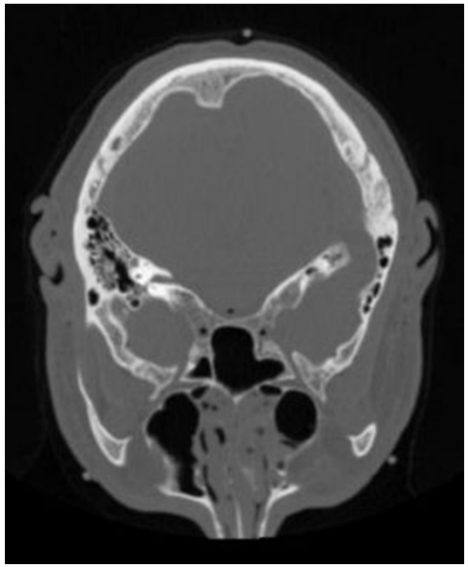


In cross-sectional imaging, parallel planar (2D) images from various levels within a 3D specimen are collected…

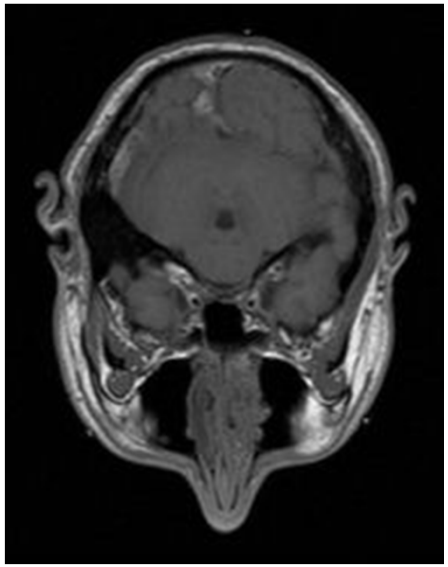
Cross-Sectional Image Acquisition

Several imaging technologies have been developed over the years that can produce cross-sectional images, such as CT and MR in radiological imaging, and confocal and two-photon techniques in optical microscopy.

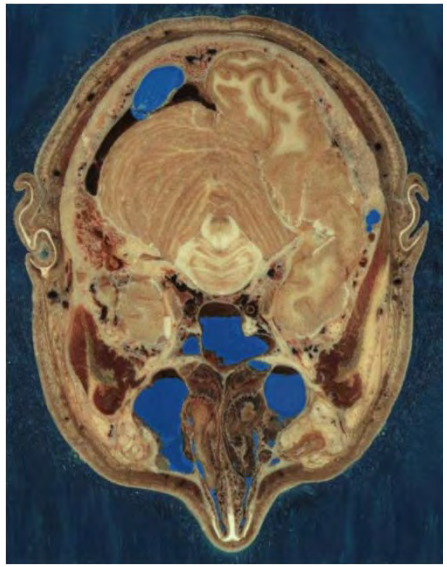
Computed Tomography



Magnetic Resonance



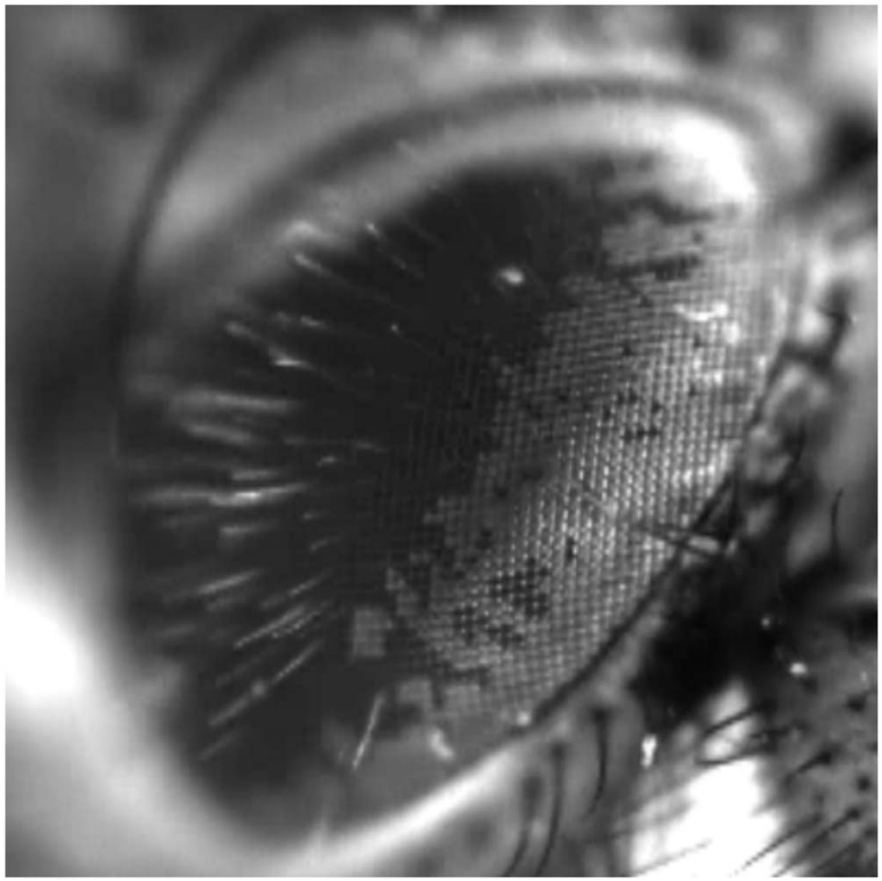
Blockface



e.g. Visible Human Project [1994], cross sections of human head.
http://www.nlm.nih.gov/research/visible/visible_human.html

Optical Microscopes have limited Depth-of-Field

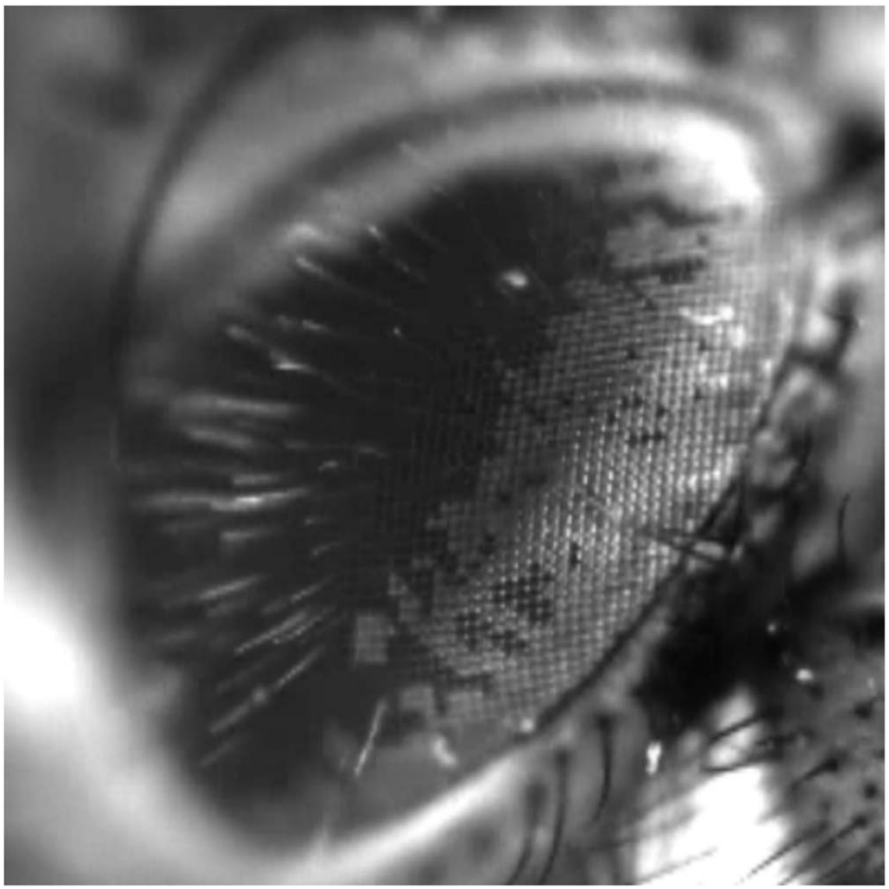
This makes it difficult to study thick specimens

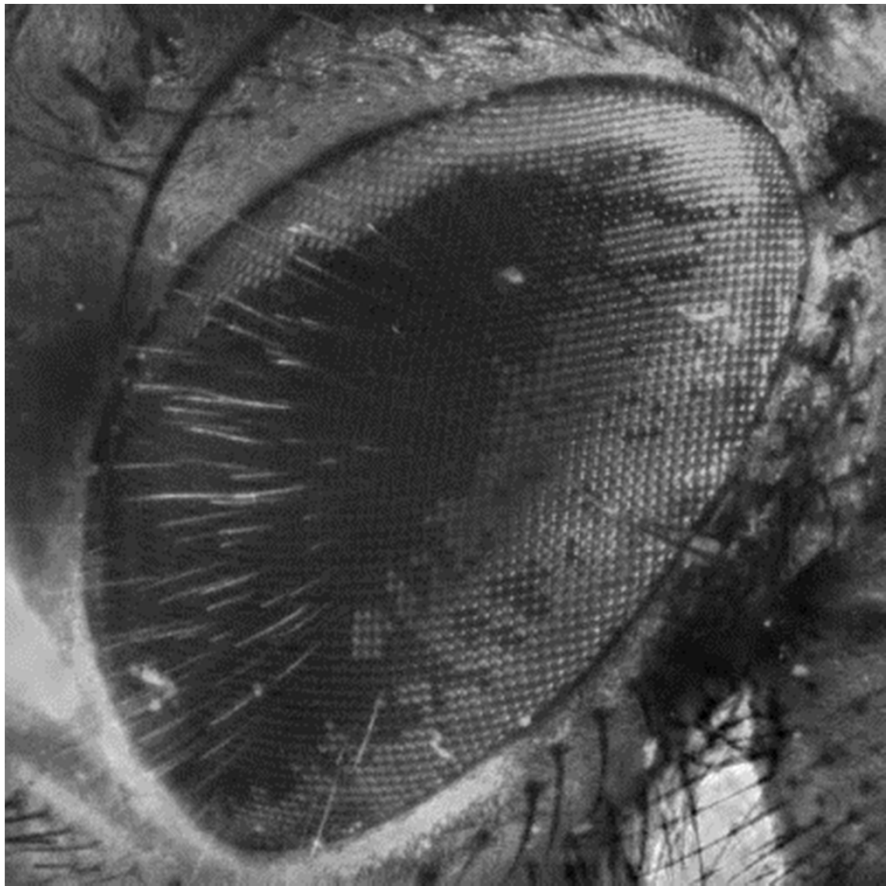


Structures closer to the focal plane are more in focus
than structures farther away from the focal plane

Extended Depth-of-Field

But you can create a projection image with extended depth-of-field

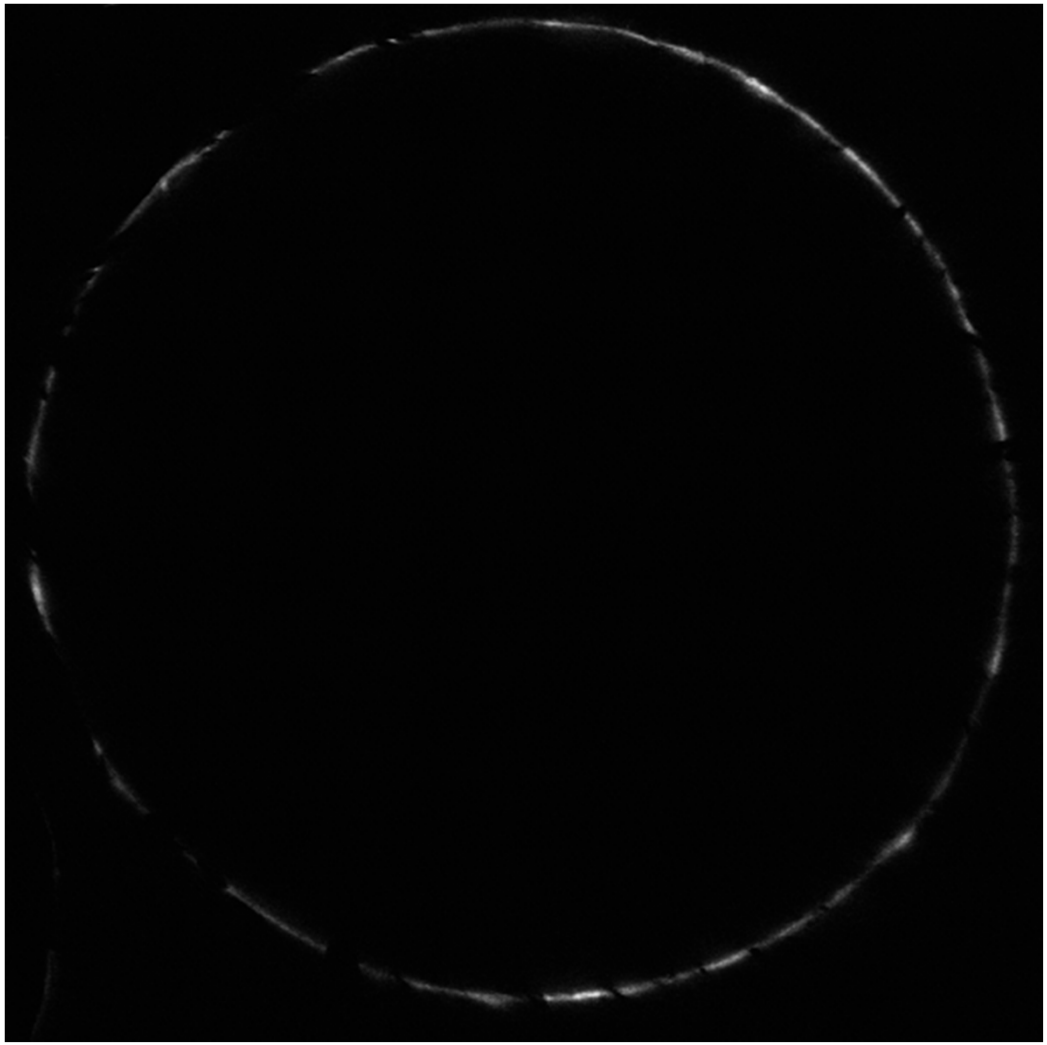




Use software to create a composite image (e.g. weighted average)
of just the in-focus portions of ALL of the images in the z stack
(e.g. used plugin for ImageJ from http://bigwww.epfl.ch/demo/edf)

Confocal microscopes have smaller depth-of-field

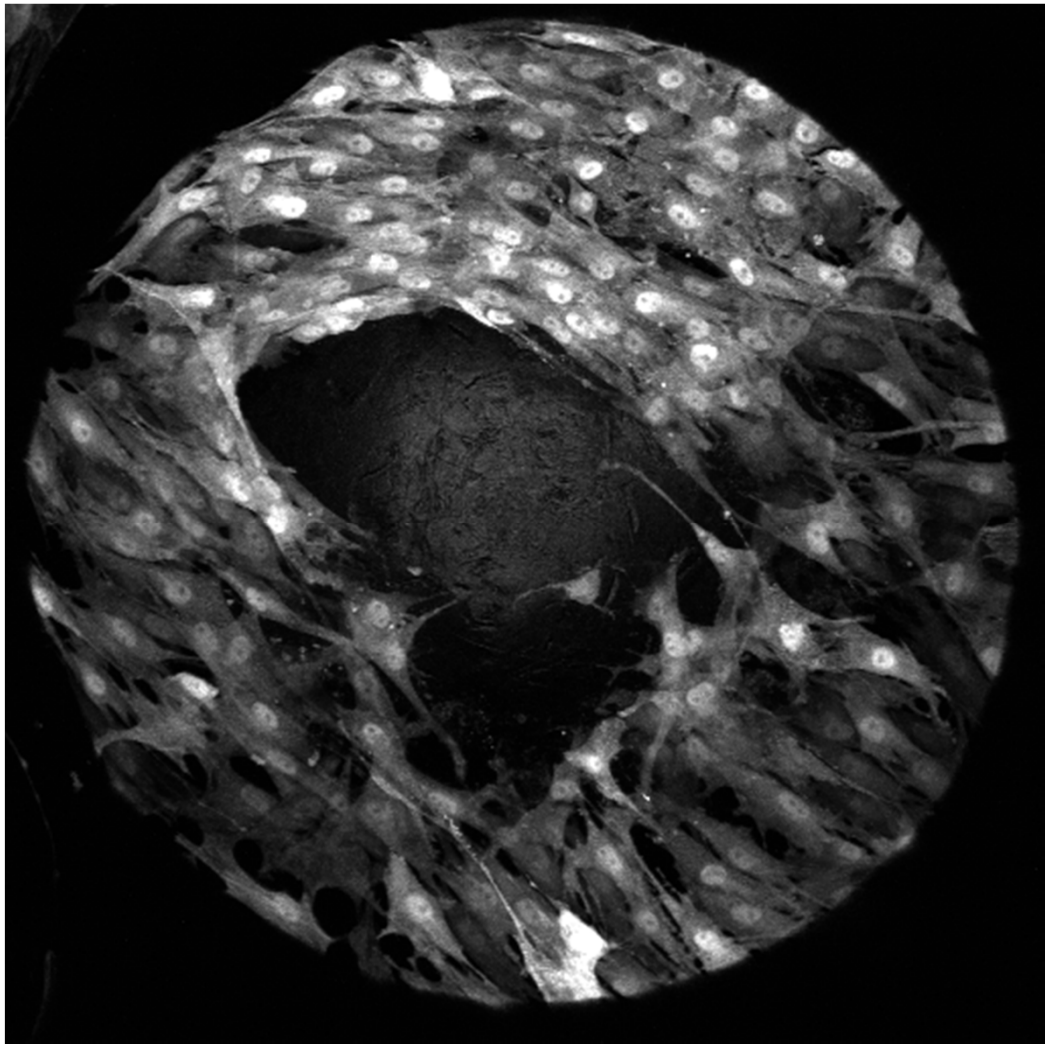
e.g. 352 LSCM images – but what is it?



Images collected on Olympus FV1000 using “super 20x” objective at the ICBM

Its… cells growing on a microsphere

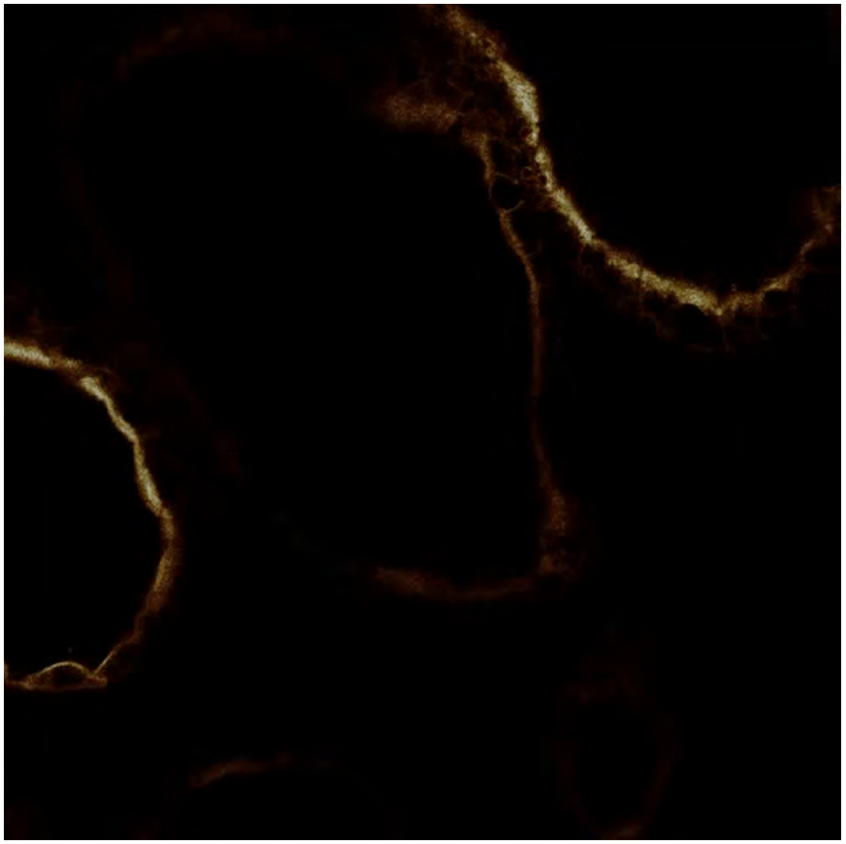
Made visible by computing maximum intensity projection



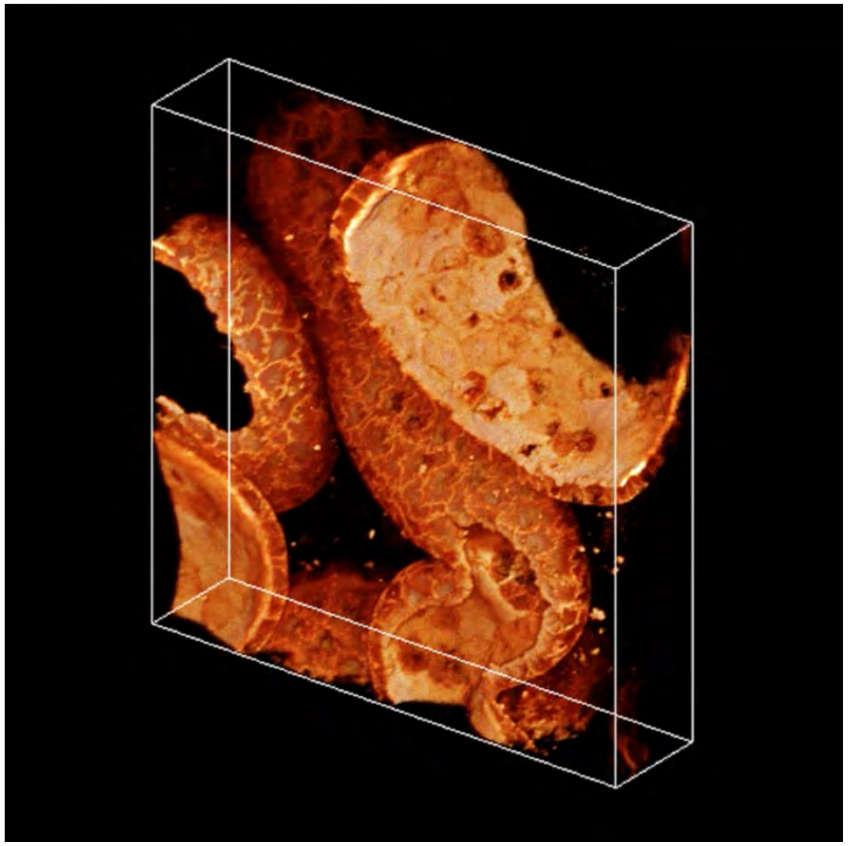
One example of 2D image compositing operation performed on the image stack

What about off-axis viewpoints?

You need 3D imaging software to compute off-axis views of z stacks



On-axis movie using ImageJ



Off-axis rendering using Voxx

Cystic kidney tubules were rendered using alpha blending

Visual Analysis (qualitative)

Volume rendering programs can be used to create 2D projection images showing the 3D stacks of cross-sectional images from various points of view.

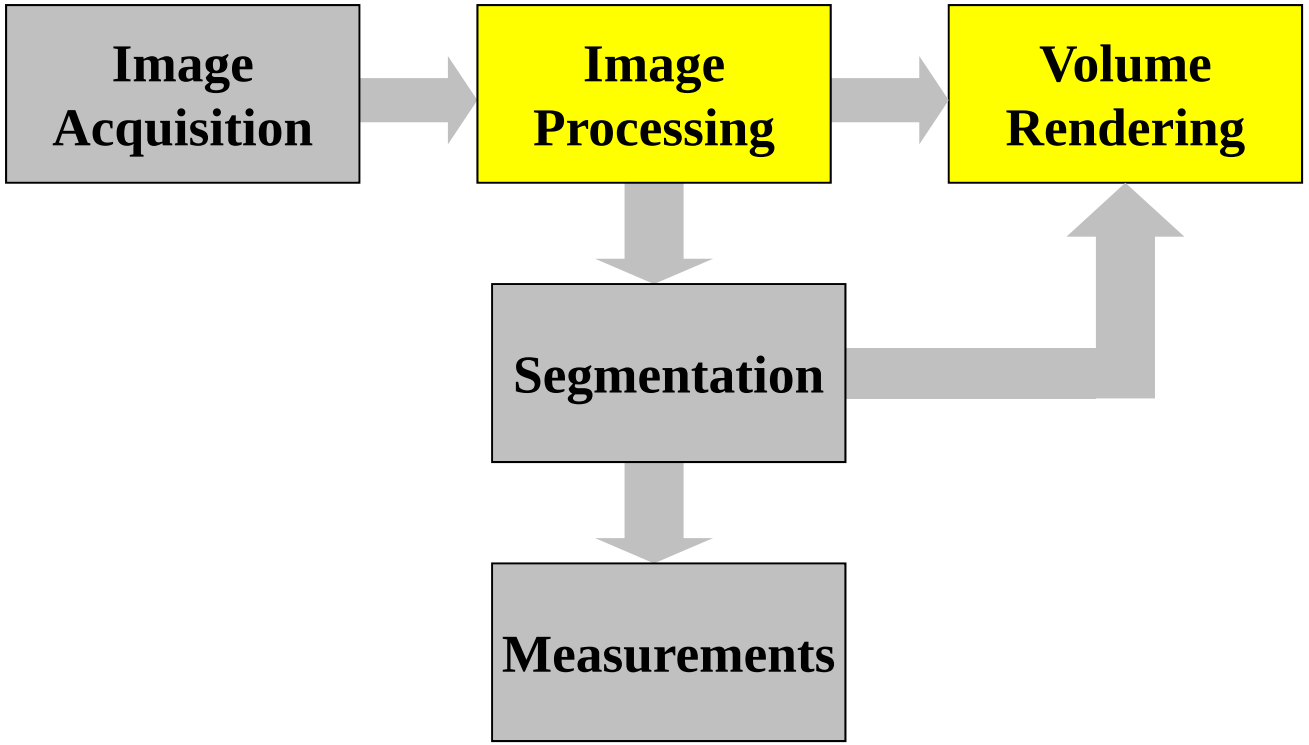
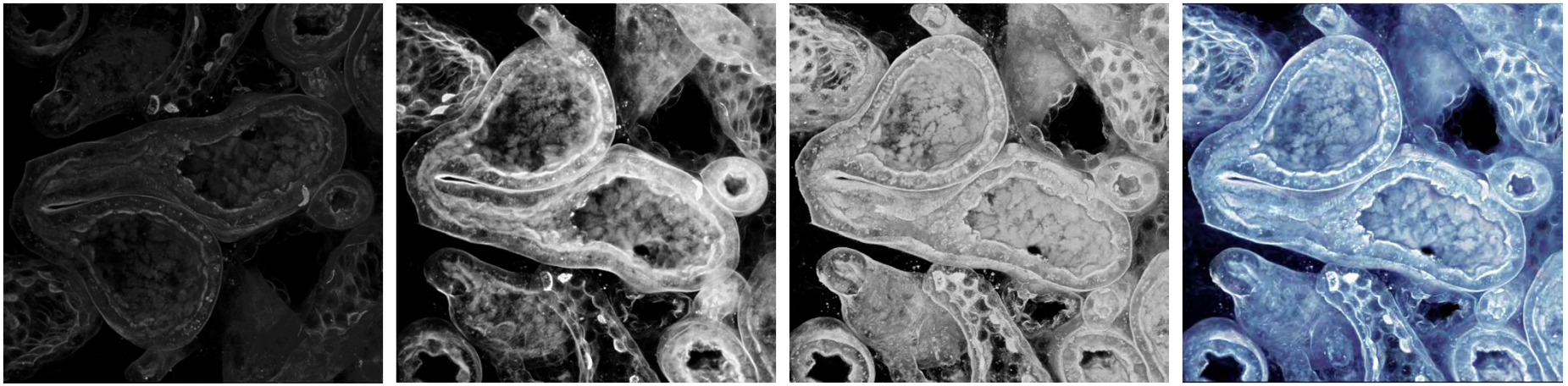


Image processing operations may need to be performed on 3D images before they are displayed or passed to image analysis software

Intensity and Color Mapping

You must carefully adjust brightness, contrast, color, and opacity to produce a high-quality 3D effect. Here is a typical sequence of operations that needs to be performed:



1) Select blending mode – alpha, sum, or max.

2) Adjust opacity – so that you can see into deeper portion of image stack. Only need for alpha blending.

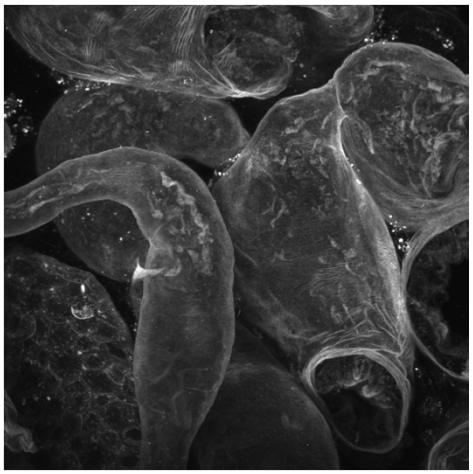
3) Adjust contrast and brightness - so that you can see monochrome specimen.

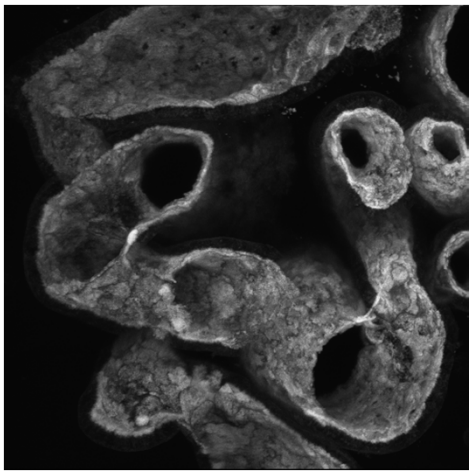
4) Colorize – to highlight structures of interest, and/or improve the 3D effect.

Image Compositing – 2P Microscopy

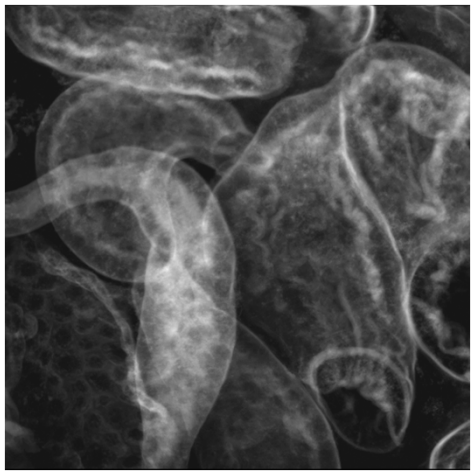
The various math operators used to combine projection images can produce very different looking volume renderings, so its important to understand how this works.

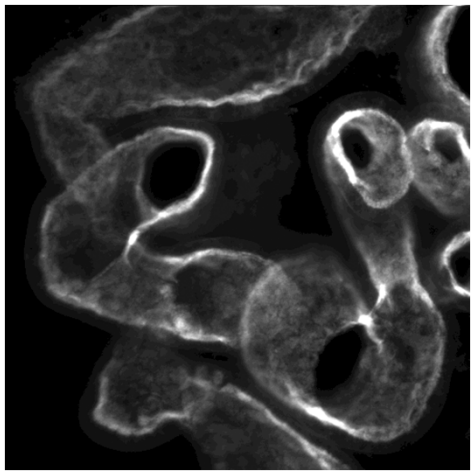
Maximum



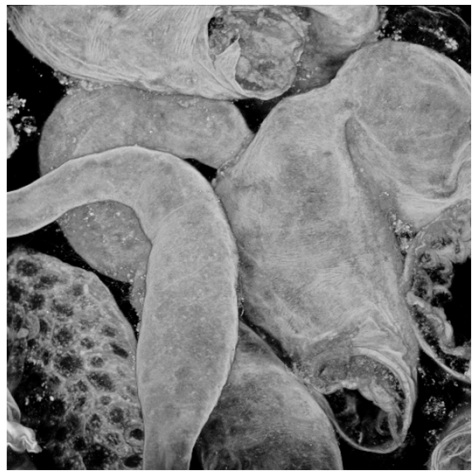


Average





Alpha blending



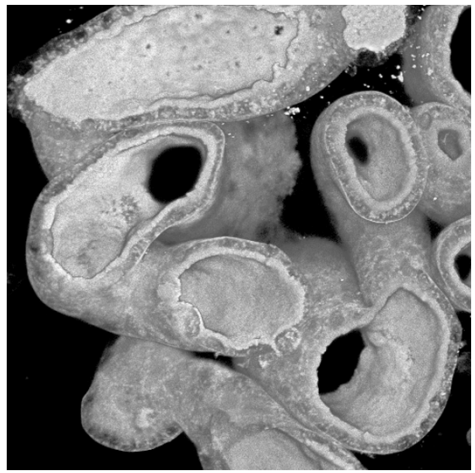
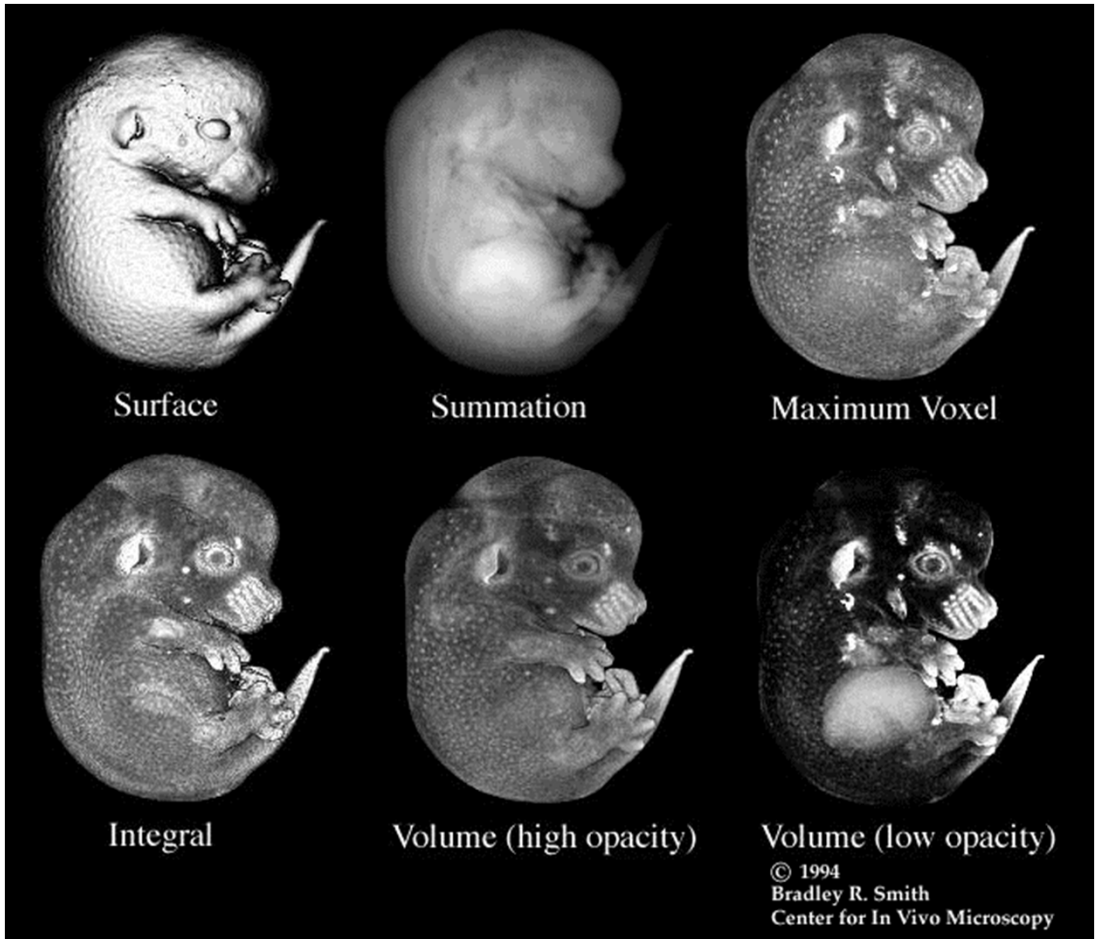


Image Compositing – Micro MRI

The various math operators used to combine projection images can produce very different looking volume renderings, so its important to understand how this works.

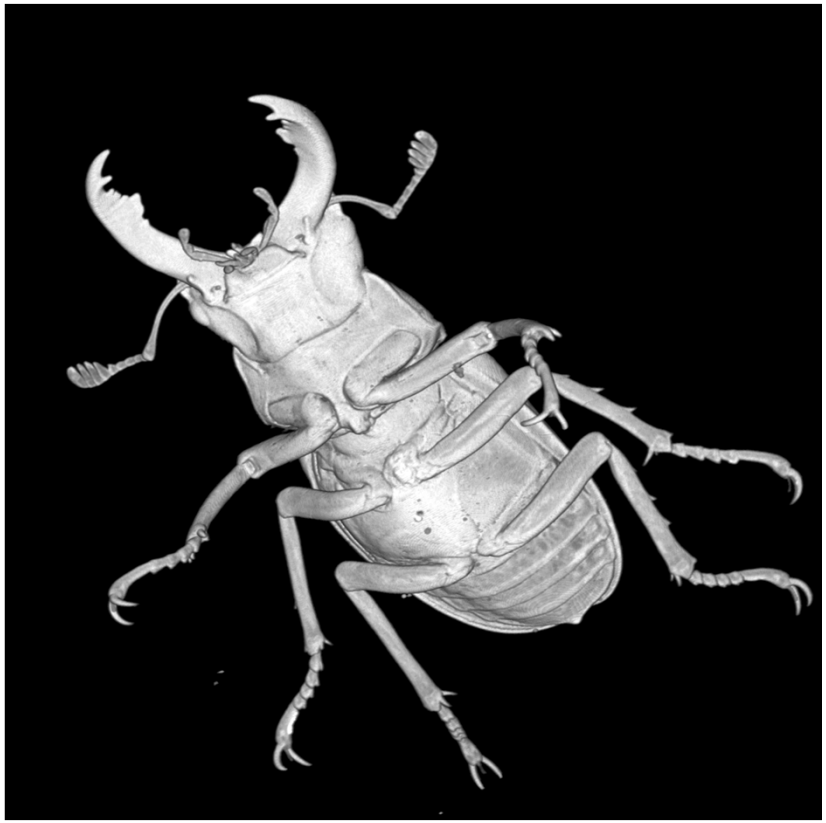


http://embryo.soad.umich.edu/animal/animalSamples/animalSamples.html

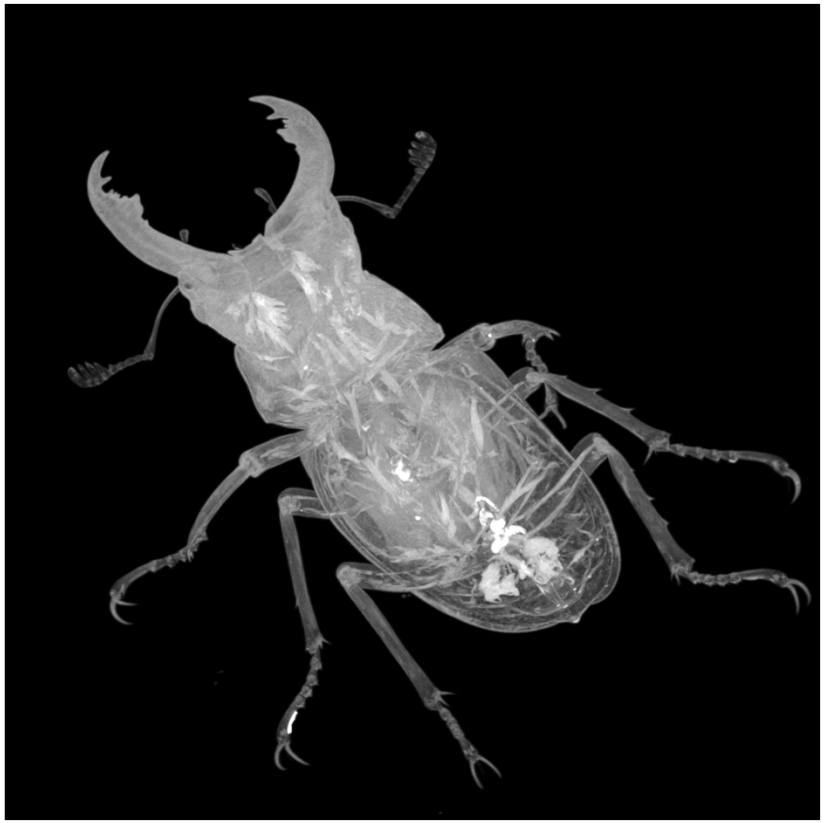
Image Compositing – Micro CT

The various math operators used to combine projection images can produce very different looking volume renderings, so its important to understand how this works.

MicroCT rendered using Voxx



Alpha blending

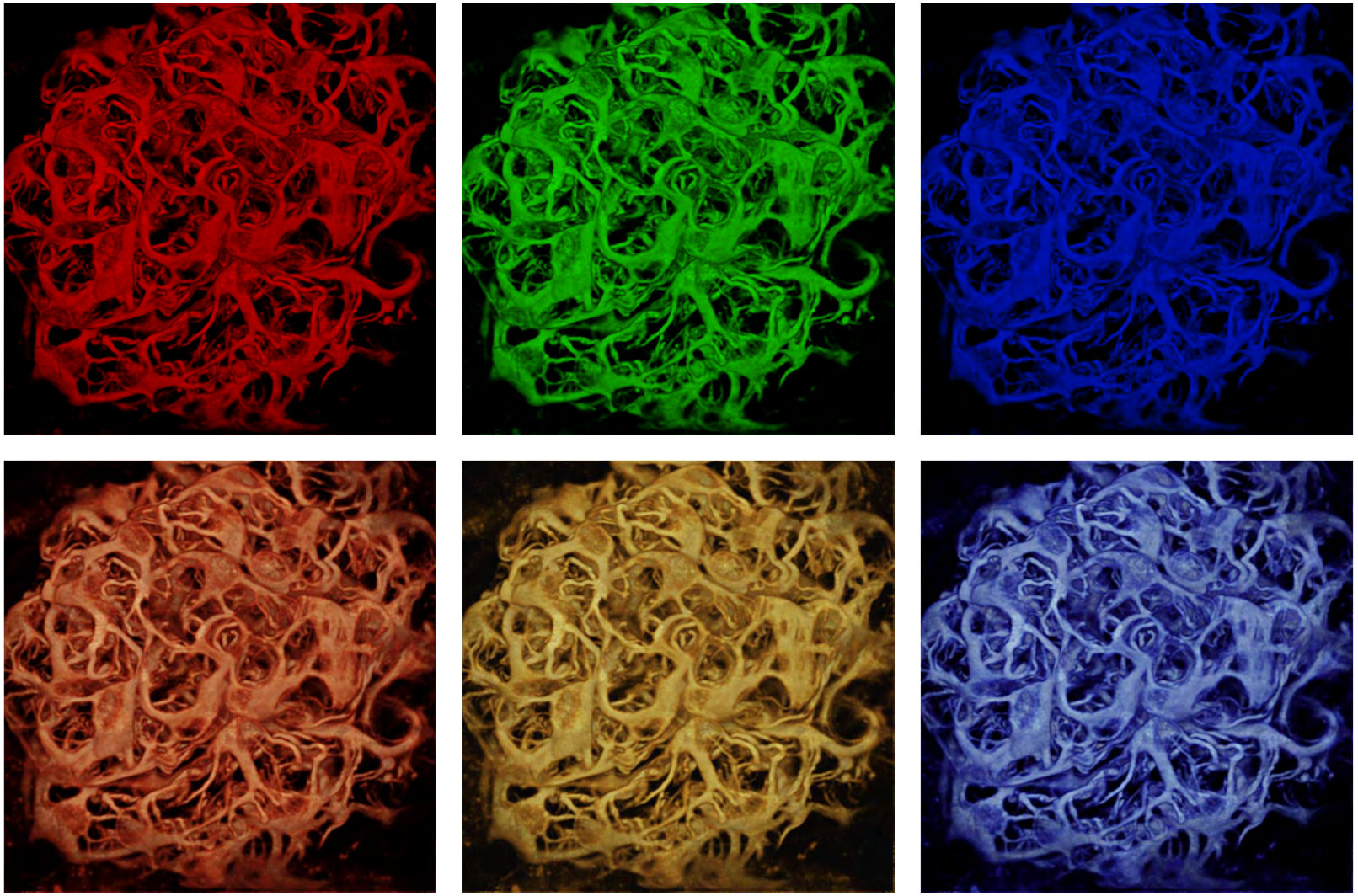


Maximum Intensity Projection

www.cg.tuwien.ac.at/research/publications/2005/dataset-stagbeetle

Colorization

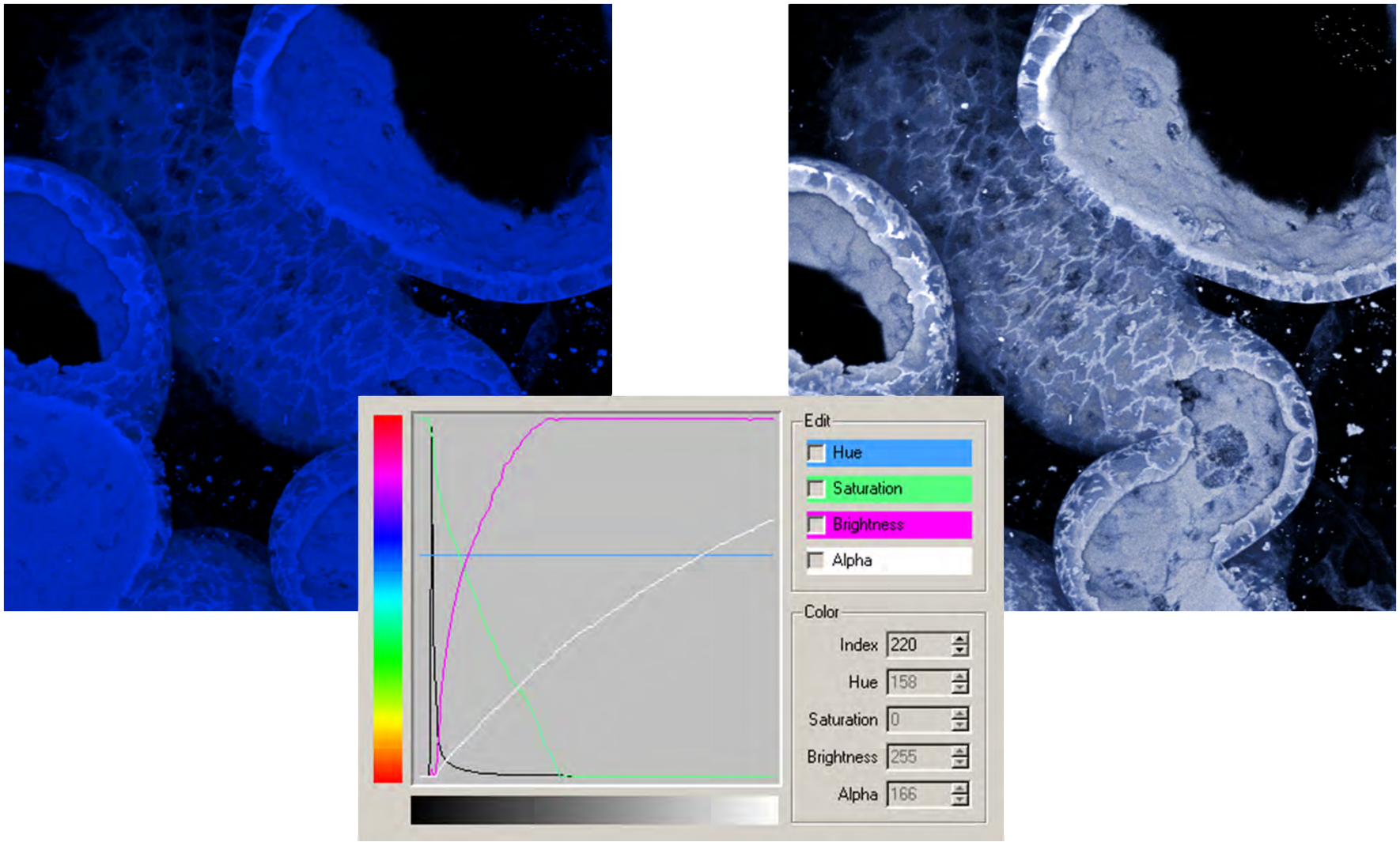
You need to assign each channel a different color when displaying multi-channel images



You must choose appropriate colors for viewing images on monitors and color prints

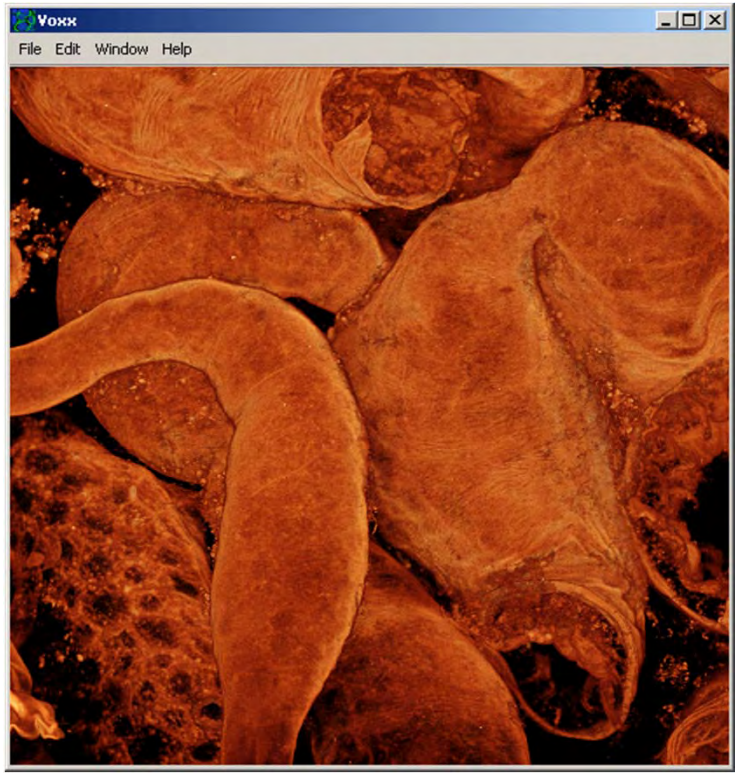
Intensity and Color Mapping

Here we improve the visibility of structural details and enhance the 3D effect, by making saturation decrease as the intensity increases in a constant hue image

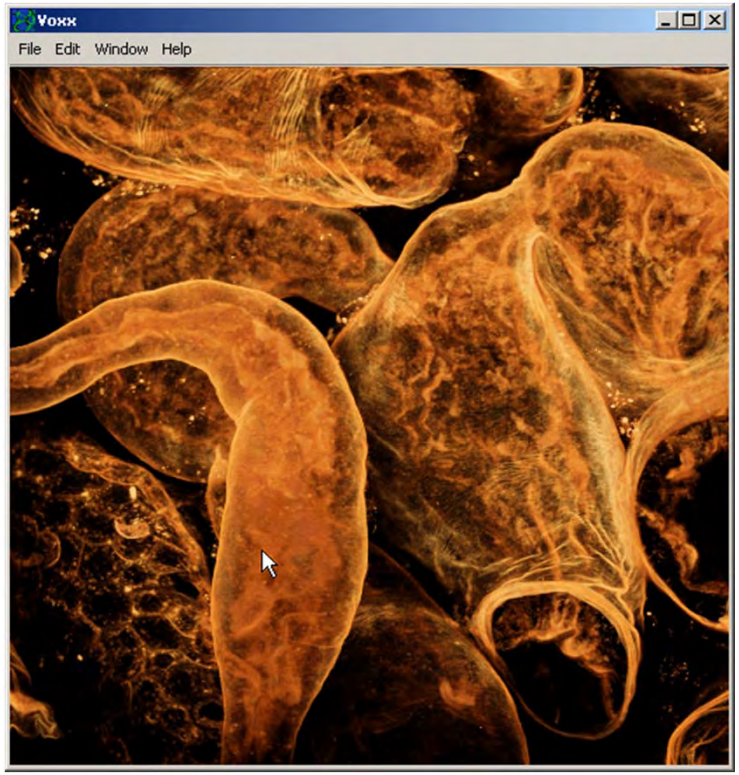


Adjust Opacity

Making alpha an increasing function of pixel intensity causes more brightly fluorescing structures to become more visible when the images are averaged



High Opacity (opaque)

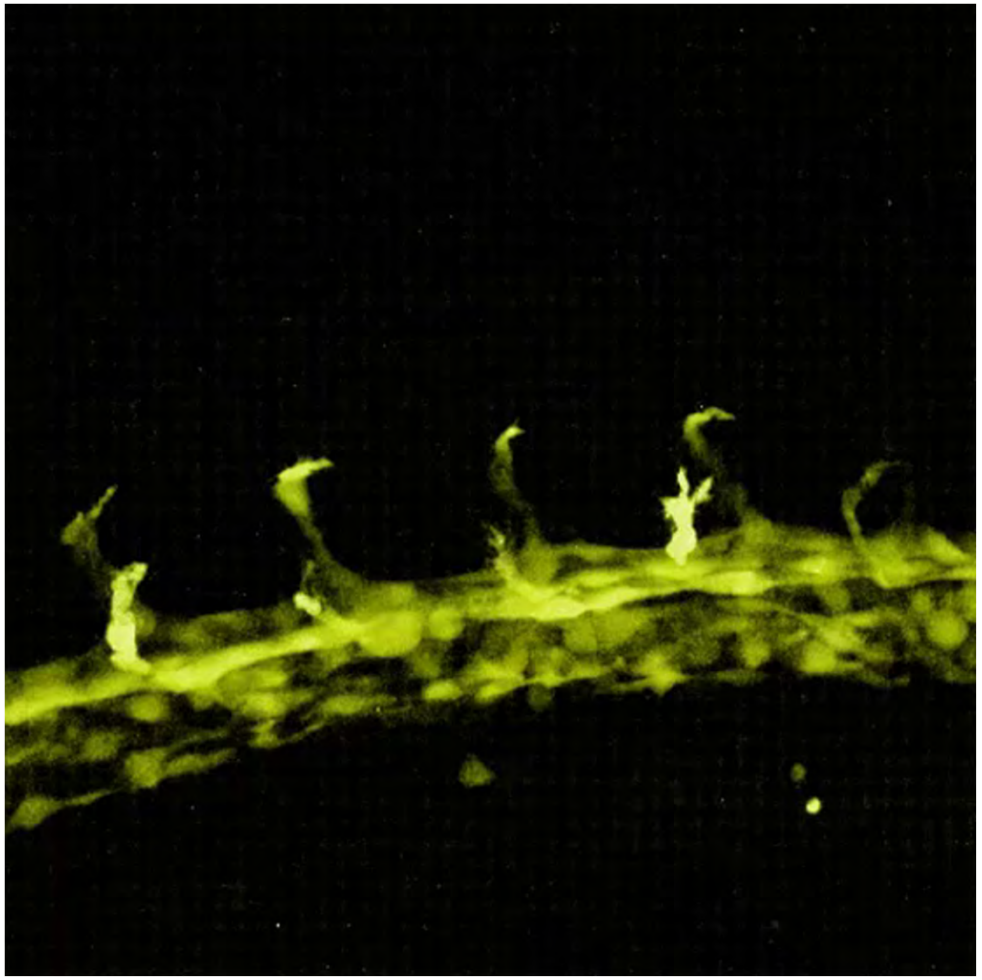


Low Opacity (translucent)

e.g. polycystic tubules in which large alpha values allow us to see details on the outer surface, while using smaller alpha values allow us to see the brush border inside the tubules...

Volume Rendering: 4D (3D + Time)

Several volume rendering programs can also display sequences of 3D images, which is useful for 3D developmental studies



e.g. 3D time series of eGFP-labeled developing vasculature in a zebrafish.

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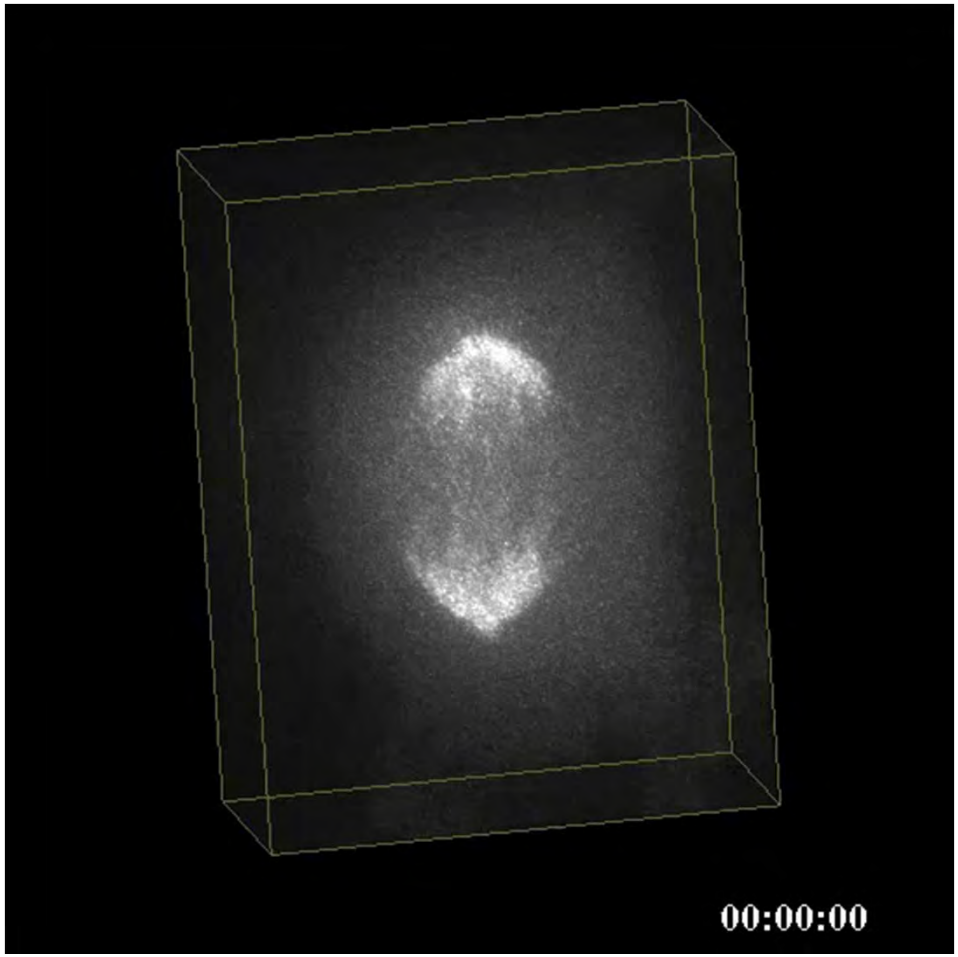


e.g. dividing cell

Lighting

Lighting can produce an improved 3D effect, by providing an additional depth cue and information about the orientation of surfaces of objects

volume rendering using Voreen
by Jonsson, et al (2013)

volume rendering using Exposure Render
by Kroes, et al (2012)

Quantitative Analysis (numerical)

Segmentation is the process of separating an image into groups of pixels associated with various structures

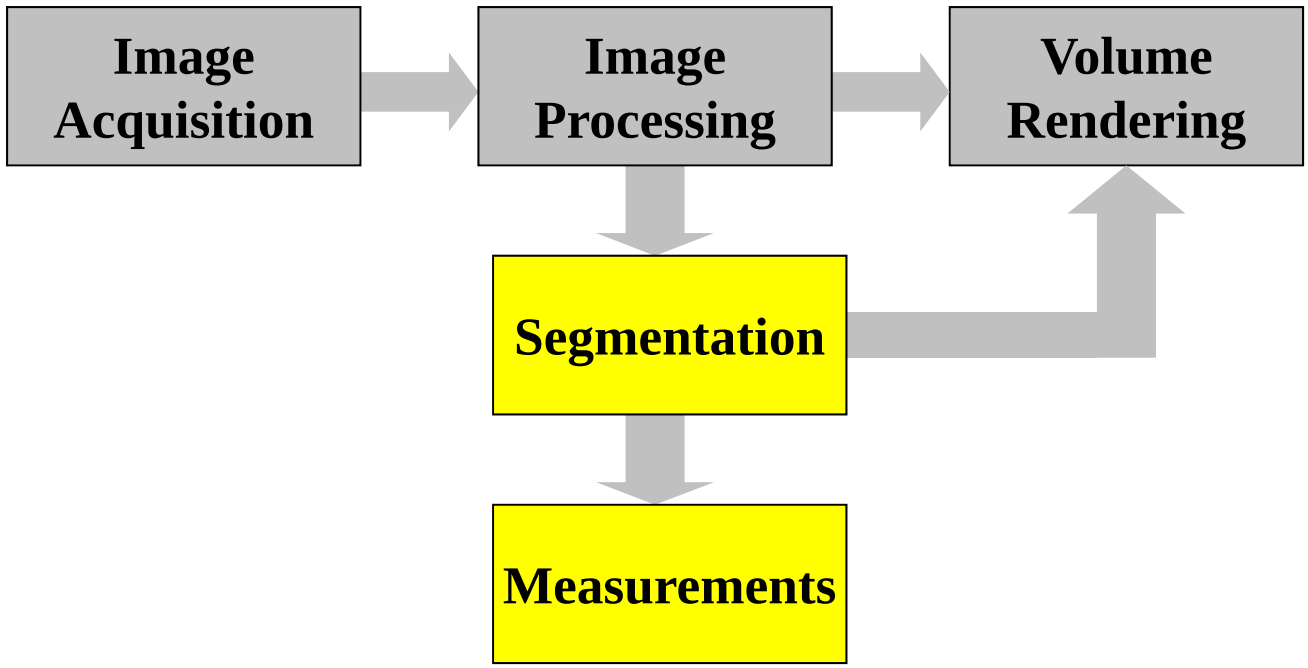
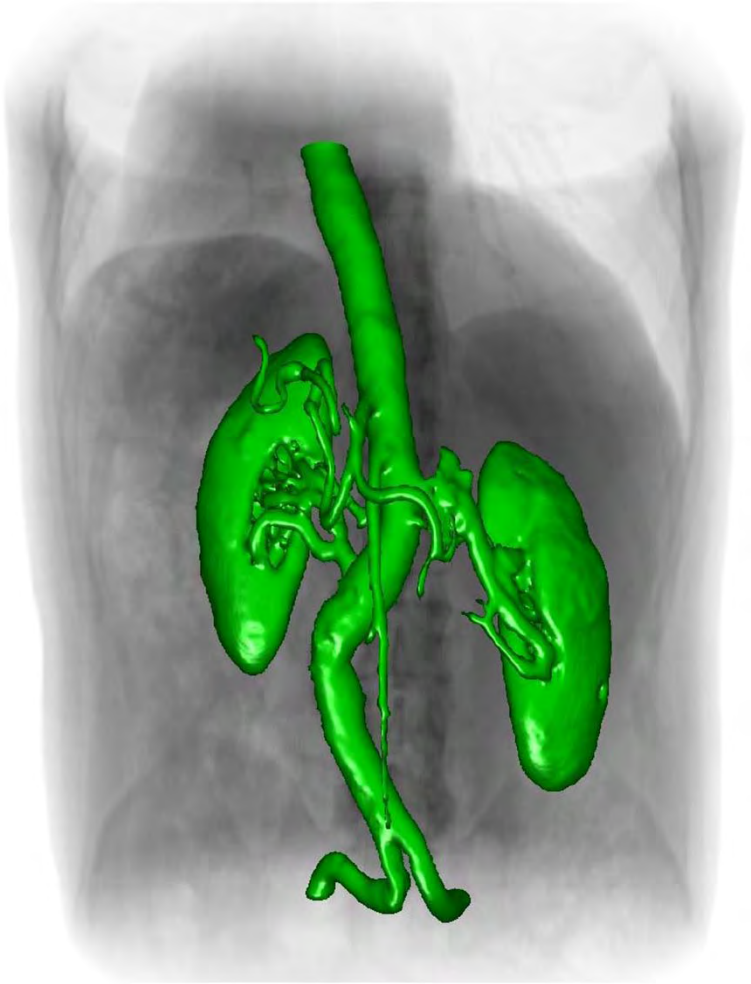


Image segmentation is the critical problem that must be solved in almost every image-based research project before quantitative image analysis can be done.

Segmentation

Just as GPUs have made real-time volume rendering practical and affordable, GPUs have the potential to also do this for 3D image segmentation



Example

Roberts et al (2010) created an optimized GPU implementation of the narrow band level set method which ran 14x faster than previous GPU level set programs.

Jalba et al (2013) developed a GPU implementation which ran 5x faster than Roberts' program.

http://graphics.stanford.edu/~mlrobert/publications/hpg_2010

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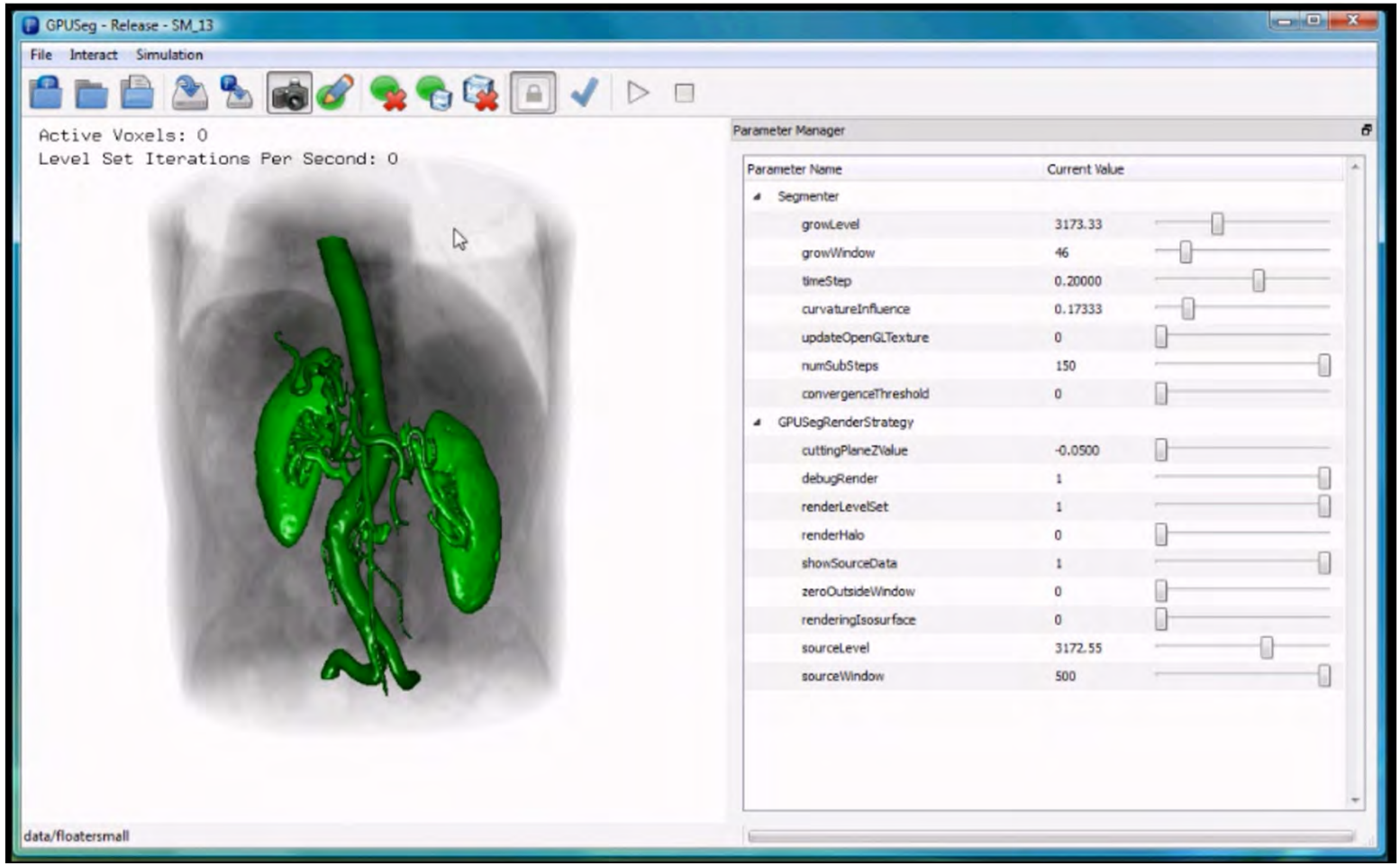

http://graphics.stanford.edu/~mlrobert/publications/hpg_2010/data/hpg_2010_movie.mov

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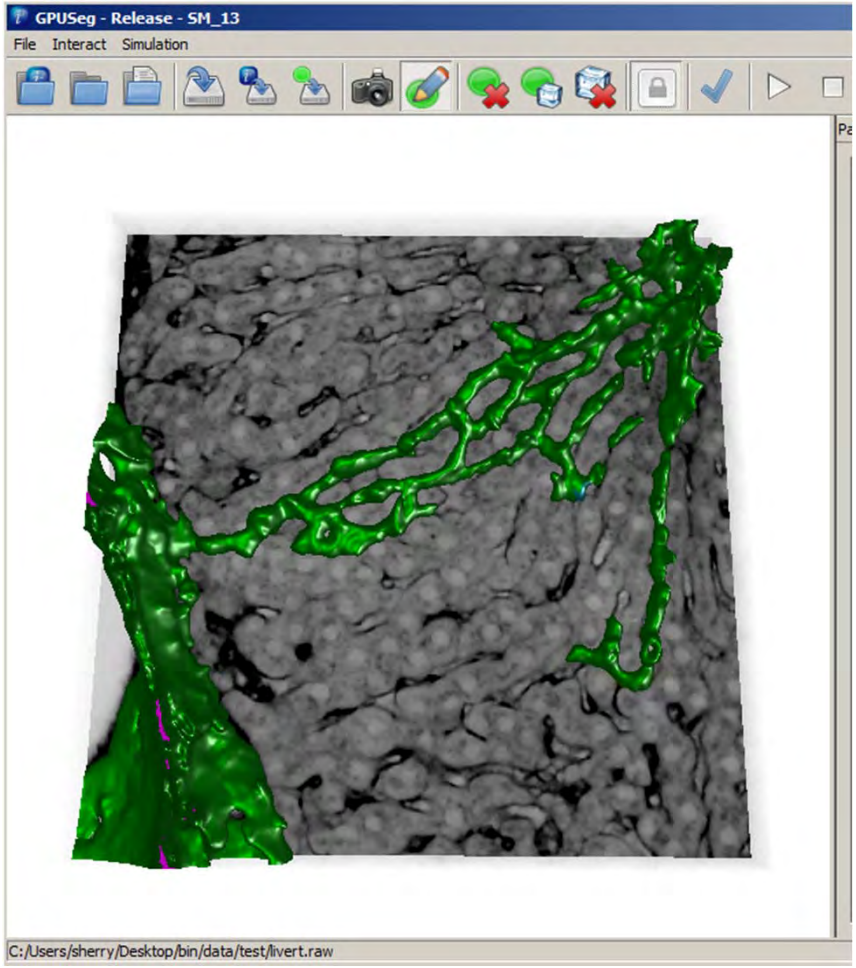


I recently had a chance to run Roberts' GPUSeg program on a GeForce Titan GPU (equipped with 6 GB of memory), and were able to process up to 512x512x400 LSCM liver images. Thats a useful image stack size for 3D microscopy…

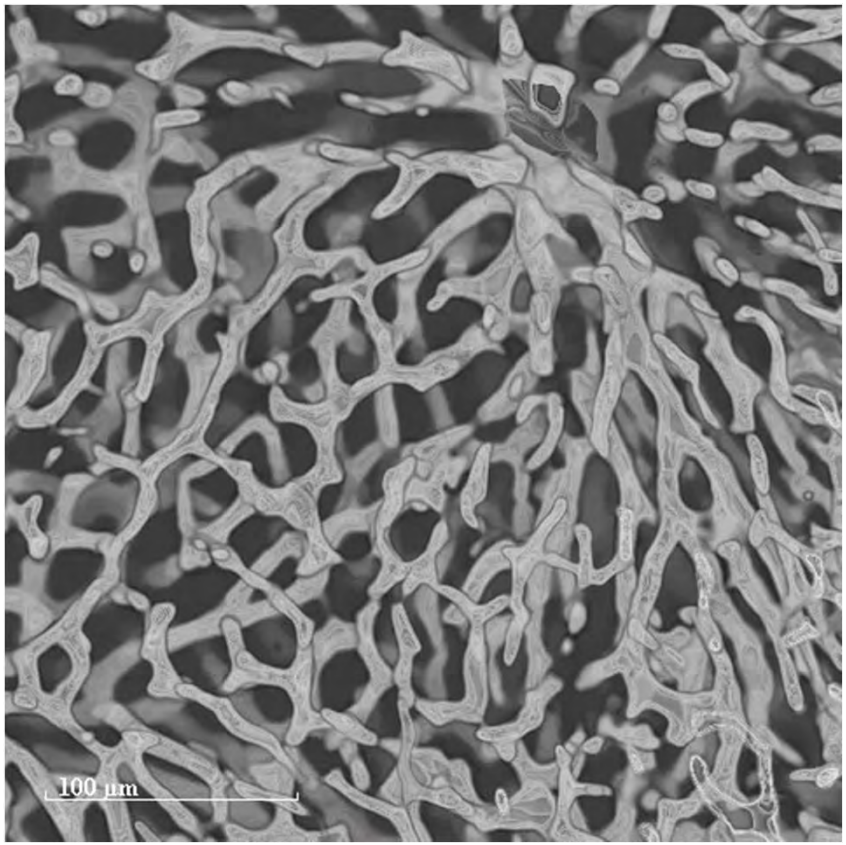
We've still not determined if level-set-based segmentation is going to work for vasculature in liver labelled with lens culinaris agglutinin, but interactively steering GPU-based segmentation software has a lot of potential…

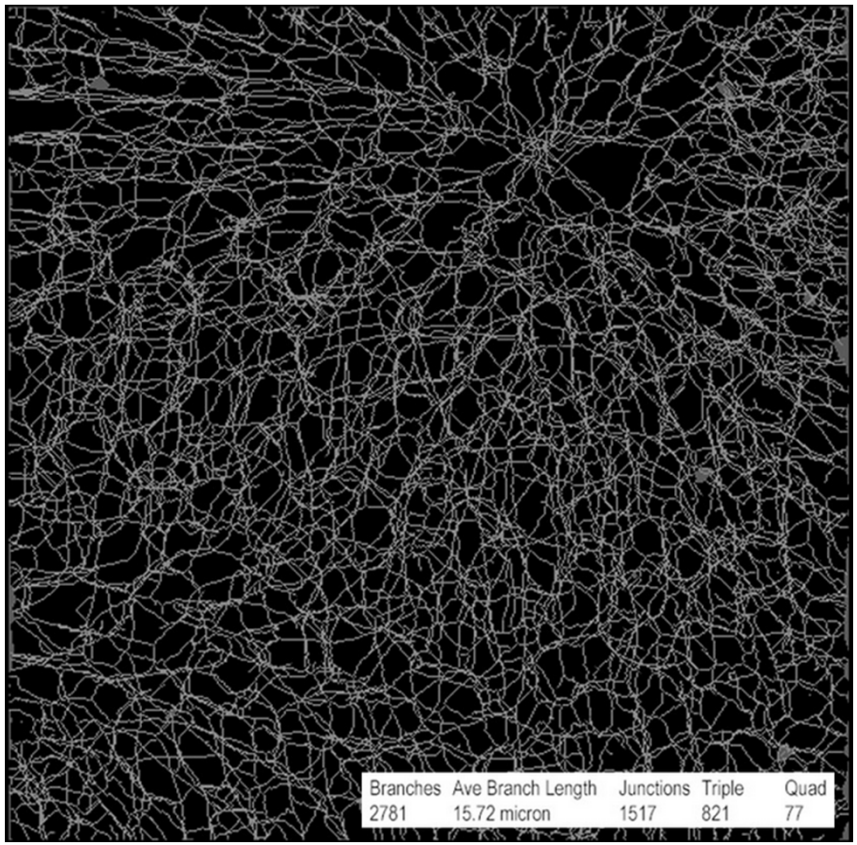
Liver data courtesy of Drs. Sherry Clendenon and Ken Dunn, Indiana University

https://github.com/mroberts3000/AWorkEfficientGpuAlgorithmForLevelSetSegmentation/

Measurements

Why are we interested in semi-automatic segmentation of liver vasculature?
Because currently someone has to manually segment vessels using TrackEM in Fiji





The segmented images are rendered using Voxx, skeletonized using Fiji, and then branch length and branch point analyses are performed (Dr. Sherry Clendenon)

Volume Imaging Software

There are many 3D image processing programs, but not many can handle the multi-channel 3D and 4D images produced by confocal and 2P microscopes.

Free	BioImageXD	www.bioimagexd.net
	ImageJ/Fiji	http://3dviewer.neurofly.de rsbweb.nih.gov/ij/plugins/volume-viewer.html
	Vaa3D	www.vaa3d.org
	Voreen	www.voreen.org
	Voxx	www.indiana.edu/~voxx
Commercial	Amira/Avizo	amira.zib.de www.fei.com/software/amira-3d-for-life-sciences/
	Huygens	www.svi.nl
	Image-Pro 3D	www.mediacy.com
	Imaris	www.bitplane.com
	Volocity	www.perkinelmer.com/pages/020/cellularimaging/products/volocity.xhtml

There is no standard file format for 3D/4D images.
Keep this in mind when selecting software to use with your microscope(s).
Consider using OME's image database OMERO (www.openmicroscopy.org).

GPU-Accelerated Video Boards

GPUs are what makes real-time 3D image processing practical

NVIDIA GeForce

GTX Titan X (12 gigabytes) $ 1000
GTX 980 (4-8 gigabytes) $ 550-
GTX 970 (3-4 gigabytes) $ 330-370
…

AMD Radeon

FirePro S9150 (16 gigabytes) $ 3100-3500
R9 290X (4-8 gigabytes) $ 300-450
R9 280X (3 gigabytes) $ 180-300
…

Choose video board based on which GPU programming language(s) your 3D imaging software uses...

If it uses CUDA, then you must use Nvidia GPUs. If it uses OpenCL or OpenGL/GLSL, then you can usually use either Nvidia or AMD. Carefully check PC system requirements.

GPU-Accelerated Video Boards

Choose video board based on the size of your largest image stack

1) Compute size of your image stack in pixels =
   image_width x image_height x image_slices x image_channels

2) Multiply size by 2 if your ADC produces images with more than 8 bits
   (e.g. 12 bits is pretty common)

3) Multiply by 3 or 4, in case gpu programs need more image memory
   (e.g. to add or subtract two 3d images, A=B+C, the program needs
   memory for 3 images)

   e.g. for a stack of 256 images, each 1024x1024 12-bits, with 3 fluors,
   (1024 x 1024 x 256 x 3) x 2 x 4 = 2,147,483,648 bytes (**2 gigabytes**)

Choose host PC based on power requirement and size of video board

1) Highest-performance video boards need HIGH-CURRENT PC power supply
   (e.g. Titan specs > 42 Amps at 12 volts!)

2) High-performance video boards are physically LARGE
   (e.g. occupy 2 PCIe x16 card slots, need full-length slots (boards typ. 10.5”))

References

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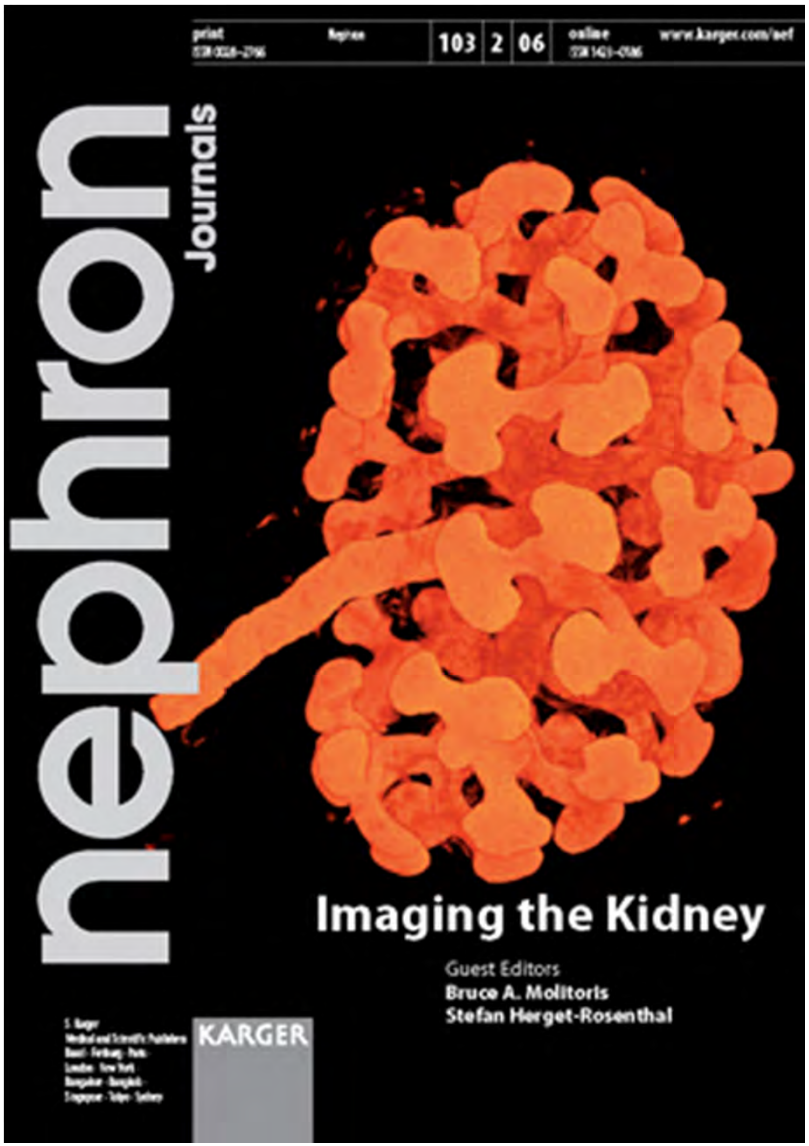
http://graphics.stanford.edu/~mlrobert/publications/hpg_2010/

Questions?



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