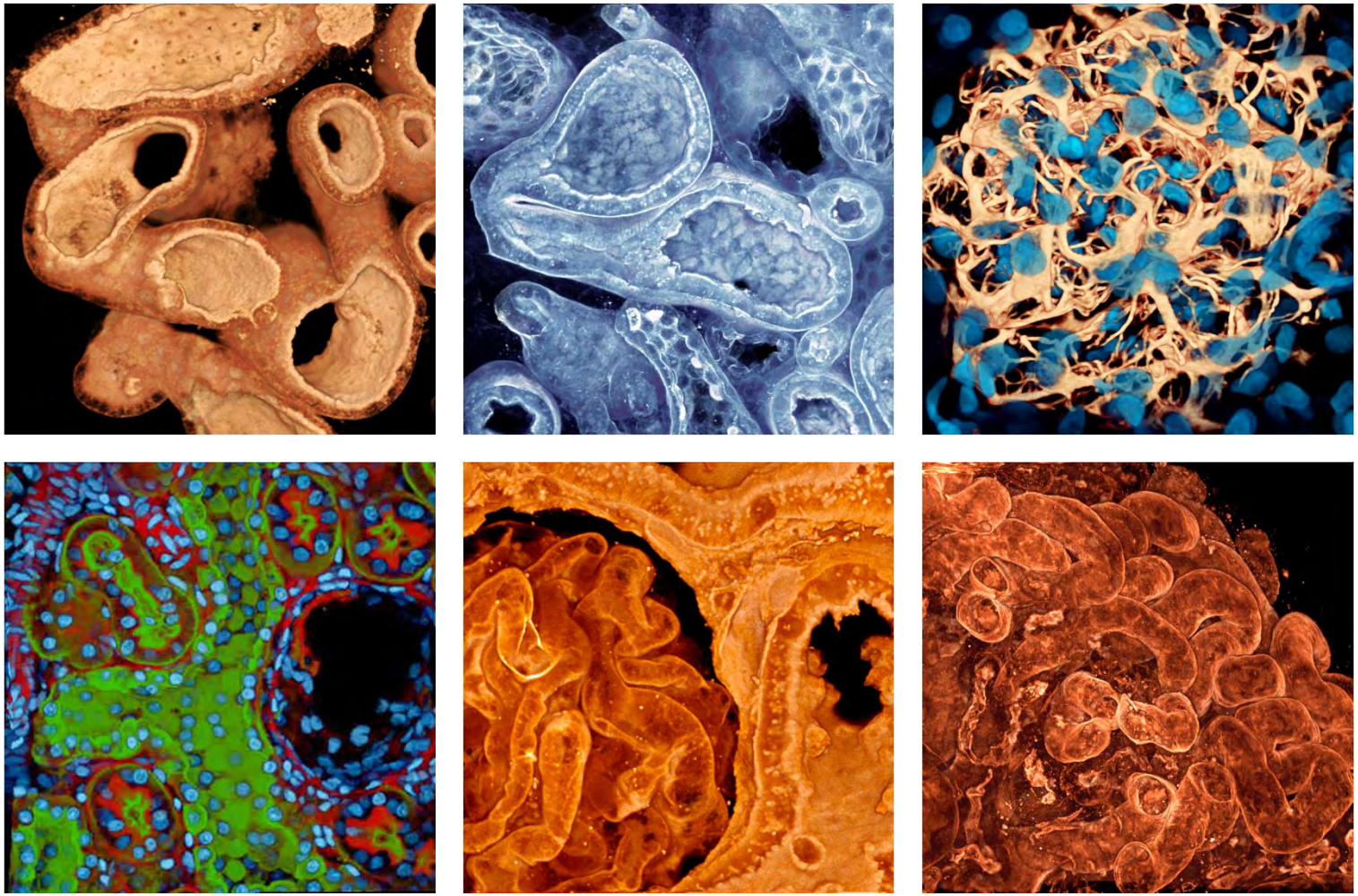
3D Visualization and Analysis

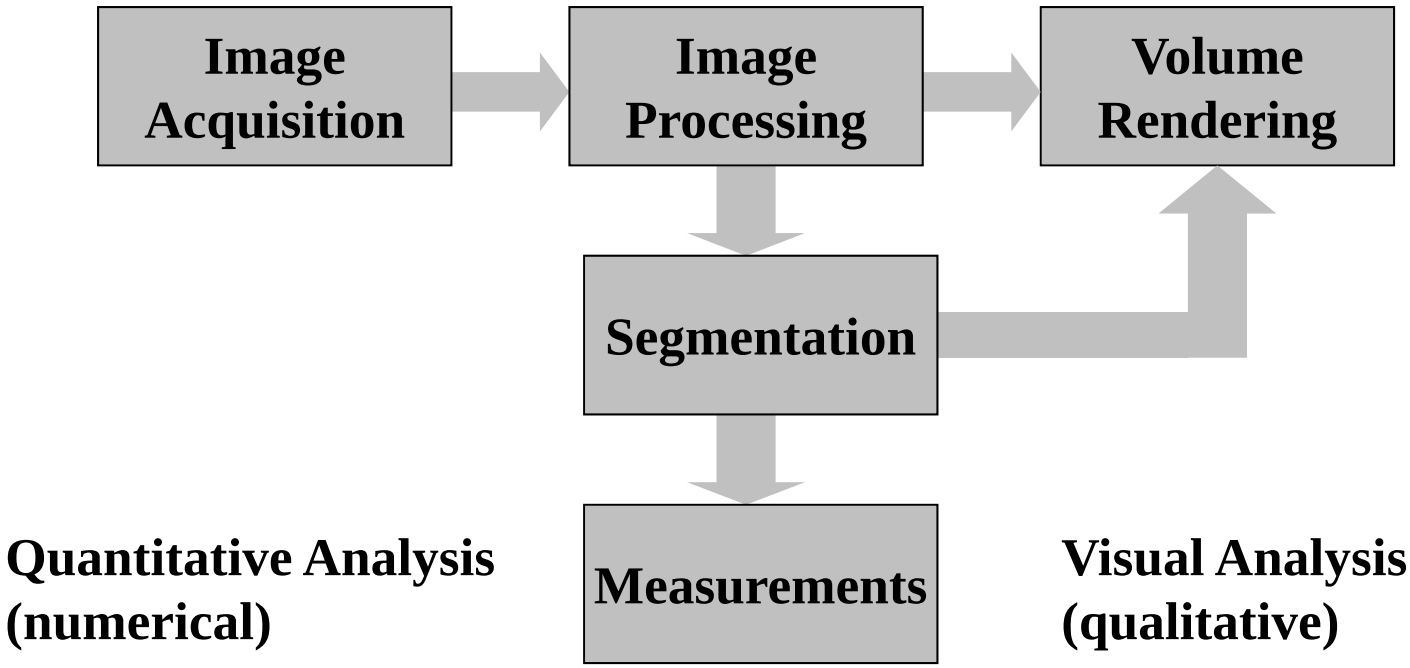
J. L. Clendenon

Aeon Imaging LLC



Data flow through the 3D imaging pipeline

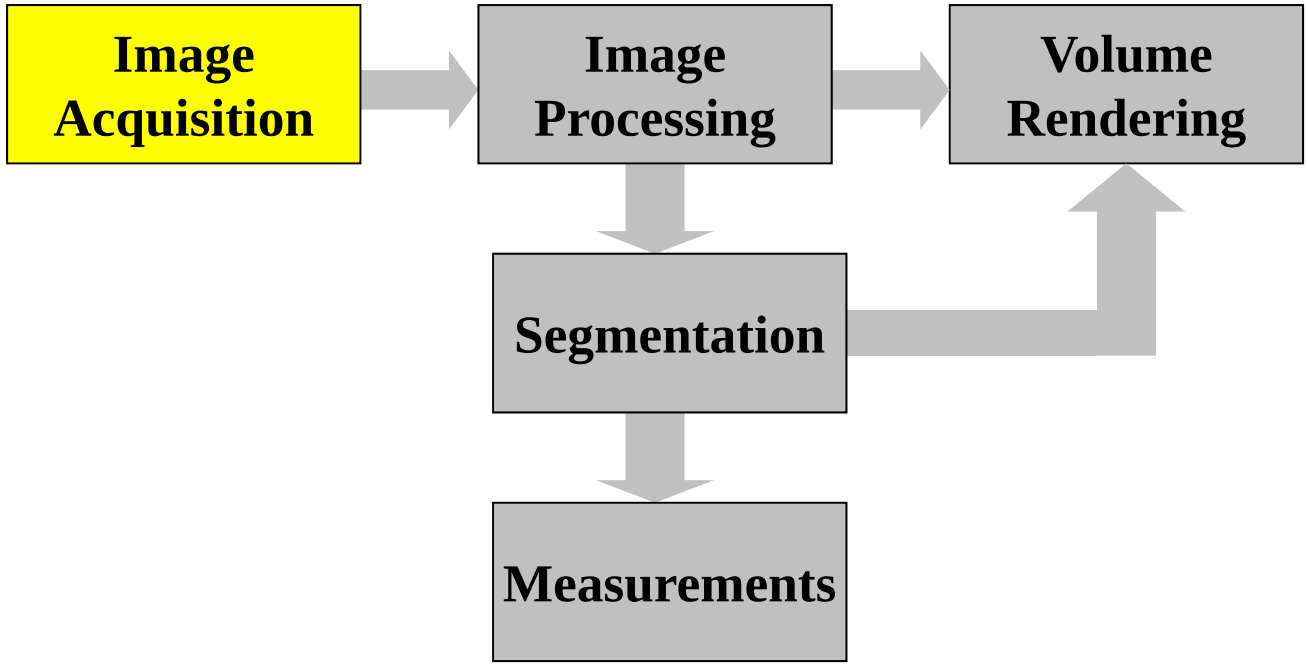
People involved in image-based research must perform several tasks: image acquisition, processing, rendering, segmentation, and measurement.



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.

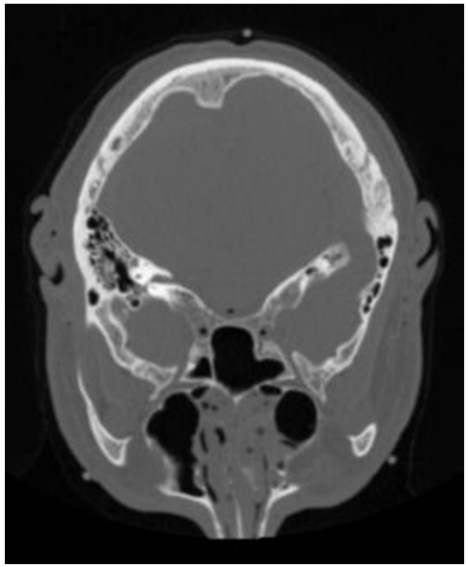


In cross-sectional imaging, parallel planar (2D) images from various levels within a 3D specimen are collected…

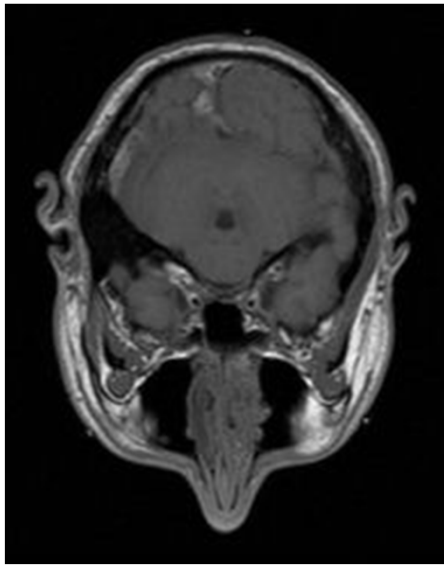
Cross-Sectional Image Acquisition

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.

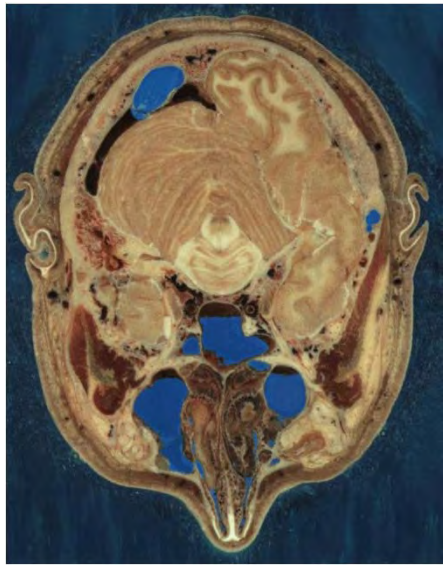
Computed Tomography



Magnetic Resonance



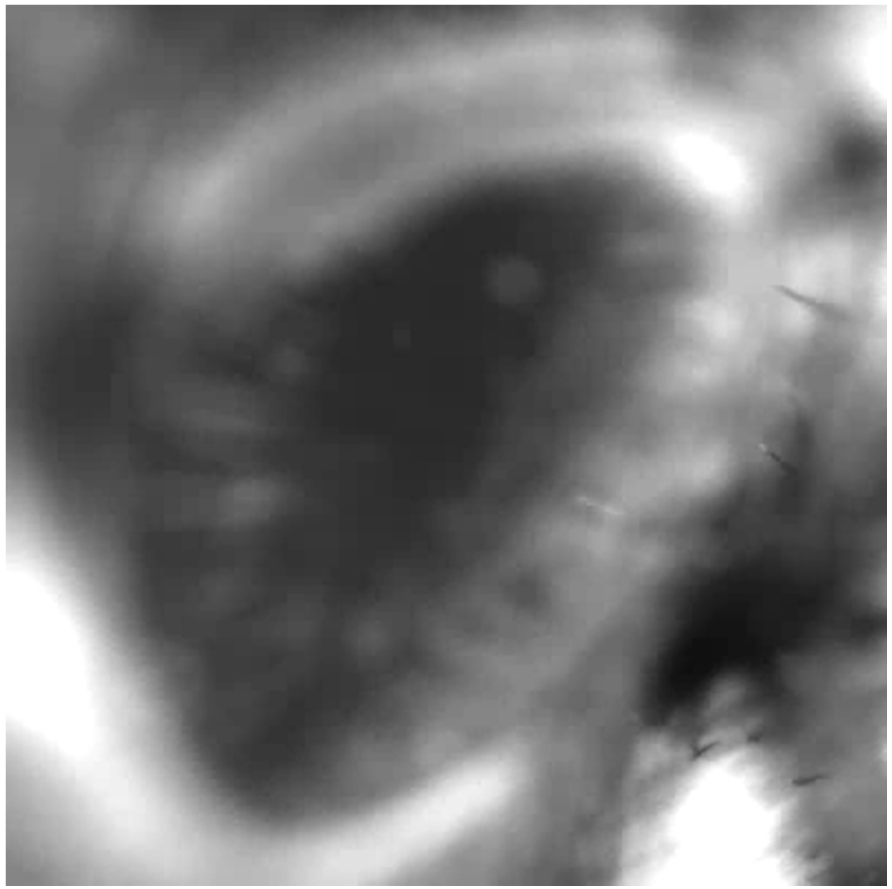
Blockface



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

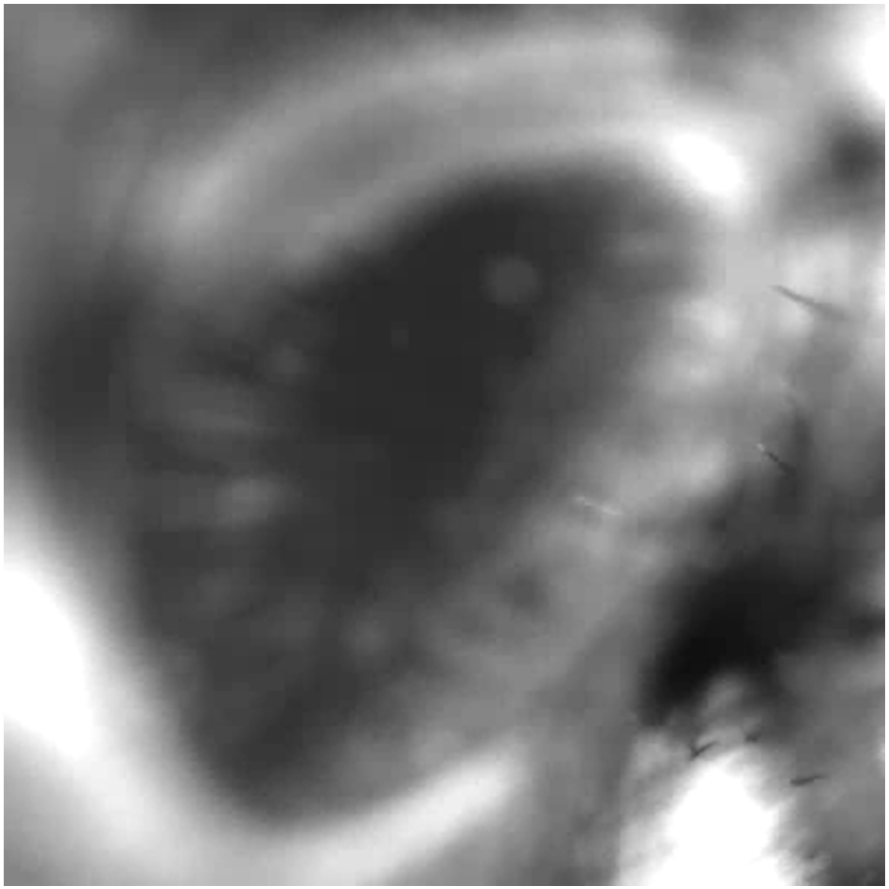
This makes it difficult to study thick specimens

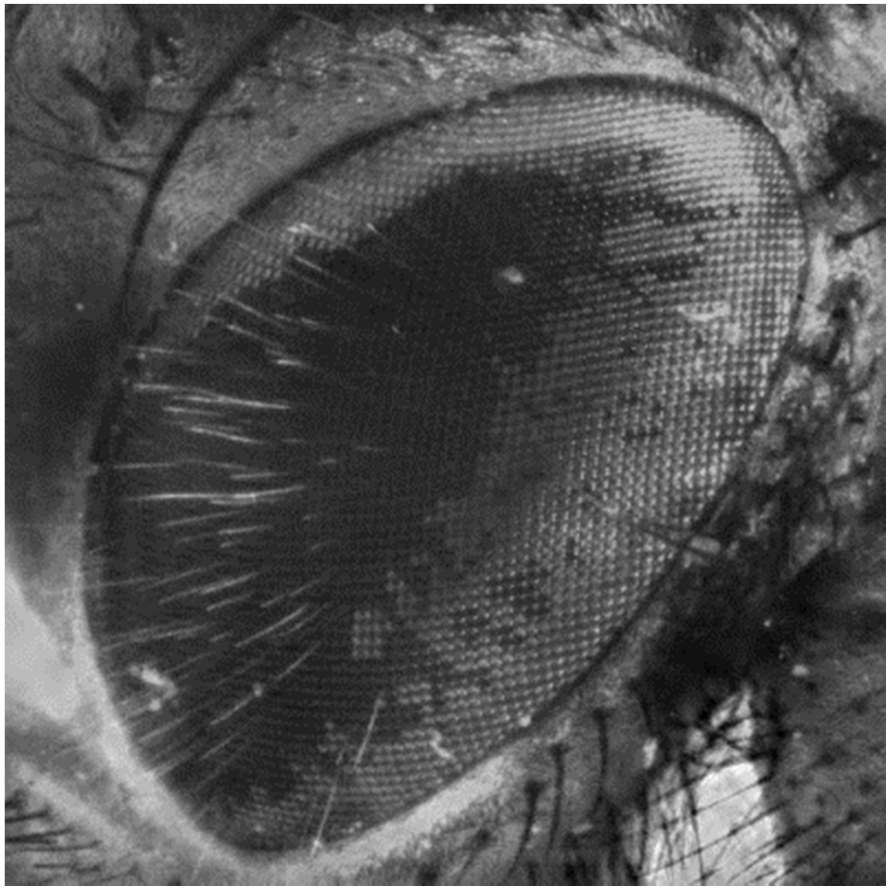


Structures closer to the focal plane are more in focus
than structures farther away from the focal plane

Extended Depth-of-Field

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

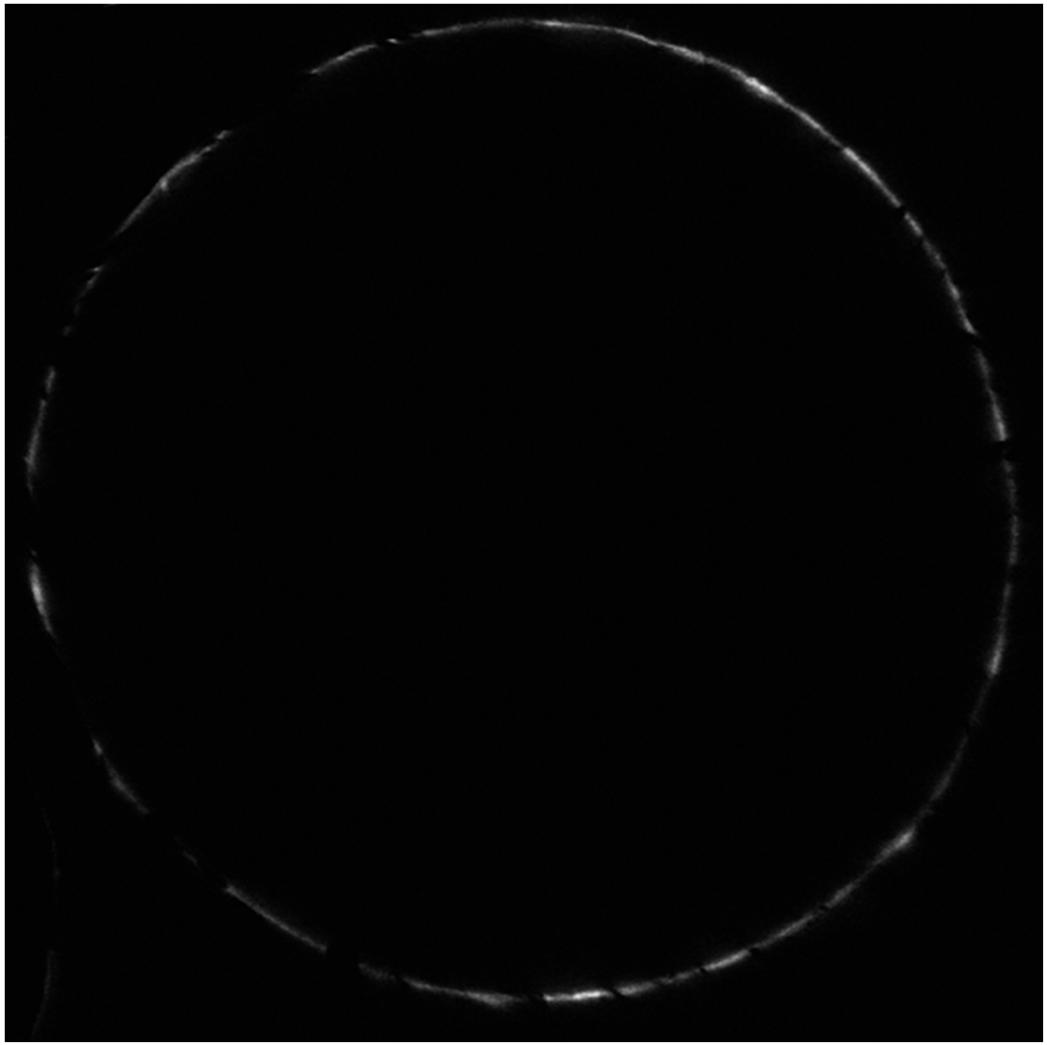




Use software to create a composite image (e.g. weighted average)
of just the in-focus portions of ALL of the images in the z stack
(e.g. used plugin for ImageJ from http://bigwww.epfl.ch/demo/edf)

Confocal microscopes have smaller depth-of-field

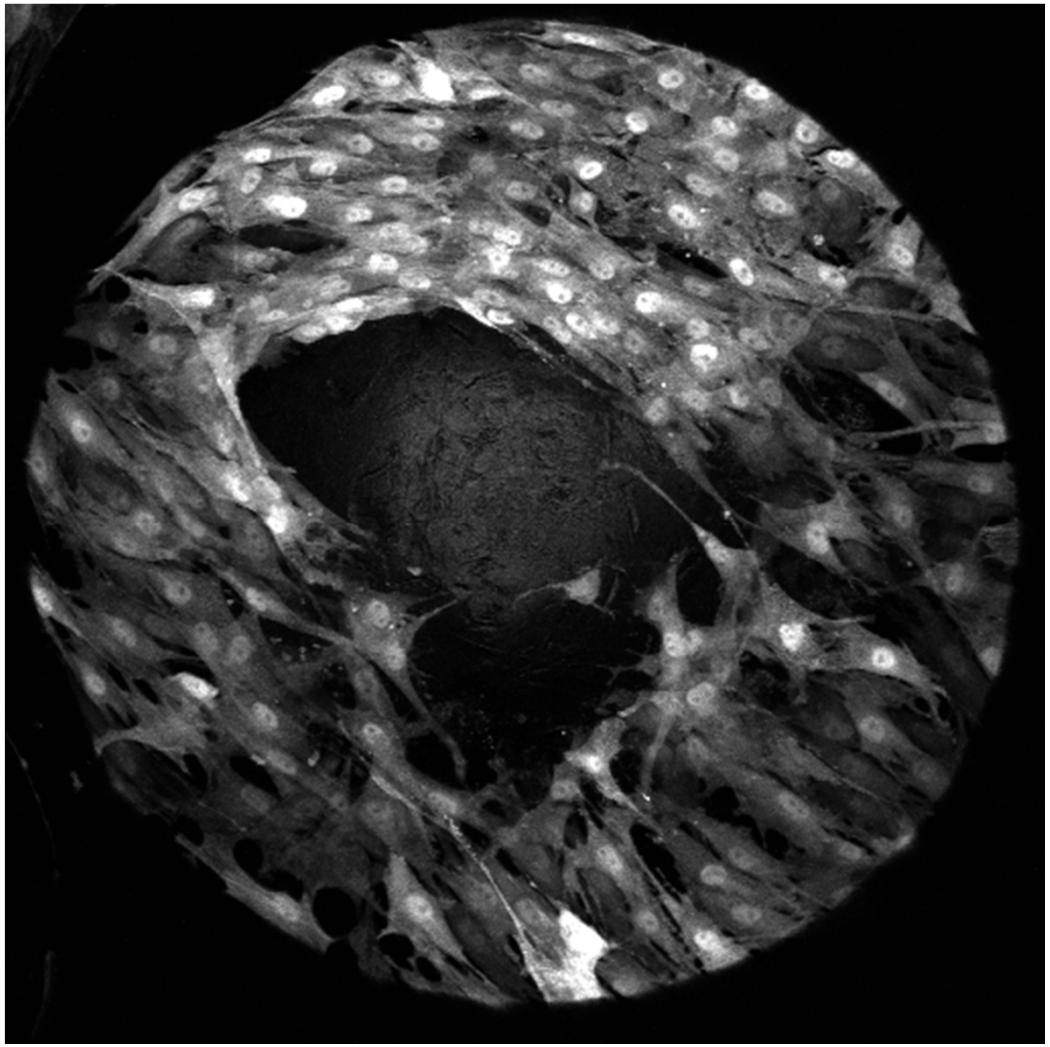
e.g. 352 LSCM images – but what is it?



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

Its… cells growing on a microsphere

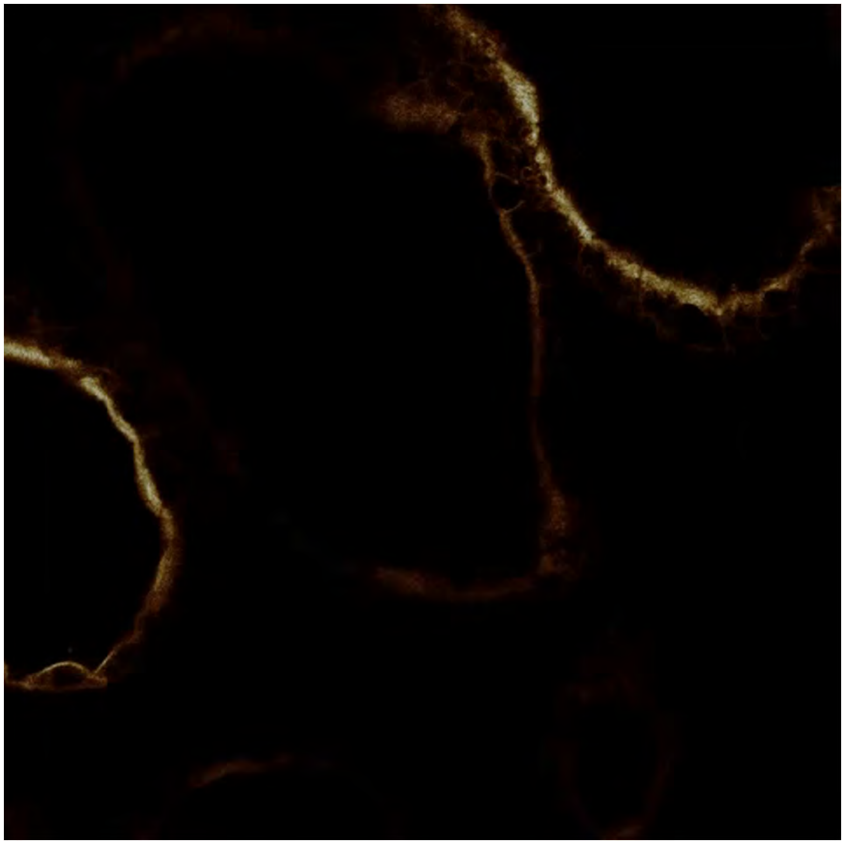
Made visible by computing maximum intensity projection

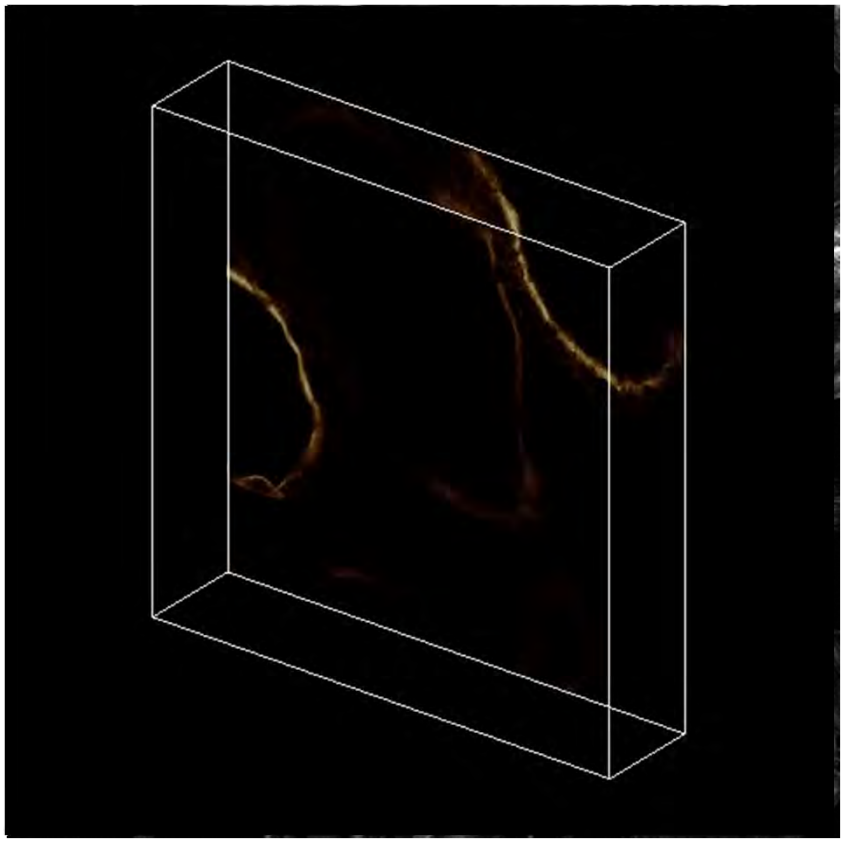


One example of 2D image compositing operation performed on the image stack

What about off-axis viewpoints?

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





These cystic kidney tubules were rendered using Voxx software.

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.

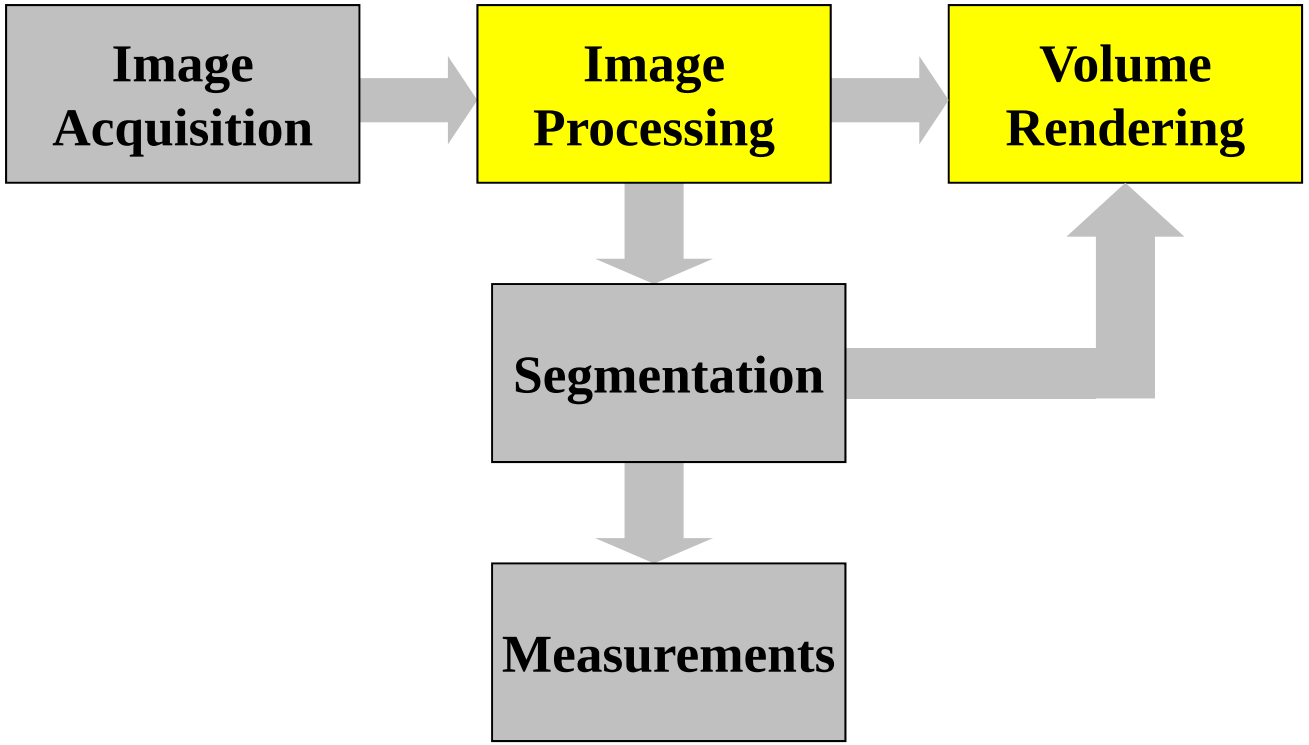
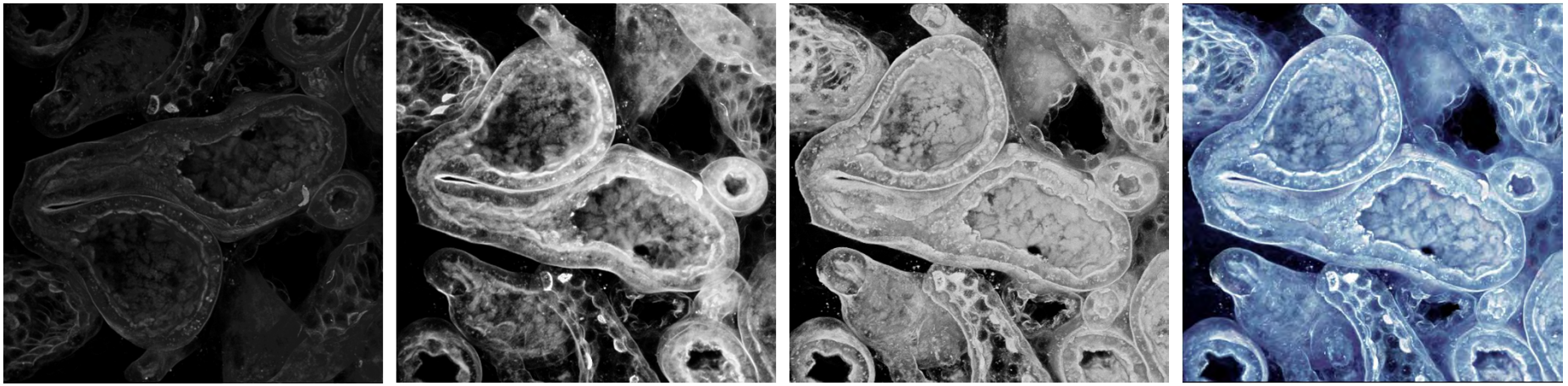


Image processing operations may need to be performed on 3D images before they are displayed or passed to image analysis software

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.

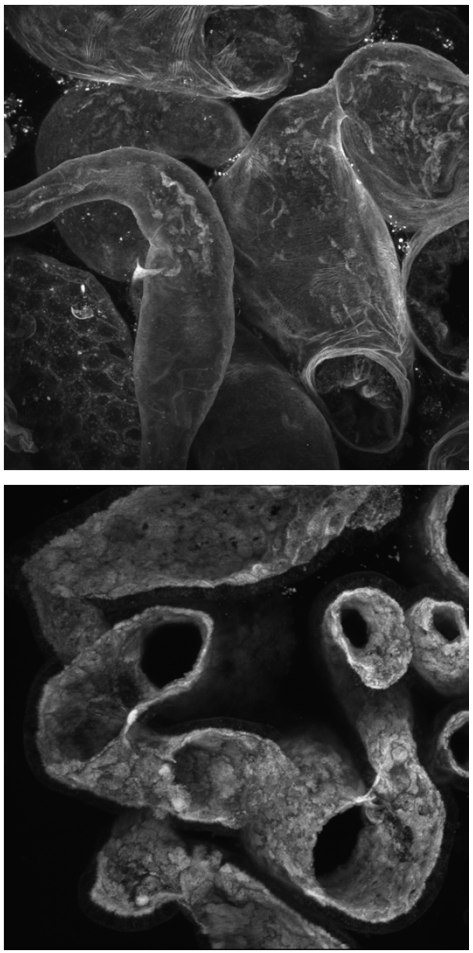
3) Adjust contrast and brightness - so that you can see monochrome specimen.

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

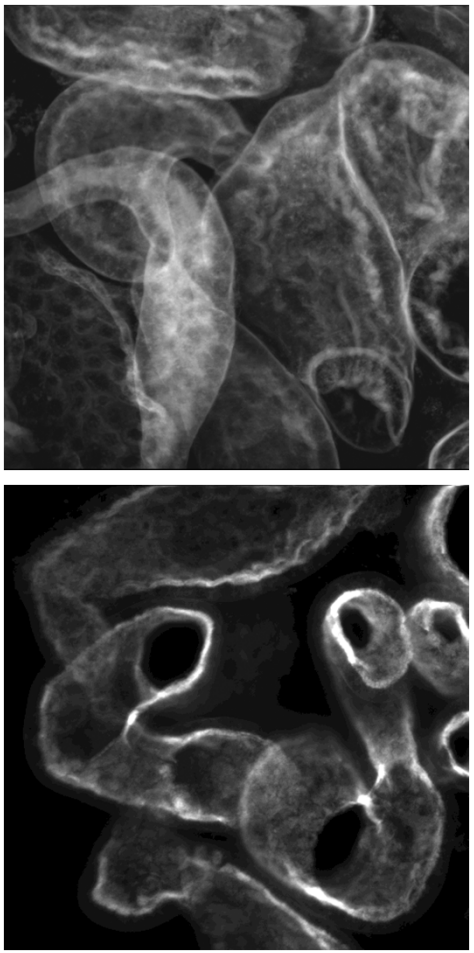
Image Compositing – 2P Micoscopy

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

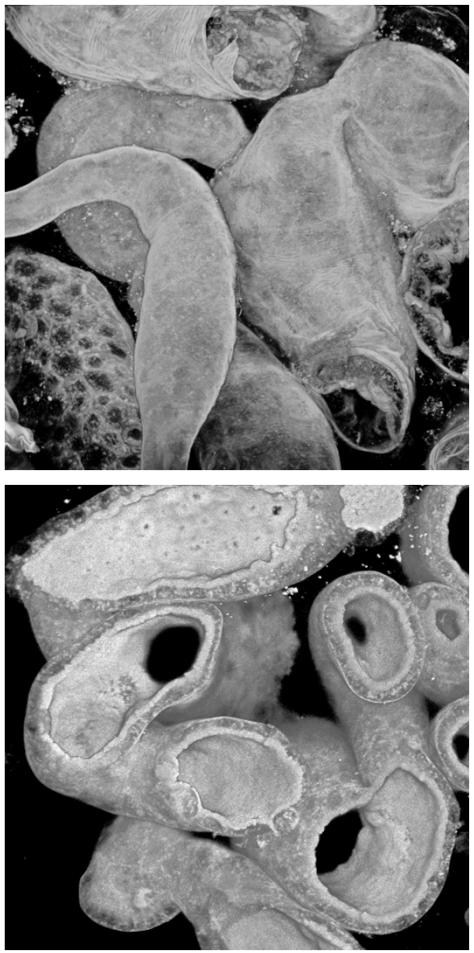
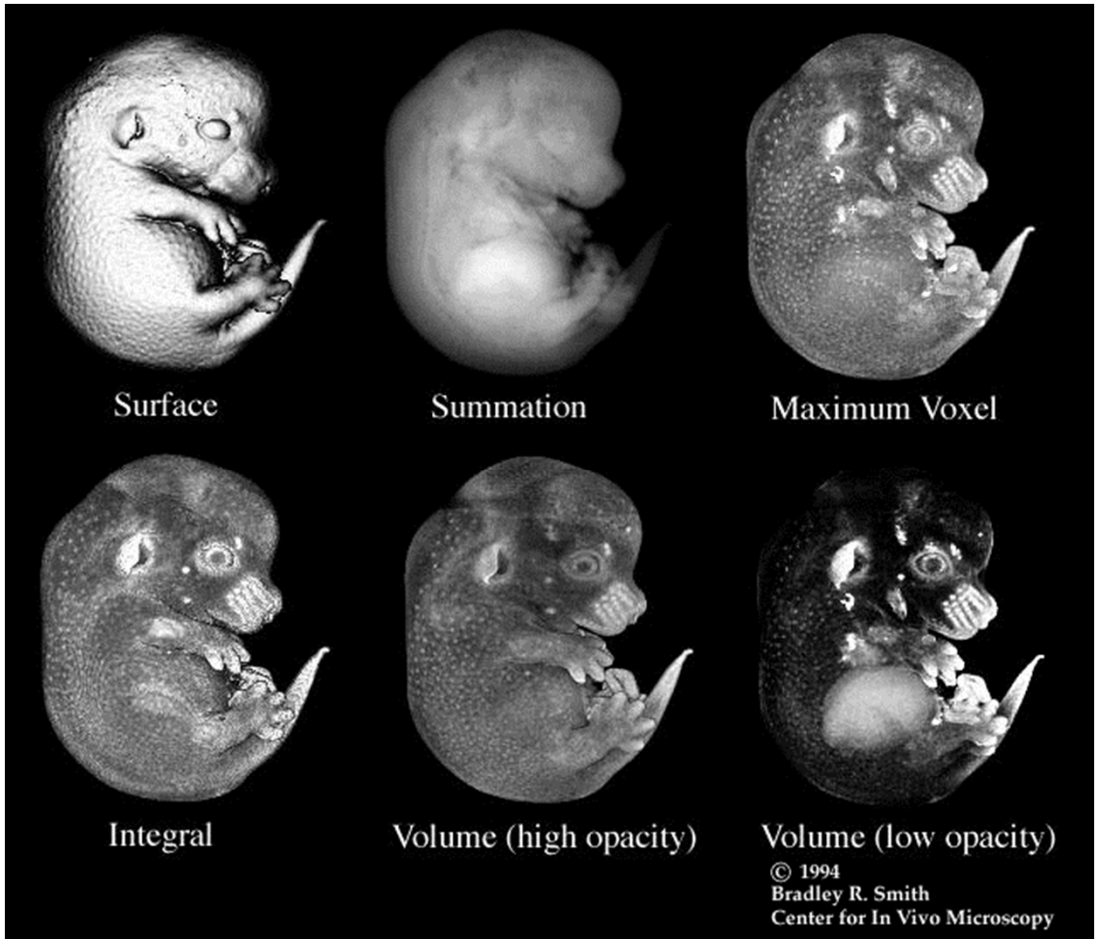


Image Compositing – Micro MRI

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

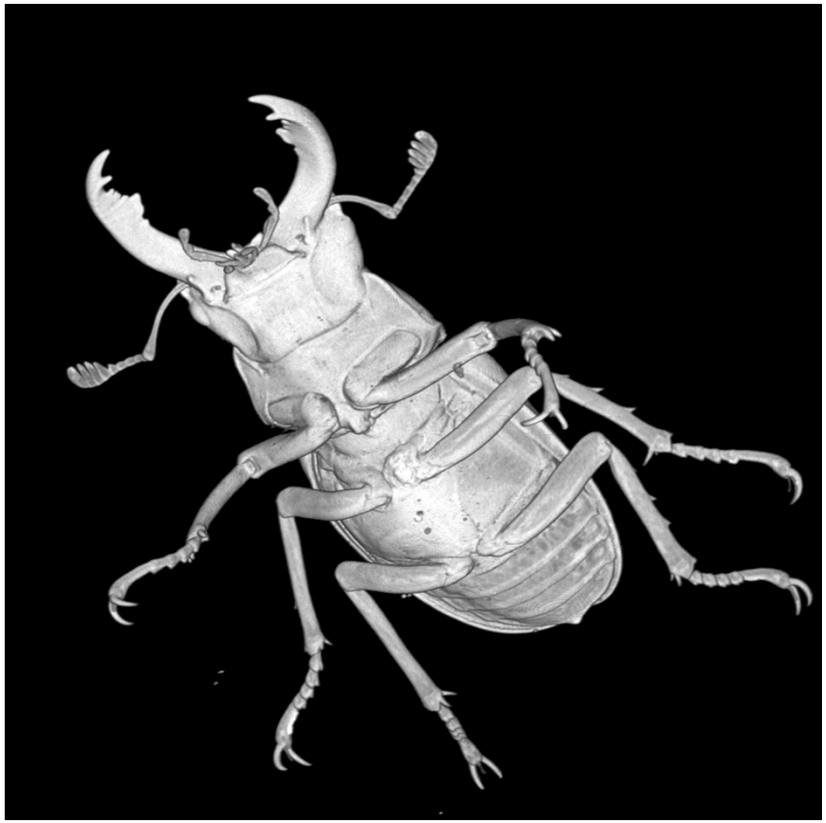


http://embryo.soad.umich.edu/animal/animalSamples/animalSamples.html

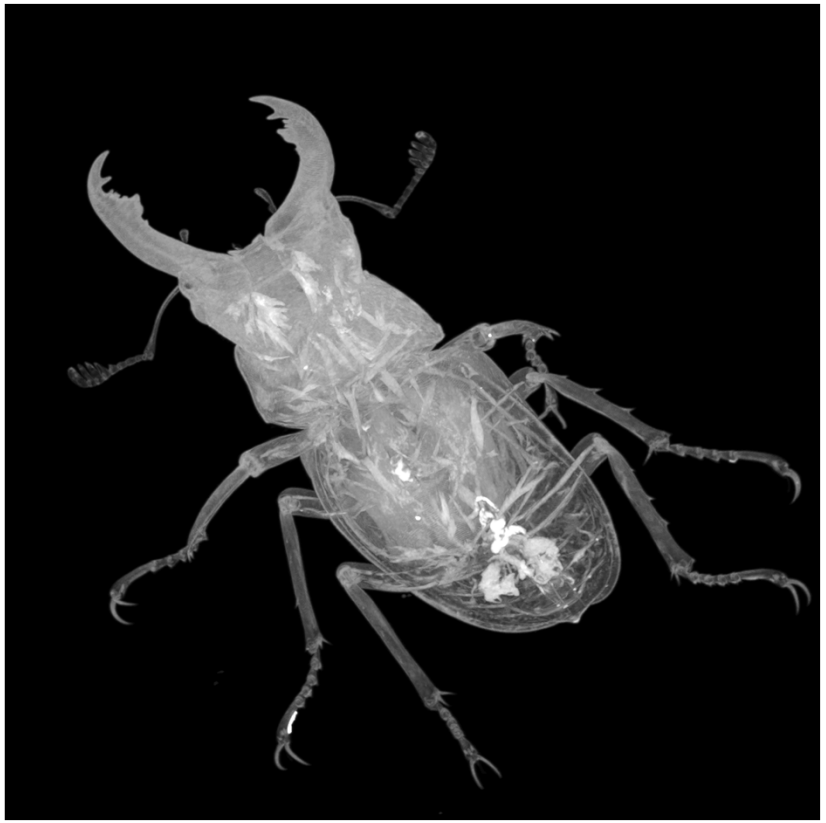
Image Compositing – Micro CT

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

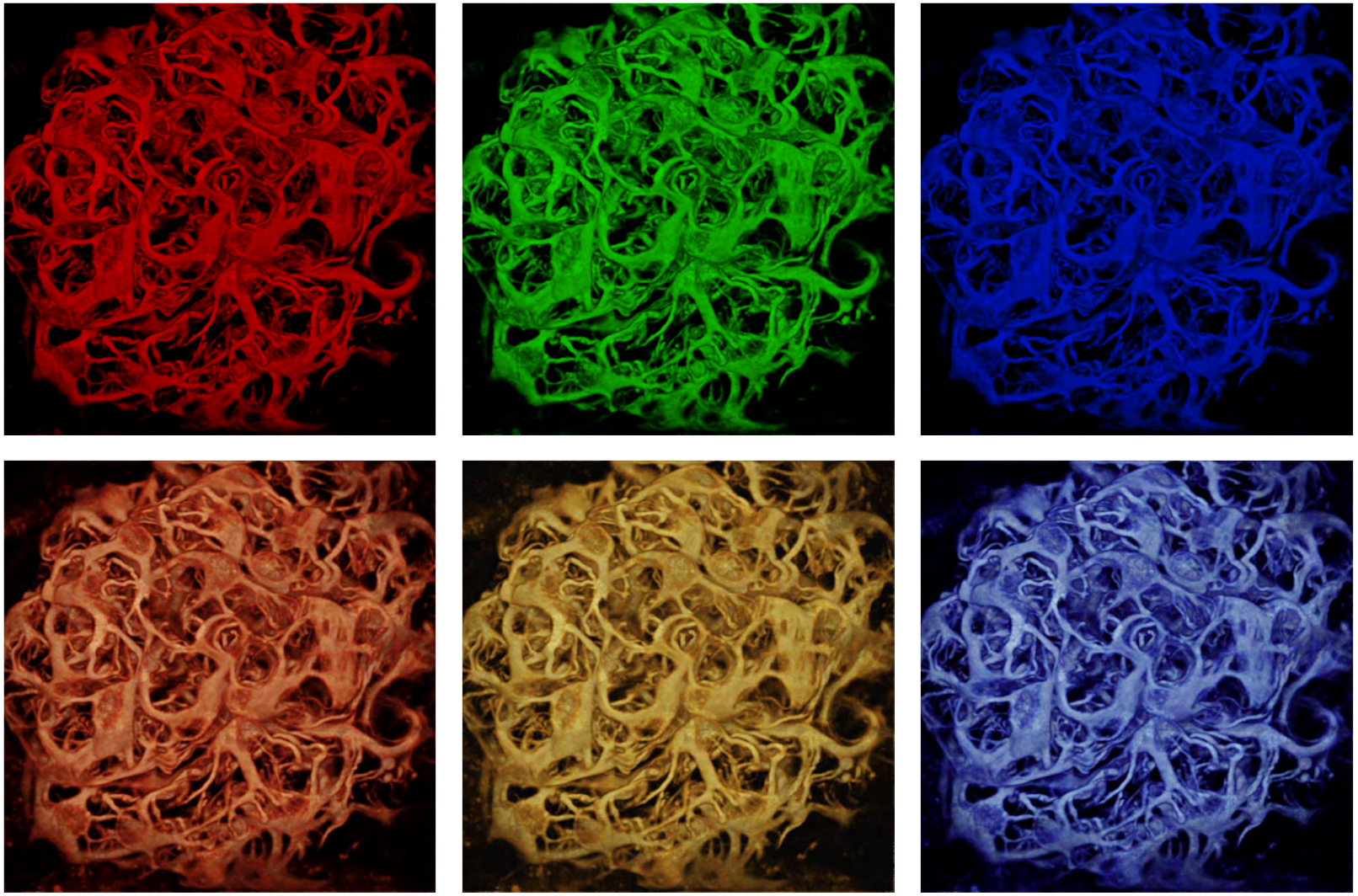


Maximum Intensity Projection

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

Colorization

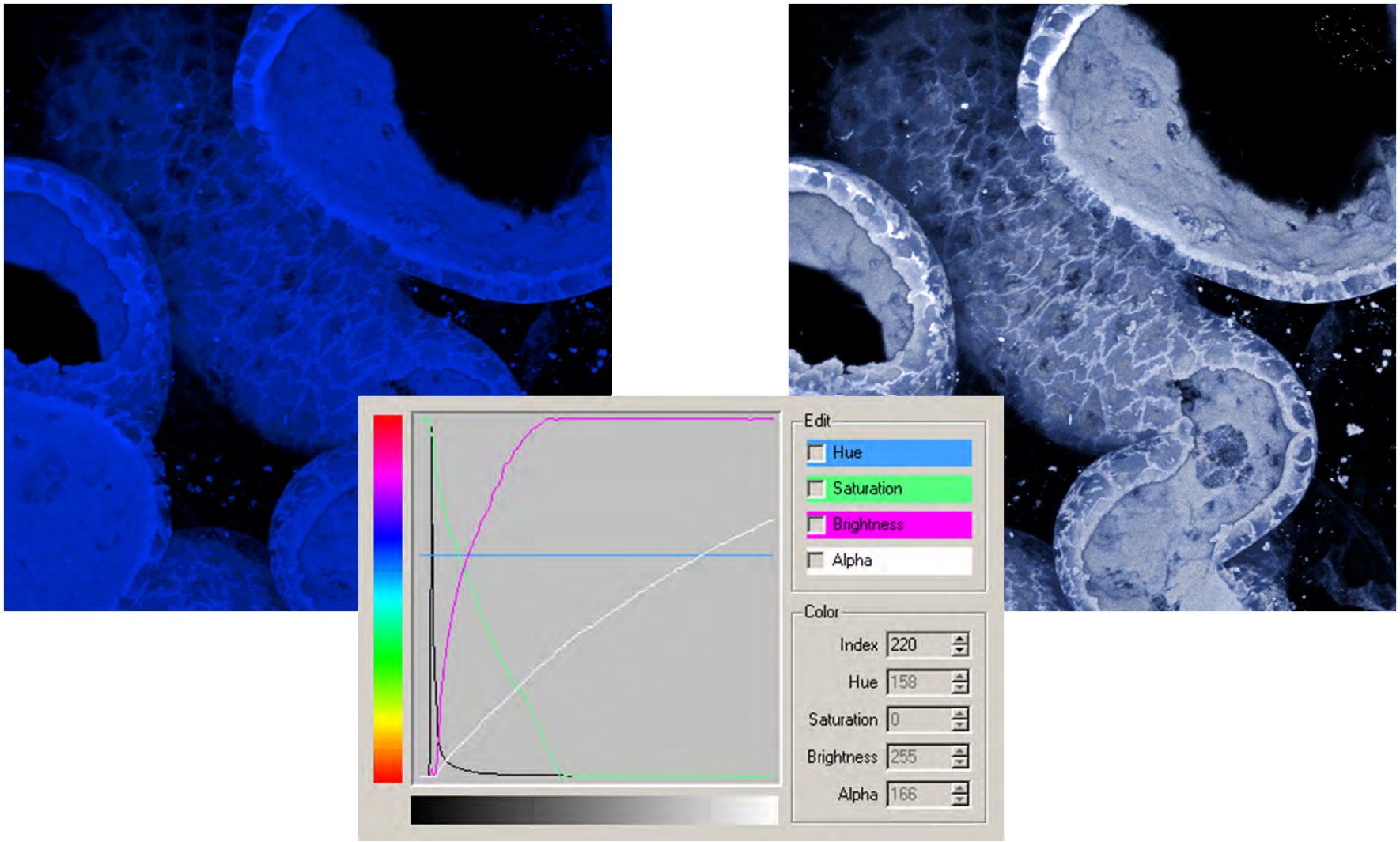
You need to assign each channel a different color when displaying multi-channel images



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

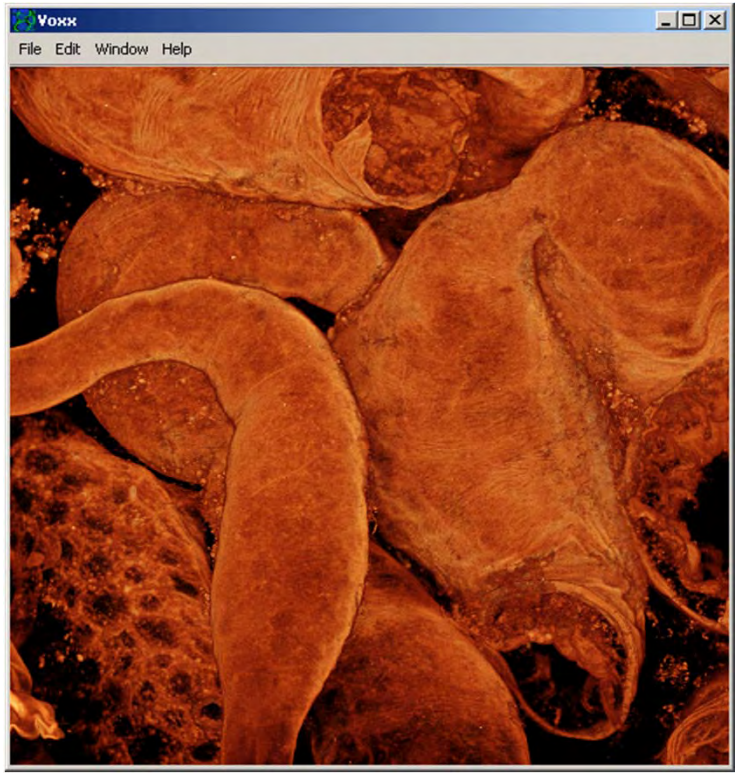
Intensity and Color Mapping

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

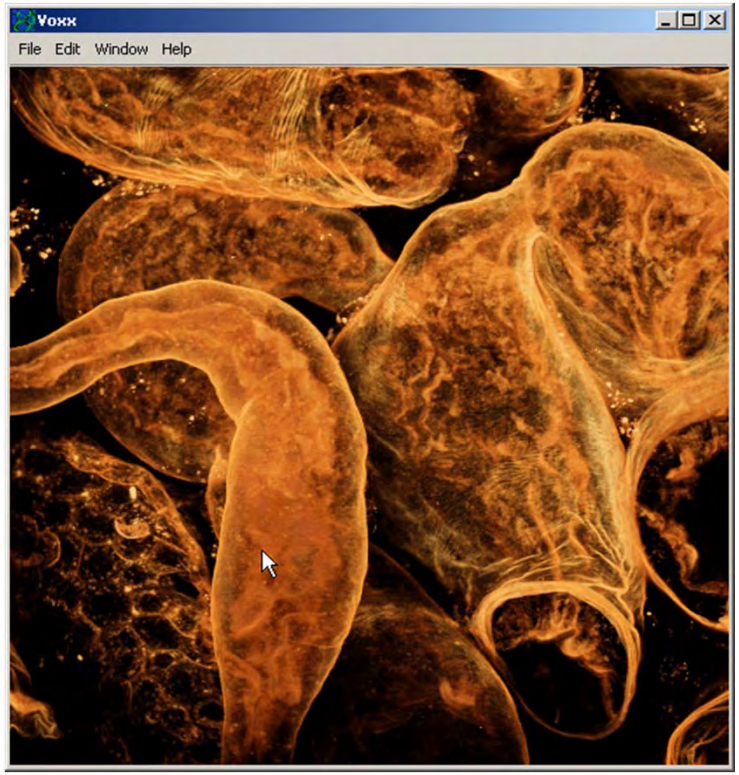


Adjust Opacity

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)

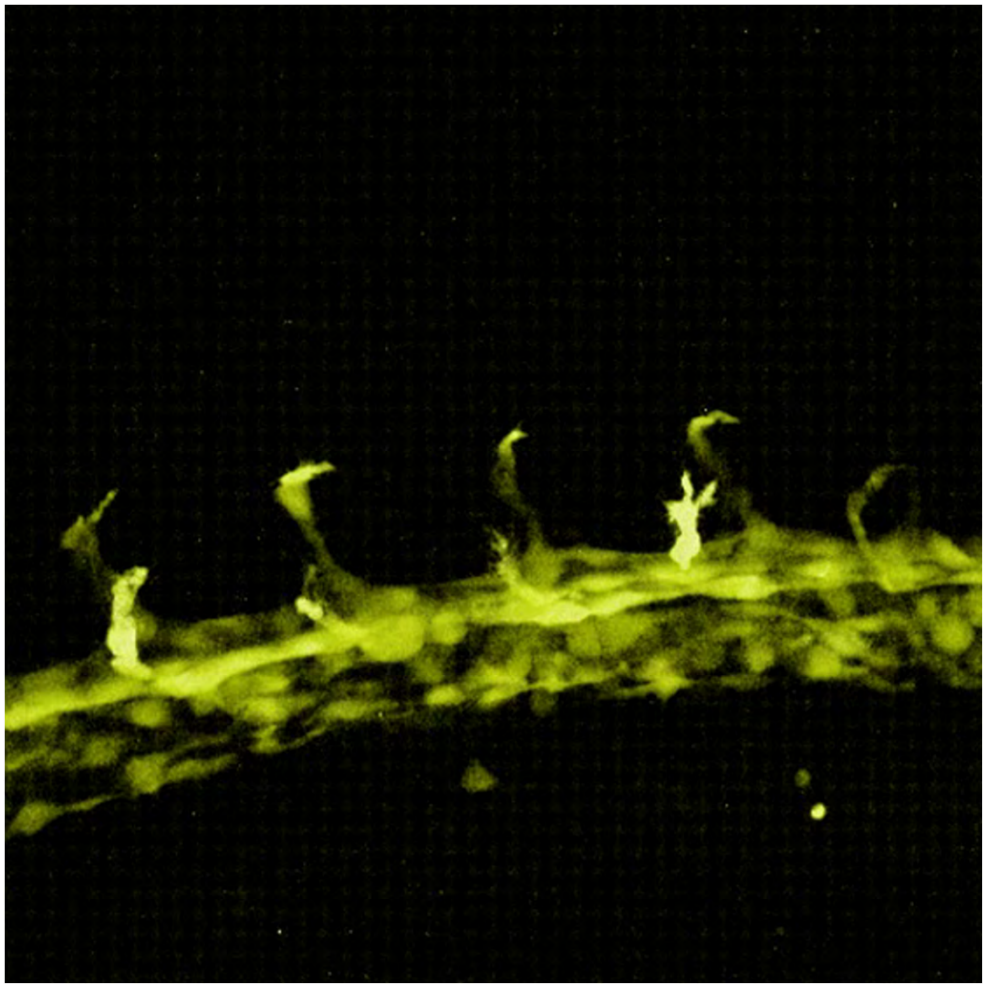


Low Opacity (translucent)

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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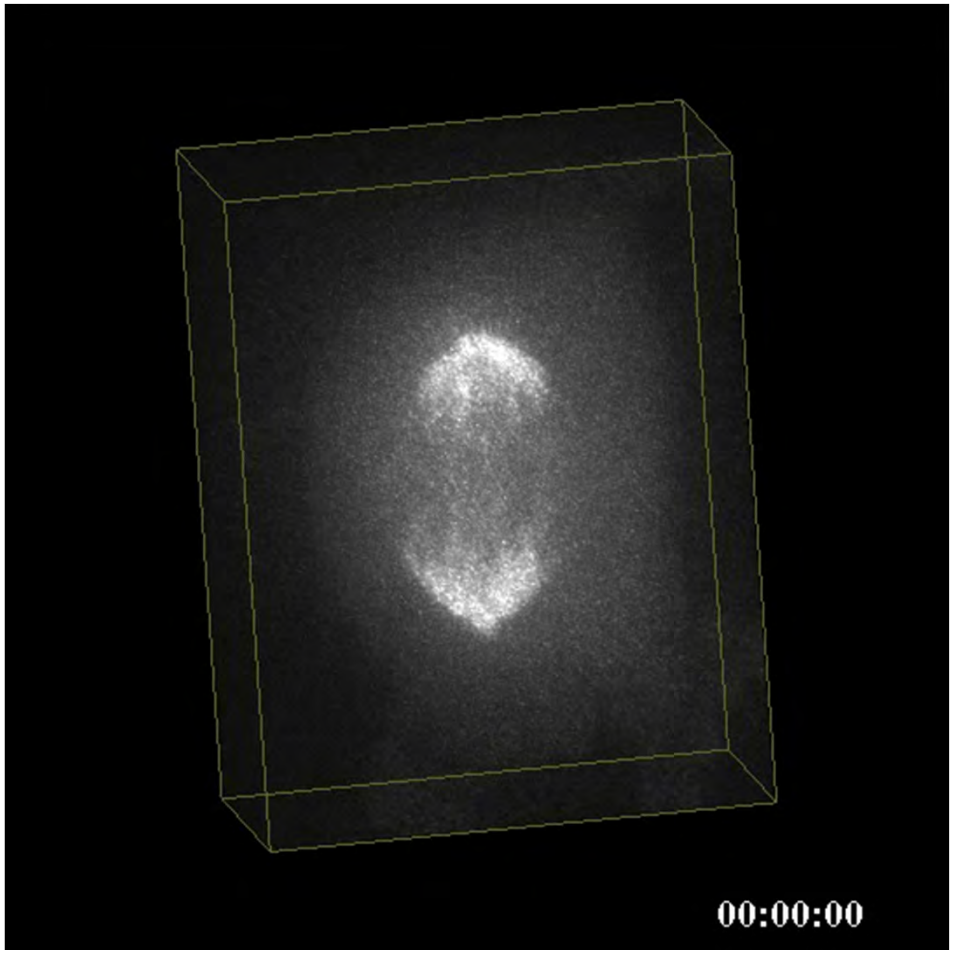


e.g. dividing cell

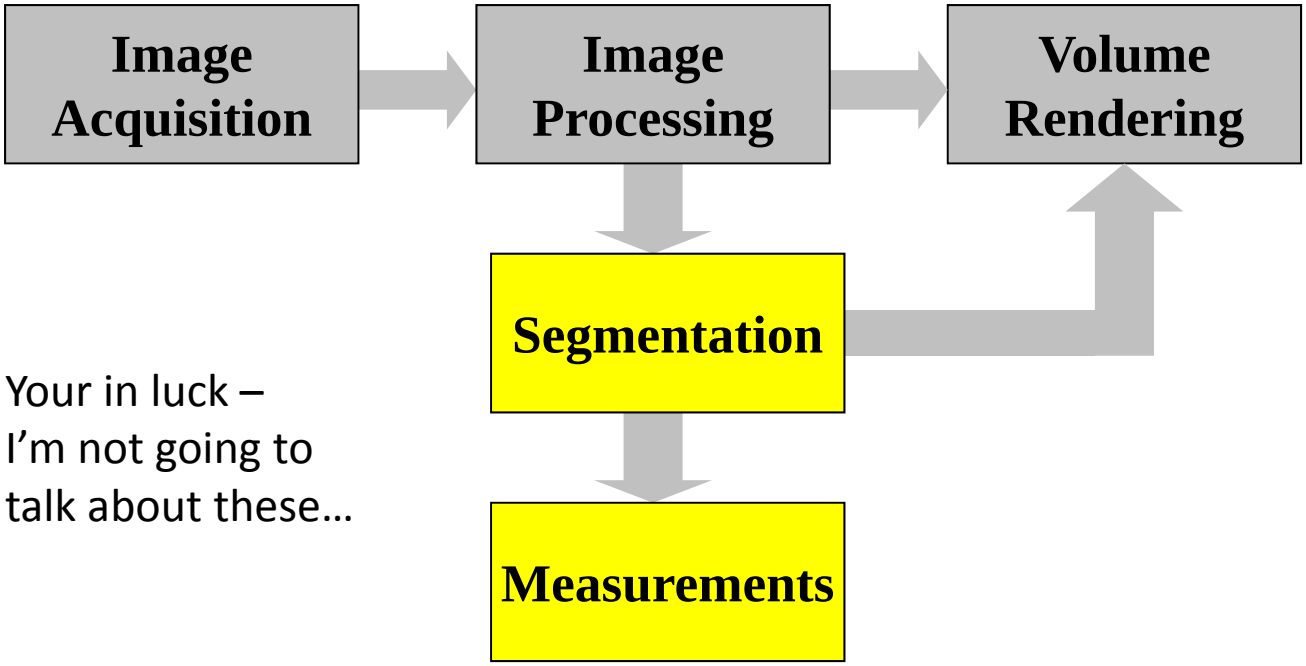
Lighting

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

e.g. volume rendering using Voreen by Jonsson, Sunden, Ynnerman, Ropinski (2013)

Quantitative Analysis (numerical)

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



Your in luck –
I'm not going to talk about these…

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

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
	Endrov	www.endrov.net
	ImageJ	http://rsbweb.nih.gov/ij/plugins/volume-viewer.html
	(Fiji)	
	Vaa3D	www.vaa3d.org
	Voreen	www.voreen.org
	Voxx	www.indiana.edu/~voxx
Commercial	Amira	www.vsg3d.com, amira.zib.de
	AutoQuant X3	www.mediacy.com
	Huygens	www.svi.nl
	Image-Pro	www.mediacy.com
	Imaris	www.bitplane.com
	Volocity	www.perkinelmer.com

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 (6 gigabytes) $ 1000
GTX 680 (2-4 gigabytes) $ 500-600
GTX 670 (2-4 gigabytes) $ 400-500
…

AMD Radeon

HD 7970 (3 gigabytes) $ 400-450
HD 7950 (3 gigabytes) $ 300-350
…

GPU-Accelerated Video Boards

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

Choose your host PC carefully…

High-performance boards are LARGE (e.g. full-size dual-slot PCIe and beyond), and need HIGH-CURRENT power supplies (e.g. Titan specs > 42 Amps at 12 volts!).

Questions?