3D Visualization and Analysis

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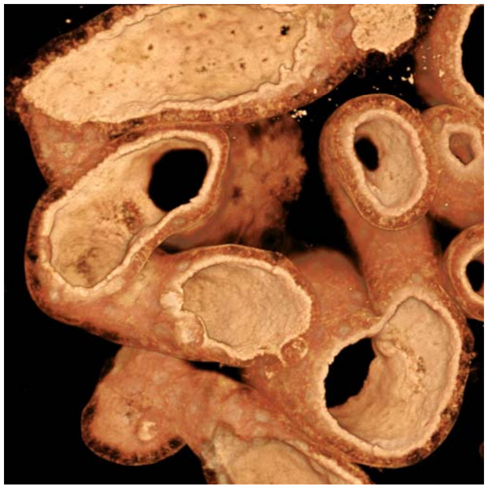
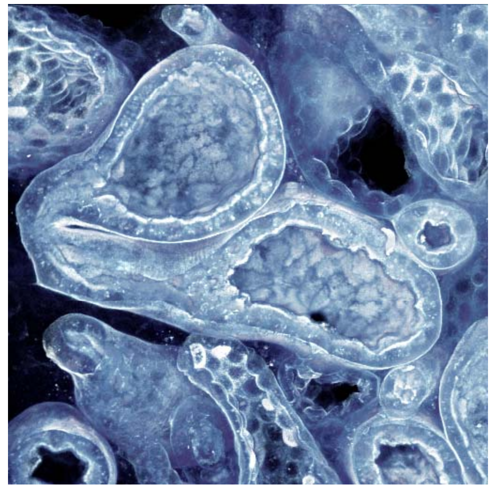
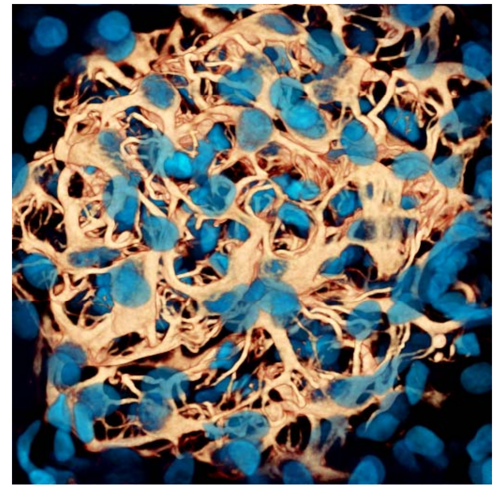
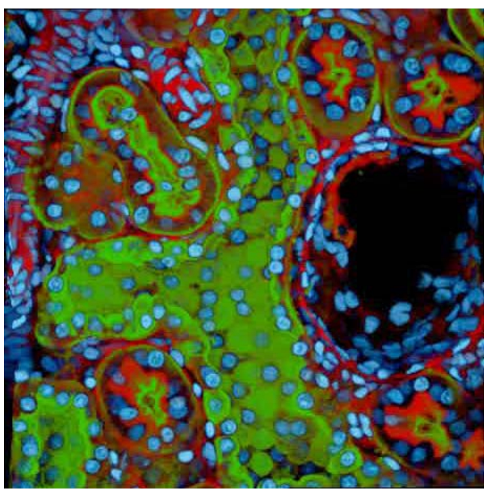
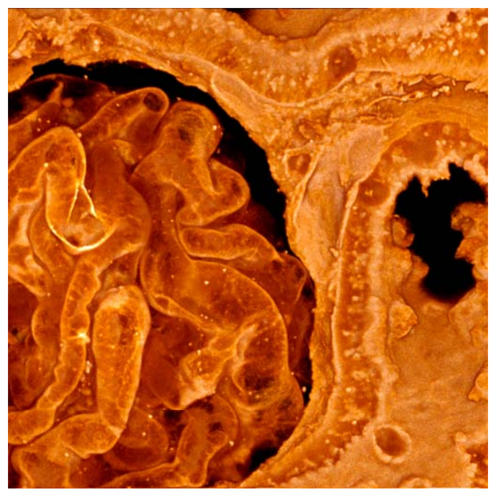
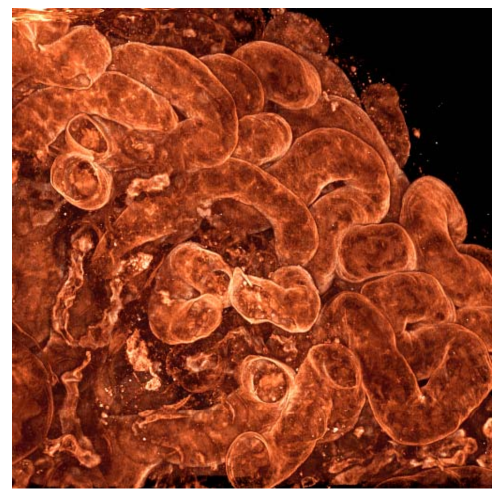
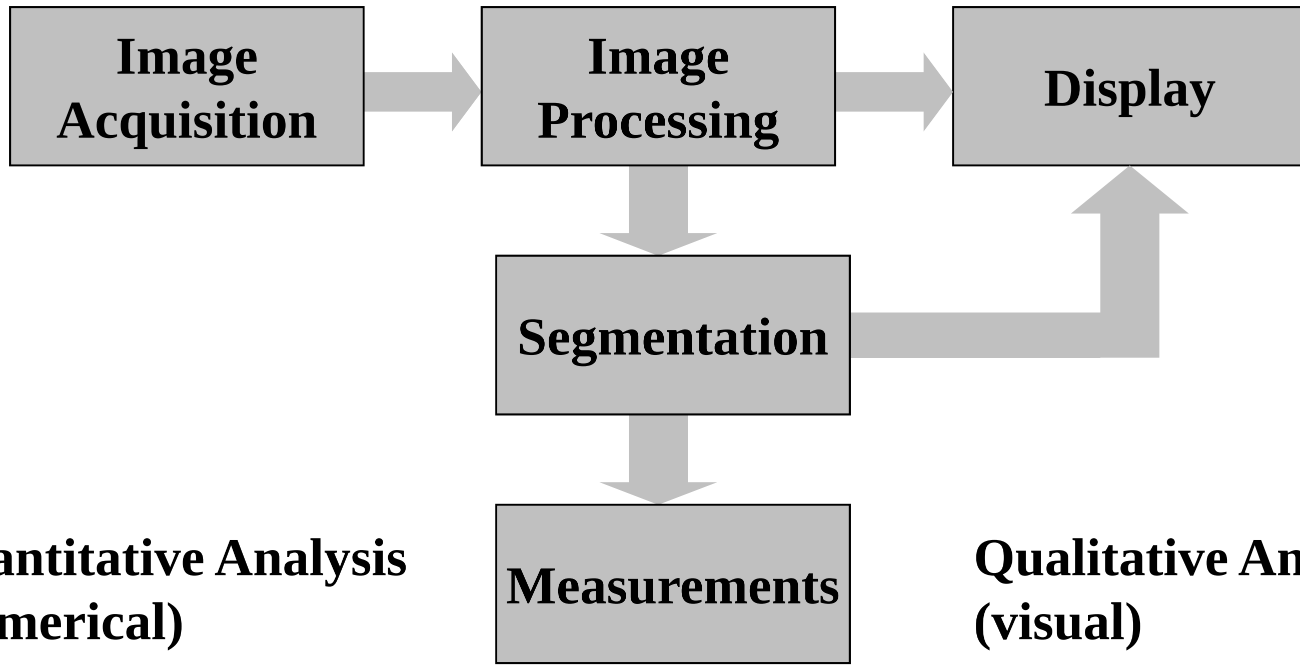







www.nephrology.iupui.edu/imaging

The Problem

Image-based research is time consuming and labor intensive



Quantitative Analysis (numerical)

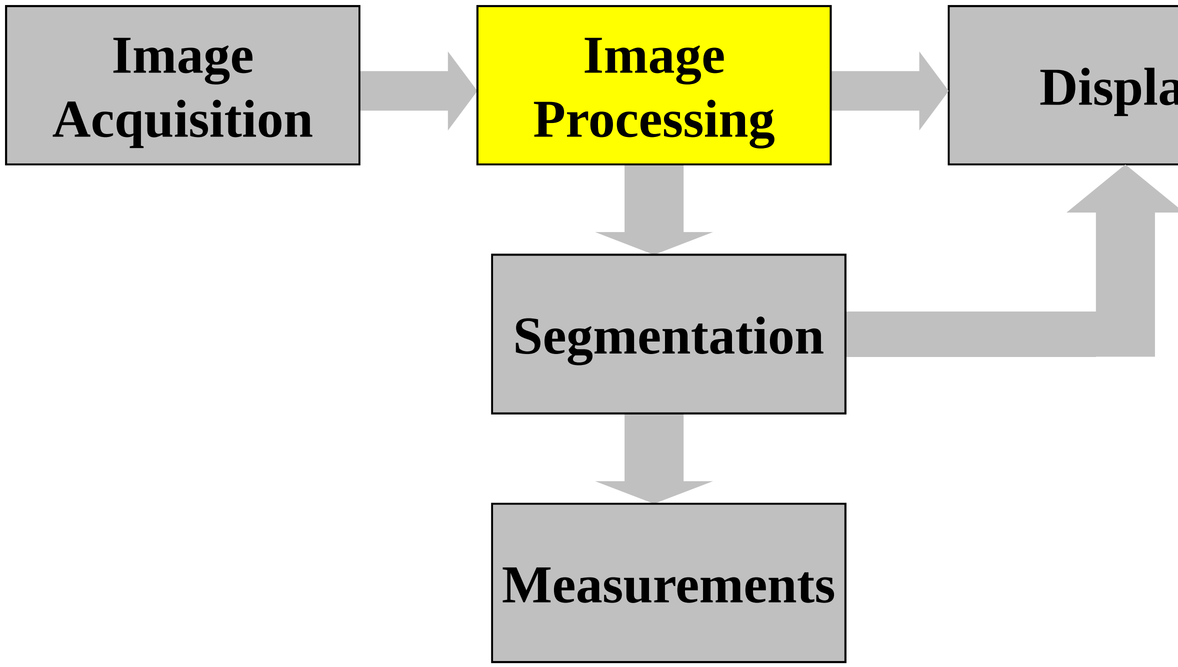
Qualitative Analysis (visual)

Image processing, segmentation, and rendering are computation-intensive tasks.
Some image analysis tasks can be fully automated.

For all of the other tasks, image analysts need (near) real-time visual feedback to perform those tasks…

Digital Image Processing

The application of any mathematical operation that modifies the original values of pixels in a digitized image



Once you've acquired some images, you often need to process them before they are displayed, or before they can be segmented and measured

Why do Image Processing? To correct for distortion

Image processing software can sometimes be used to compensate for distortion introduced by instruments and/or tissue during image acquisition

- Limited spatial resolution
- Nonuniform illumination
- Geometric distortion
- Poor signal-to-noise ratio
- Crosstalk
- Chromatic aberration
- etc.

Image acquisition hardware has limitations

Why do Image Processing? For "image enhancement"

Image processing software can sometimes be used to improve the visibility of biological structures of interest

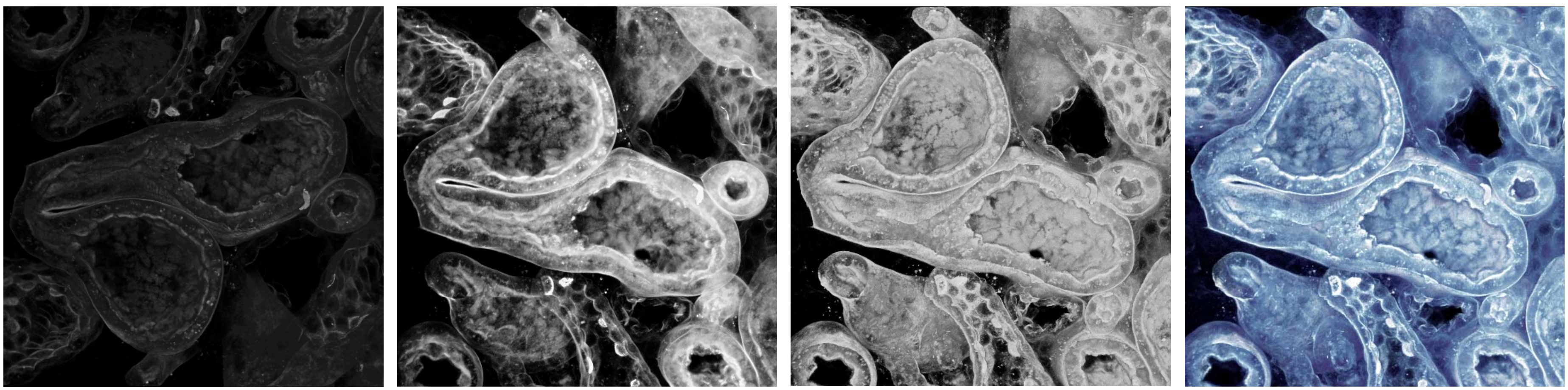


Image display hardware and the human visual system have limitations

Why do Image Processing? For Segmentation

Before imaging software can be used to measure things, you first have to isolate the structures that you want to measure. That procedure is called segmentation

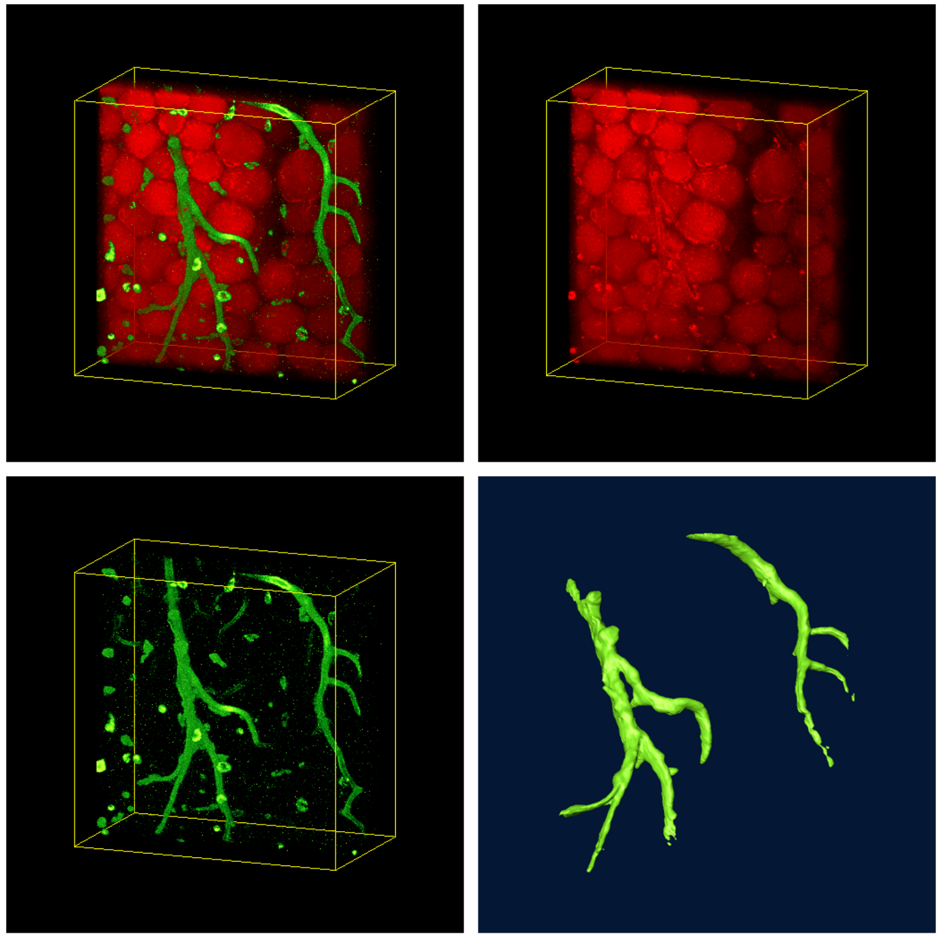
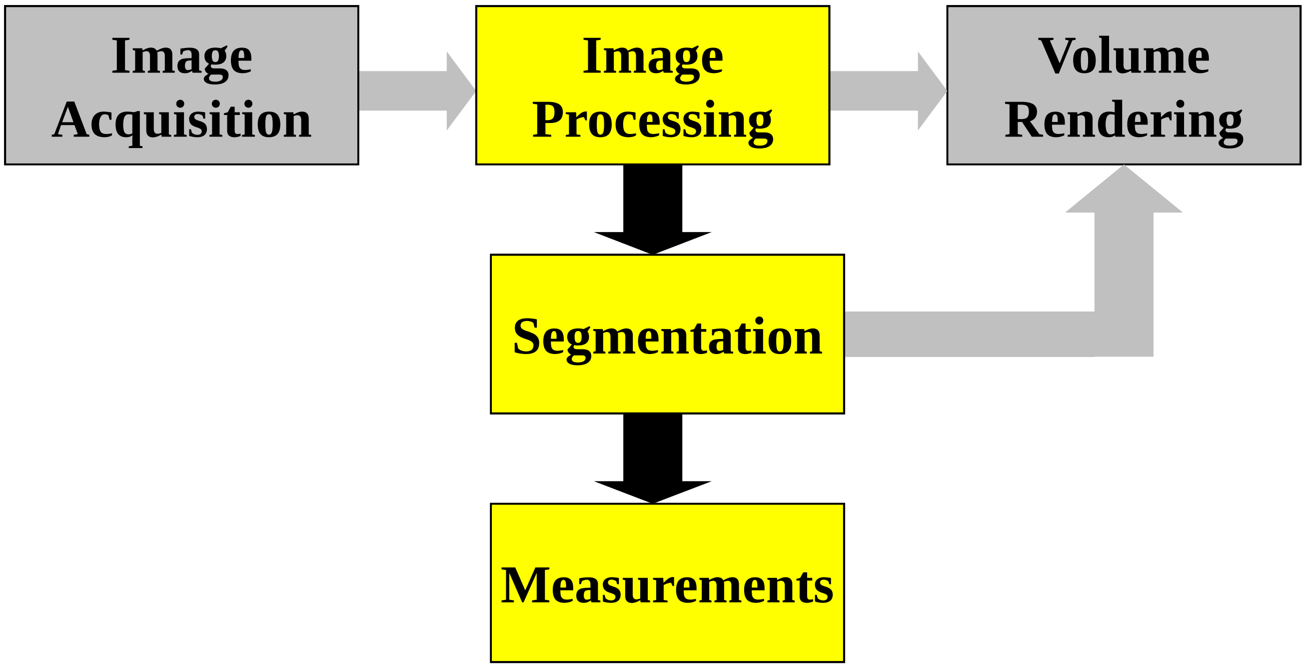


Image processing is required before/during segmentation, to assist in the separation of biological structures from their surroundings

Automatic Image Segmentation

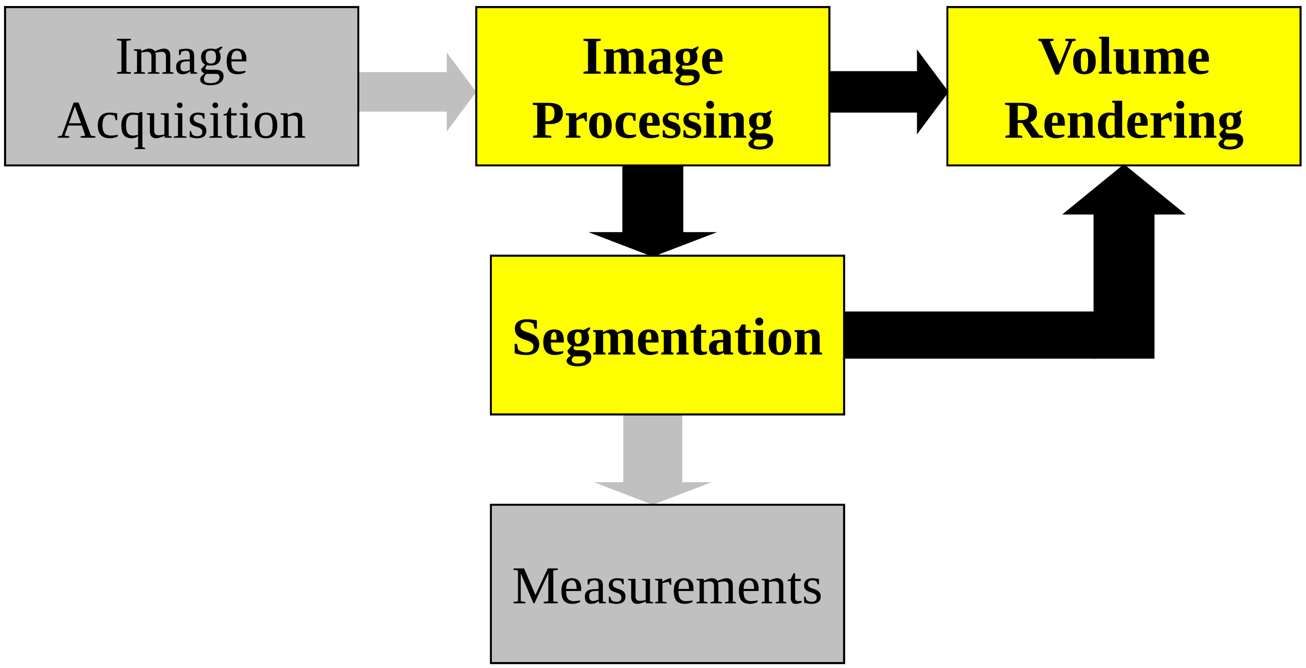
The holy grail for image analysts – reliable, fully automatic segmentation



Quantitative image analysis requires that biological structures in 3D images be recognized and labeled, before measurements can be made

Interactive Image Segmentation

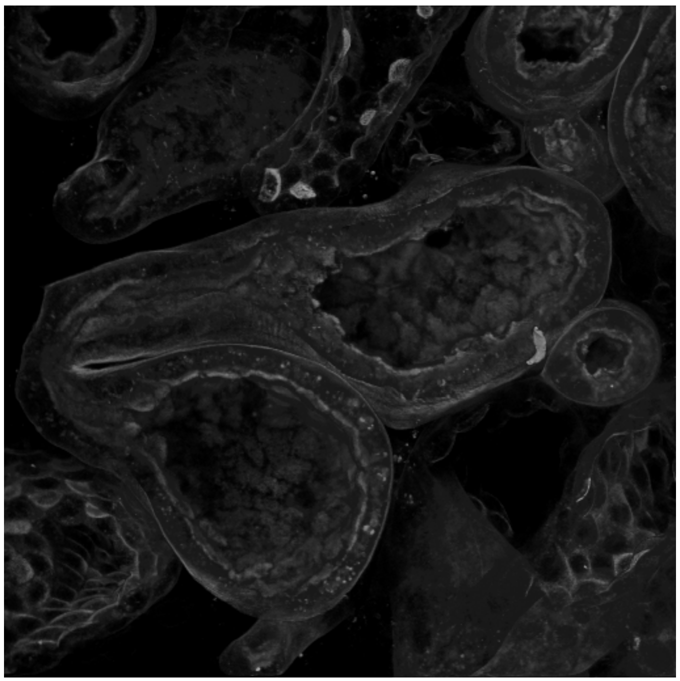
Interactive segmentation – when fully automatic segmentation isn't feasible

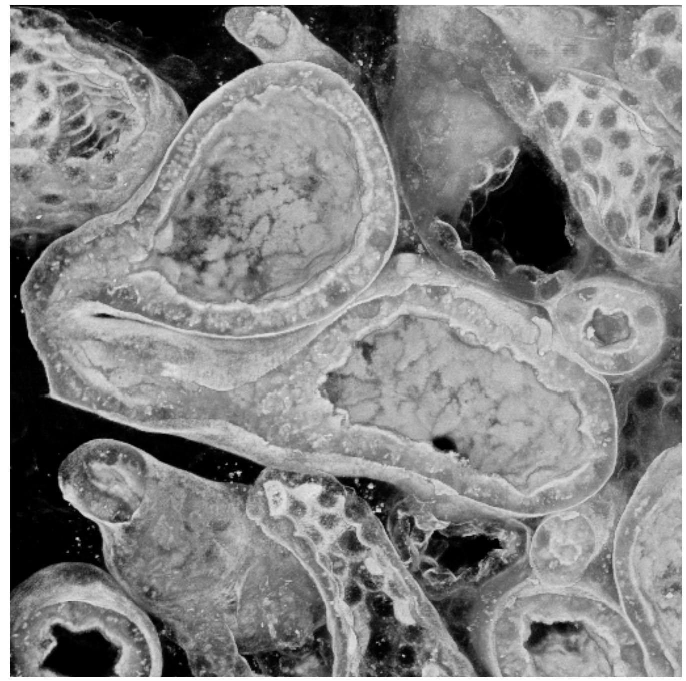


More advanced volume rendering involves both image preprocessing and voxel classification/segmentation. But voxel classification is often still done manually

Image Processing using Intensity Transforms

The human eye cannot detect small differences in intensity between regions within an image, even though the pixels have measurably different values

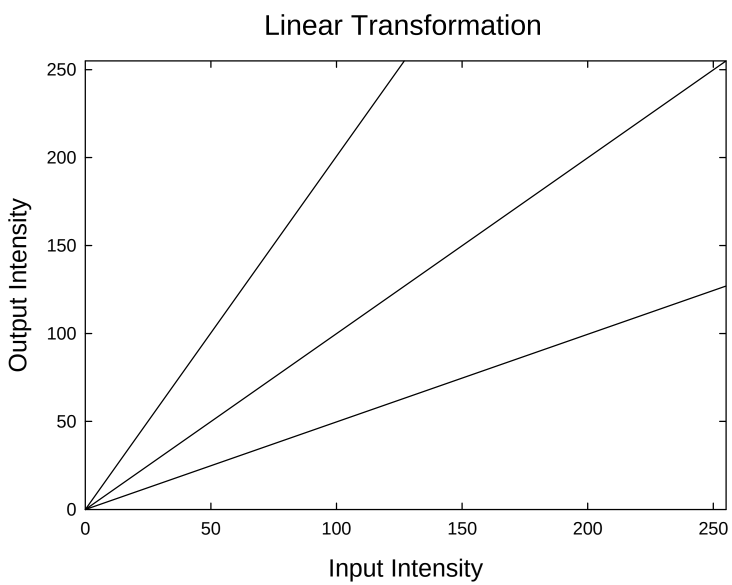


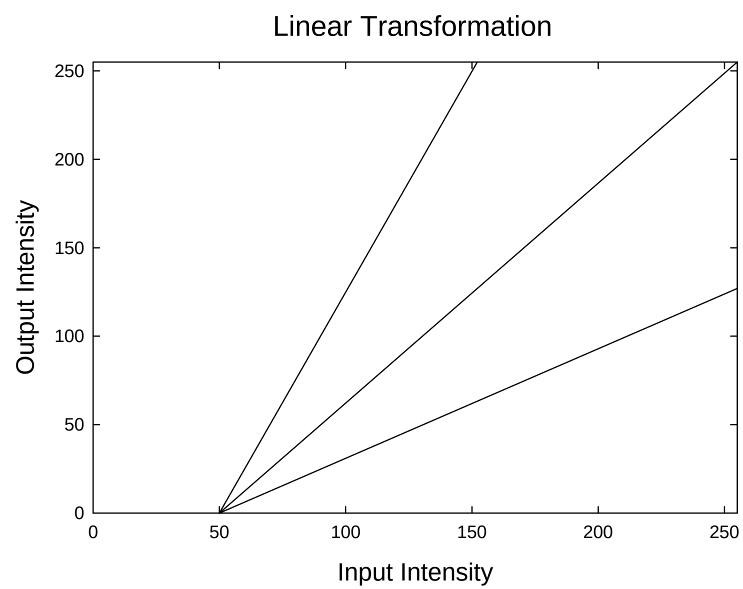


Brightness and contrast stretching operations can make those small differences in intensity visible...

Intensity Transforms

The most common problem encountered when rendering confocal and two-photon image stacks is that they are dim and low-contrast. Intensity transformations can often be used to improve the visibility of structures.



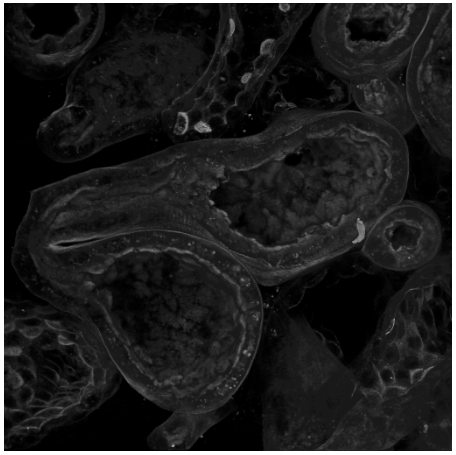


Linear transfer function **y = m x.** Setting slope (m) > 1 will increase the contrast of all pixel values.

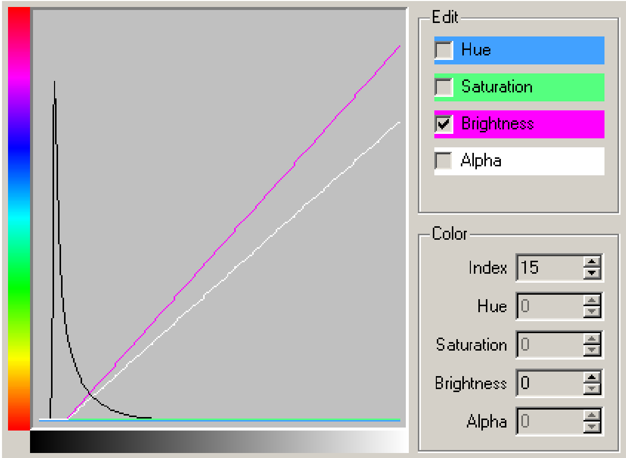
Because of the nonzero background intensity values, you may need to shift the x-axis intercept slightly to the right of the background value(s).

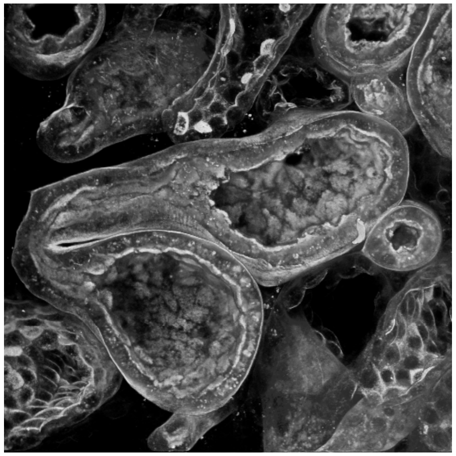
Intensity Transforms

Example of linear transfer functions. Increasing the slope of brightness curve from 1 to 3 increases the brightness and contrast of all pixel values. Also note that x-axis intercept was shifted slightly to the right of the background peak

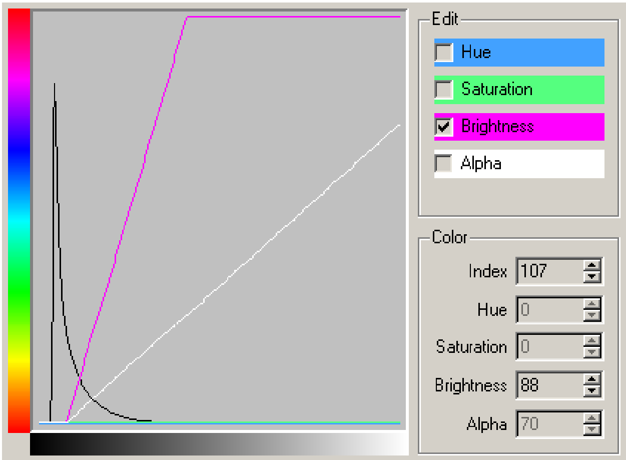


Slope=1



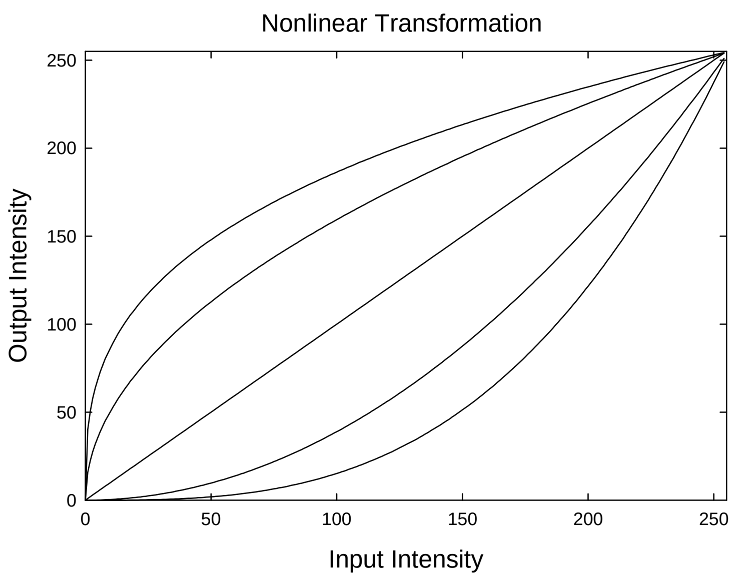


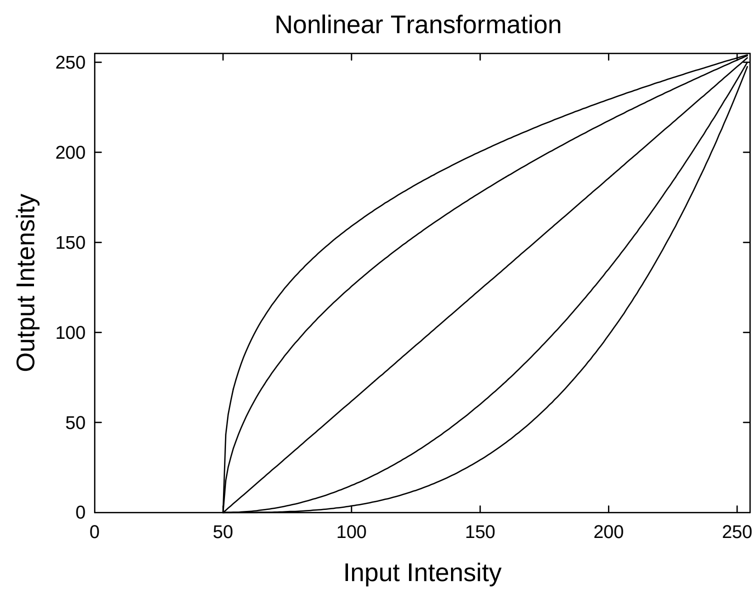
Slope=3



Intensity Transforms

The most common problem encountered when rendering confocal and two-photon image stacks is that they are dim and low-contrast. Intensity transformations can often be used to improve the visibility of structures.



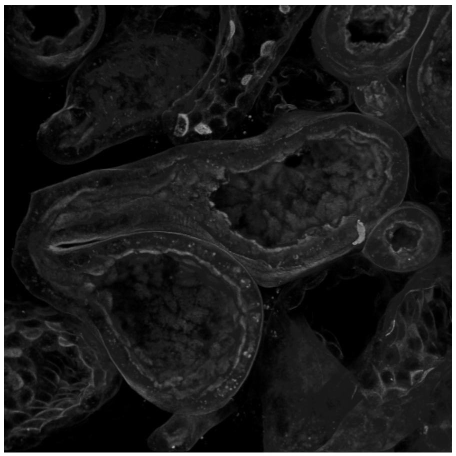


Power-law transfer function $\mathbf{y = x^a}$. Setting power (a) < 1 will increase the contrast of dim pixels and decrease the contrast among bright pixels.

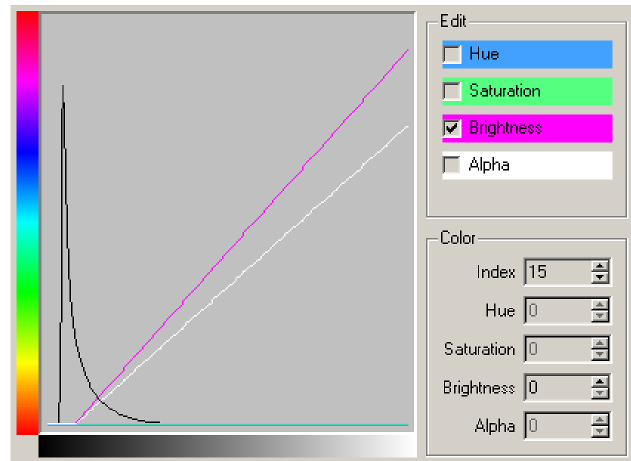
Because of the nonzero background intensity values, you may need to shift the x-axis intercept slightly to the right of the background value(s).

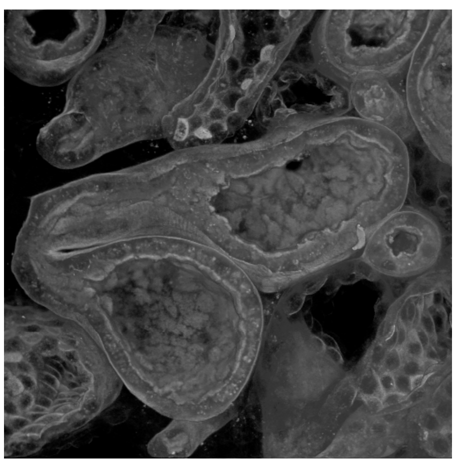
Intensity Transforms

Example of power-law transfer functions. Decreasing the power will increase the brightness and contrast of dim pixels and decrease the contrast among bright pixels.

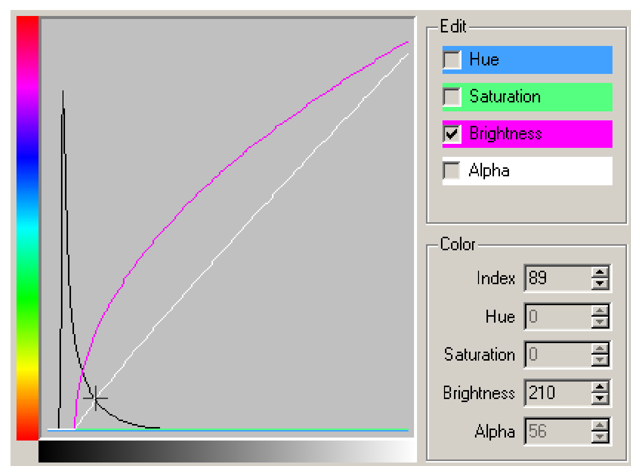


Power=1





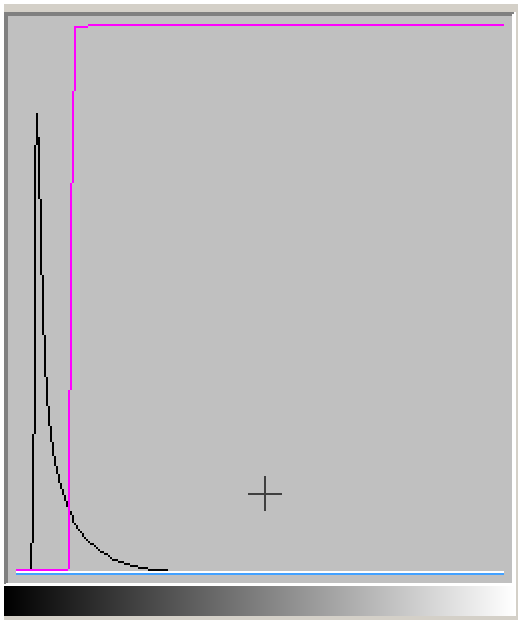
Power=0.5



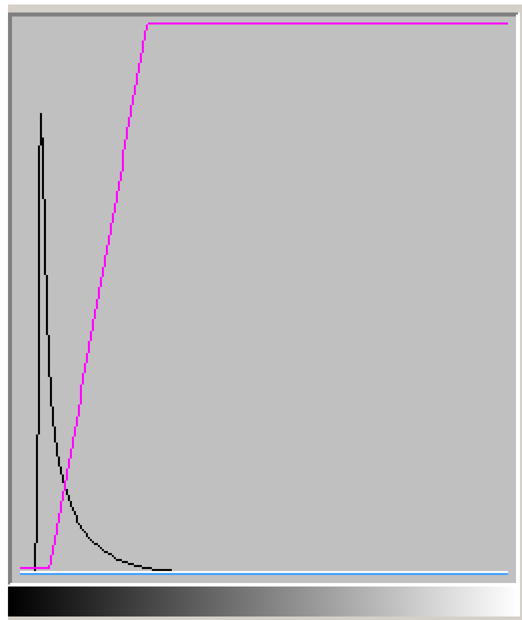
Intensity Transforms: Hard vs Soft Thresholding

Intensity transformations used to modify brightness and contrast can also be used to implement thresholding, by replacing low-intensity pixel values with zero.

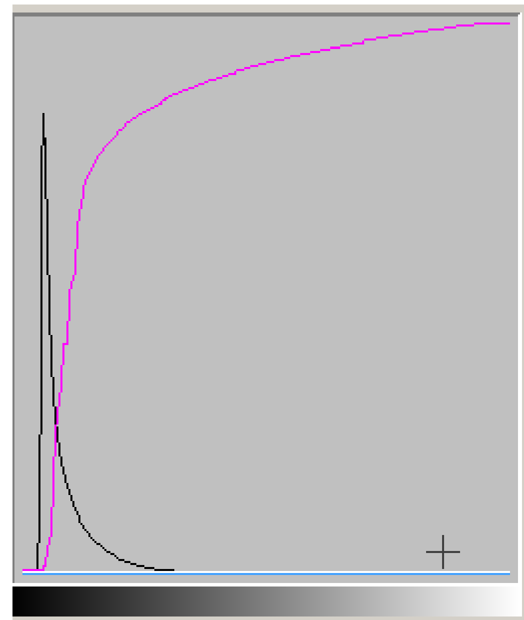
Hard Threshold



Soft Threshold



Softer Threshold

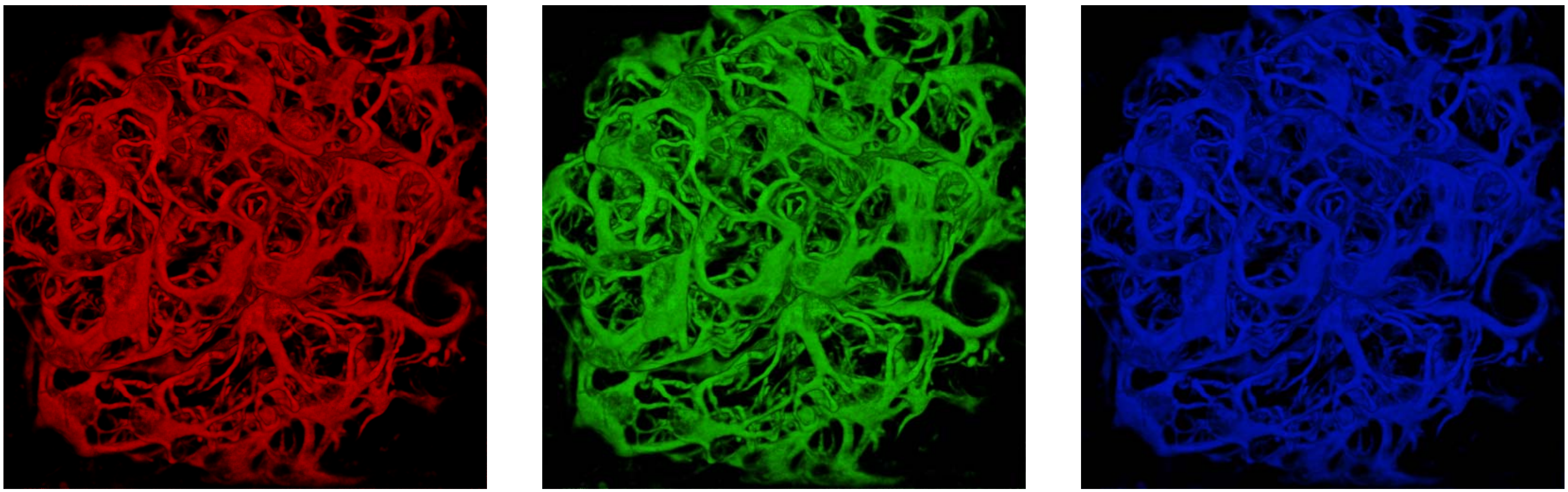


"Hard thresholding" typically replace all pixel values below a given value with zero, and replaces all pixel values greater than or equal to that value with a nonzero value.

"Soft thresholding" has a transition region, instead of a single threshold value, in which pixel intensities are made to rapidly increase from zero to some nonzero value

Color Transforms

Your choice of colors affects the visibility of structures, and whether the printed image will look more or less like the on-screen image

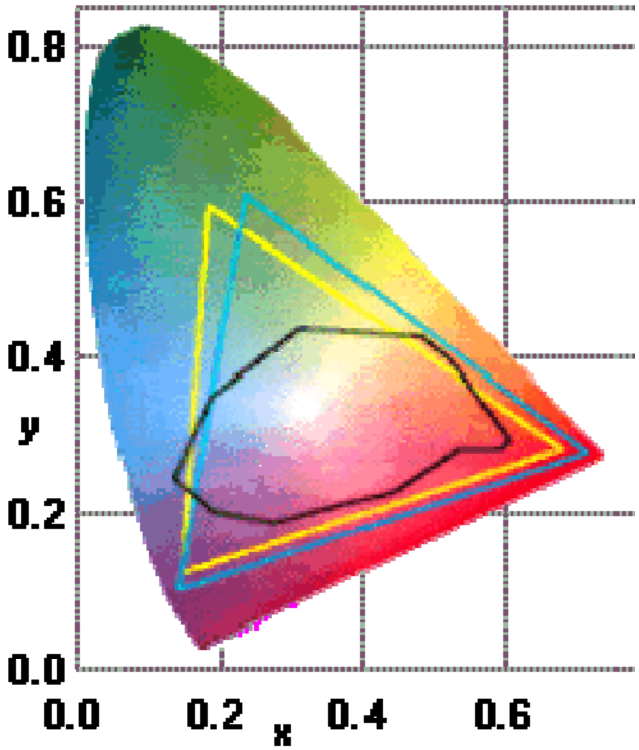


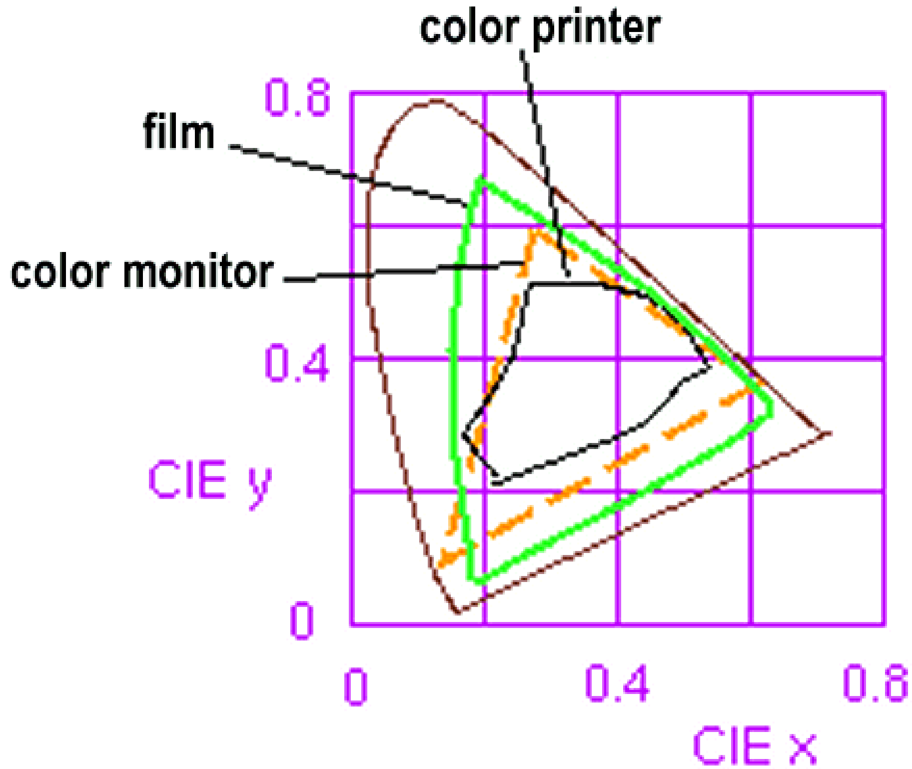
Red and blue objects can be difficult to see in on-screen images, because the human eye is less sensitive to red and blue, and more sensitive to green. Try using a mixture of the primary RGB colors (e.g. reddish-orange instead of red for Rhodamine, cyan instead of blue for DAPI).

And it can be difficult to get printed images to look like the on-screen image when using highly saturated red, green, or blue. Monitors can display colors than are not reproducible on printers. Try slightly reducing color saturation...

Colorization

The choice of colors affects the visibility of structures, and whether the printed image will look more or less like the on-screen image





It can be difficult to get printed images to look like the on-screen image when using highly saturated red, green, or blue. Monitors can display colors than are not reproducible on printers. Try slightly reducing saturation in HSV, to move a color inside the printer's color gamut...

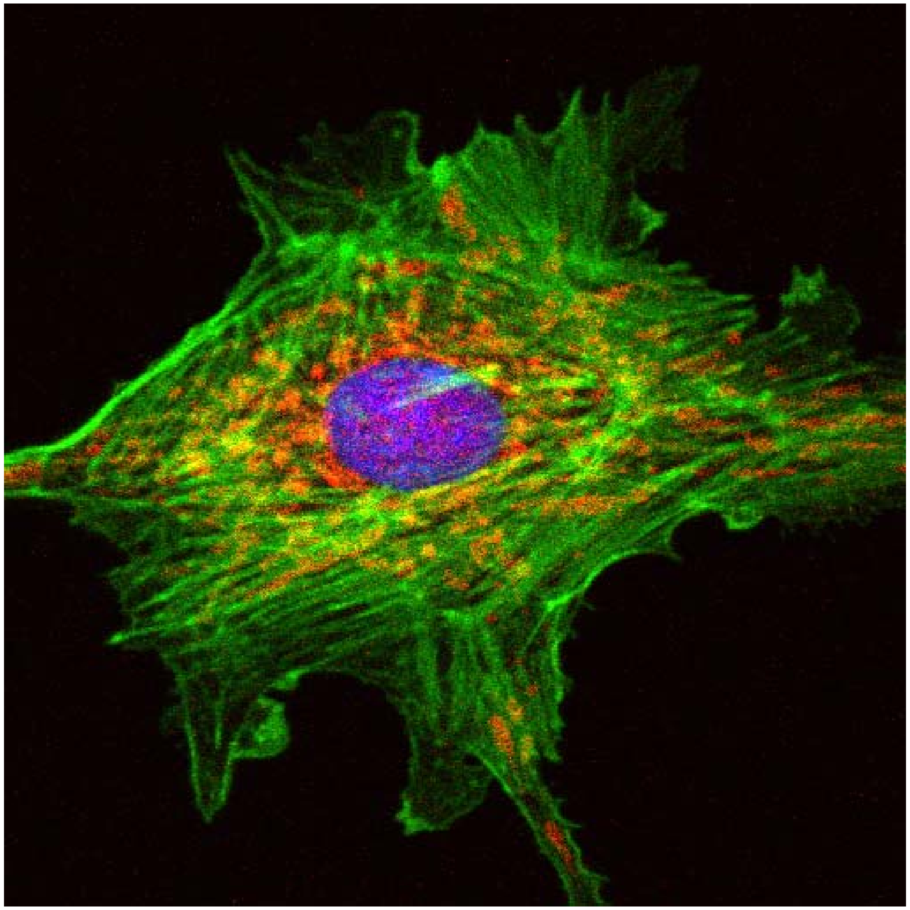
Image Processing using Filters

Filters use the values of neighboring pixels to compute a new/filtered value for each pixel in an image

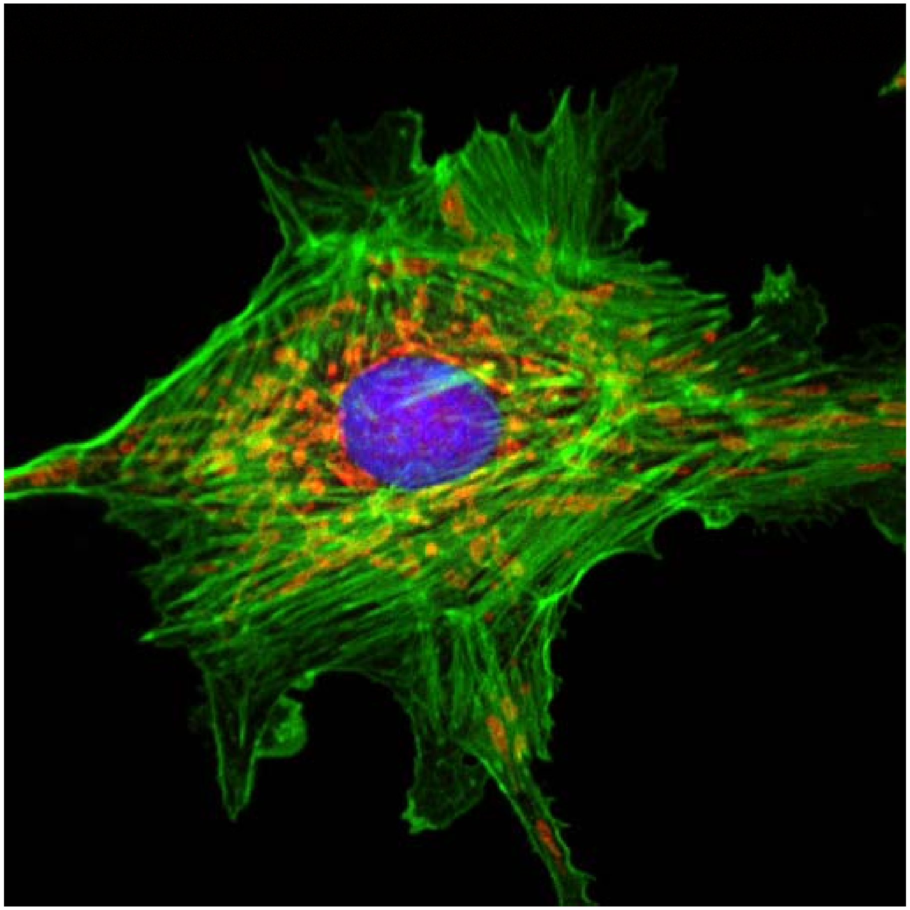
Convolution:	1) arbitrary filter kernels, non-separable 2) special cases, separable kernels 3) special cases, recursive
Diffusion:	1) nonlinear isotropic 2) nonlinear anisotropic 3) reaction-diffusion
Transform-based:	1) FFT or Hartley 2) wavelets
Deconvolution:	1) constrained iterative methods 2) transform-based methods
Morphological:	1) grayscale morphological (min, max, etc) 2) binary morphological (erode, dialate, etc)

Noise Reduction

Some kinds of image processing are performed during image acquisition
(e.g. filters that perform denoising using clever forms of averaging)



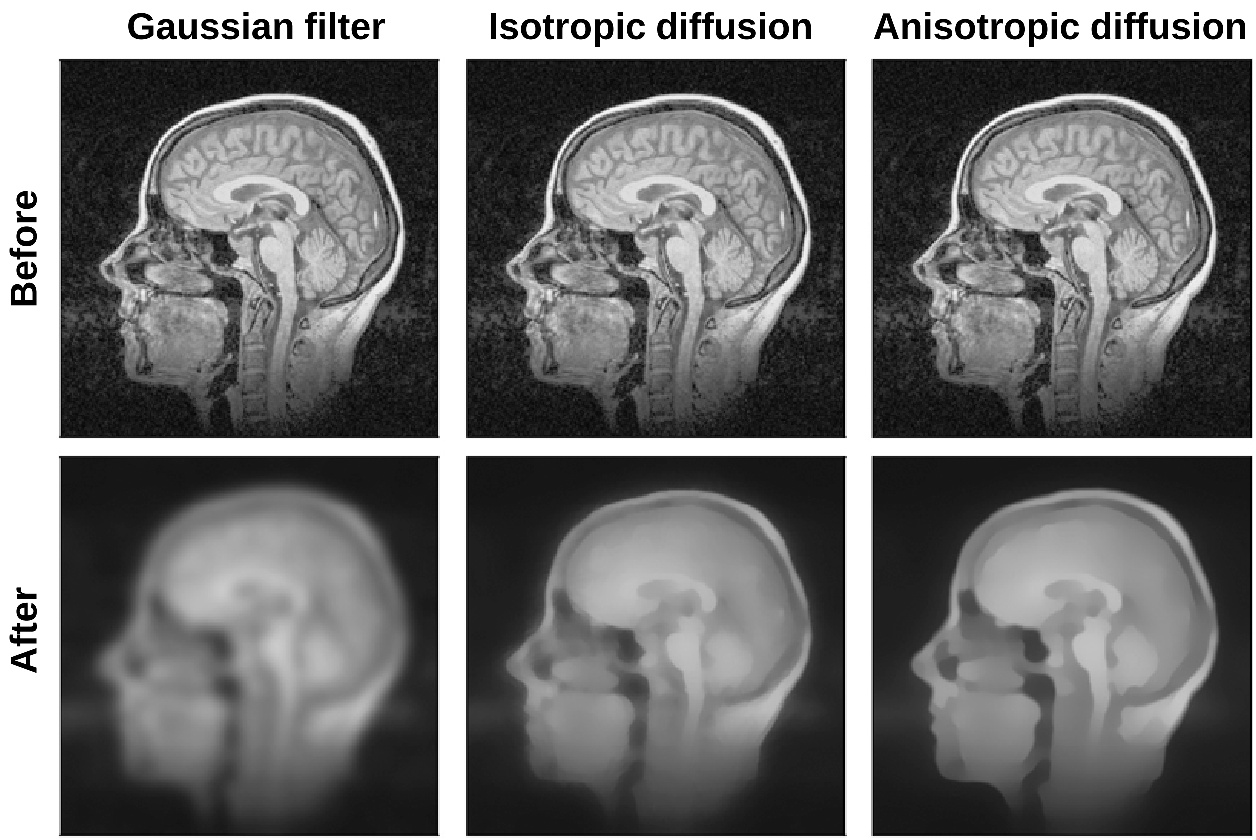
Noisy Image of Cell



Averaged Image

Smoothing (Averaging) Filters

Iterative smoothing filters based on nonlinear diffusion equations preserve the boundary between different structures better than Gaussian filters

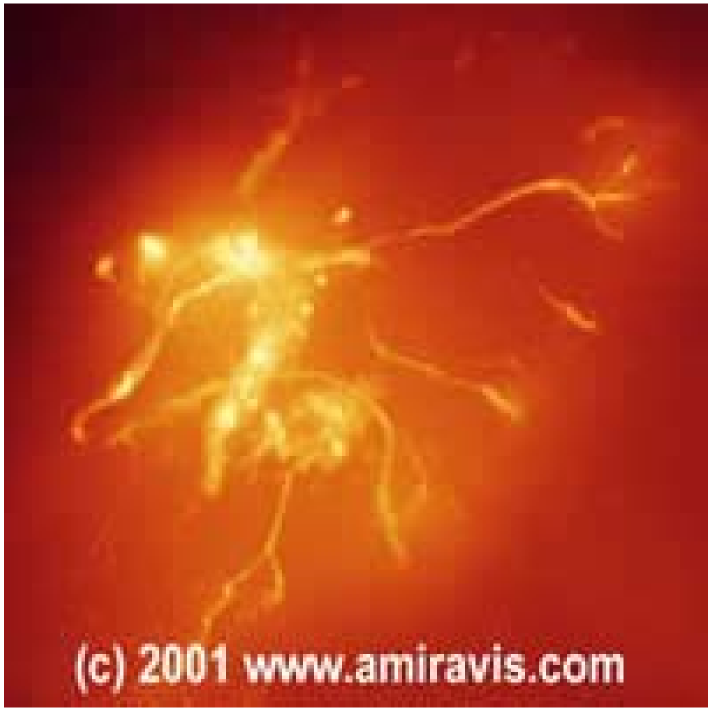


Original work on nonlinear diffusion filters in 2D image processing done by Perona and Malik (1990). Above images by Langer (2004).

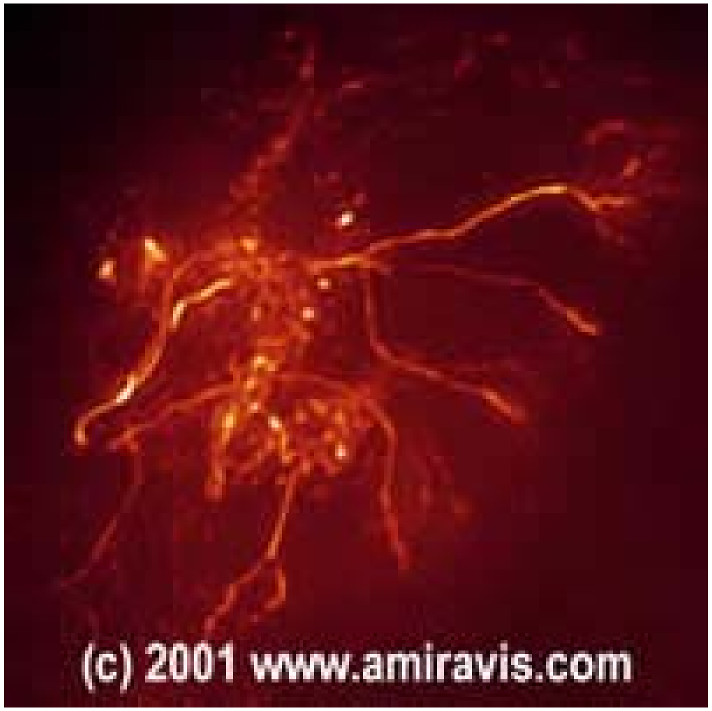
Deconvolution Filters

Some kinds of image processing are performed after image acquisition
(e.g. deconvolution, crosstalk correction)

Before Deconvolution

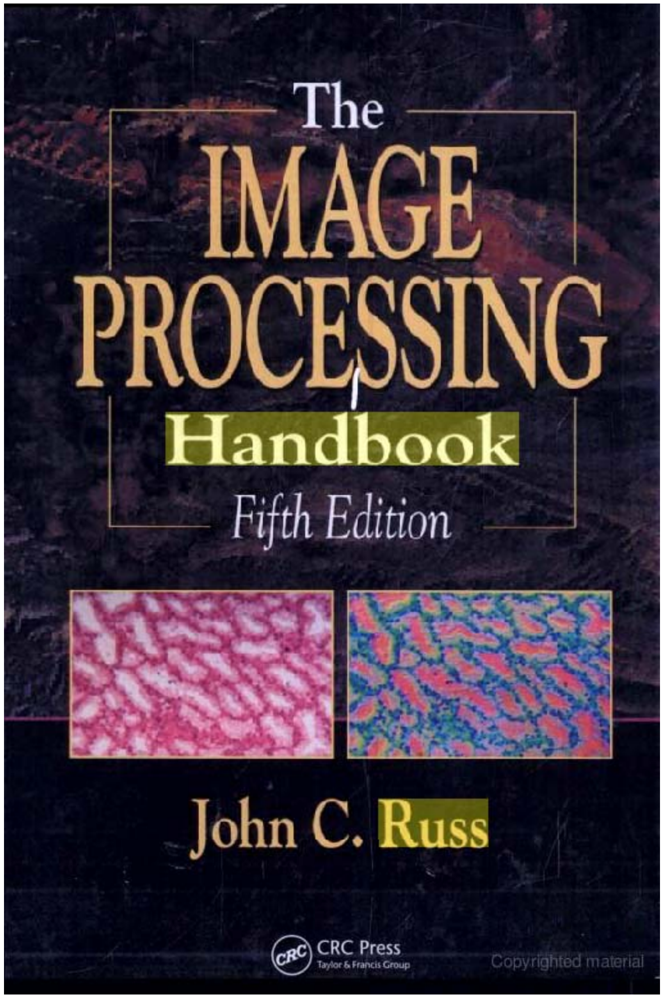


After Deconvolution



3D deconvolution software can be used to compensate to some degree for the poor axial resolution of microscope optics. It can also improve brightness and contrast.

A Good Introduction to Image Processing – with the minimum of math



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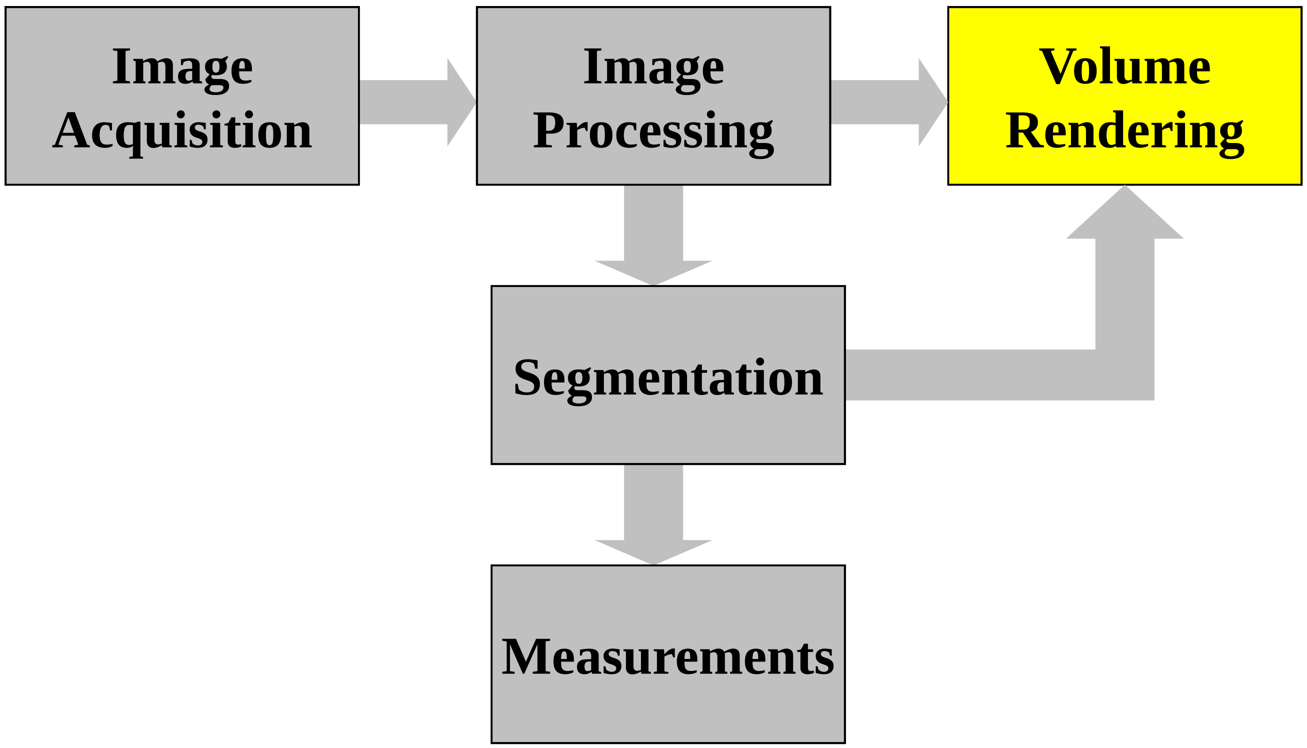
ISBN-13: 978-0849372544

Volume Rendering

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

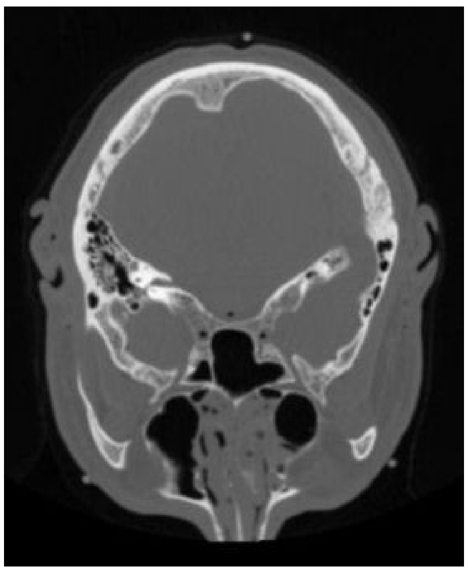


Volume renderers can display individual cross sections, but they can also display many cross sections simultaneously, which makes it easier to study 3D morphology

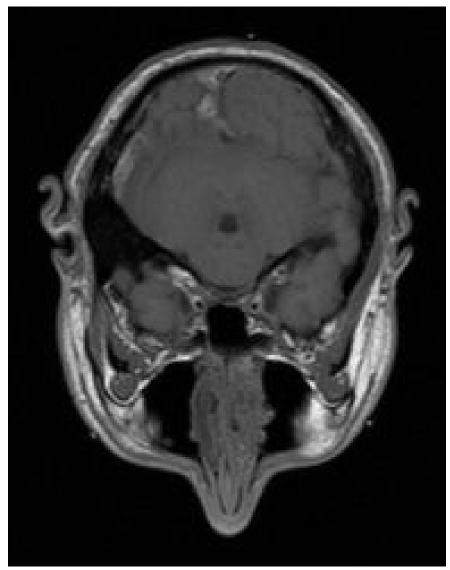
Cross-Sectional Imaging

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.

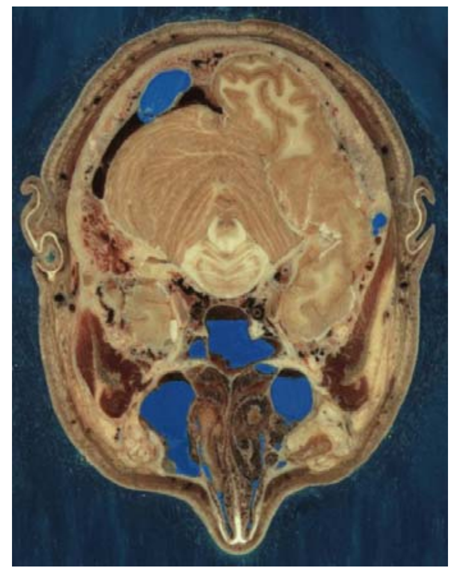
Computed Tomography



Magnetic Resonance



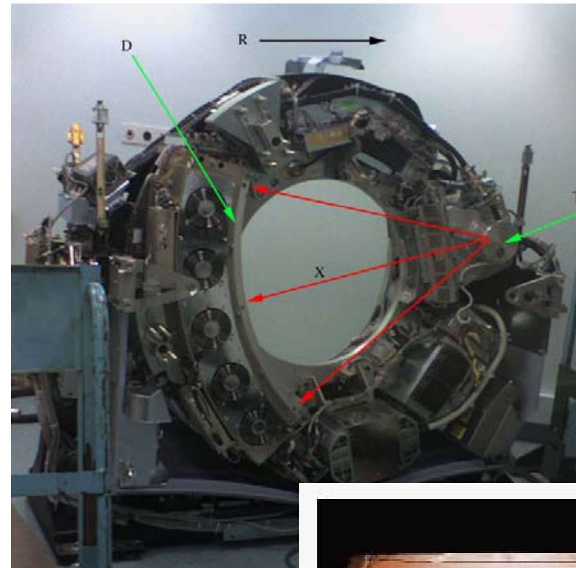
Blockface

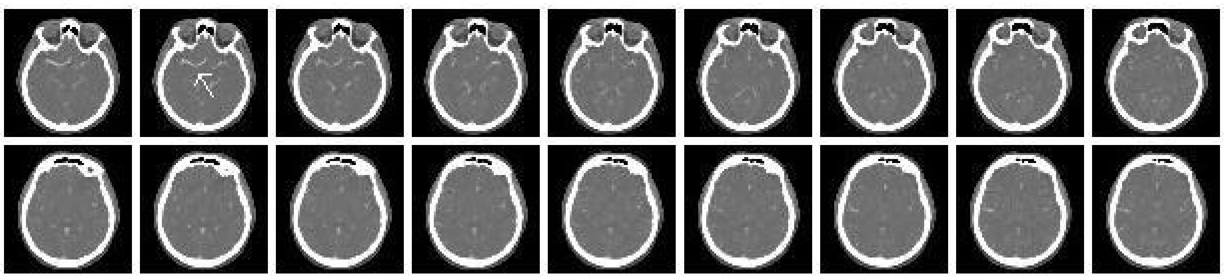


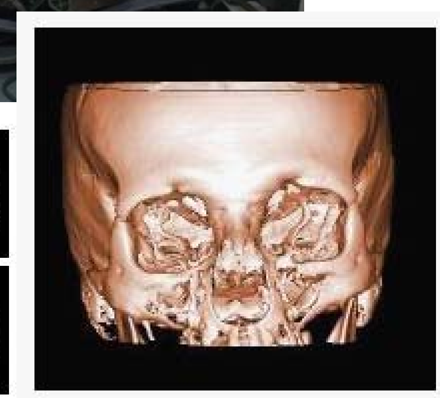
e.g. Visible Human Project [1994], cross sections of human head.
http://www.nlm.nih.gov/research/visible/visible_human.html

CT Image Acquisition

In computed tomography (CT), a rotating gantry carrying x-ray sources and detectors measures x-ray absorption from several directions within a thin slab of tissue. This 1D data is converted into a cross-sectional (2D) image using reconstruction software…



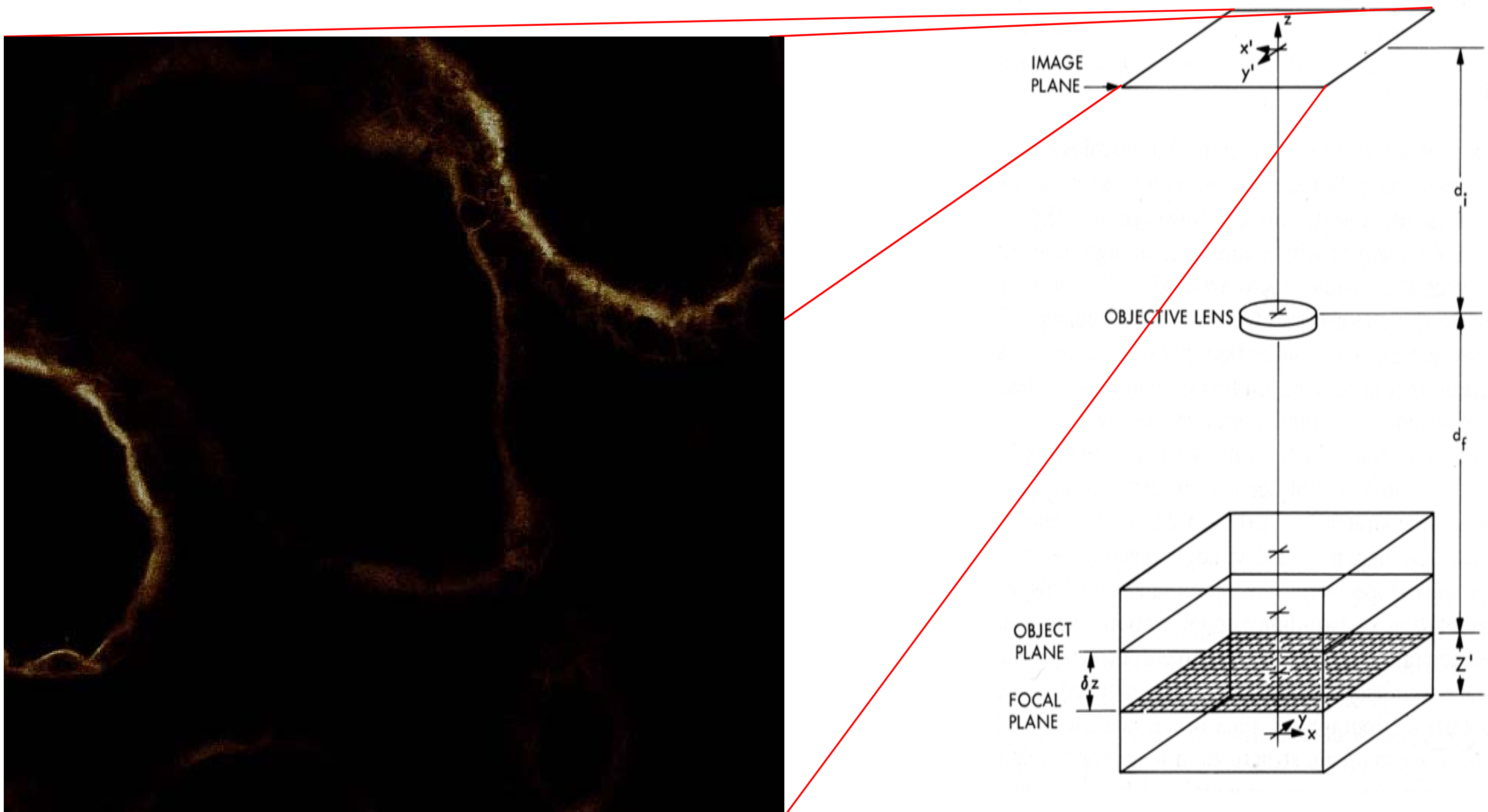




A motorized table then moves the subject to the next position. A set of parallel 2D images is collected by repeating the procedure. Volume rendering software displays them in 3D.

Image Acquisition using Light Microscopy

Several kinds of optical microscopes use laser scanners and clever optics
to collect a set of very thin parallel planar (2D) images, including:
laser scanning confocal microscopes, spinning disk confocal, two-photon, etc.



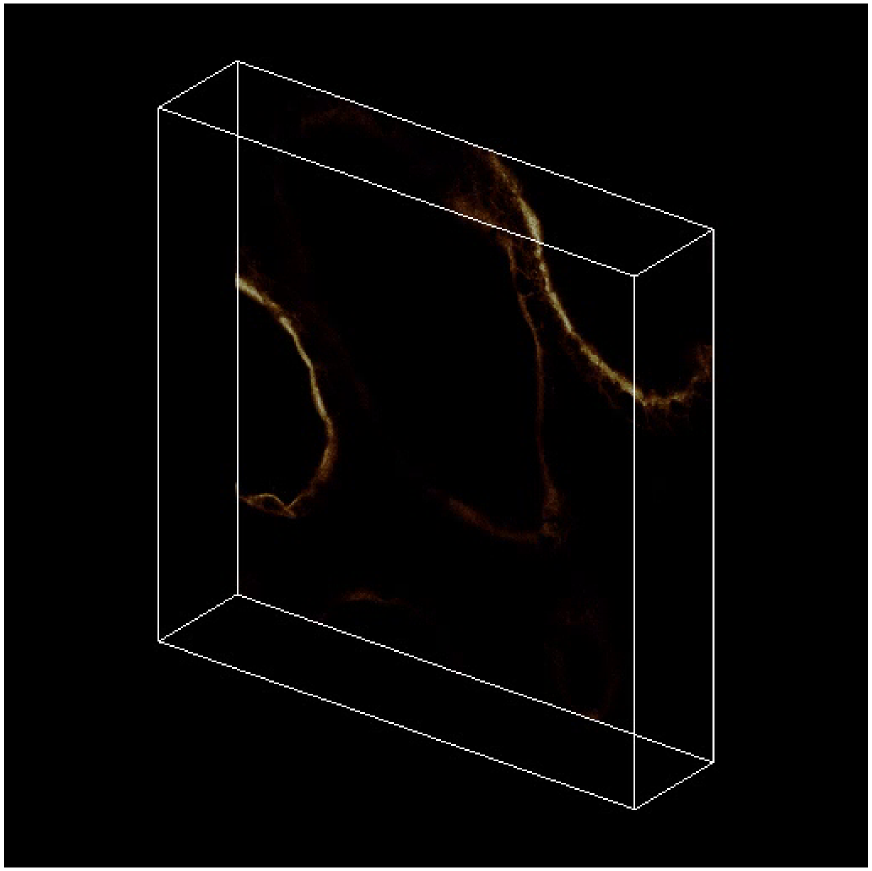
movie

K. R. Castleman,
Digital Image Processing
(c) 1979 Prentice Hall

Volume Rendering: How It Works

The 2D projections of your cross-sectional images are combined as they are rendered in back-to-front order, forming a 2D composite image that is displayed on the monitor

Laser Scanning Confocal Microscopy

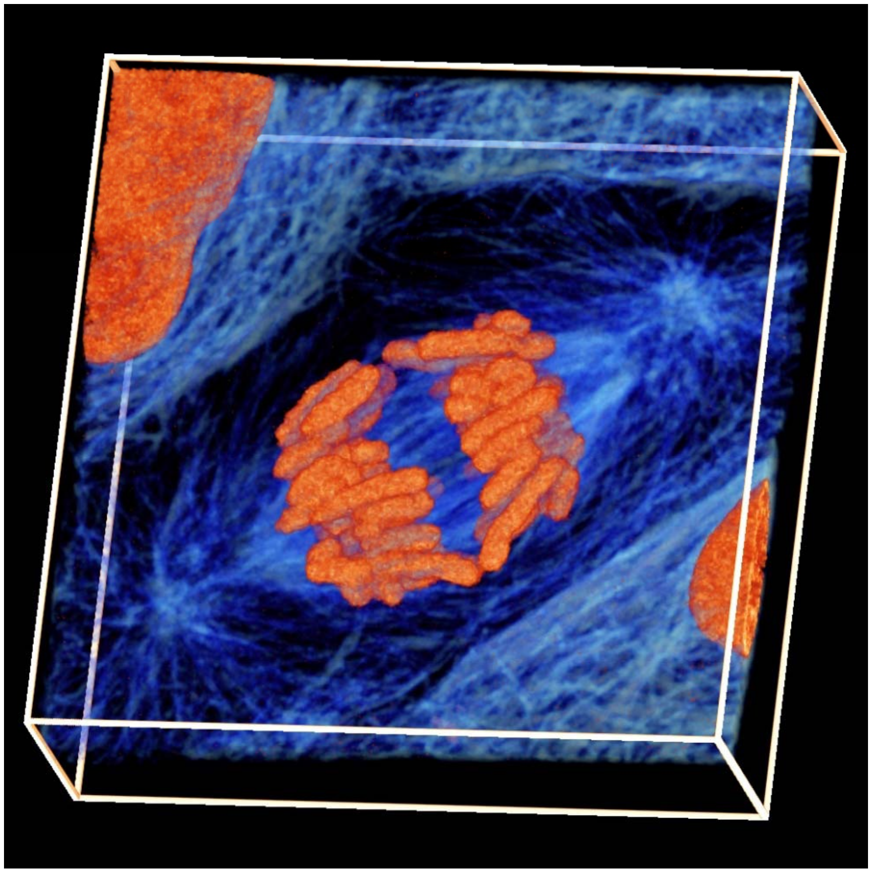


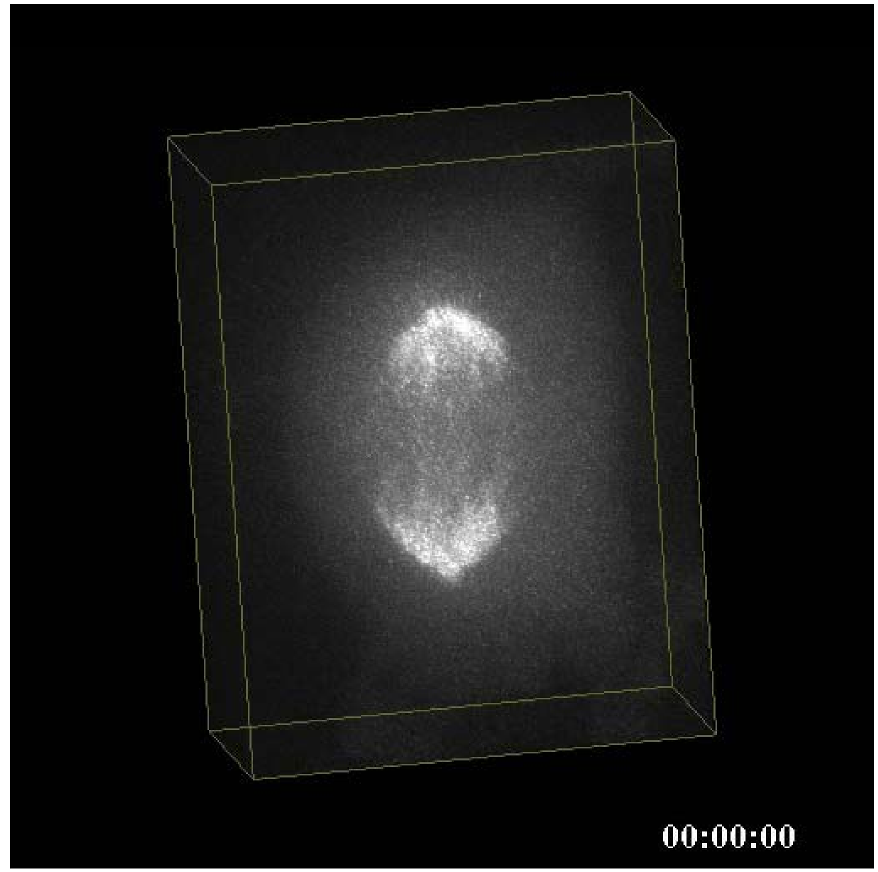
movie

You select a function (e.g. max, mean, etc.) that is used to combine the projection images.

4D Imaging (3D + Time)

You can collect and display sequences of 3D images, which is useful for developmental studies

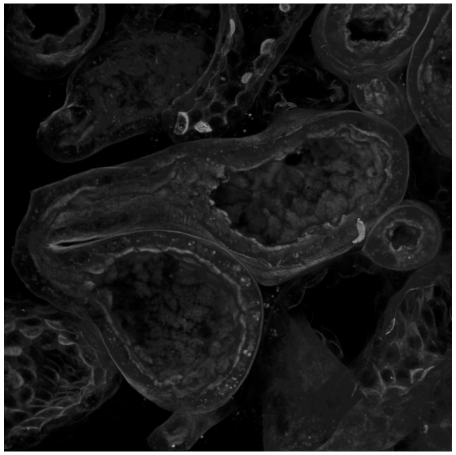




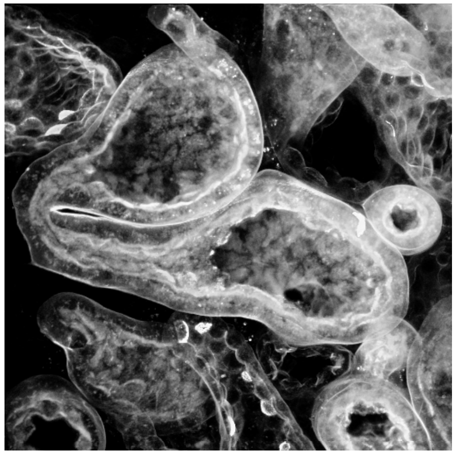
e.g. dividing cell (chromosomes and mitotic spindle)

Volume Rendering: Setup

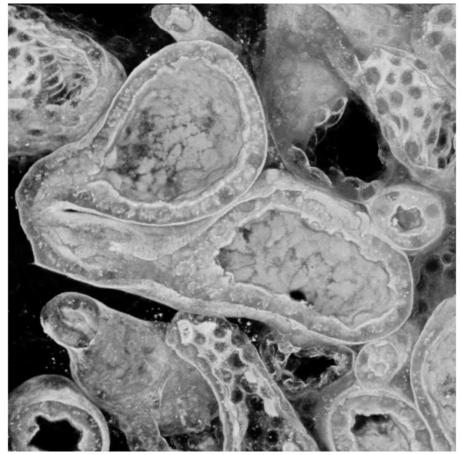
Volume rendering programs must be carefully configured to produce a high-quality 3D effect. Here is a typical sequence of operations that needs to be performed:



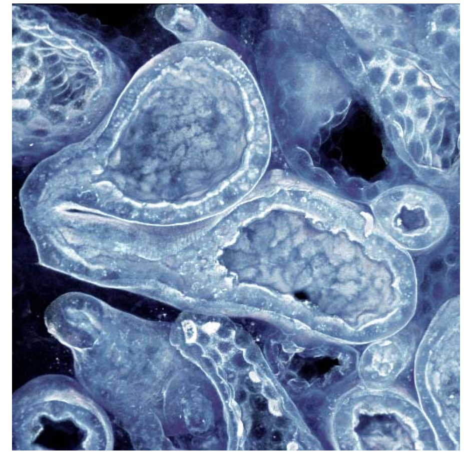
1) **Select blending mode** – alpha, sum, or max.



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



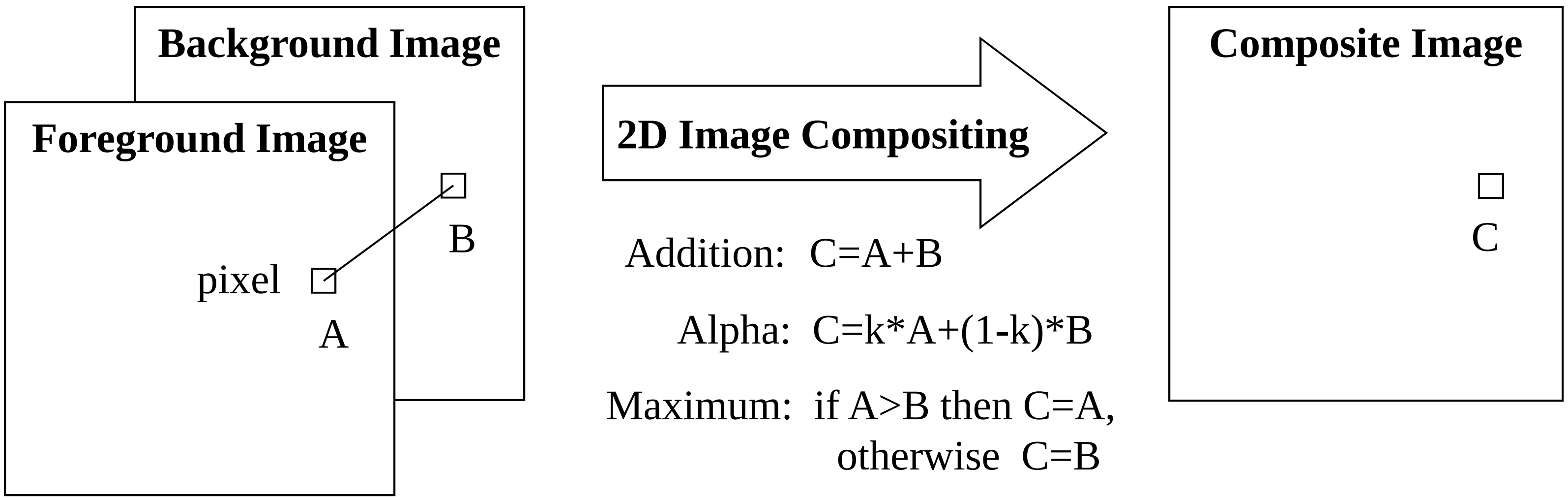
3) **Adjust contrast and brightness** - so that you can see monochrome specimen.

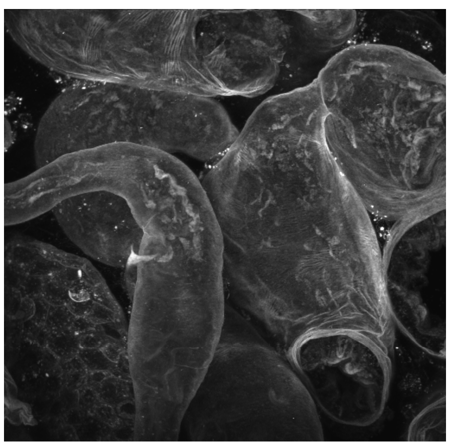


4) **Colorize** – to highlight structures of interest, and/or improve the 3D effect.

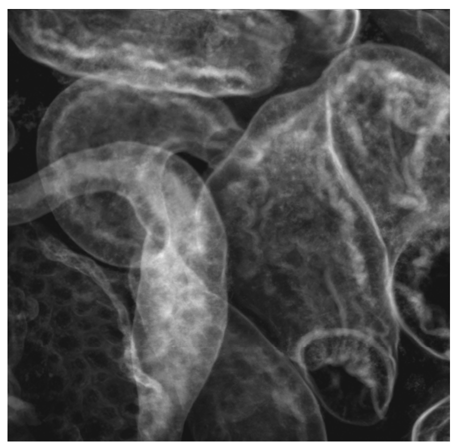
Select Blending Mode (Max, Sum, Alpha)

The 2D projections of cross-sectional images are combined as they are rendered in back-to-front order, forming a 2D composite image that is displayed on the monitor





Maximum



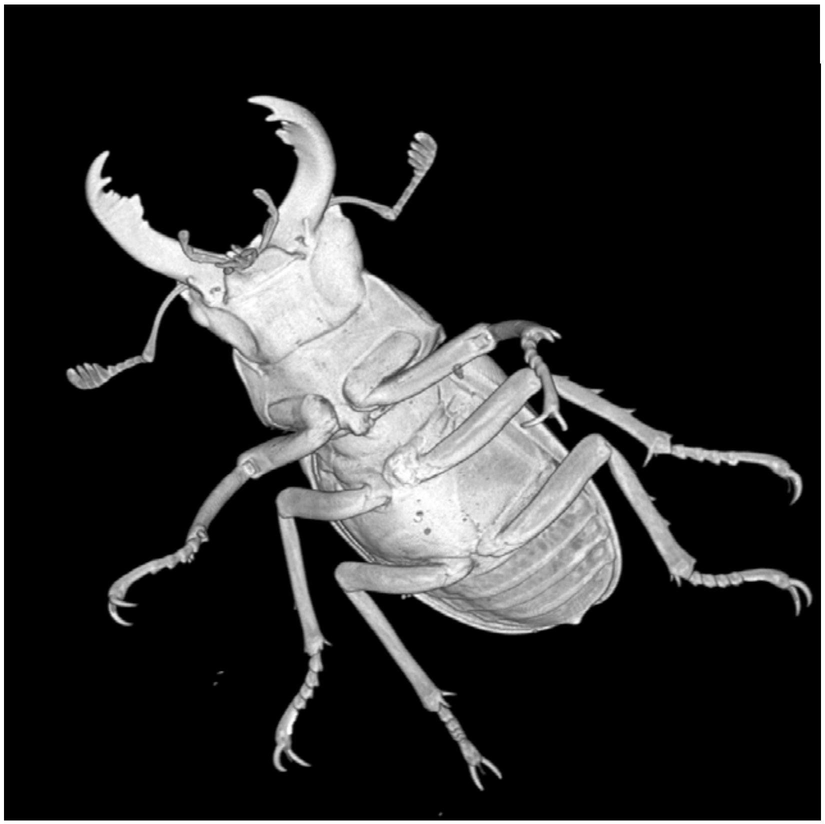
Addition

Alpha blending

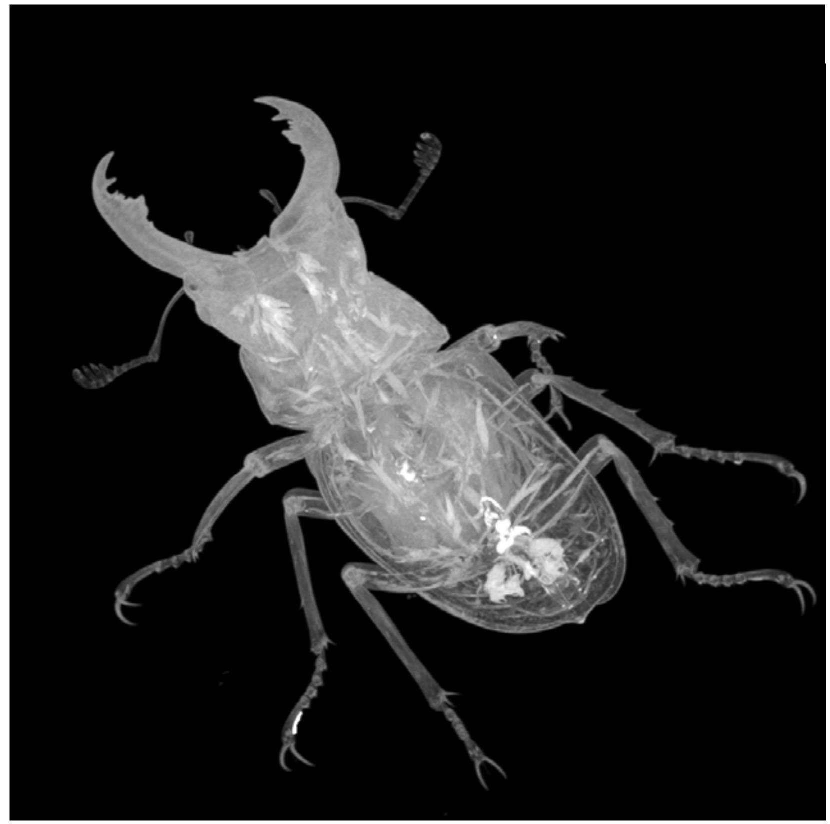
Select Blending Mode

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 of stag beetle



Alpha blending



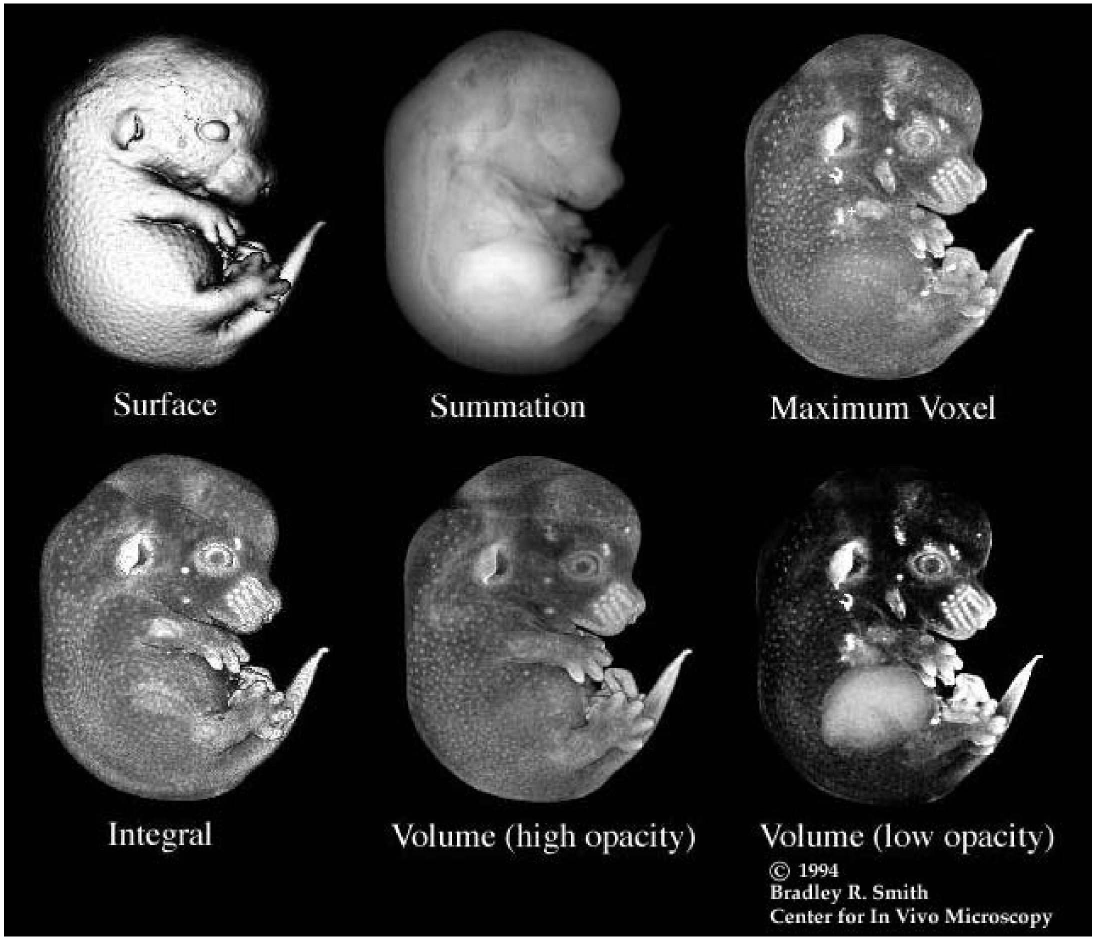
Maximum Intensity Projection

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

Select Blending Mode

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

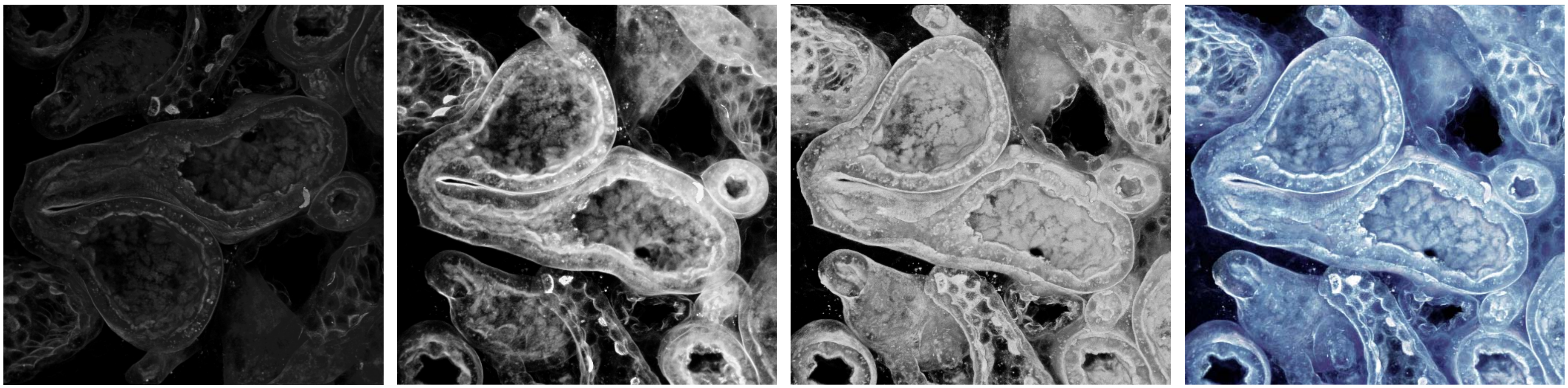
Magnetic Resonance Microscopy



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

Adjust Opacity (only for alpha blending)

Volume rendering programs must be carefully configured 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.

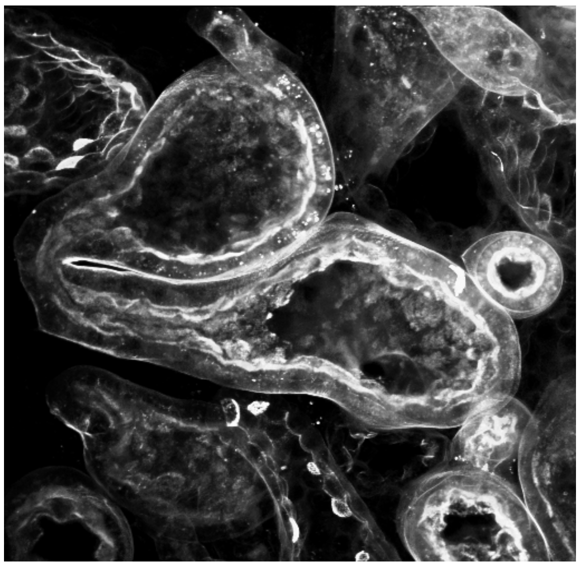
3) **Adjust contrast and brightness -** so that you can see monochrome specimen.

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

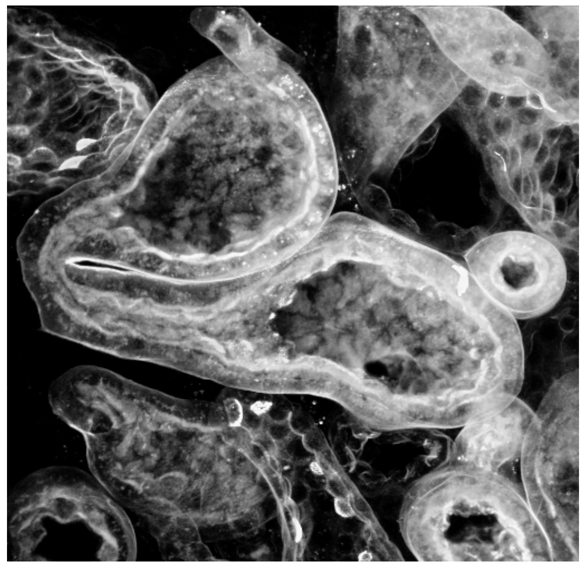
Adjust Opacity

Alpha blending is a weighted averaging procedure used in volume rendering that makes some structures appear more or less opaque

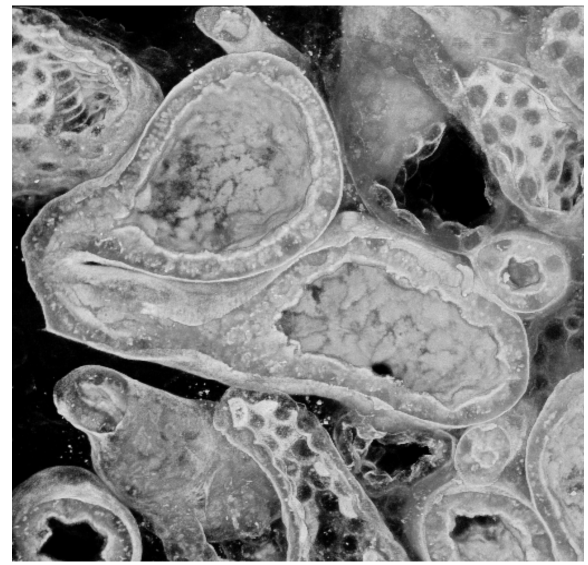
Small Alpha



Medium Alpha



Large Alpha



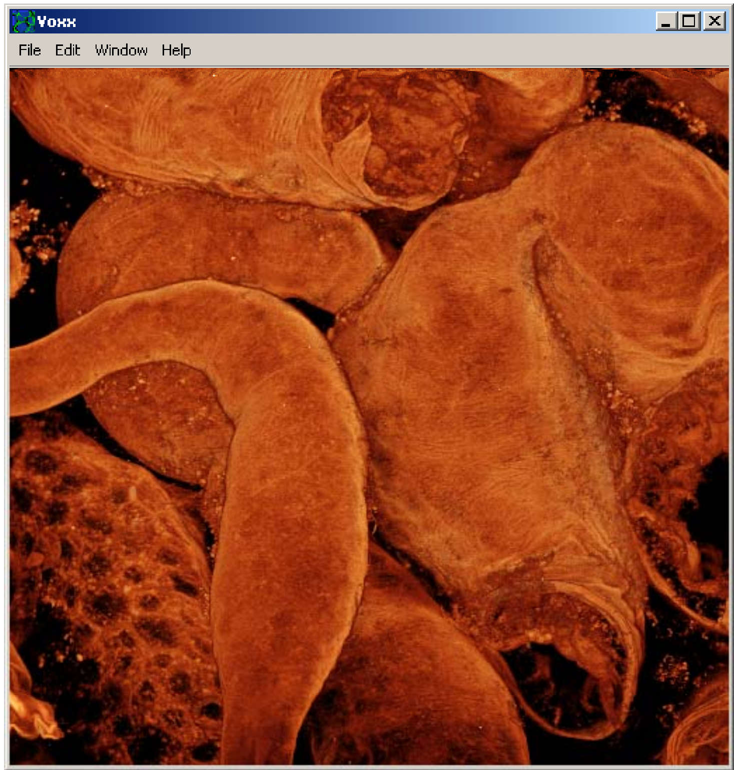
Each pixel in every cross-sectional image has a weight (alpha) associated with it.

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

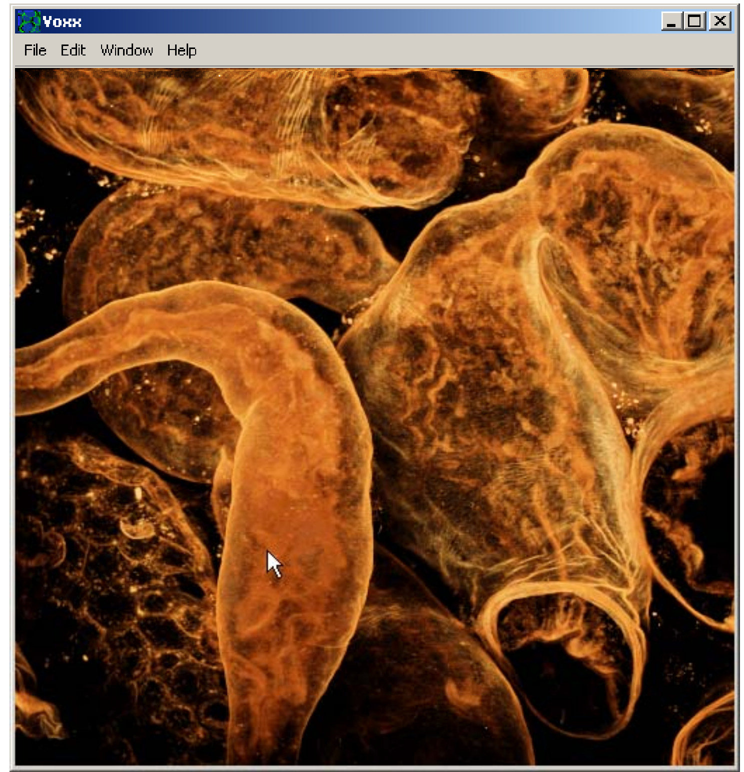
Because images are averaged in back-to-front order, bright foreground structures obscure lower intensity background structures – i.e. brighter objects appear “opaque”.

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)

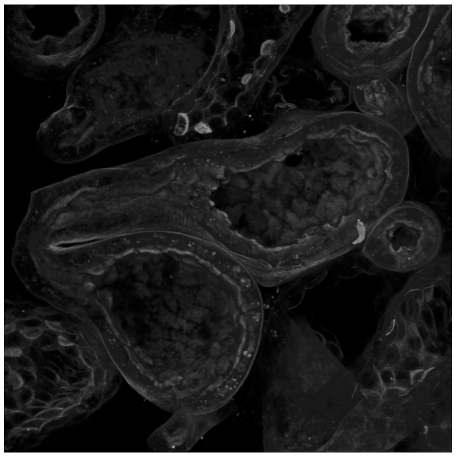


Low Opacity (translucent)

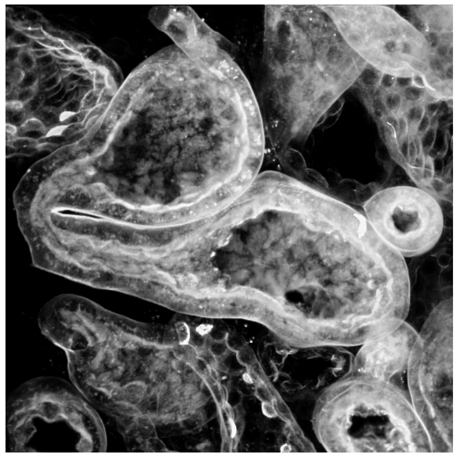
e.g. polycystic tubules in which large alpha values allow us to see details on the outer surface, while using smaller alpha values allow us to see the brush border inside the tubules...

Adjust Brightness and Contrast

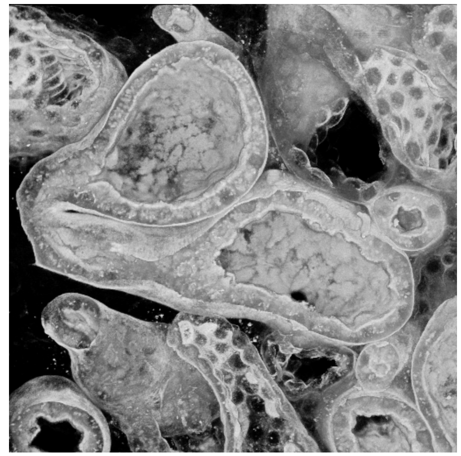
Volume rendering programs must be carefully configured to produce a high-quality 3D effect. Here is a typical sequence of operations that needs to be performed:



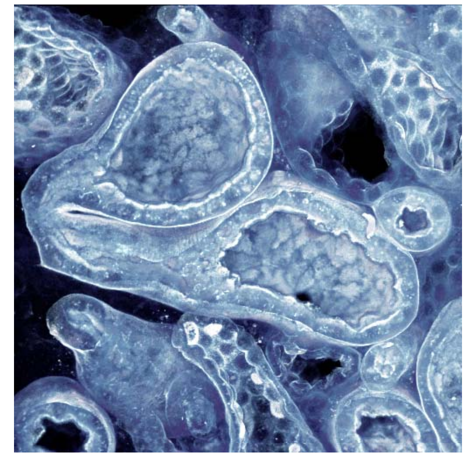
1) **Select blending mode** – alpha, sum, or max.



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



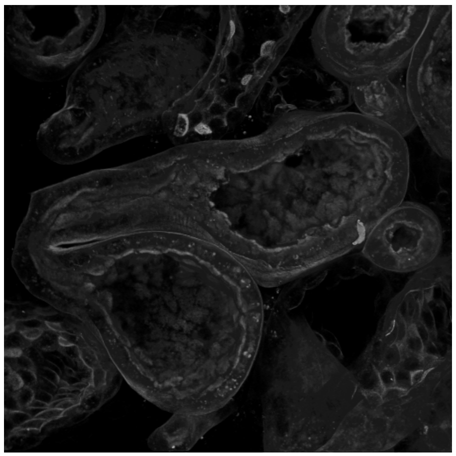
3) **Adjust contrast and brightness -** so that you can see monochrome specimen.



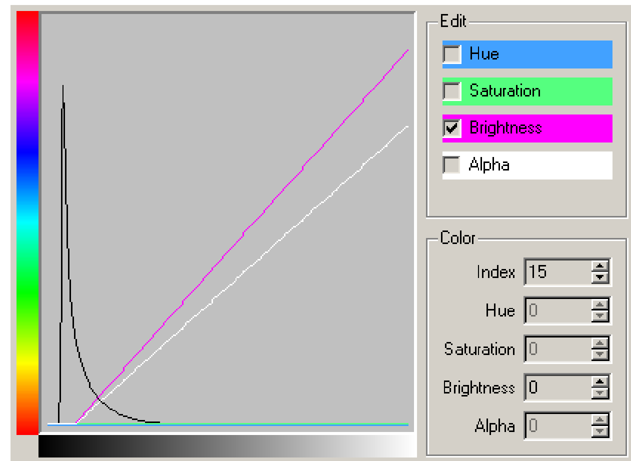
4) **Colorize –** to highlight structures of interest, and/or improve the 3D effect.

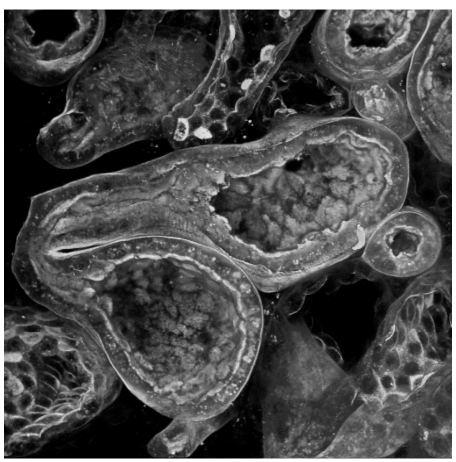
Adjust Brightness and Contrast

Example of linear transfer functions. Increasing the slope of brightness curve from 1 to 3 increases the brightness and contrast of all pixel values. Also note that x-axis intercept was shifted slightly to the right of the background peak

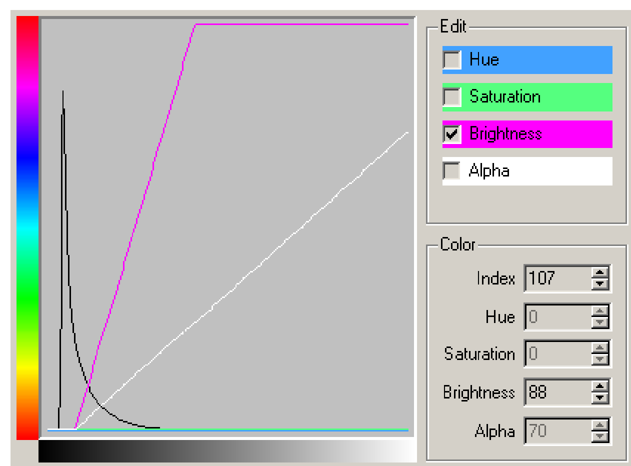


Slope=1



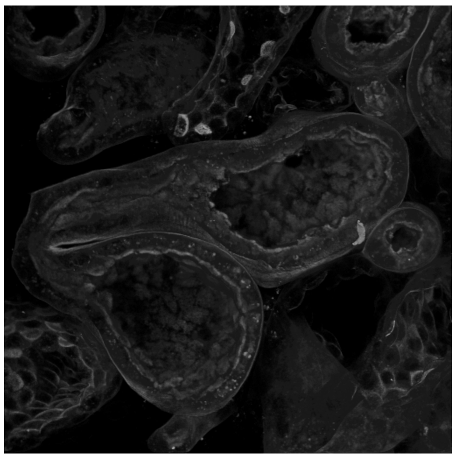


Slope=3

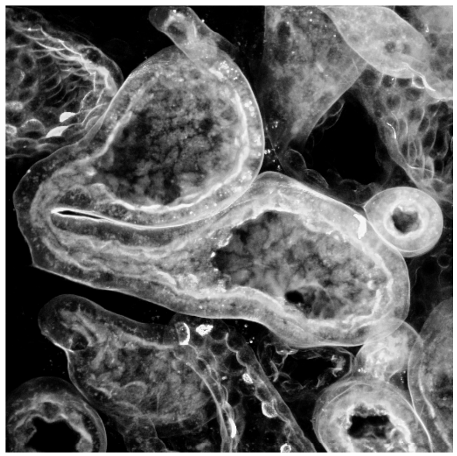


Adjust Color

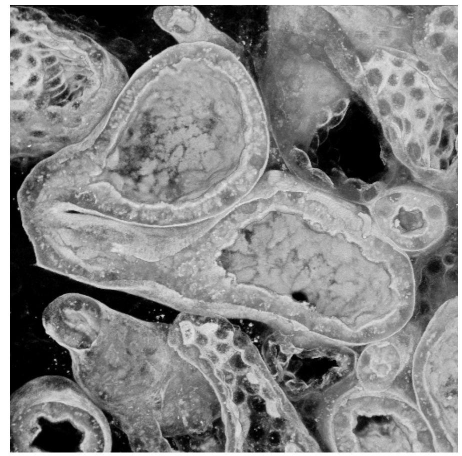
Volume rendering programs must be carefully configured to produce a high-quality 3D effect. Here is a typical sequence of operations that needs to be performed:



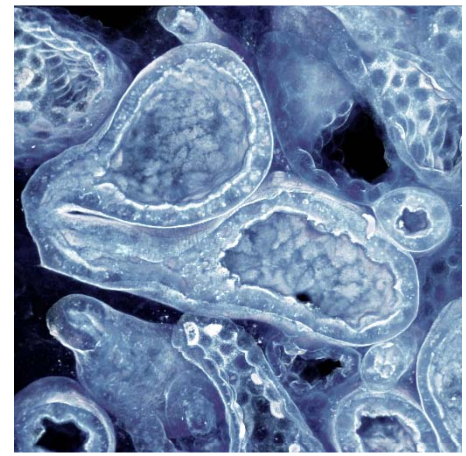
1) **Select blending mode** – alpha, sum, or max.



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



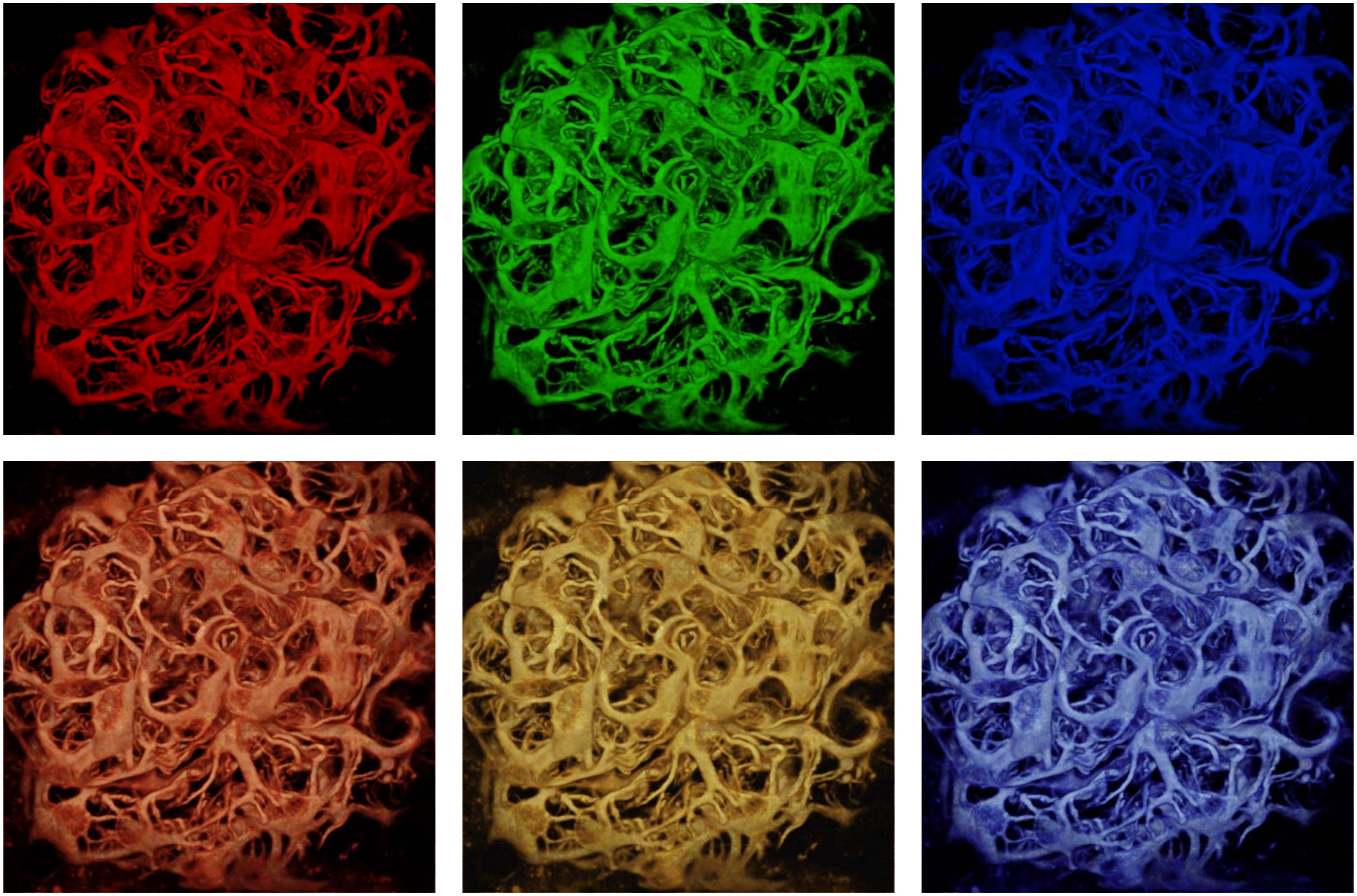
3) **Adjust contrast and brightness -** so that you can see monochrome specimen.



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

Colorization

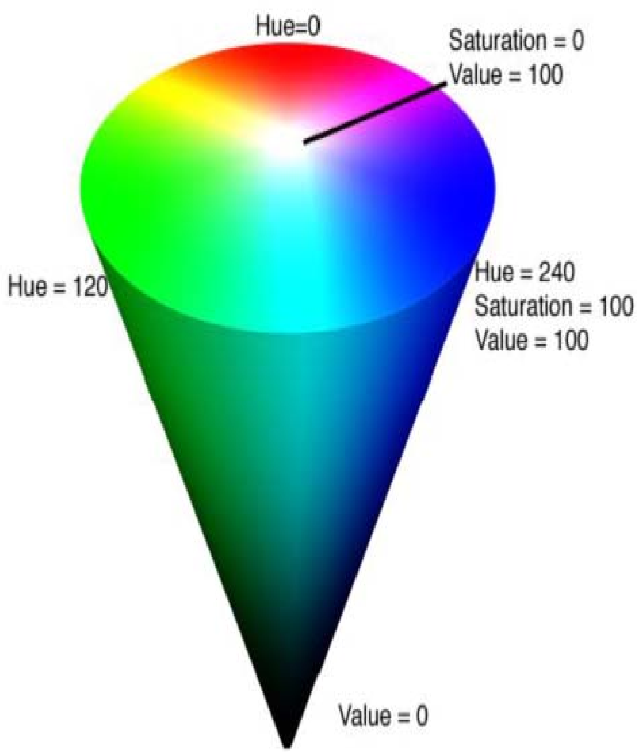
You need to assign each channel a different color when displaying multi-channel images



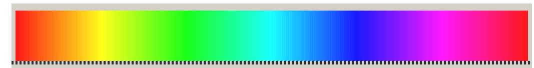
You must choose appropriate colors for viewing images on monitors and color prints

Colorization: Adjust Hue and/or Saturation

The RGB representation of color used in monitors and video boards is not easy for people to work with. So other color representations have been developed.



Hue



determines which spectral color is used. It is often specified as an angle between 0 and 360 degrees, but some programs use 0-1 or 0-255 scales. On a 0-1 scale, red=0, green=120/360, blue=240/360, etc.

Saturation

determines how much white is added to produce the color. S=0 for a totally desaturated color (i.e. no color), S=1 for a pure color (i.e. no white).

Value

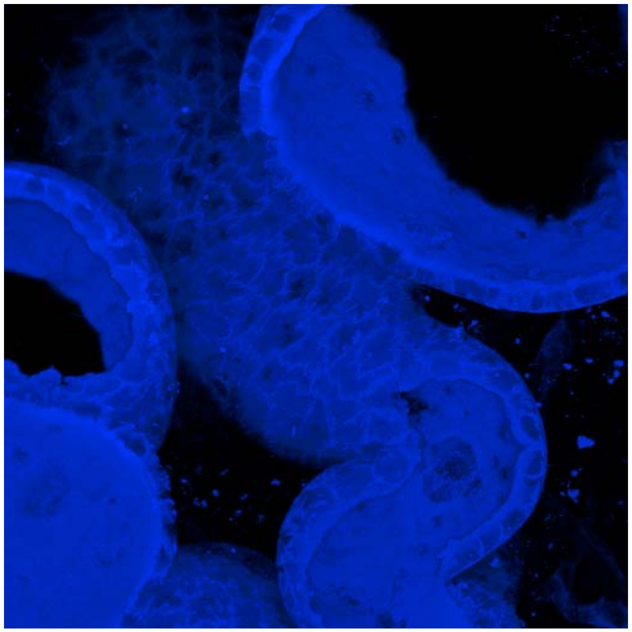
determines the brightness of the color. V varies between 0 (black) and 1.

Hue, Saturation, and Value (HSV) is commonly used instead of RGB, then converted into RGB by the imaging programs.

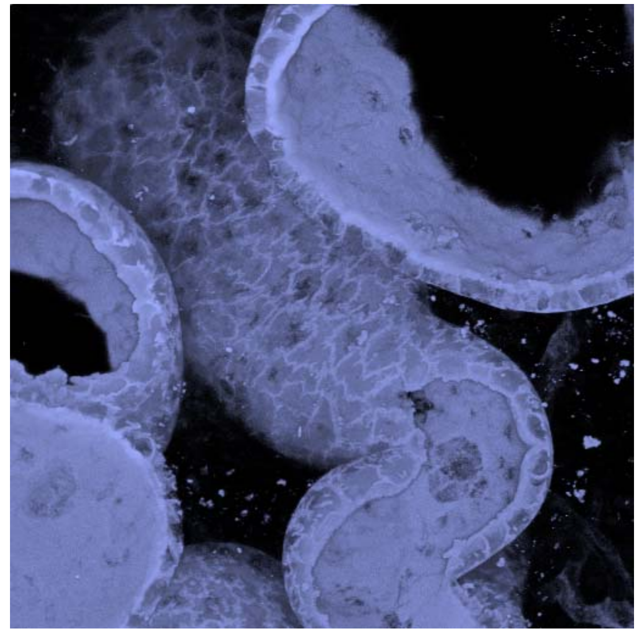
Colorization: Adjust Hue and/or Saturation

The simplest color choice: pick constant hue and saturation,
independent of the intensity of the image

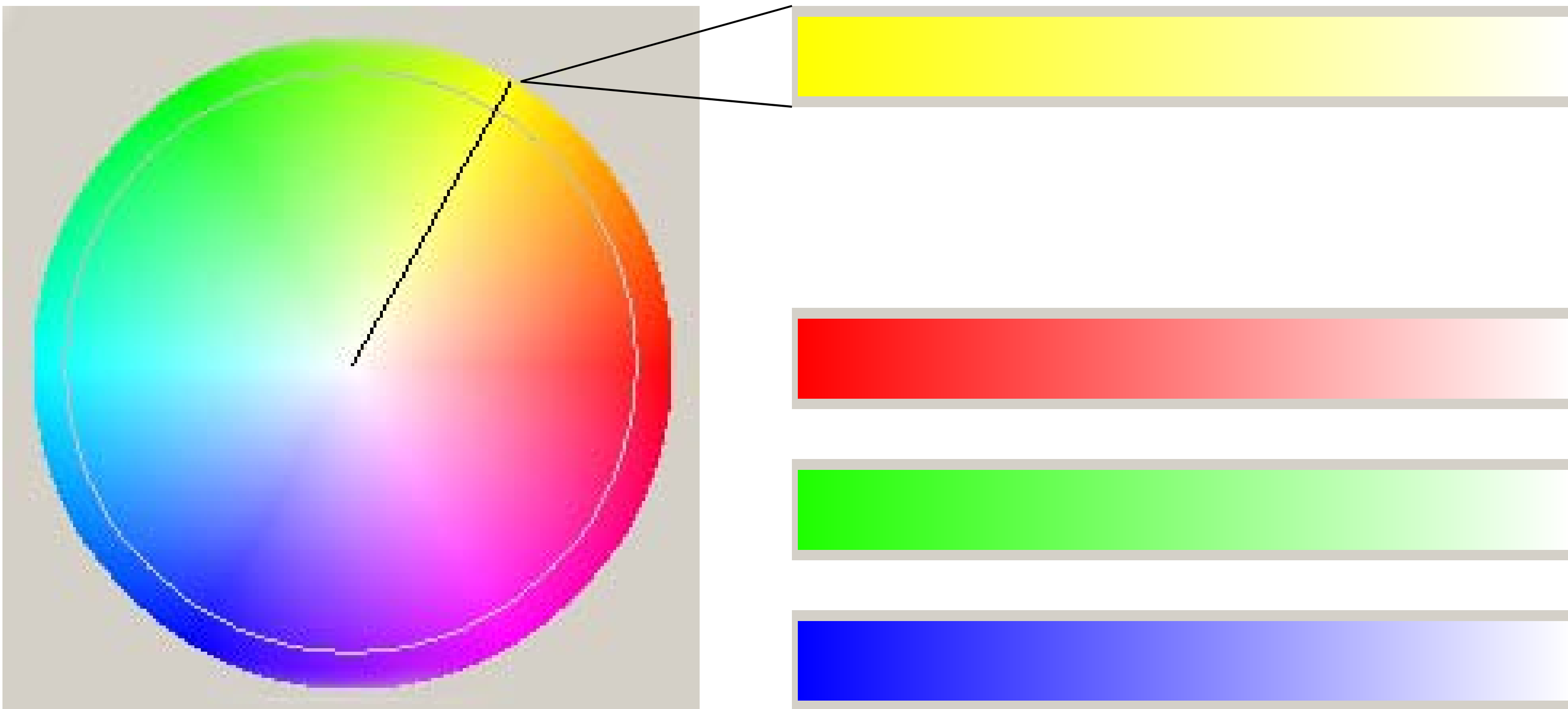
Constant Hue



Constant Saturation



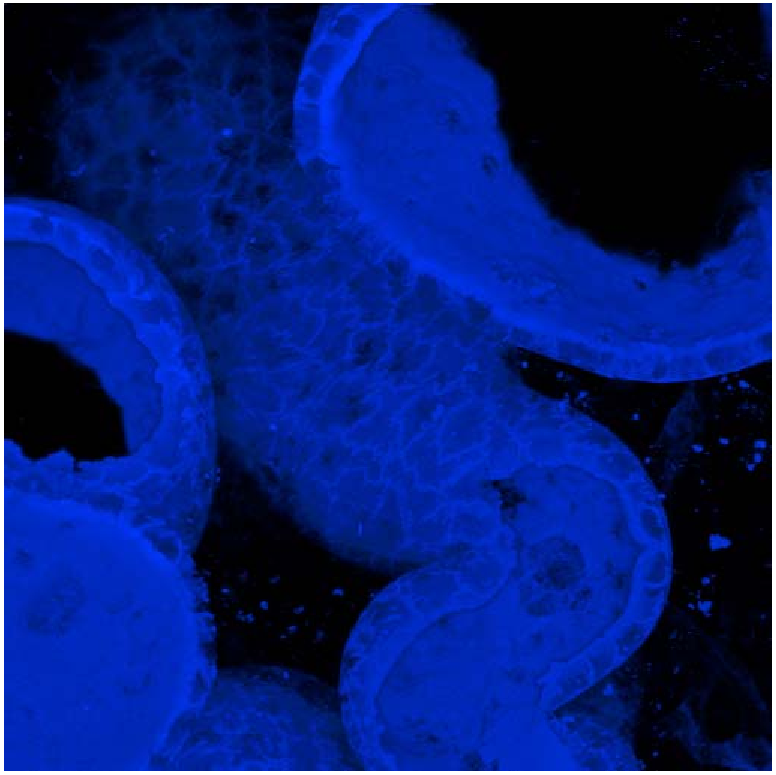
Colorization: Make Saturation a Function of Intensity



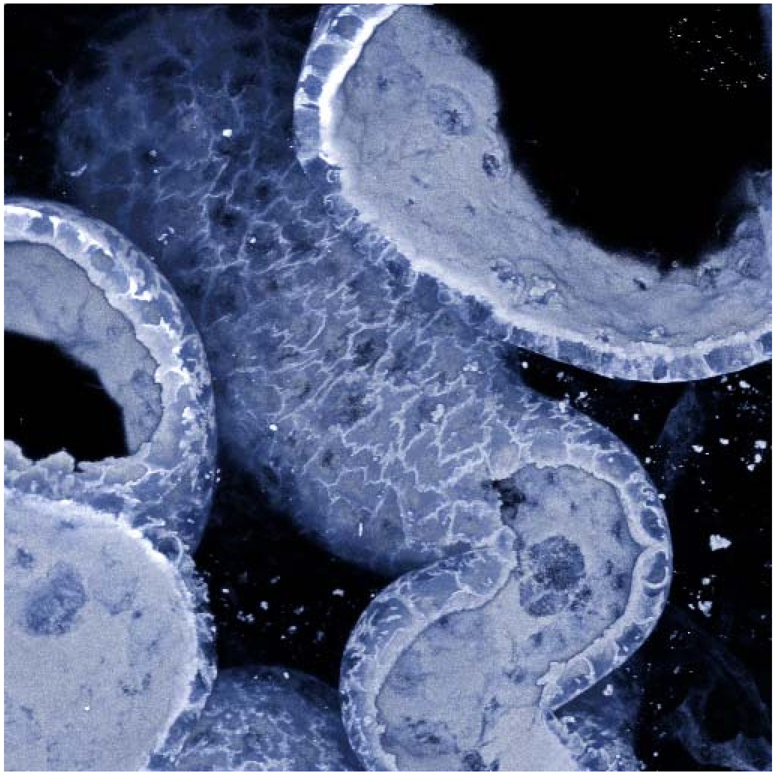
Color scales can provide an additional (weak) depth cue. Darker saturated colors are associated with more distant objects, while lighter less-saturated colors are associated with nearer objects .

Colorization: Varying the Saturation

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

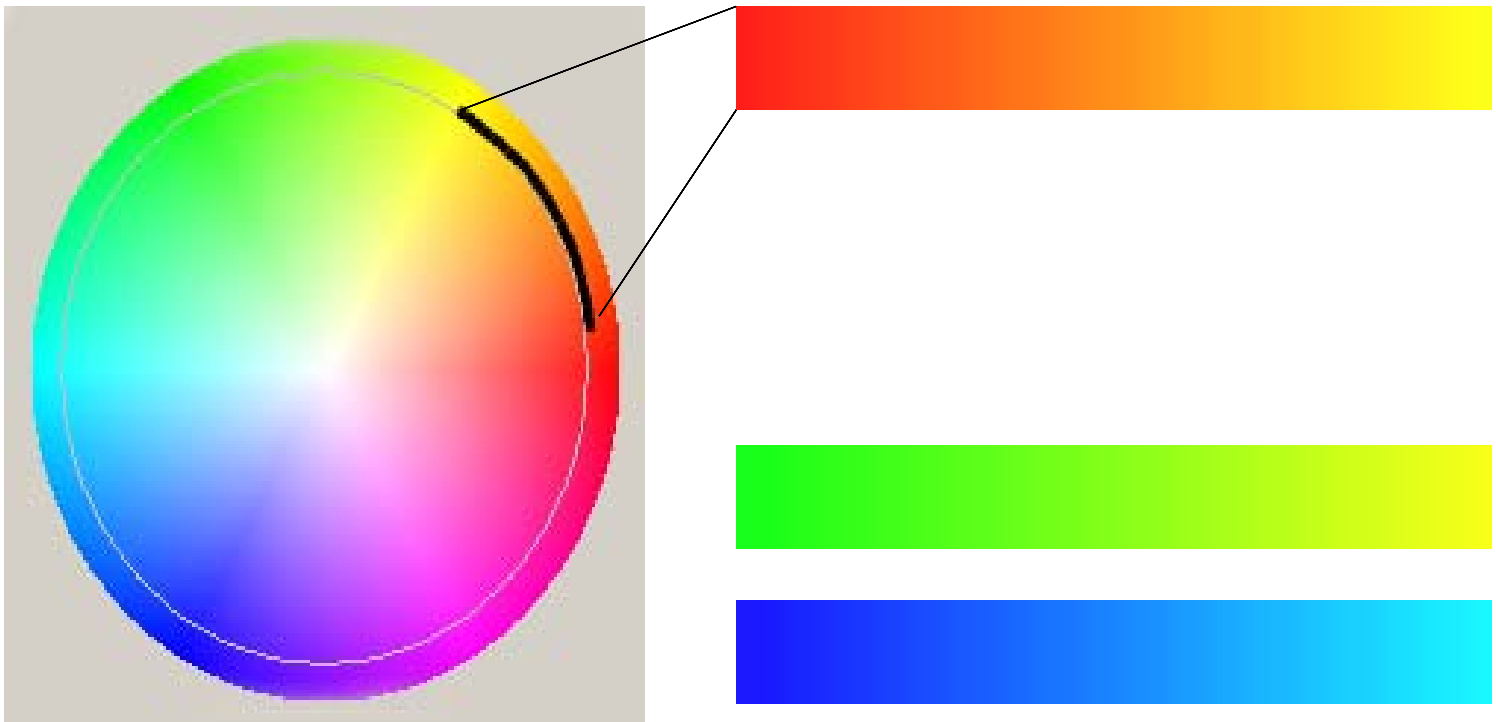


Both hue and saturation are constant



Hue is constant, but saturation varies

Colorization: Make Hue a Function of Intensity

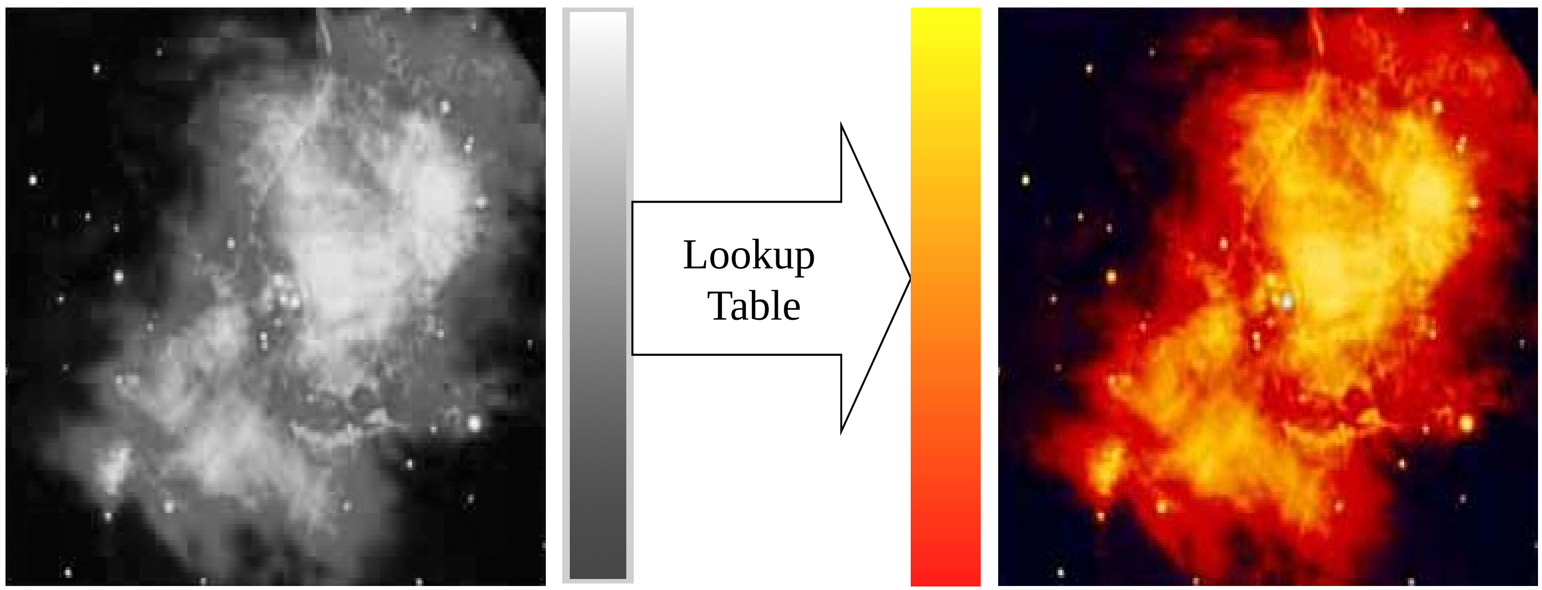


Color scales can provide an additional (weak) depth cue. Darker saturated colors are associated with more distant objects, while lighter less-saturated colors are associated with nearer objects .

Hue-Based Color Scales

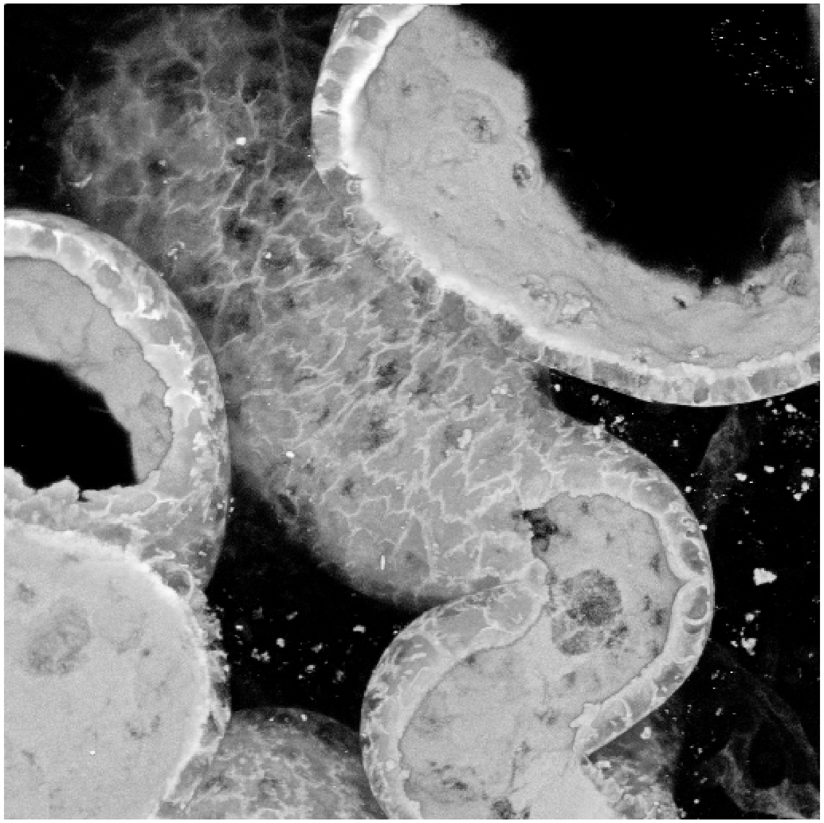
Simultaneously changing the hue and brightness in color scales can produce a better intensity-to-color mapping. Commonly done outside of fluorescence microscopy.

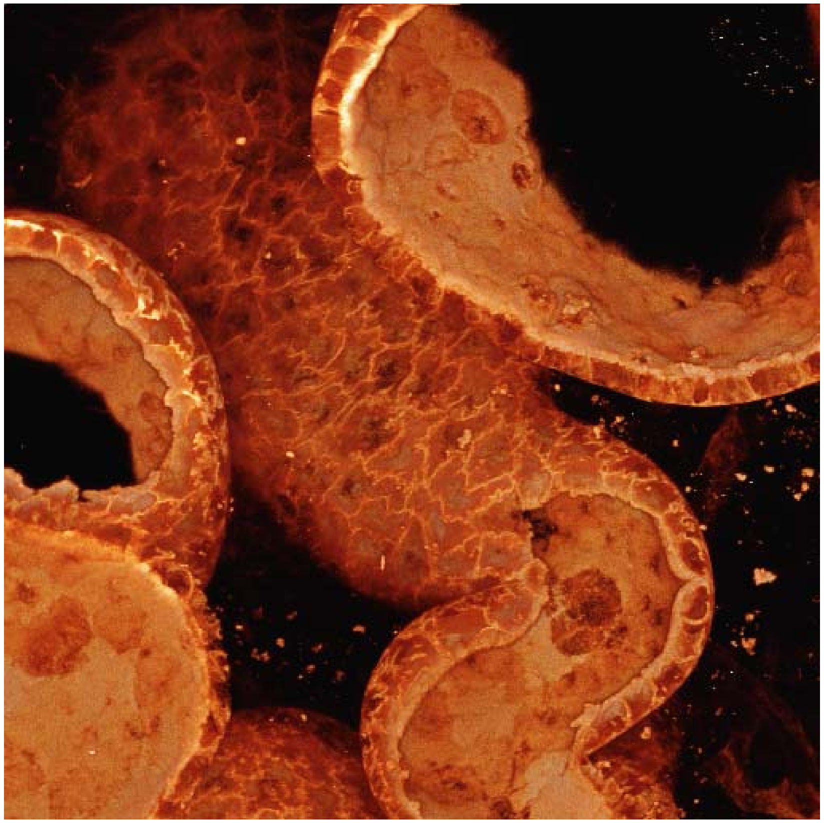
Crab Nebula (infrared) **Colorized Crab Nebula**



Modulate Hue and Saturation

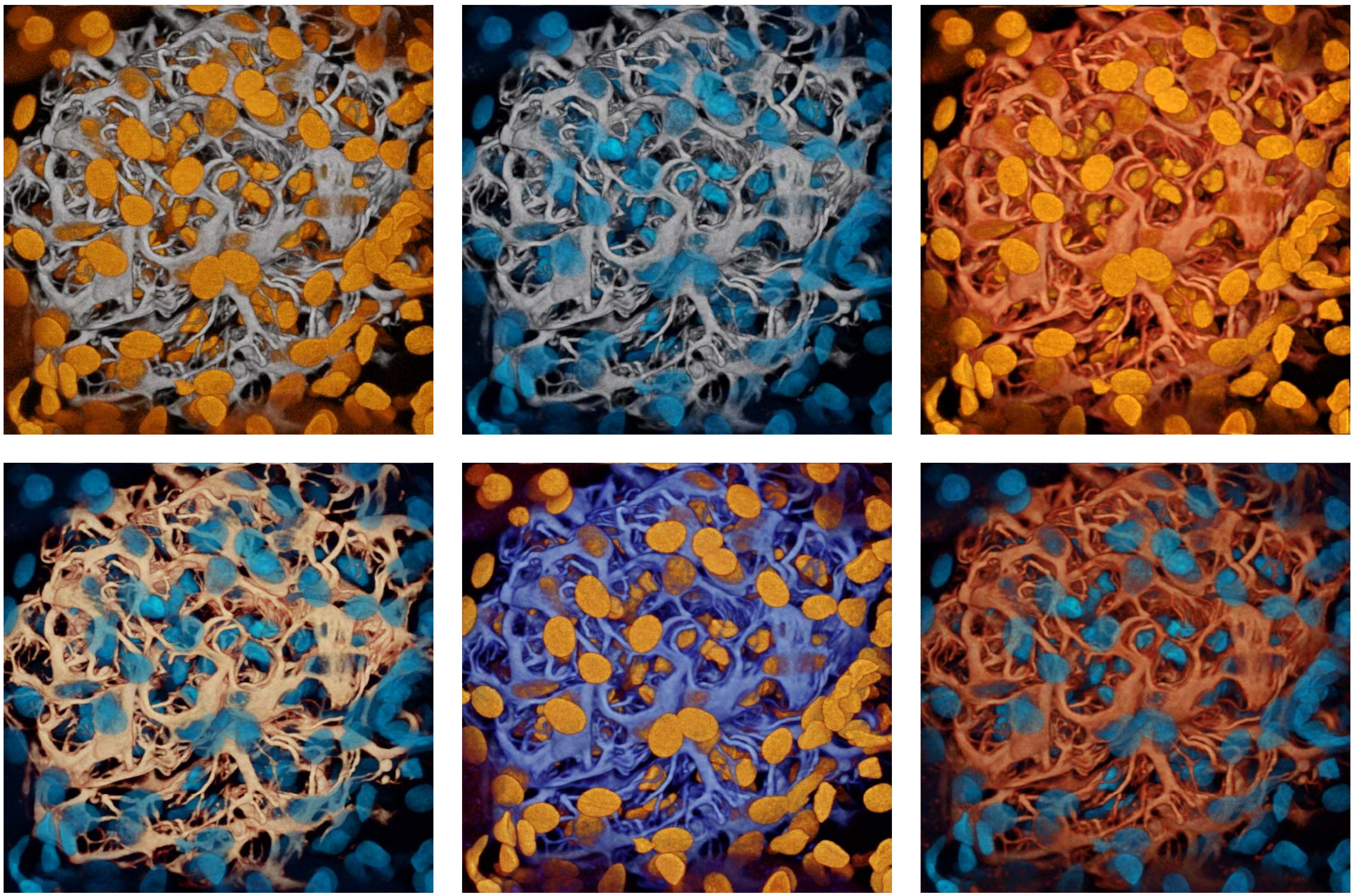
Examples of simultaneously changing hue and saturation as a function of brightness, to improve the visibility of structural details and to enhance the 3D effect





Colorization: Select Complimentary Colors

One should choose complementary colors when colorizing different structures in images – to improve their visibility, and for aesthetic reasons



Colorization: Select Complimentary Colors

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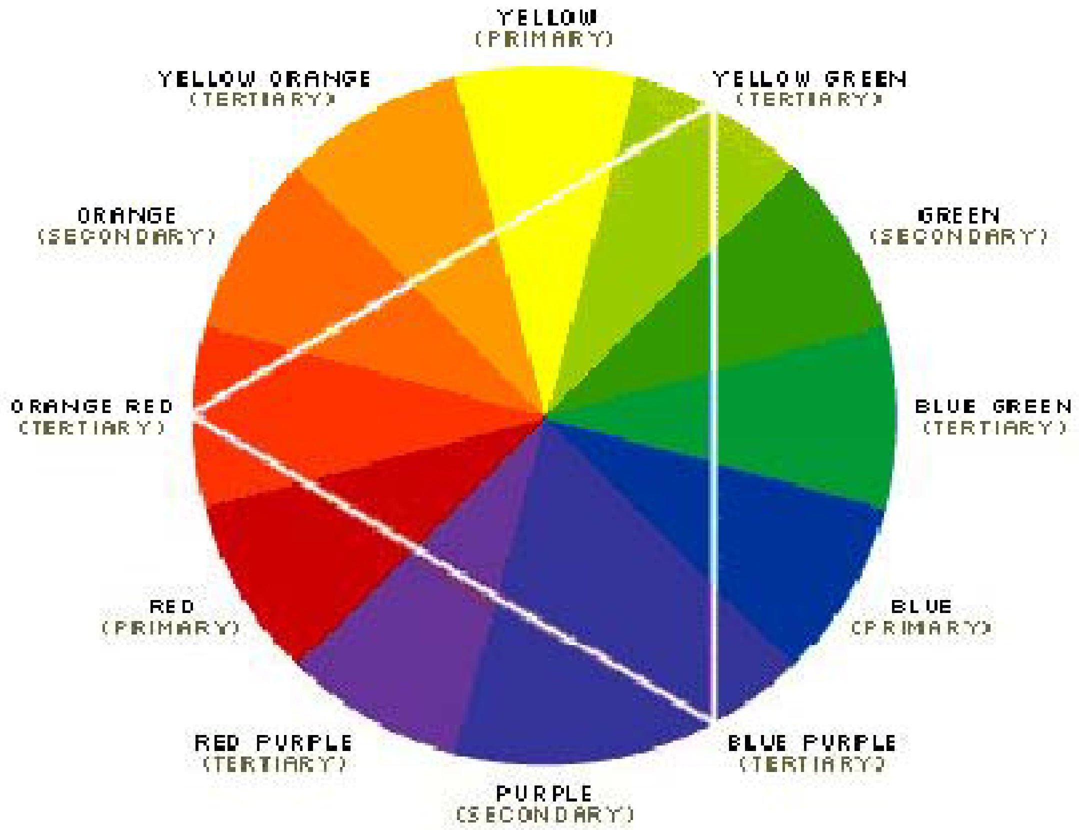
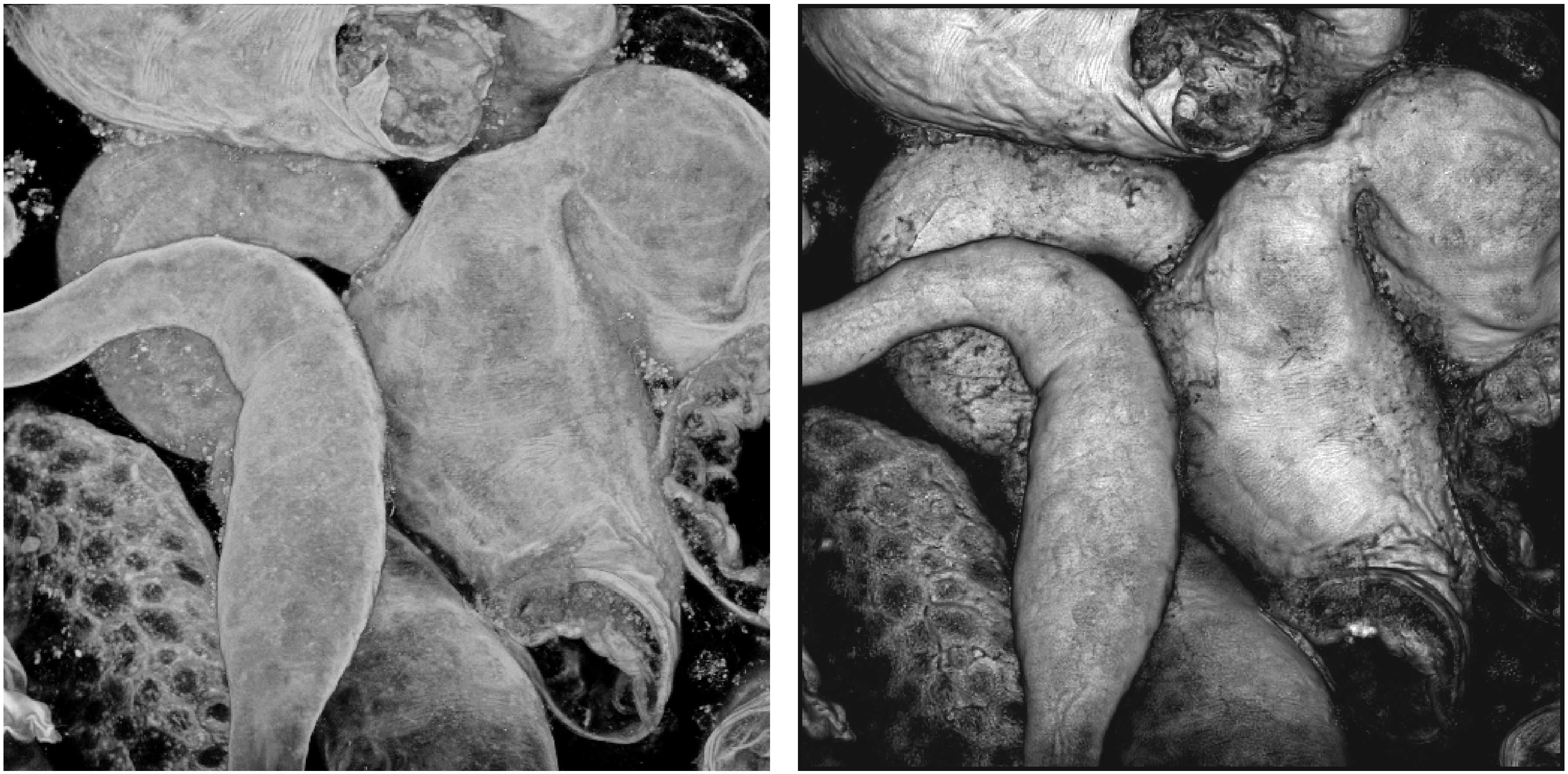
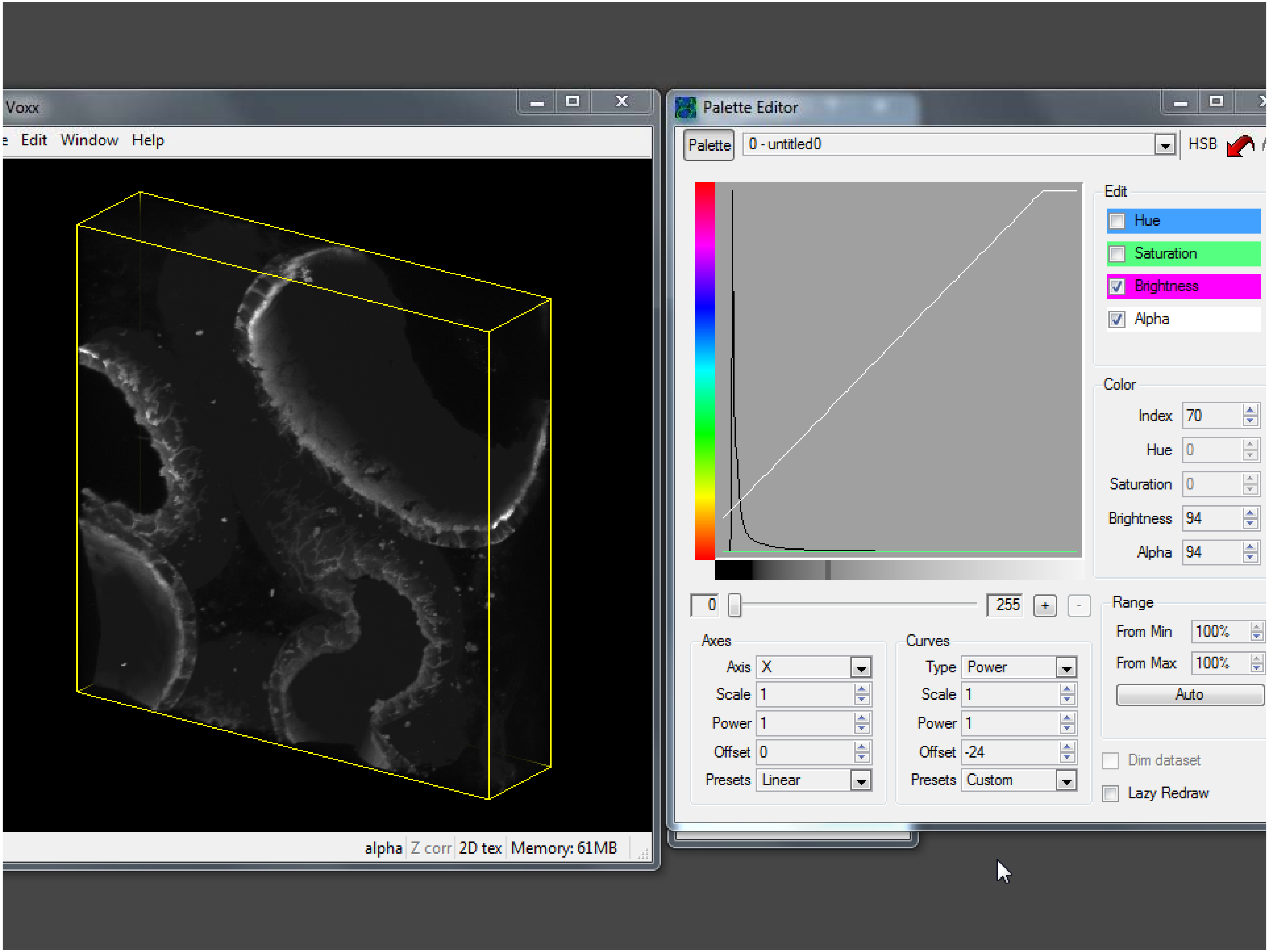


Lighting

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



Unlit alpha-blended image of cystic proximal tubules in kidney (left), and simulated illumination using Lambertian model (right).



Voxx
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100%
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Axes
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Power
1
Offset
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Presets
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Curves
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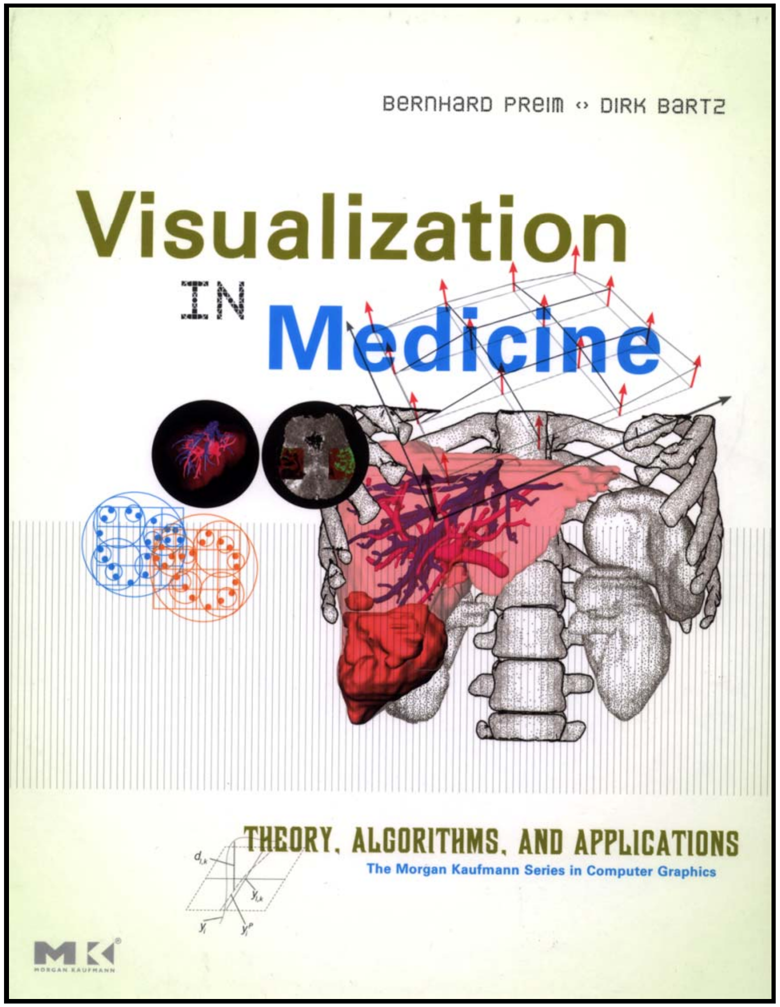
Scale
1
Power
1
Offset
-24
Presets
Custom
Dim dataset
Lazy Redraw

Are there any good books on this stuff ?



B. Preim, D. Bartz
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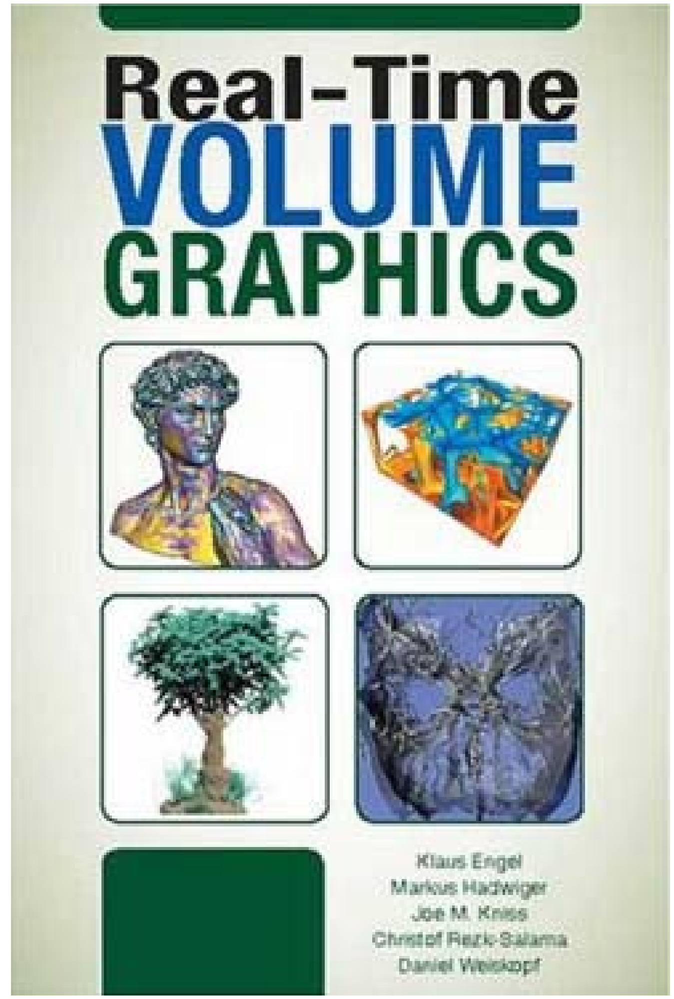

K. Engel, M. Hadwiger, et al
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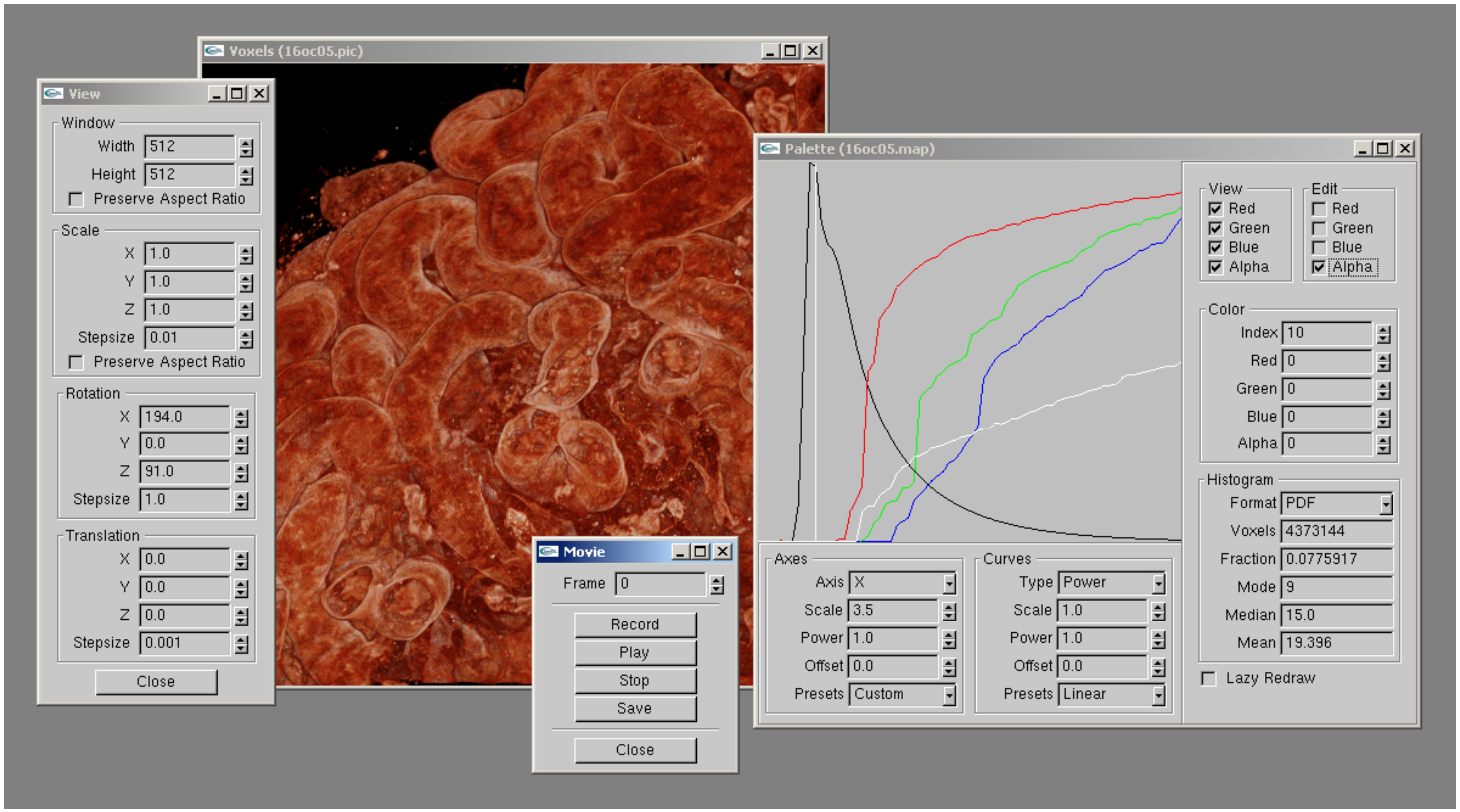
So what kind of microscopy-oriented 3D imaging software is available ?

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. Here are a few:

Free	**BioImageXD**	www.bioimagexd.net
	Endrov	www.endrov.net
	ImageJ	http://rsb.info.nih.gov/ij
	VisBio	www.loci.wisc.edu/visbio
	Voxx	www.nephrology.iupui.edu/imaging/voxx
	V3D	http://penglab.janelia.org/proj/v3d
Commercial	**Amira**	www.amiravis.com
	AutoQuant X	www.mediacy.com
	Huygens	www.svi.nl
	Image-Pro Plus	www.mediacy.com
	Imaris	www.bitplane.com
	Metamorph	www.moleculardevices.com
	Volocity	www.improvision.com/products/volocity

Voxx

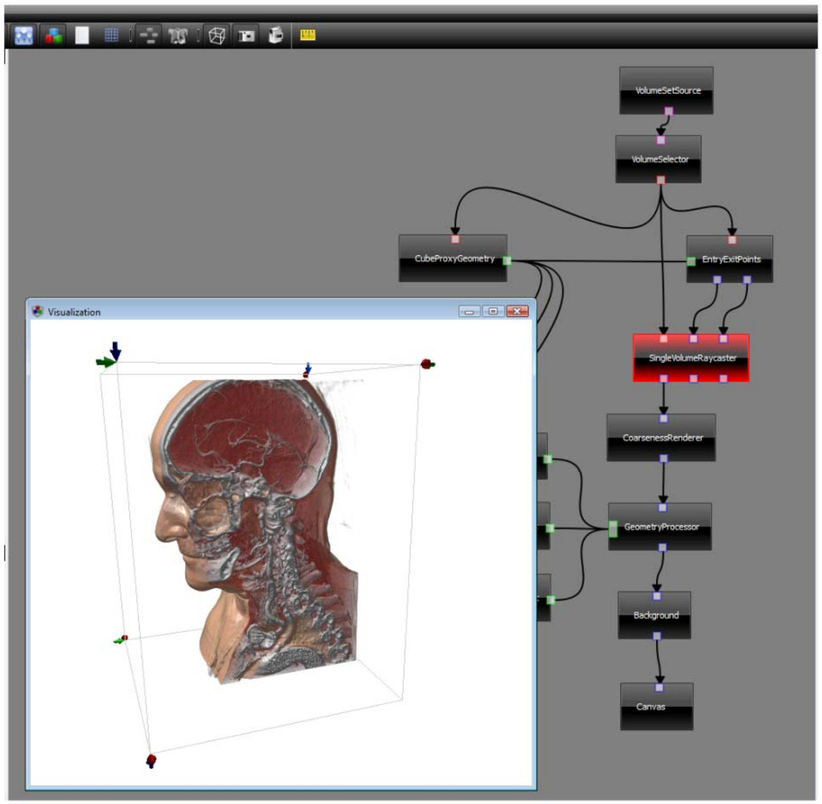
If any of you want to experiment with a volume renderer using confocal microscope images - you could download Voxx, along with sample data sets…



http://nephrology.iupui.edu/imaging/voxx
http://nephrology.iupui.edu/imaging/voxx/sample_data

Voreen and Slicer

If any of you want to experiment with a volume renderer using CT or MRI images - you can download Voreen or 3D Slicer, along with sample data sets…



www.slicer.org

www.voreen.org
www.voreen.org/108-Data-Sets.html

And what kind of hardware do I need ?

In the early days of high-performance 3D imaging (1990s), volume rendering programs had to be run on very expensive Silicon Graphics workstations if you wanted real-time rendering. Typical prices for SGI's systems ranged from $30K to $200K+.

Now, any PC with a low-cost ($100-400) video board equipped with a GeForce or Radeon graphics processor can do real-time 3D rendering.

Questions?