



Deconvolution of Microscopy Images

Issues to Consider for Widefield and Confocal Microscopy

from images to ANSWERS[®]

If oversubscribed please email jjirroi@mediacy.com to reschedule.

Webex support: 408-435-7088
866-229-3239

Presented by:

Jonathan Girrior
3D Applications Manager
Media Cybernetics

Overview

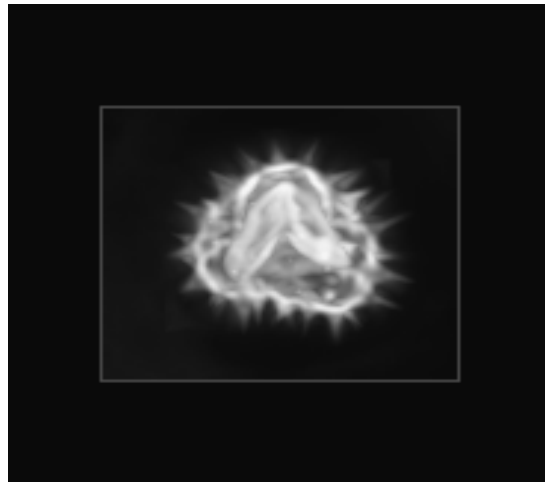


- Image Formation
- Types of Image Restoration
- Blind vs. Non-blind Deconvolution
- Spherical Aberration
- Resources

Image Formation



The object

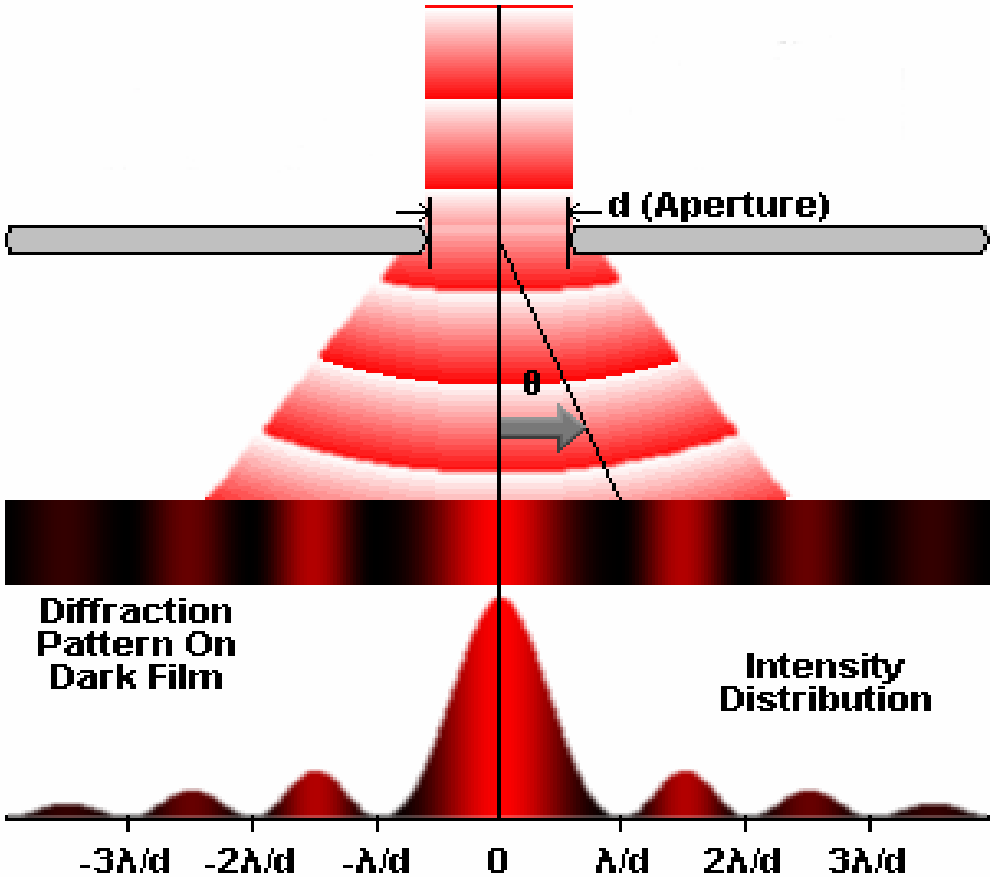


The image

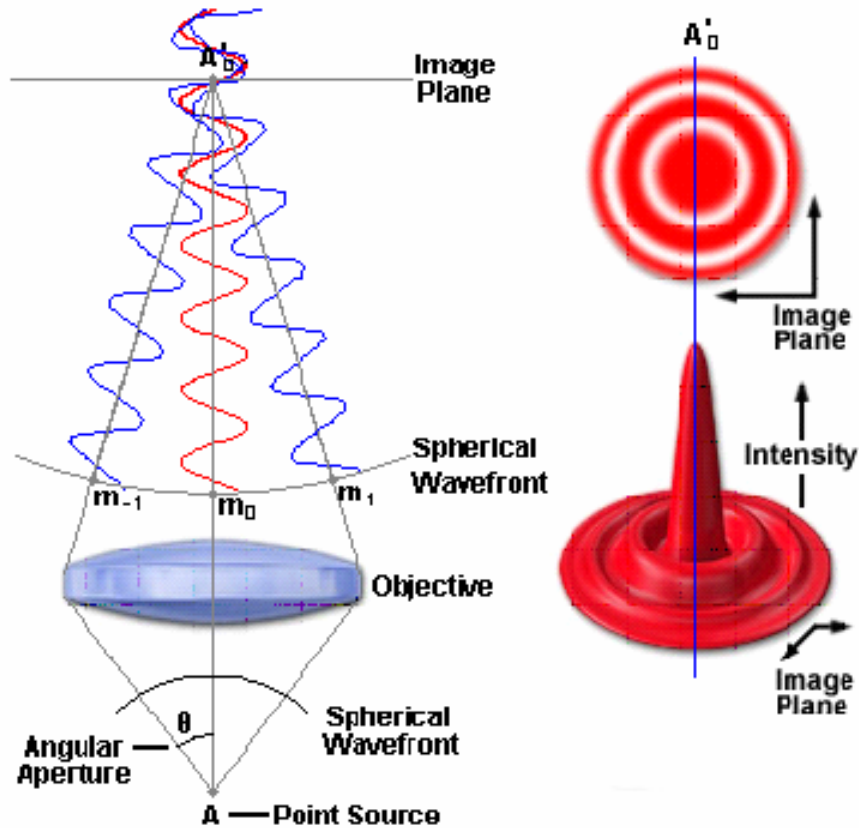


What happened?

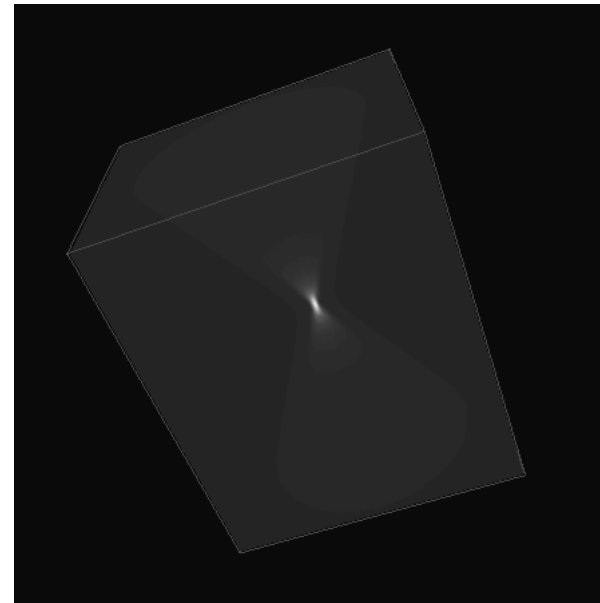
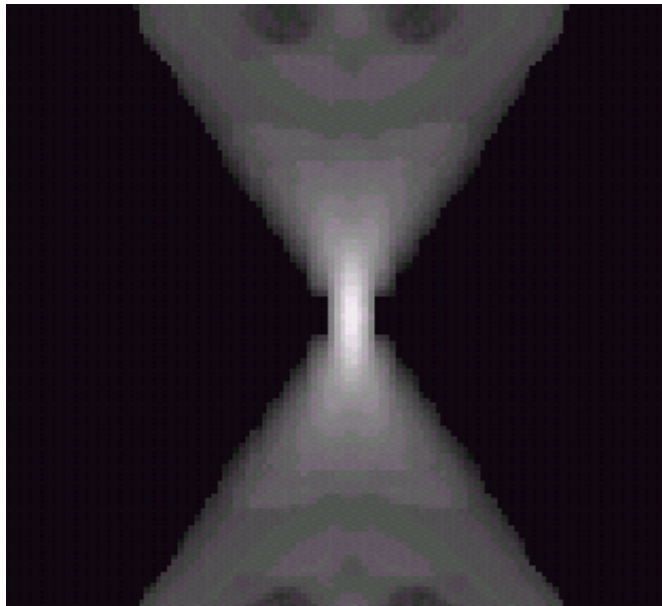
One Dimension - Diffraction



Two Dimensions - Airy Disk



Three Dimensions – Point Spread Function (PSF)

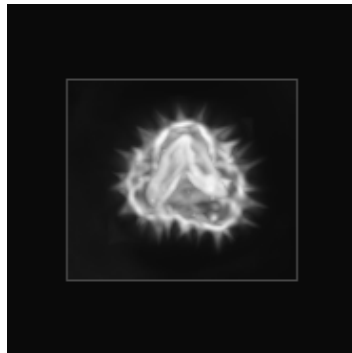


- An impulse response.
- Characterizes your microscope.

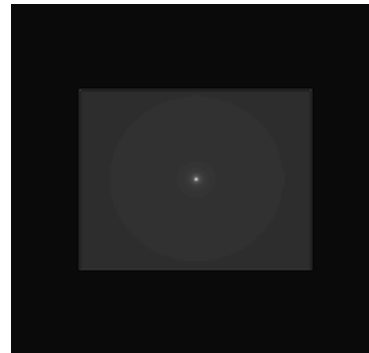
Effects of the PSF



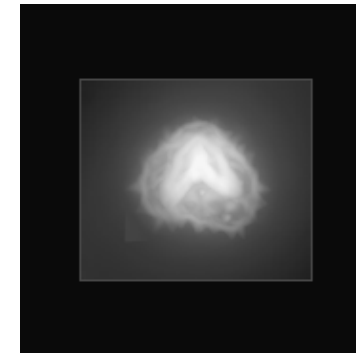
Object



PSF



Image



- An object is a collection of point sources.
- Mathematical operation called a convolution.
- The Microscope is a convolution operator.

Qualitative vs. Quantitative

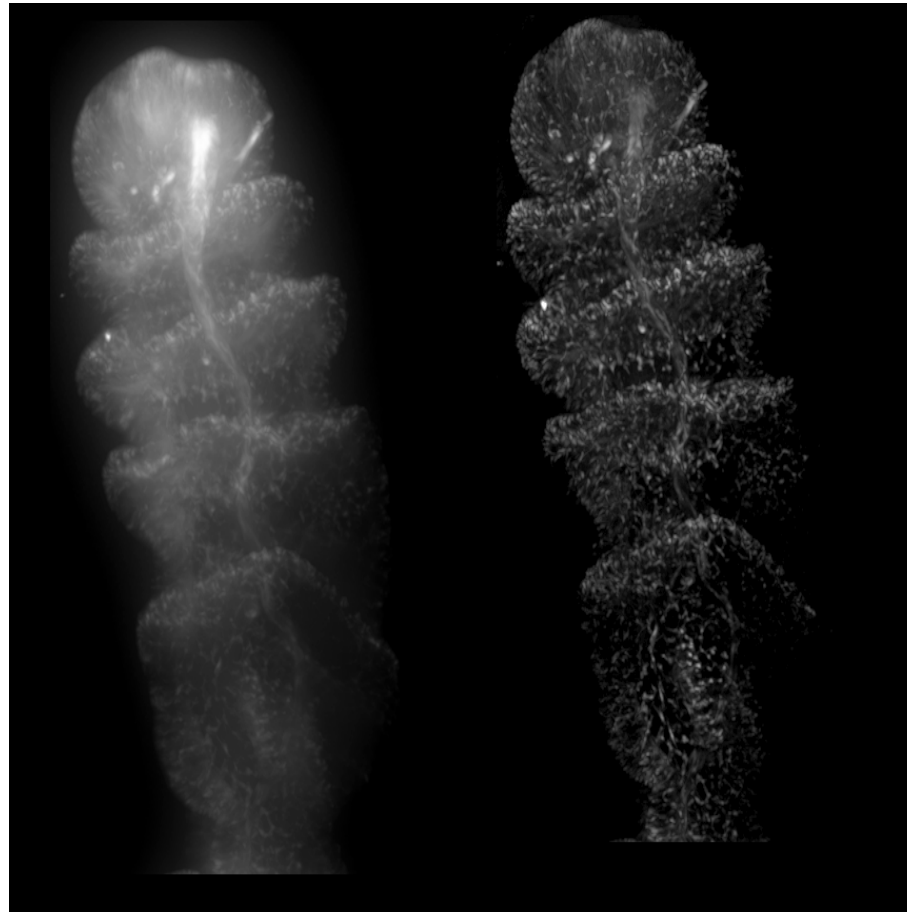


No-Neighbors	Single slice
Nearest-Neighbors	Several optical sections
Inverse Filtering	Whole volume, one pass

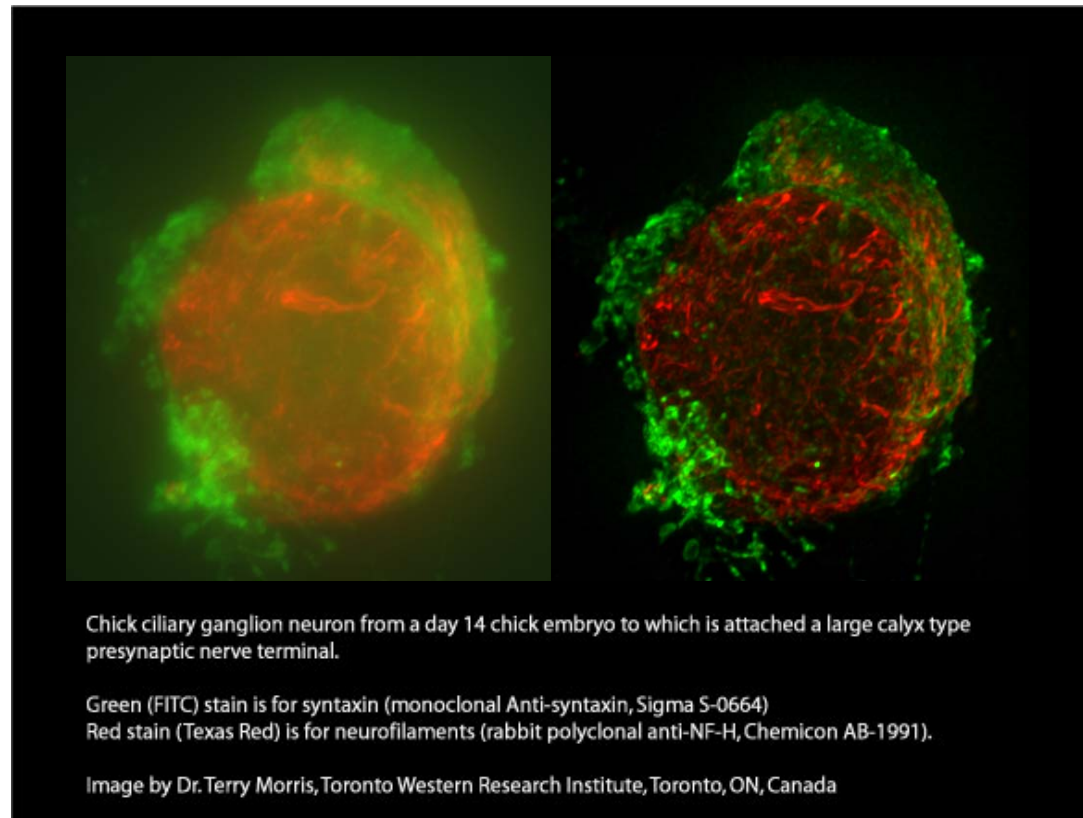
Non-blind Deconvolution	Whole volume, multi-pass, known PSF
--------------------------------	--

Blind Deconvolution	Whole volume, multi-pass, adaptive PSF.
----------------------------	--

Widefield Deconvolution



Widefield Deconvolution

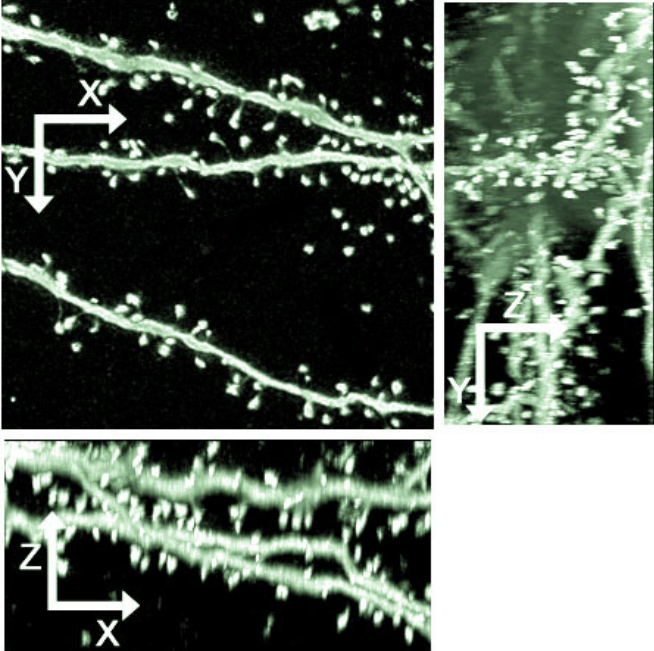
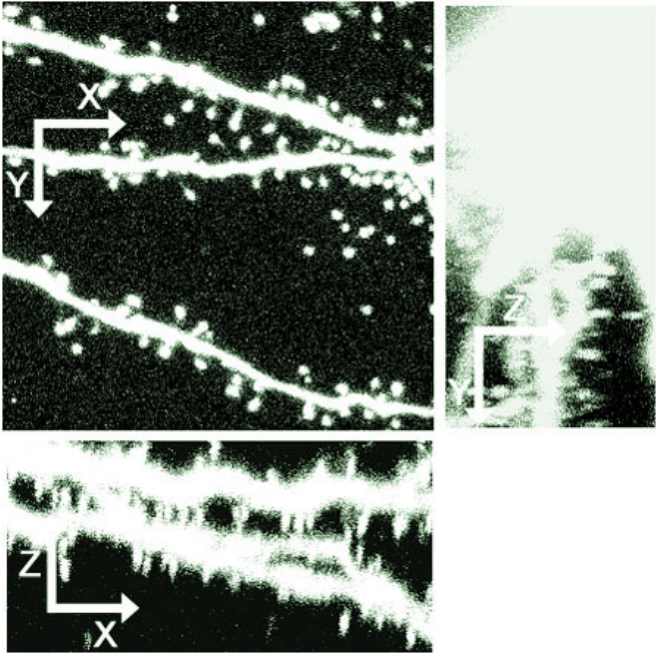


Chick ciliary ganglion neuron from a day 14 chick embryo to which is attached a large calyx type presynaptic nerve terminal.

Green (FITC) stain is for syntaxin (monoclonal Anti-syntaxin, Sigma S-0664)
Red stain (Texas Red) is for neurofilaments (rabbit polyclonal anti-NF-H, Chemicon AB-1991).

Image by Dr. Terry Morris, Toronto Western Research Institute, Toronto, ON, Canada

Confocal Deconvolution



MOUNT SINAI
SCHOOL OF
MEDICINE

COMPUTATIONAL NEUROBIOLOGY
and IMAGING CENTER



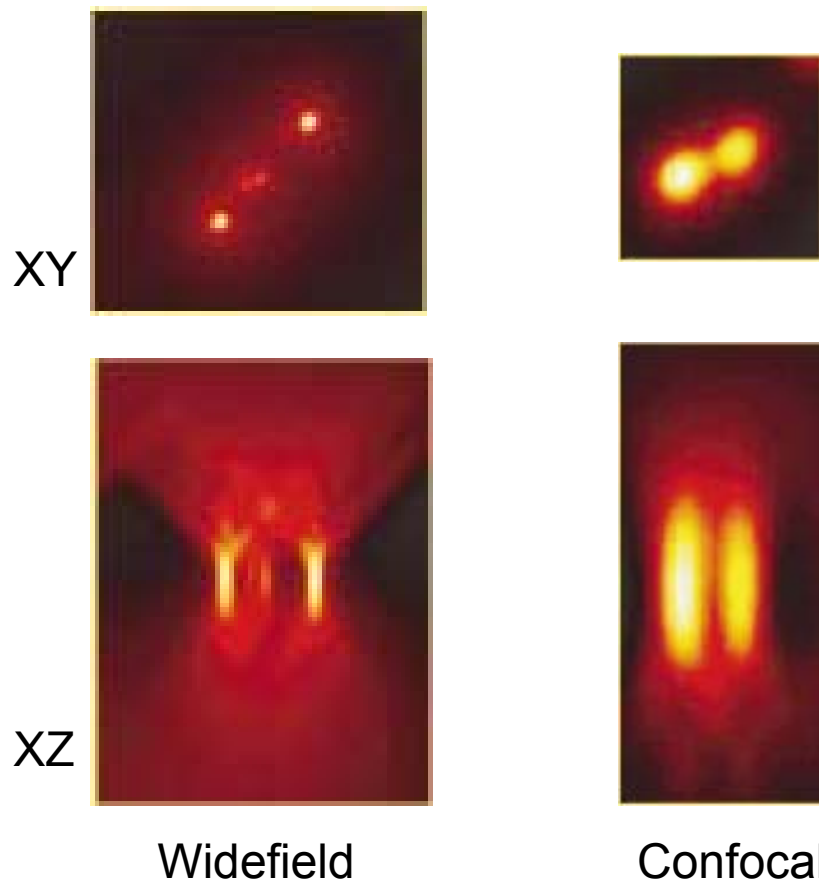
Partners in Innovation™

Microimaging Applications Group



Dial in: (319) 741-8100 Access: 627827# Unmute/mute: #6

Widefield vs. Confocal PSF



No-Neighbors Deblurring



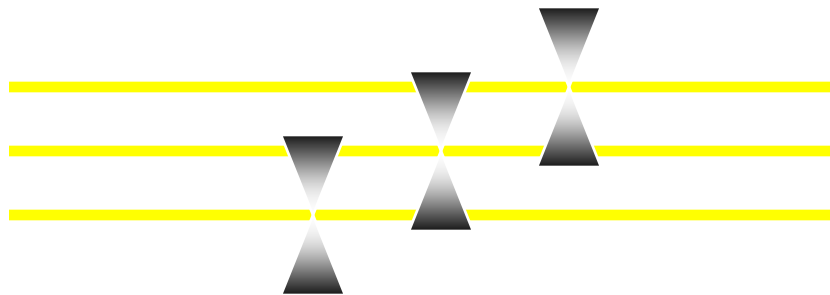
- Uses only current slice.
- Blurs current slice with PSF to approximate blur contribution from adjacent slices.
- Subtract away the blur.
- Deblurs one slice at a time
- Very fast, 2D, only qualitative



Nearest Neighbors Deblurring



- Uses center slice and two adjacent slices
- Estimate amount of blur that adjacent slices contribute to center slice
- Subtract away the blur
- Deblurs one slice at a time
- Fast, 2D, only qualitative



Inverse Filter



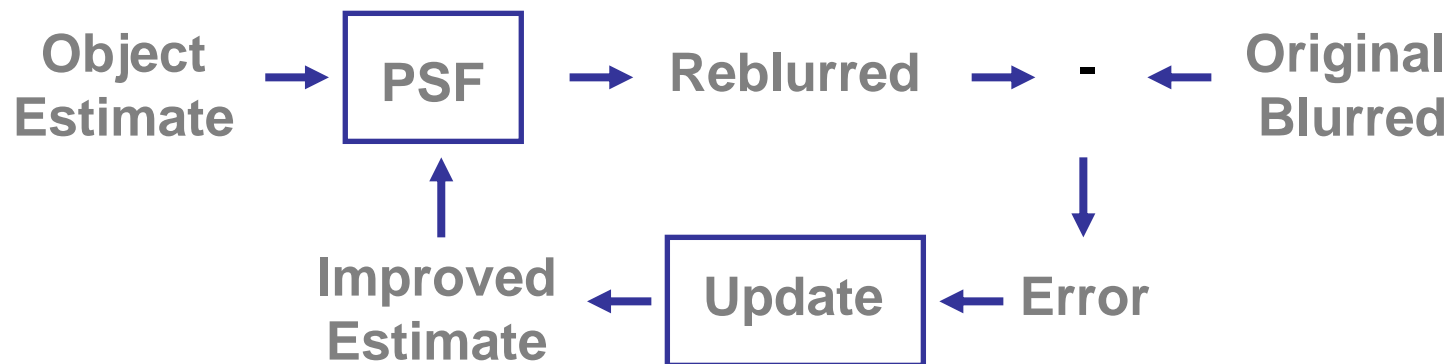
- Restoration algorithm – looks at whole structure
- Works well on large objects
- Fast, 3D, qualitative
- Regularization/Stability

$$\text{Image} \otimes \frac{1}{\text{PSF}} = \text{Deblurred}$$

3D Non-blind Deconvolution



- Requires PSF (theoretical or measured)
- Iteratively improve object estimate
- Impose constraints (non-negativity)
- Requires highly optimized imaging system



3D Blind Deconvolution

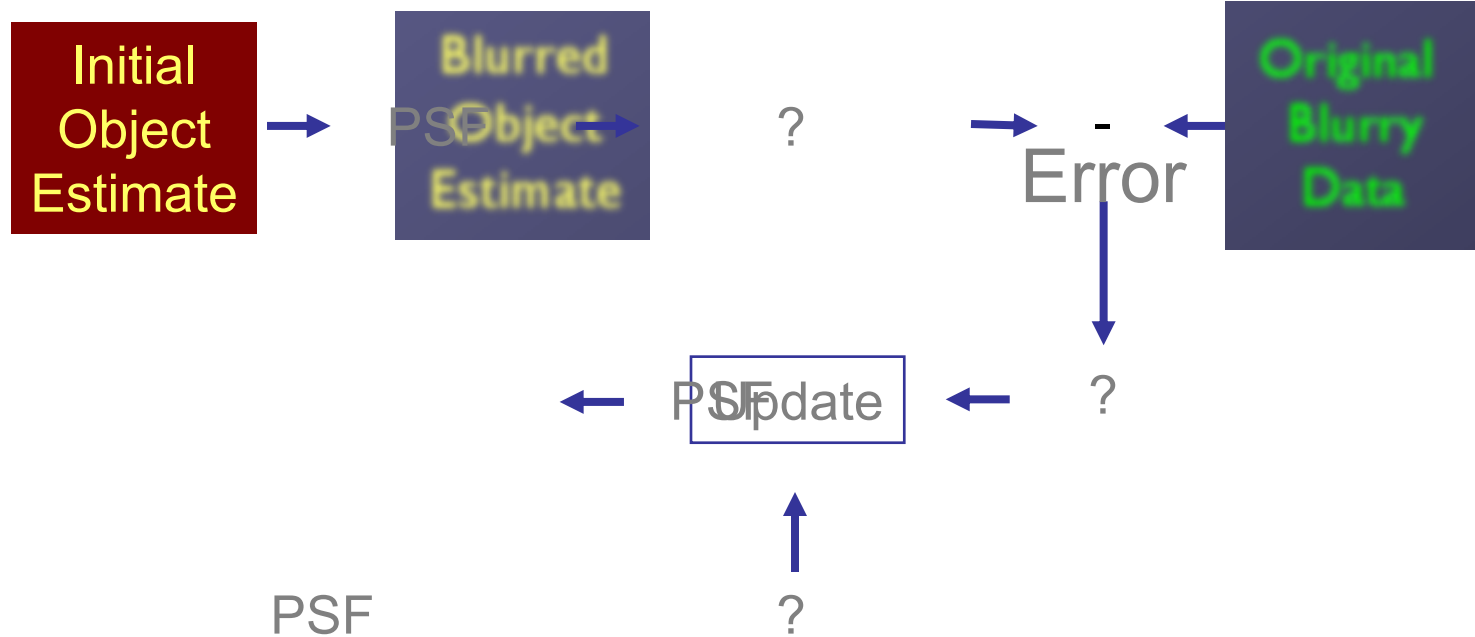


- Estimate both PSF and Object!
- Need microscope parameters (NA, RI, xyz, λ)
- Theoretical PSF with spatial/spectral constraints.
- Object with non-negativity and noise constraints.

3d Blind Deconvolution



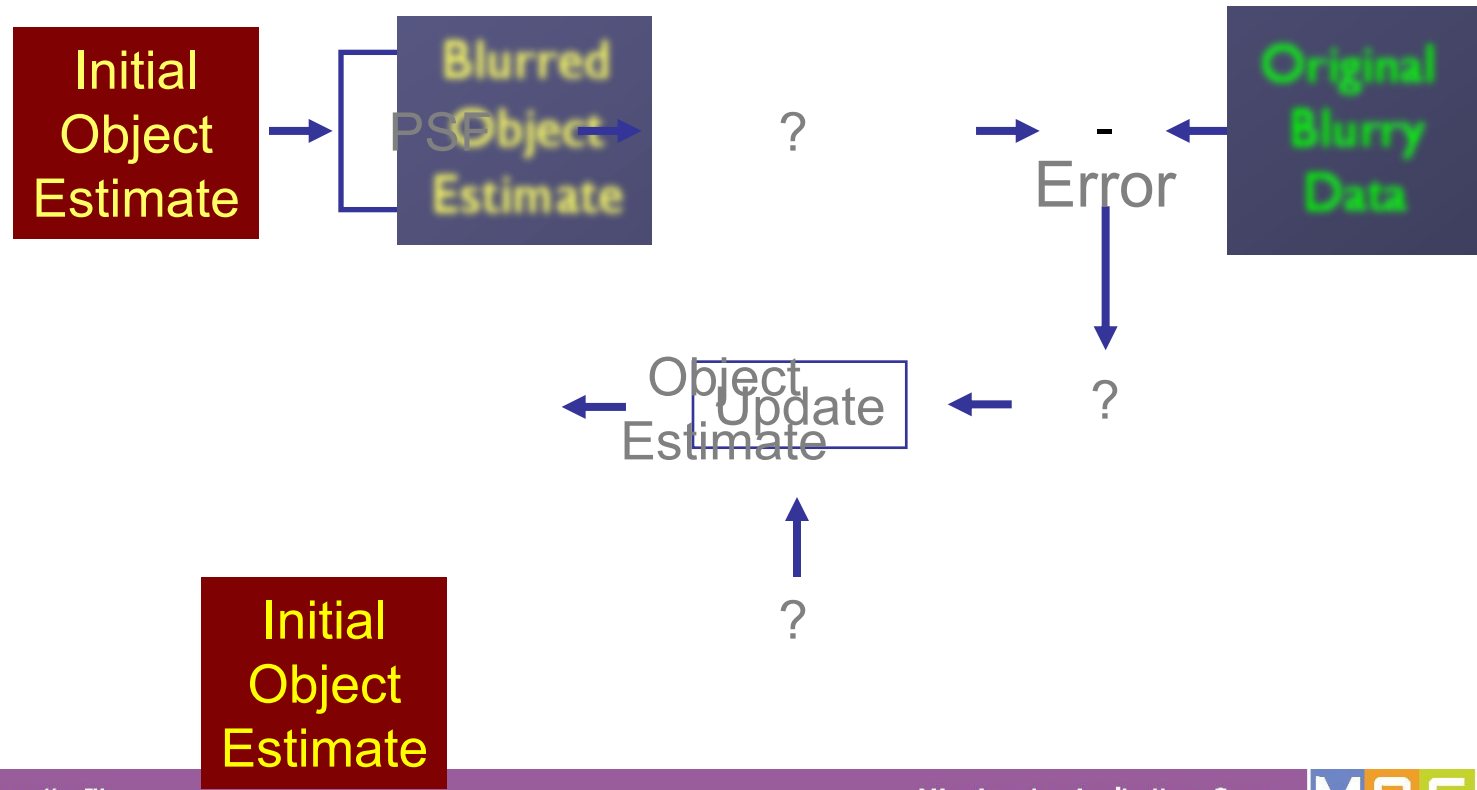
Iteration 1 - PSF



3D Blind Deconvolution



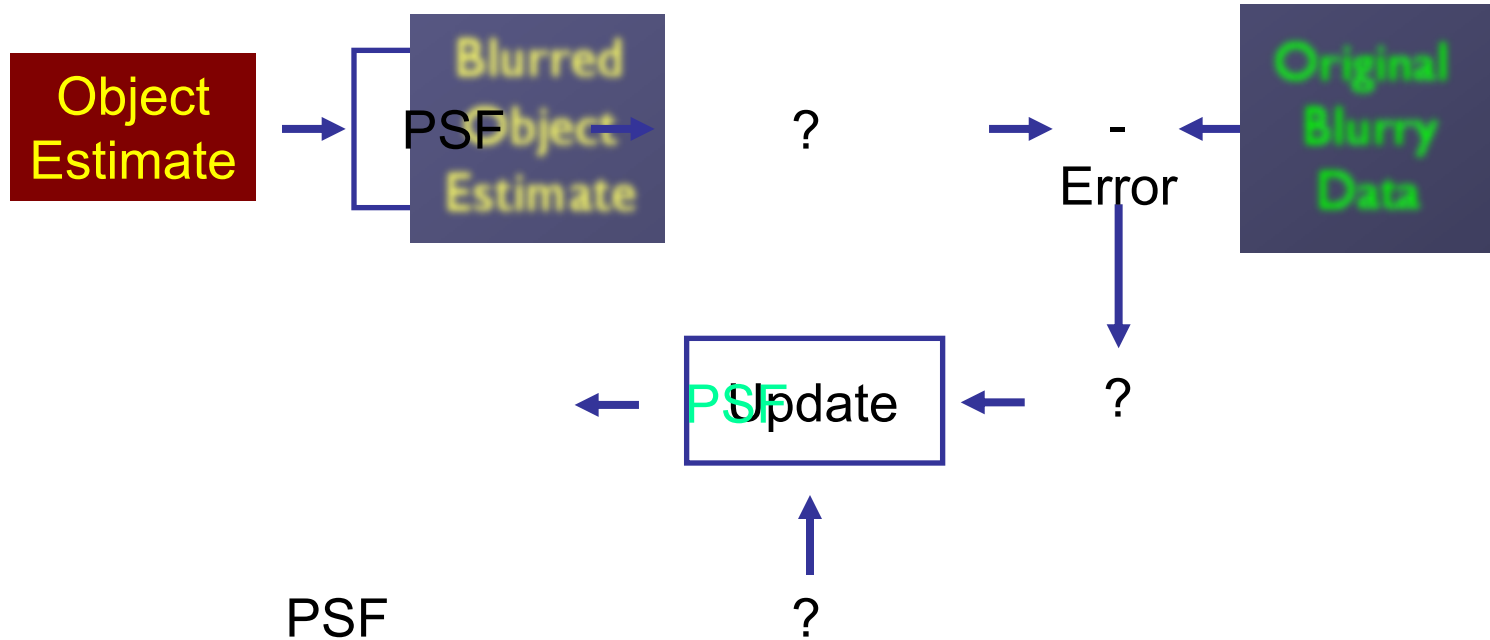
Iteration 2 - Object



3D Blind Deconvolution



Iteration 2 - PSF



Blind vs. Non-Blind Deconvolution



- Non-blind
 - Must maintain library of PSFs.
 - PSFs need to be optimally measured and noise free.
 - Faster because only estimating image.
- Blind
 - Does not require imaging of beads.
 - Can respond to changes in optical model: Specimen, thermal expansion, aberrations and noise.

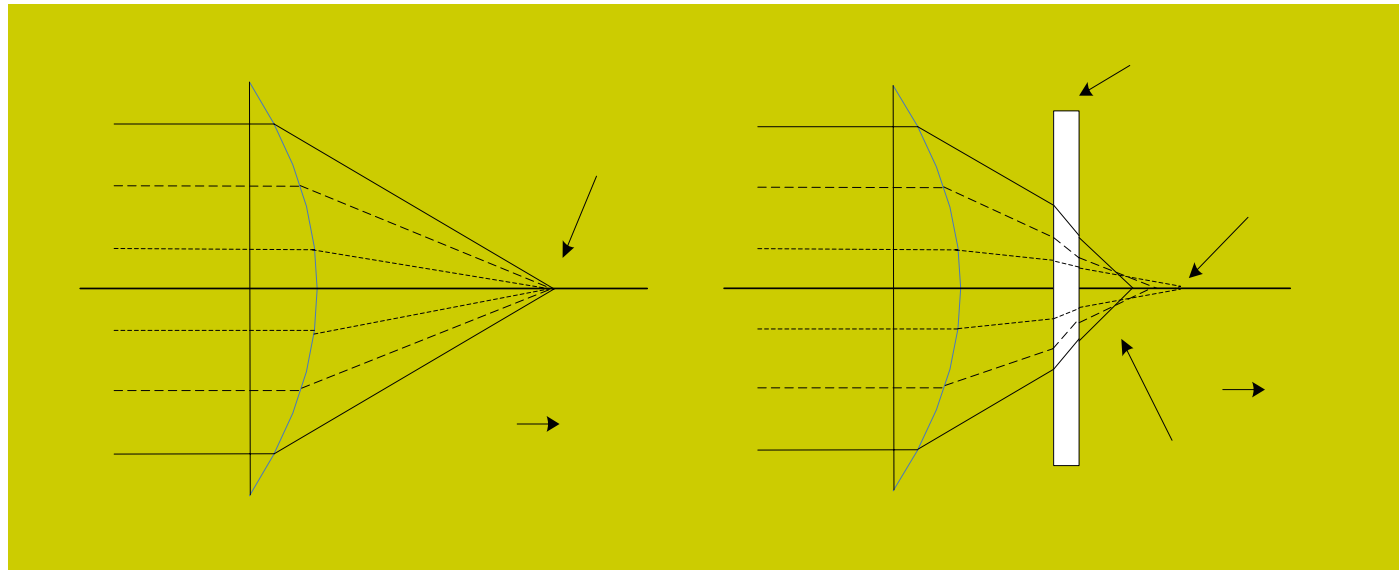
AutoQuant X Products



- AutoDeblur Widefield: \$5,950 US
- AutoDeblur Confocal: \$5,950 US
- AutoDeblur Widefield & Confocal: \$9,400 US
- 10% Discount for all Webinar Attendees!
- Contact sales@mediacy.com

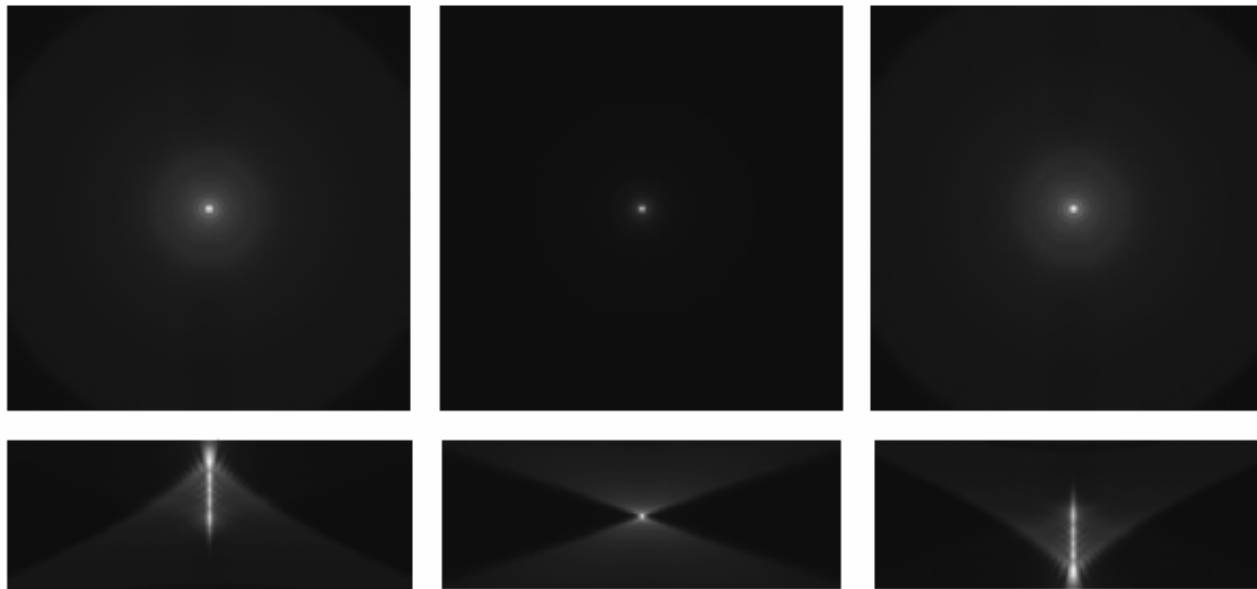
- FREE 30-day demo:
www.mediacy.com/leads/demos.asp

Spherical Aberration



- Changes in refractive index
- Incorrect cover slip thickness
- Thick specimens

Effects of Spherical Aberration



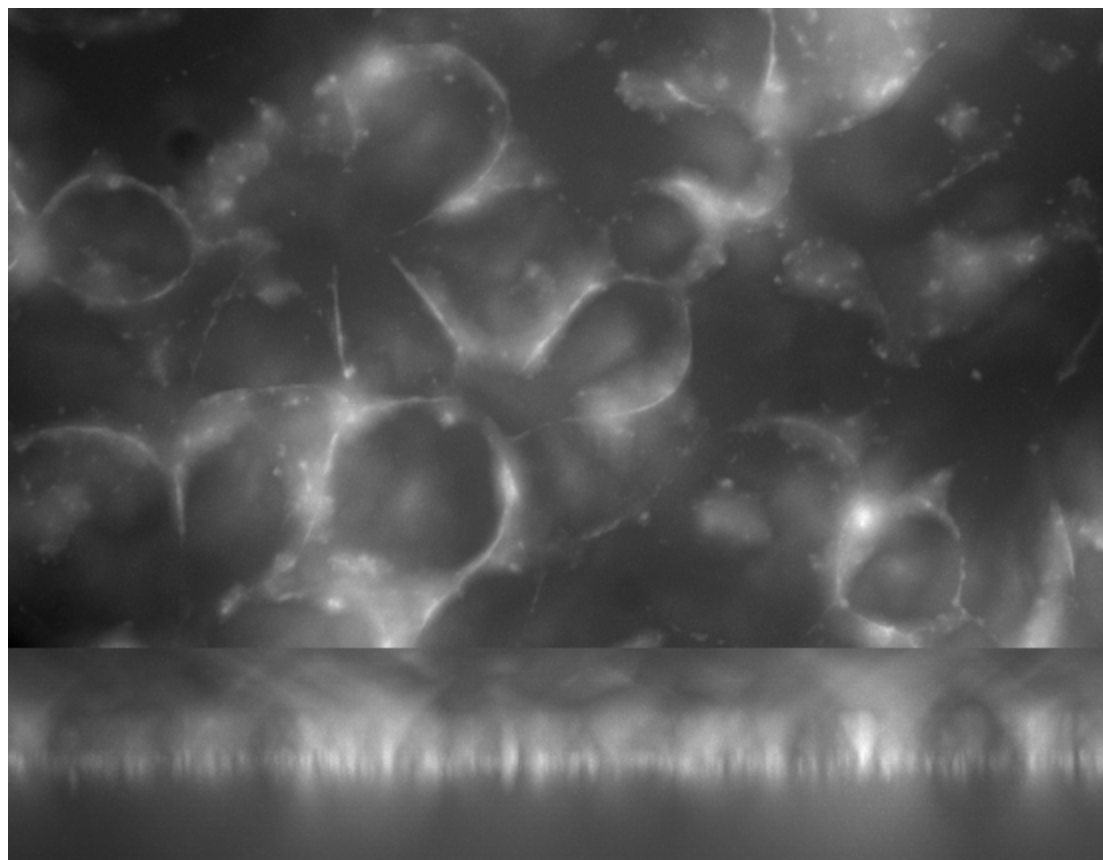
- Results in an asymmetry of the PSF.
- Adds significantly more blur.
- Reduces signal.

Correction of Spherical Aberration

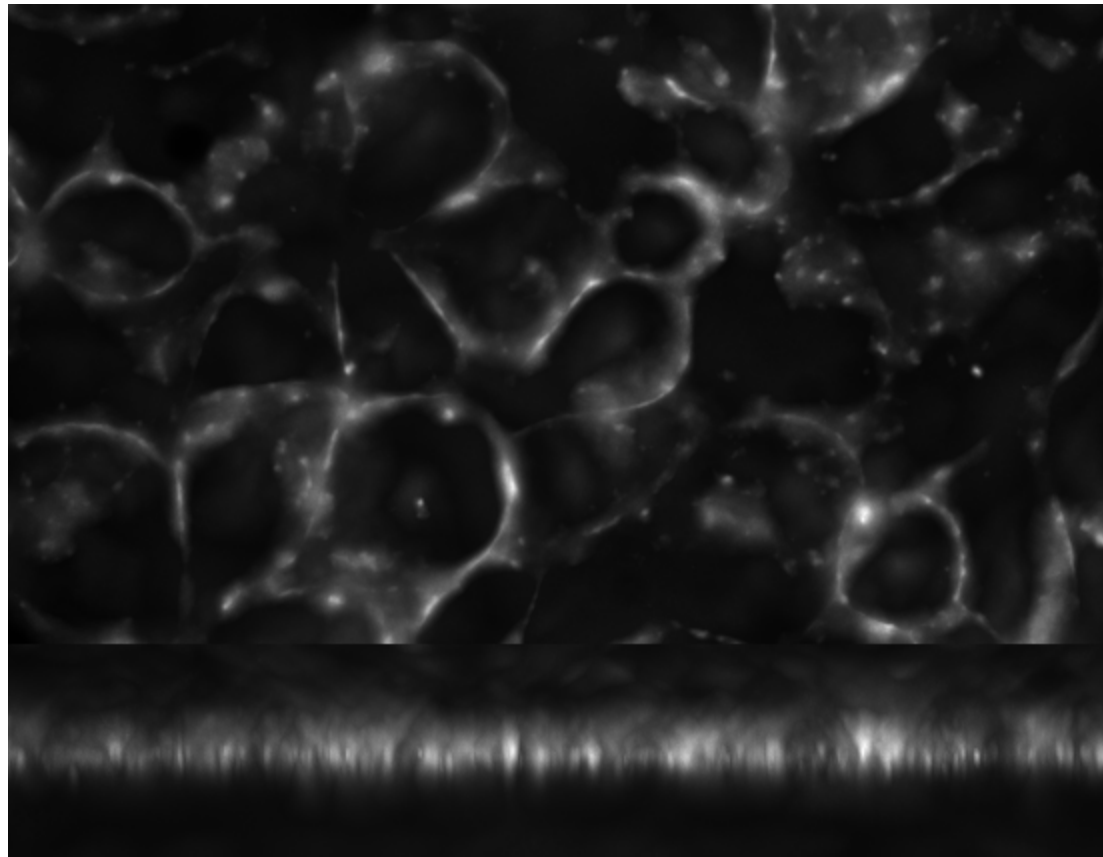


- Match refractive indexes.
- Use a SA correction collar.
- Bias the PSF with similar aberration and deconvolve.
- Bias the PSF and allow it to adapt using blind deconvolution.
- Correct with optics in infinity space.

Raw Image
max = 2,614

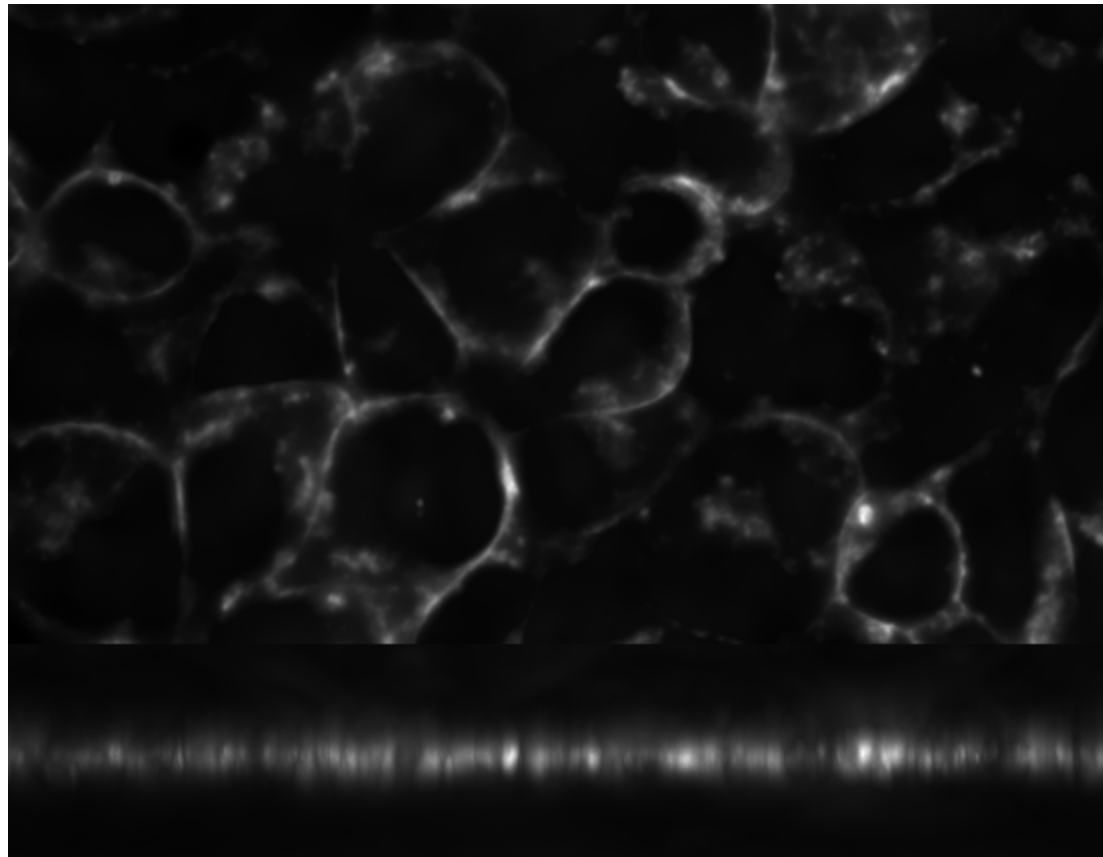


Deconvolved no SA
max = 12,134



Deconvolution with SA Correction

max = 23,938



Noise and Maximum Likelihood



- Comes from photon detection & instrument noise.
- Update step is important.
- Most companies smooth every few iterations.
- Maximum likelihood.
 - Statistical approach which tests what is the most likely to occur in the specimen using a known noise model.
 - Finds the “most likely” possibility for each portion of the image.
 - Models noise extremely well.
 - Can actually remove noise instead of suppress.

Upcoming events



- Oct. 11th - Imaging Series Webinar #3 - "3D Imaging: Issues to Consider for Biological Microscopy". FREE
- Nov. 5th AutoQuant training in San Diego during Neuroscience. Contact Kathy Hrach (kathy@mediacy.com). \$295
- Dec. 5th - AutoQuant training in Washington D.C. during Cell Biology. Contact Kathy Hrach (kathy@mediacy.com). \$295

Questions?



- Biggs D. (2004) Clearing Up Deconvolution. Biophotonics Feb 2004.
- Holmes TJ. Light Microscopic Images Reconstructed by Maximum Likelihood Deconvolution. Chapter 24 in The Handbook of Biological Confocal Microscopy, 2nd Edition, James Pawley, Editor, Plenum Press, New York, 1995.
- Swedlow JR. (2001) A working person's guide to deconvolution in light microscopy. Biotechniques 31:1076-1097.

Thank you



For more information contact:

Jonathan Girrior

Media Cybernetics

541-383-4133

jgirrior@mediacy.com

FREE 30-day demo:

www.mediacy.com/leads/demos.asp

Slideshow at

[ftp.mediacy.com/uploaded/AQX/DeconWebinar.pdf](ftp://ftp.mediacy.com/uploaded/AQX/DeconWebinar.pdf)

Content



- Deconvolution has proven to be a powerful new tool for performing high-resolution multi-dimensional imaging of biological specimens. It is used for increasing the sensitivity and improving the resolution of standard widefield microscopes and for improving the resolving capabilities of confocal systems. Attendees of this live, no-cost webinar will learn about the latest technology for performing deconvolution on a variety of microscope images and understand better the relative strengths and weaknesses of several popular algorithms. Bring your questions to this live, interactive web-based seminar.
 - What is deconvolution and how does it work?
 - Discussion on empirical vs. derived point-spread functions
 - Uses in fluorescence and brightfield modalities
 - Difference from confocal imaging
 - How do I choose one over the other?
 - Pro and cons- where deconvolution works and where it doesn't
 - Challenges and advice
 - A few before/after examples using Image Pro 3D Constructor