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Deconvolution

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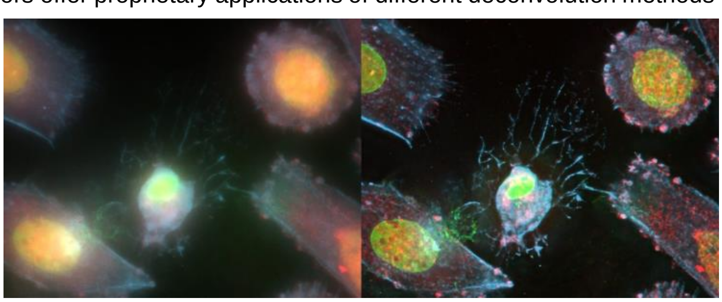
Deconvolution

Computational method for improving contrast and resolution of digital images
Multiple different methods exist, utilising different approaches and algorithms
Often applied to wide-field images, however can be applied to any microscopy image
Many vendors offer proprietary applications of different deconvolution methods



Example from Microvolution

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Causes of Image degradation

Four main cause

-Blur

-Noise

-Scatter

-Glare

Blur – the main target for deconvolution; non-random spreading of light through the imaging system

Noise – is either a result of the sample (Poisson distribution) or the imaging system (Gaussian distribution)

Scatter – is the random disturbance of light through regions of different refractive index

Glare – is the random disturbance of light through optical components of the imaging system

Sourced from Professor Pablo Artal

Blur

Non-random spreading of light through the imaging system

Caused by diffraction, *an image only limited in resolution by blur is called diffraction-limited*

Is a result of the imaging system, and mostly the imaging objective

Can be modelled mathematically relatively easily.

Deconvolution is the process of reversing the blur model of an optical system.

Original Image | Blurred Image | Deblurred Image by Blind Deconvolution

Mane and Parwar, 2015

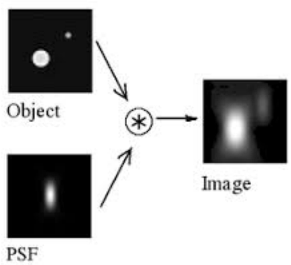
The Point Spread Function (PSF)

Imagine an infinitely small point source of light.

The microscope only capture a very small proportion of the light being emitted by this point-source, and is unable to focus it into a 3D object.

This single point-source will appear as a spread out diffraction pattern spread out in the z-axis

The optimal diffraction pattern by a single point-source of light is a PSF.



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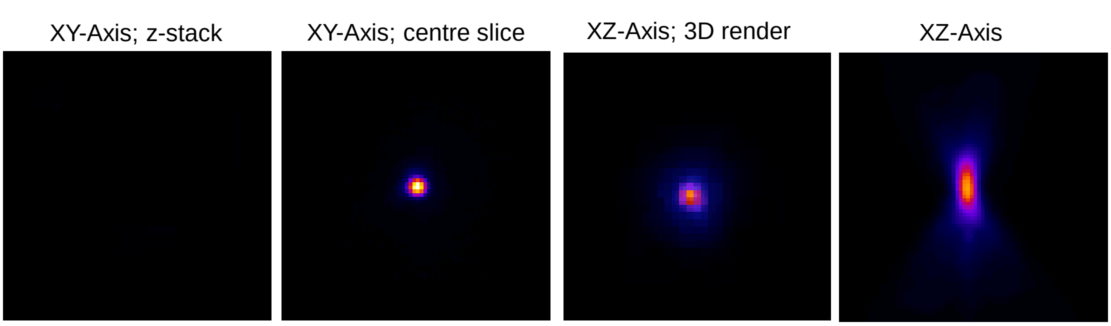
The Point Spread Function (PSF)

Imaging of a point source of light below the diffraction limit of the optical system will result in a PSF

For most systems we use 100nm fluorescence beads

Z-stacks are imaged at least twice the Nyquist criterion (~50nm/slice) & imaged greater than the visible signal of the bead.



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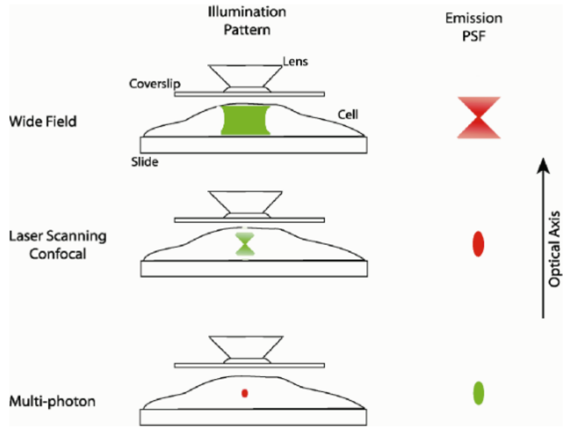
PSFs for different imaging systems

- PSFs will appear differently for different microscopes
- Widefield systems capture out of focus light within the image
- Confocal microscopes via the use of a pin-hole remove out of focus light

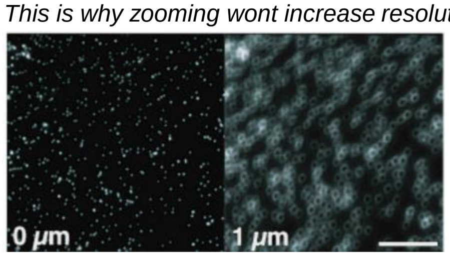


Andrews, Harper Swedlow, 2002

The PSF is the base unit of an image

Since the PSF represents a single point-source of light, an image is just made up of PSFs

The best an image can be is an assembly of PSFs, zooming or stretching wont change the resolvable single point-sources of light. *This is why zooming wont increase resolution.*



Consider the image above. The left hand panel is in focus, while the right hand panel is 1µm out of focus.

It is an image made up of multiple PSFs, the multiple rings are the aberrations of the imaging system in the optical axis.

Convolution

The mathematical model applied to the captured image as a result of the blurring induced by imaging system.

Is the application of the PSF to the point-source of light from each part of the image.

Convolution causes each point in an object of interest being imaged to be blurred.

Remember the PSF is 3-dimensional, and so is the blur in your image.

Deconvolution is just the reverse of this blur caused by the PSF. Since we can capture a PSF or theoretically generate one, we apply this to the convolved images using the PSF.

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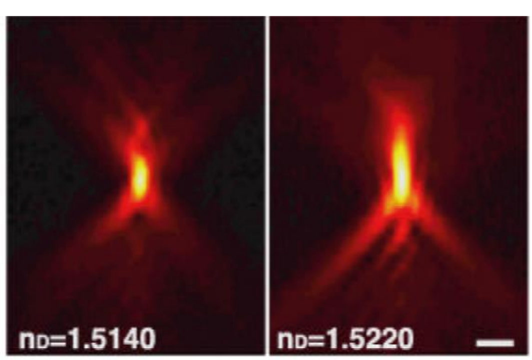
Spherical aberration

No imaging system is perfect, a captured PSF is always better than a theoretical PSF.
A mis-match of refractive indexes will result in spherical aberrations in your image.

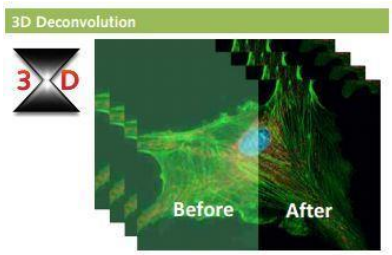


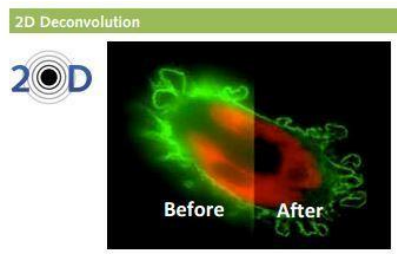
Types of deconvolution

There are two main types of deconvolution

-Deblurring - 2D based

-Image Restoration – 3D Based





Sourced from AutoQuant

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De-blurring algorithms

-Nearest Neighbour (blurs the above and below slices and subtracts them from the slice of interest)

-Multi-Neighbour (blurs a user definable number of slices above and below before subtraction)

-No-Neighbour

-Un-sharp masking

- Computationally economical
- Introduce more noise by combining that of the slices above and below with the slice of interest
- Overall reduce signal via subtraction of associated planes
- Overly enhance objects that appear in the same XY position across multiple stacks
- May ‘shift’ objects in XY if they have higher intensity in different slices.

Before and After Nearest Neighbor Deconvolution Analysis

(a) (b)

Figure 1

Olympus LifeScience

Image Restoration algorithms

Deal with blur in 3D

Instead of subtracting blur, they attempt to 're-assign' blur in 3D

Work with large matrices (3D image stacks) however deal with this by instead using the Fourier-transformed image.

Simplistically it is the multiplication of the FT-PSF with the FT-image.

Types:

Inverse Filters

Constrained Iterative

Blind Deconvolution

Inverse Filters

"Wiener Deconvolution", "Regularised Least Squares", "Linear Least Squares", "Tikhonov-Miller regularisation"

Works by dividing the FT-Image by the FT-PSF

Is very fast, but increases noise directly proportionally to the noise in the FT-PSF image.

Makes assumptions of the shape of the objects within the image (regularisation)

Iterations of regularisation remove 'noise' or 'roughness' in the objectives at the edges of the FT image (usually beyond the resolution of the image)

Usually works on a trade off of noise amplification to smoothing.

Constrained iterative algorithms

Are cyclic in how they run (iterative)

Have set limits (user defined) on possible solutions (constraints)

Creates an estimation of the object by comparing the deconvolved image with the Raw image and allows a certain degree of difference between the two, up to a set value 'figure of merit'.

The figure of merit is used to alter the estimate in a way to reduce error.

This continues looping until a threshold or limit is hit on the error value.

Constraints often are smoothness or regularisation.

Nonnegativity is also employed. Any value that becomes negative is set to 0 (background) as fluorescence in the Raw image could never be negative.

Blind Deconvolution

Is an alteration of the maximum likelihood estimation whereby both the PSF and the object are estimated

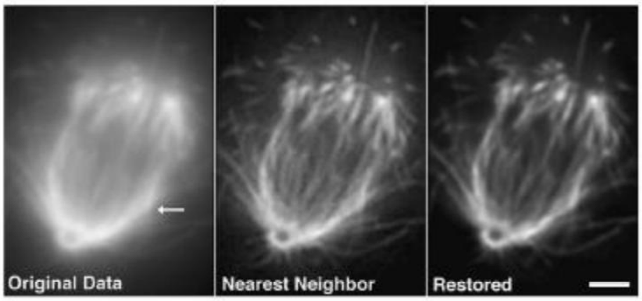
Uses the details of the imaging system to predict the PSF.

Using the predicted object and PSF an estimate is made from the blurred image and is refined with each iteration.

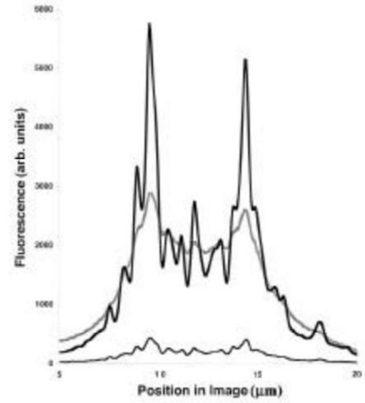
It begins with a theoretical PSF, but the model is refined with each iteration

Is useful for both high-quality images, and very noisy or spherically aberrated images.

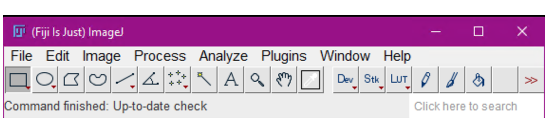
Comparison of the two main methods

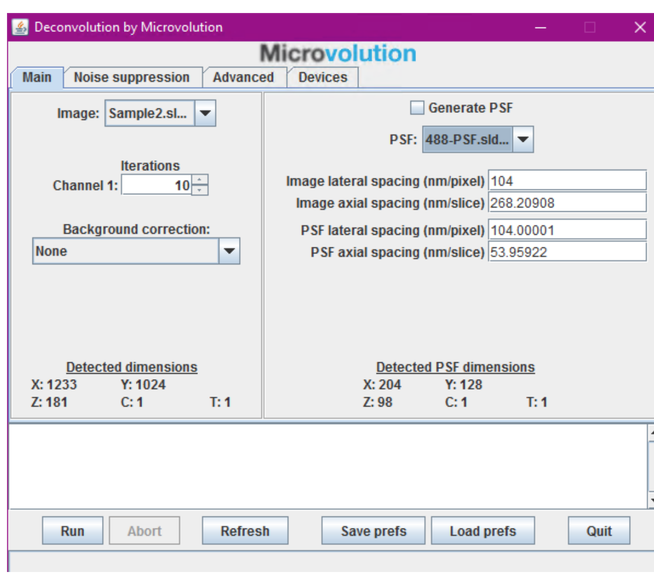


B.



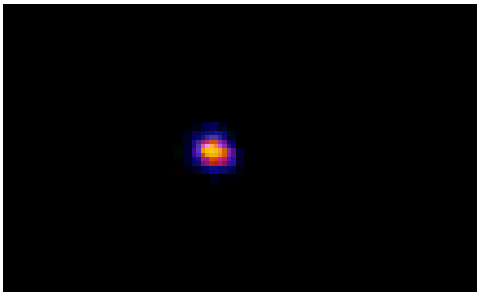
Deconvolution @ IMB



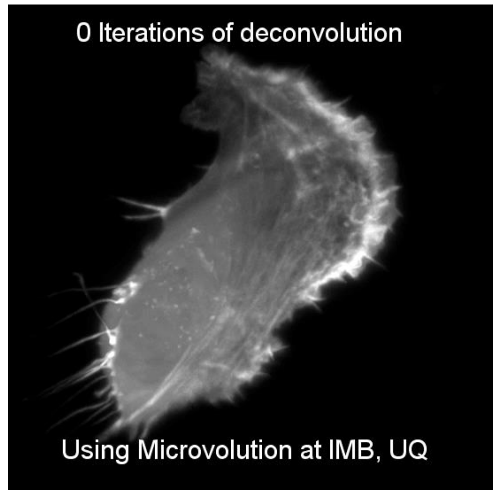


Runs in FIJI as a plugin (subscribed)
Supports multi-GPU Deconvolution
Staight-forward GUI

Lattice Light-sheet deconvolution



Example LLS Sample Deskew



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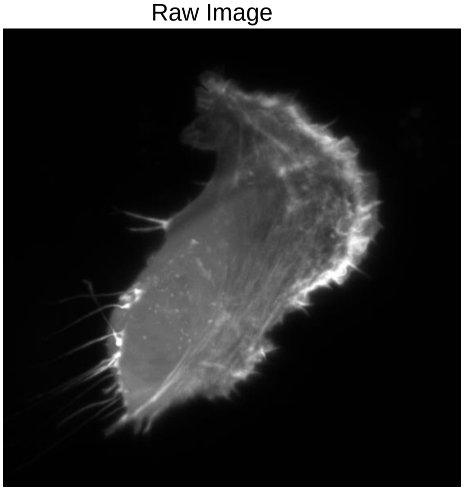
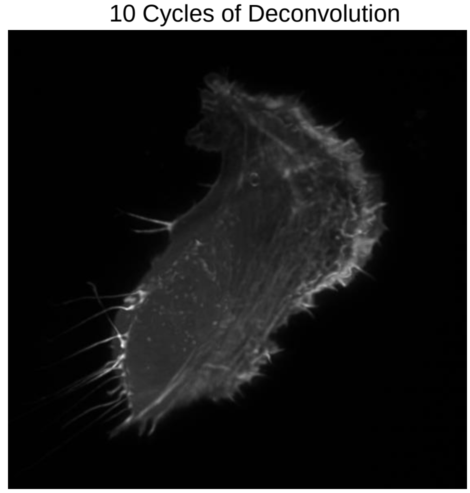
Lattice Light-sheet deconvolution – Maximum Projected

Raw Image



10 Cycles of Deconvolution



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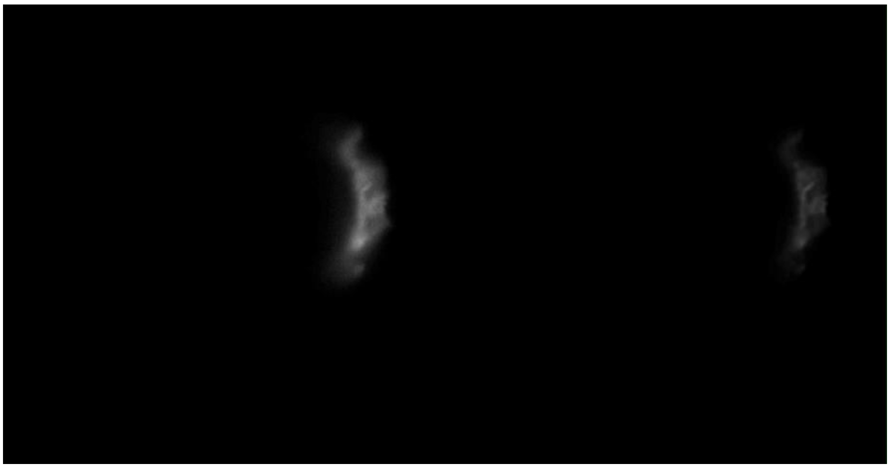
Lattice Light-sheet deconvolution

Raw Image

10 Cycles of Deconvolution



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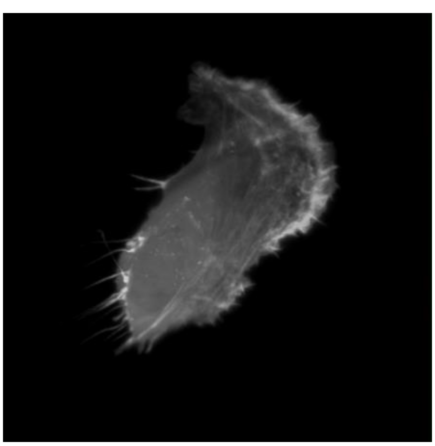
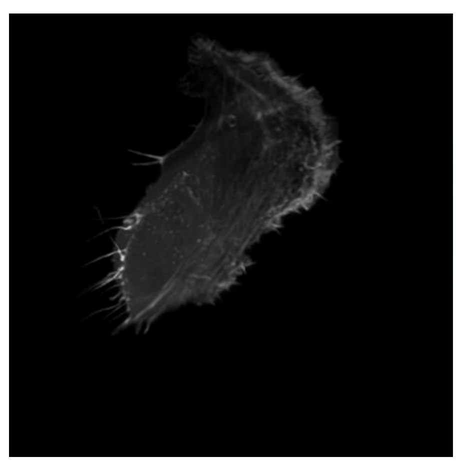
Lattice Light-sheet deconvolution – 3D Movie

Raw Image

10 Cycles of Deconvolution





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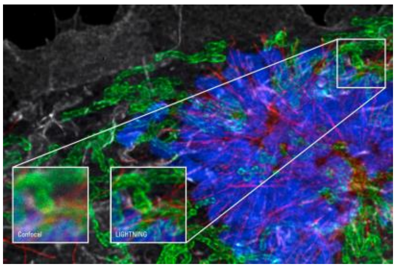
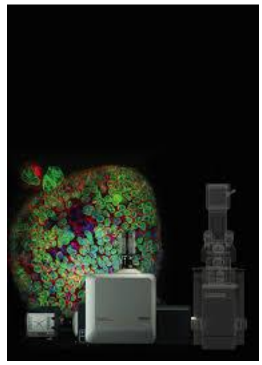
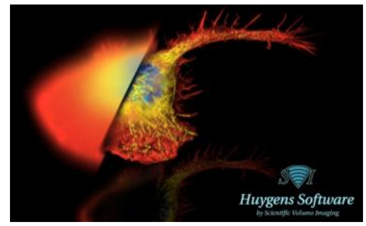
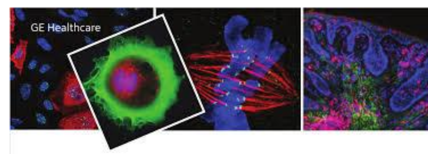
Other Deconvolution Programs @ IMB


Leica Lightning


Andor Fusion



Huygens (SVI)



Softworx Deltavision

+ Lots of free FIJI plugins

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Thank you

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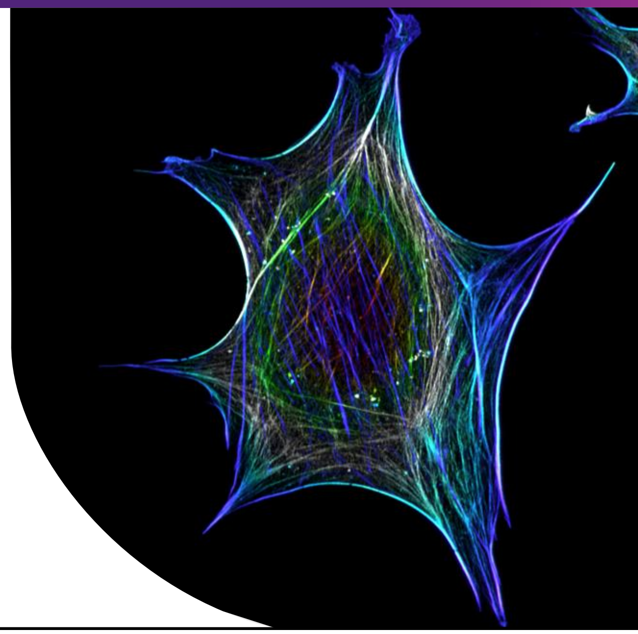

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