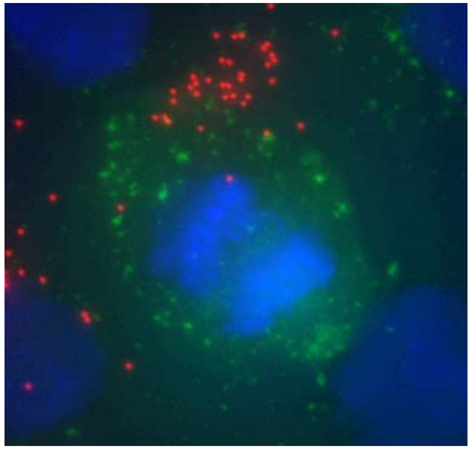
Imperial College London

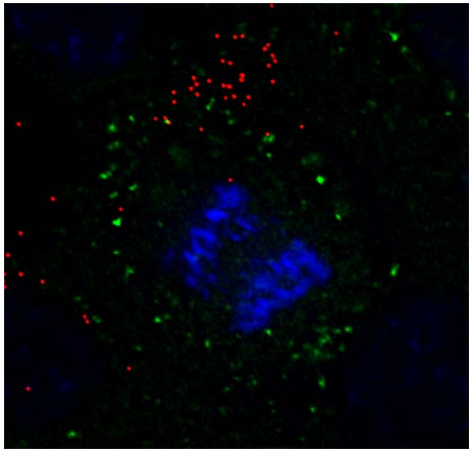
Facility for Imaging by Light Microscopy

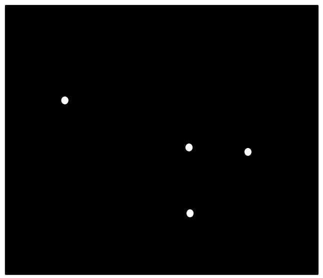
Observing Life As It Happens

"Microscopic image restoration by deconvolution"

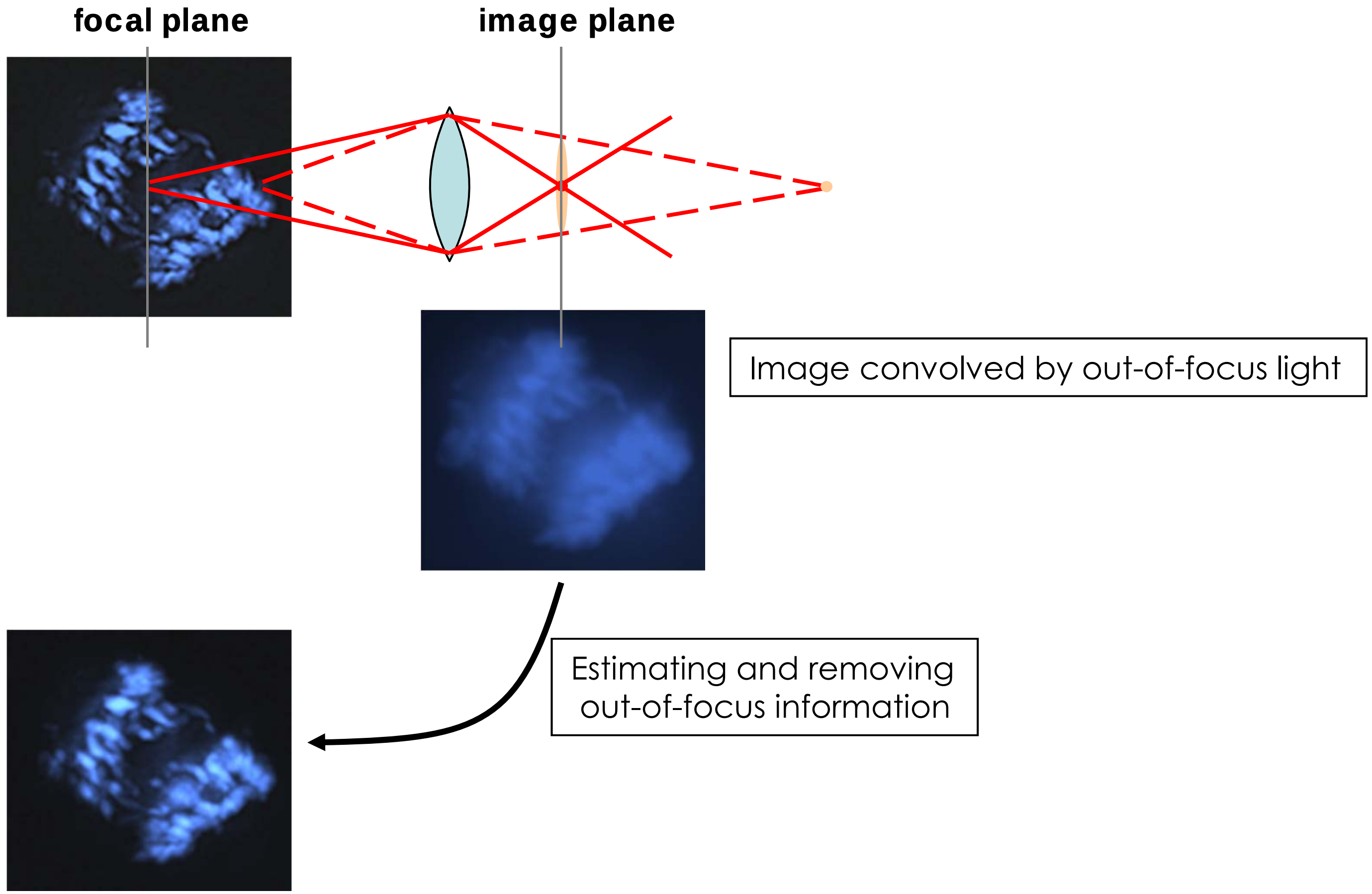
Martin Spitaler







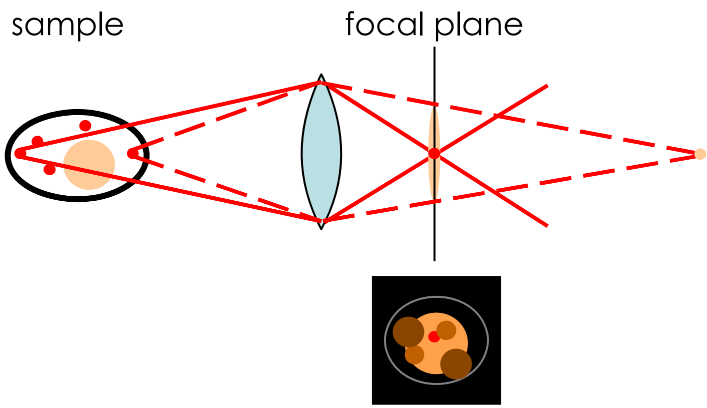
What is deconvolution?



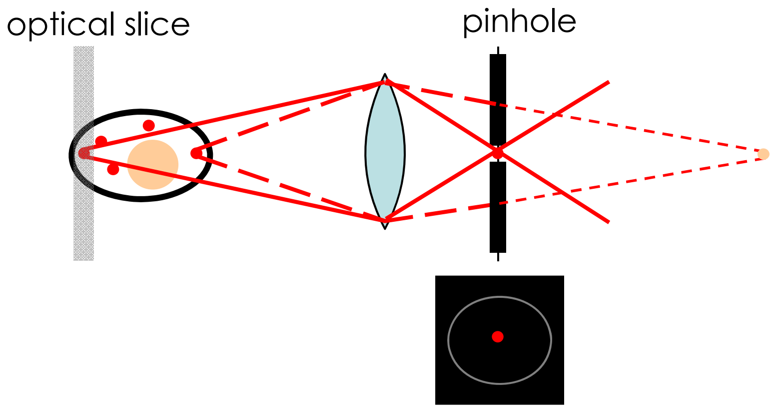
Convolution:
"Mathematical term for combining two signals to form a third signal."

Why deconvolution?

Standard (widefield) microscope

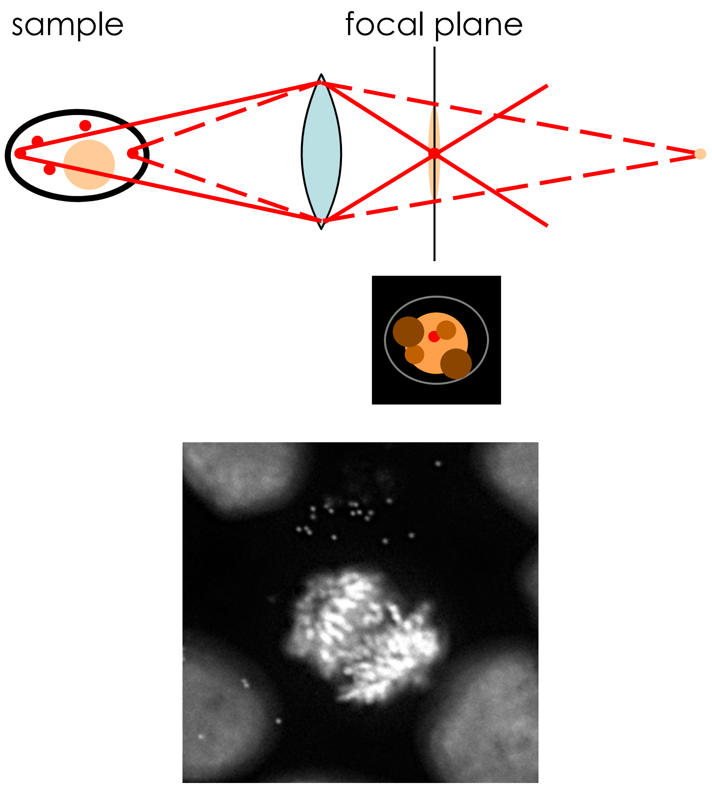


Confocal microscope

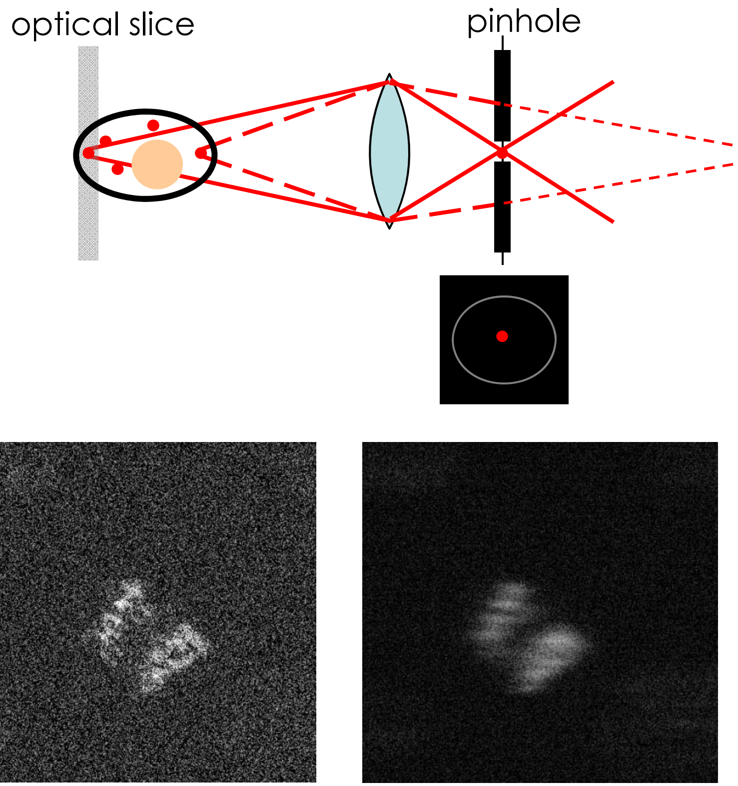


Why deconvolution?

Standard (widefield) microscope



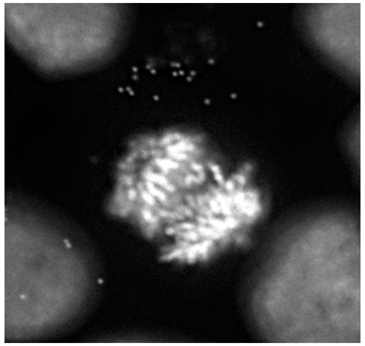
Confocal microscope



Why deconvolution?

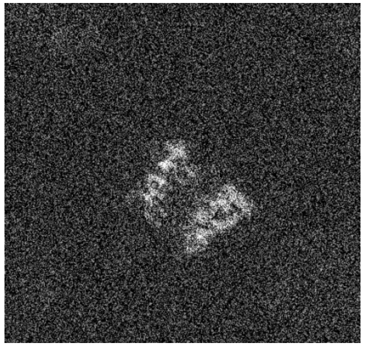
Standard (widefield) microscope

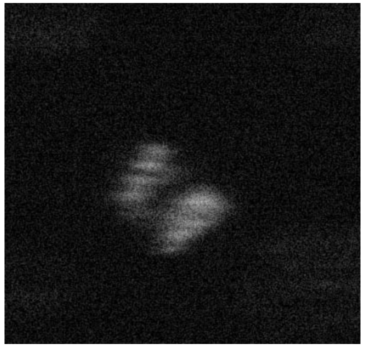
- 3D information convolved by out-of-focus light
- high sensitivity:
  - Low light exposure
  - Good signal-to-noise ratio
- fast
- (high resolution)



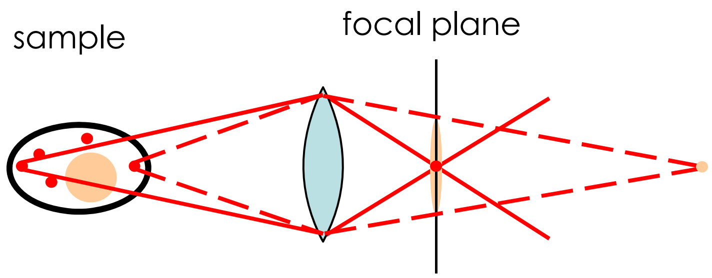
Confocal microscope

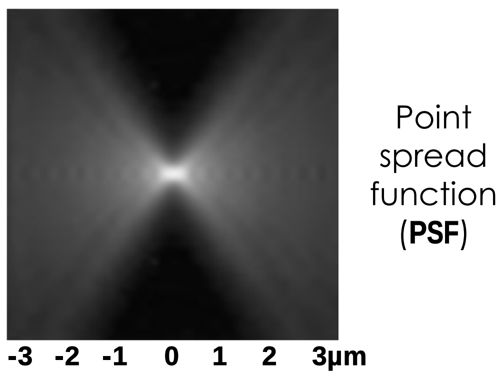
- intrinsic 3D information
- scanning technique → slow
- modest sensitivity / high phototoxicity:
  - Strong laser intensities
    (8x averaging // 40 stacks // 1min per timepoint // 10 min = 3200 light exposures *!!*)
    → bleaching, phototoxicity
- (Modest resolution)



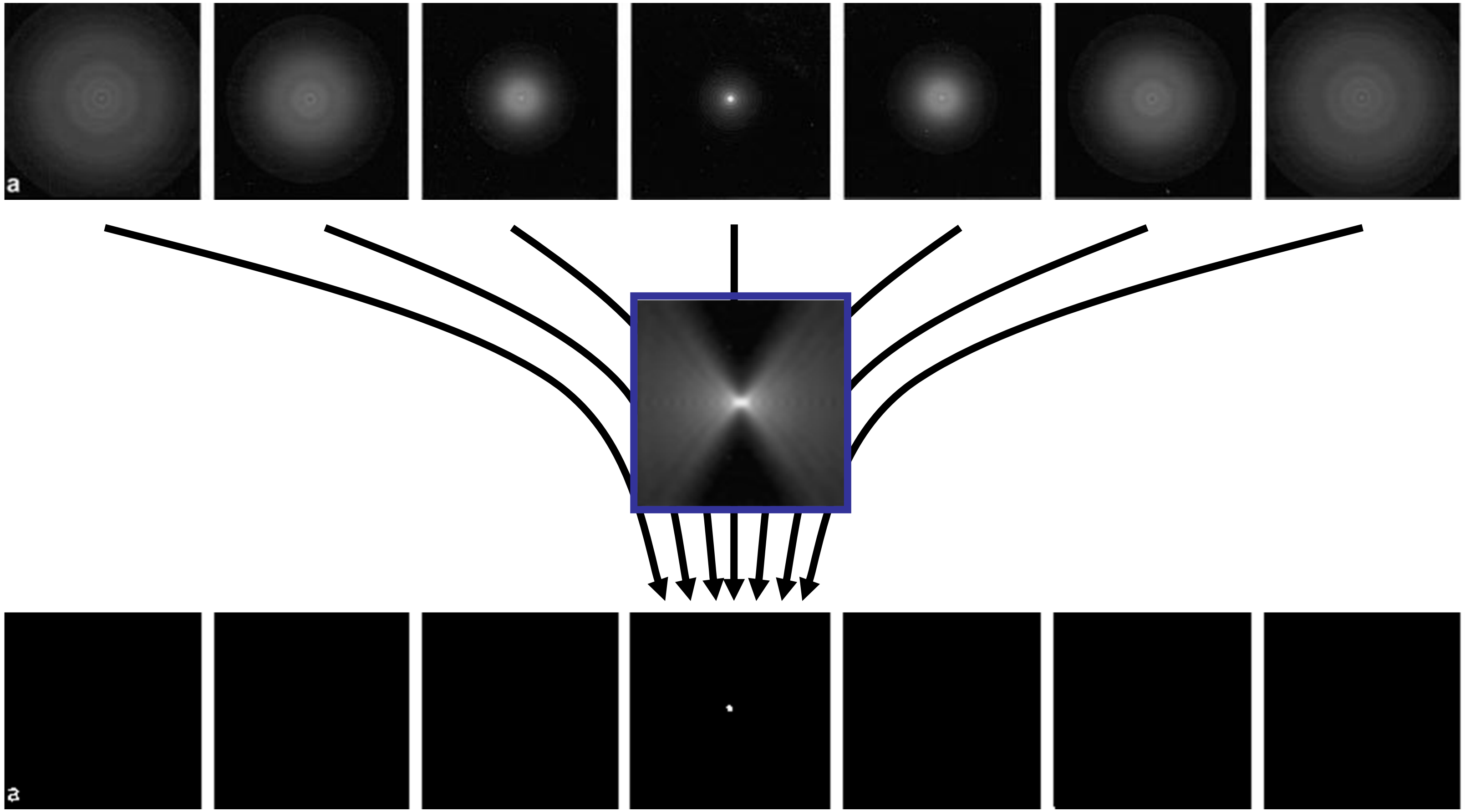


Principles of deconvolution



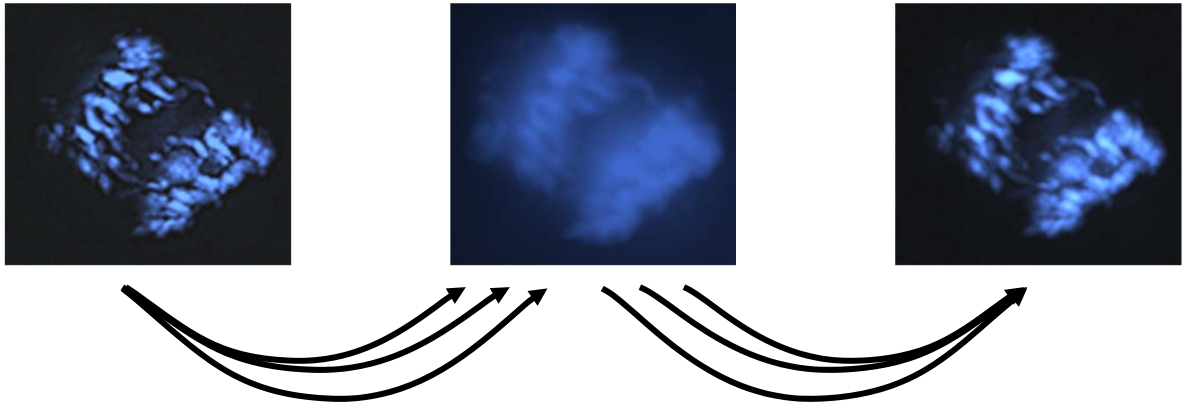


Principles of deconvolution



Out-of-focus information is moved back to its estimated origin
(no information is lost in the process!)

Principles of deconvolution



Imaging (convolution)

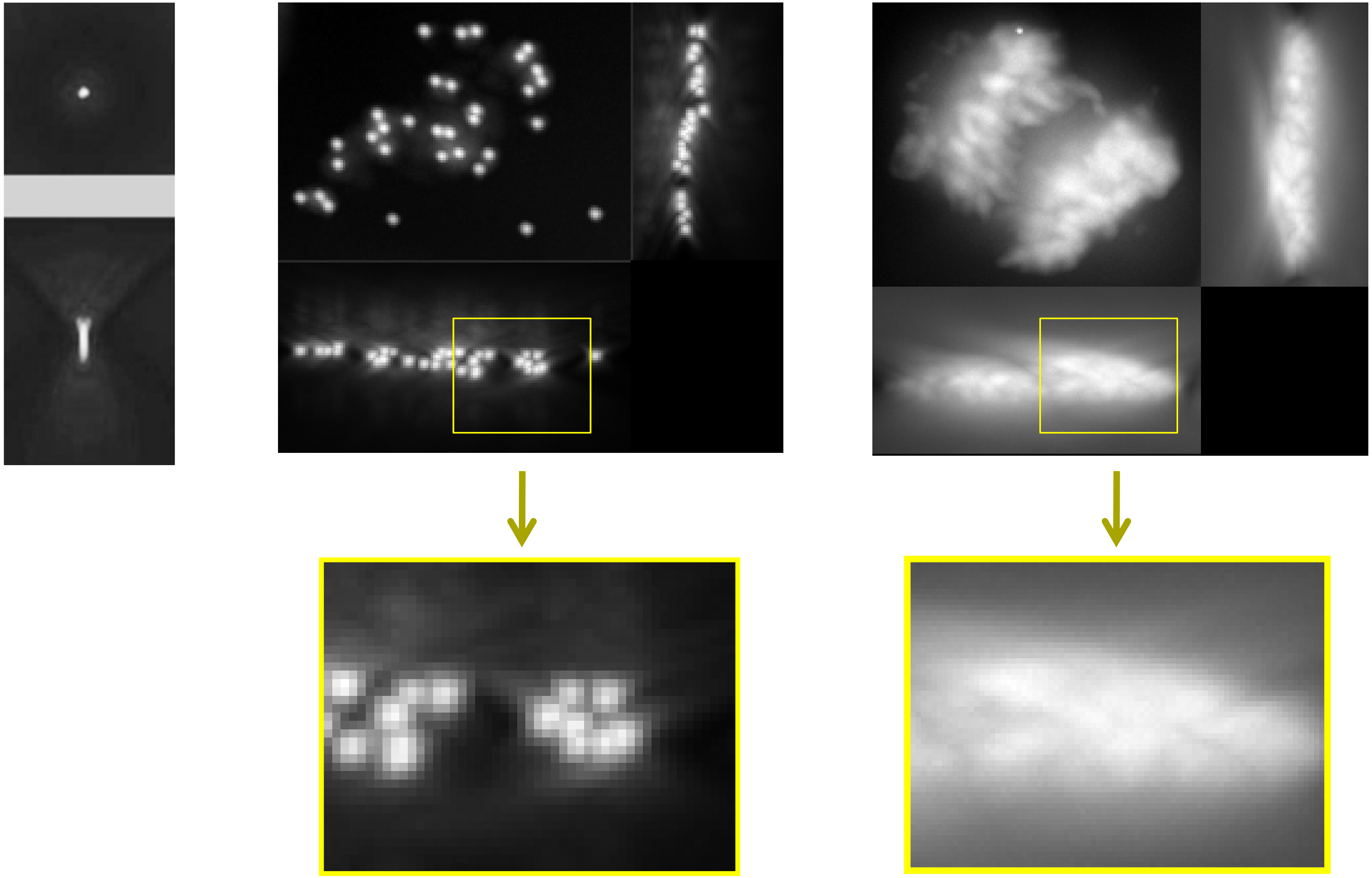
$$I(X,Y,Z) = S(X,Y,Z) \otimes PSF(X,Y,Z)$$

Reconstruction (deconvolution)

$$S = I/PSF$$

Principles of deconvolution

Challenge: complex 3D structures

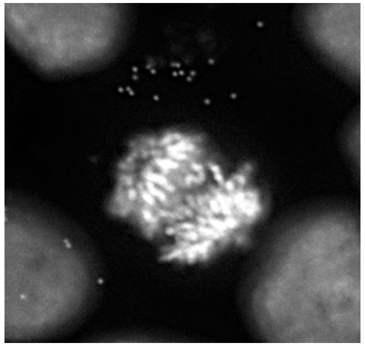


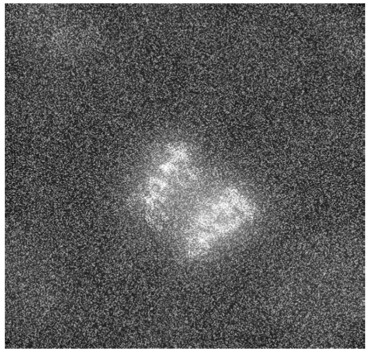
Principles of deconvolution

Challenge: Noise

Problem:
- Adds additional unknown, random component to the image

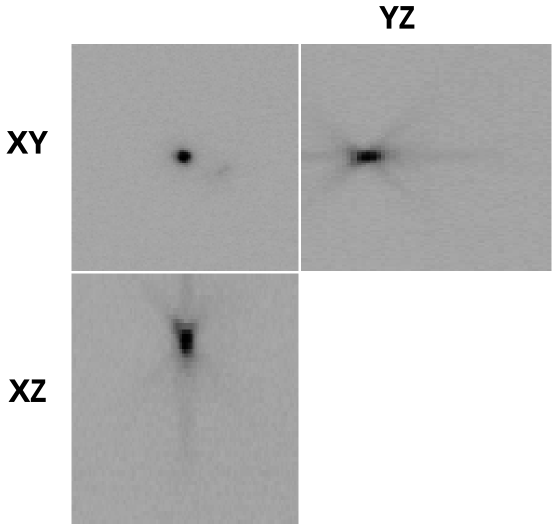
Types of noise:
- Photon noise (statistically irregular photon detection at very low light intensity)
- Detector noise (dark noise, readout noise, amplifier noise), increases with temperature and gain

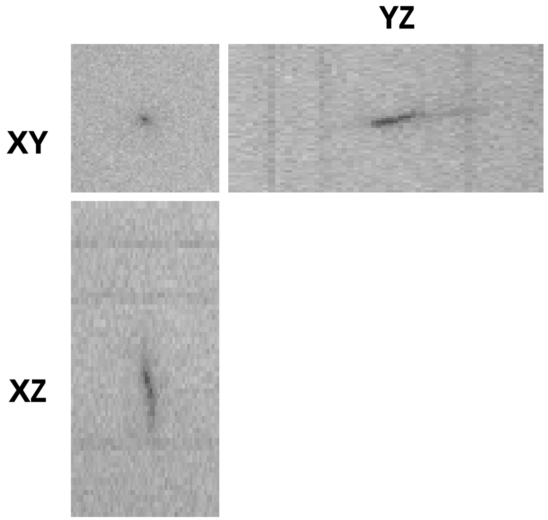




Principles of deconvolution

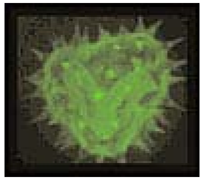
Challenge: Optical aberrations

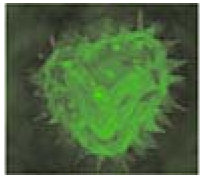




Principles of deconvolution: Algorithms

One-step linear methods





No/Nearest Neighbour:
•deblurring one 2D image slice at a time, comparing it with the one above and below (nearest neighbour)
•approximation that the out-of-focus contribution in the image slice is equal to a blurred version of the collected adjacent slices
•fast but imprecise, heavily affected by noise

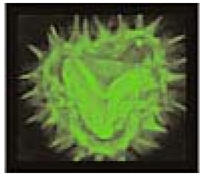
Inverse Filter (Wiener filter):
•image process dividing the captured image by the PSF
•fast and effective to remove the majority of the blur
•noise is managed through adjustable smoothing operation

Advantage:
- Fast (real time)

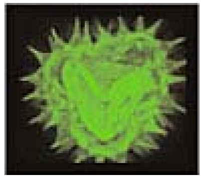
Disadvantage:
- Imprecise
- Removes information (not quantitative)
- Heavily affected by noise and imaging aberrations

Principles of deconvolution: Algorithms

Iterative constrained methods (statistical image restoration)



Non-Blind:
•requires a measured PSF
•PSF is assumed to be accurate



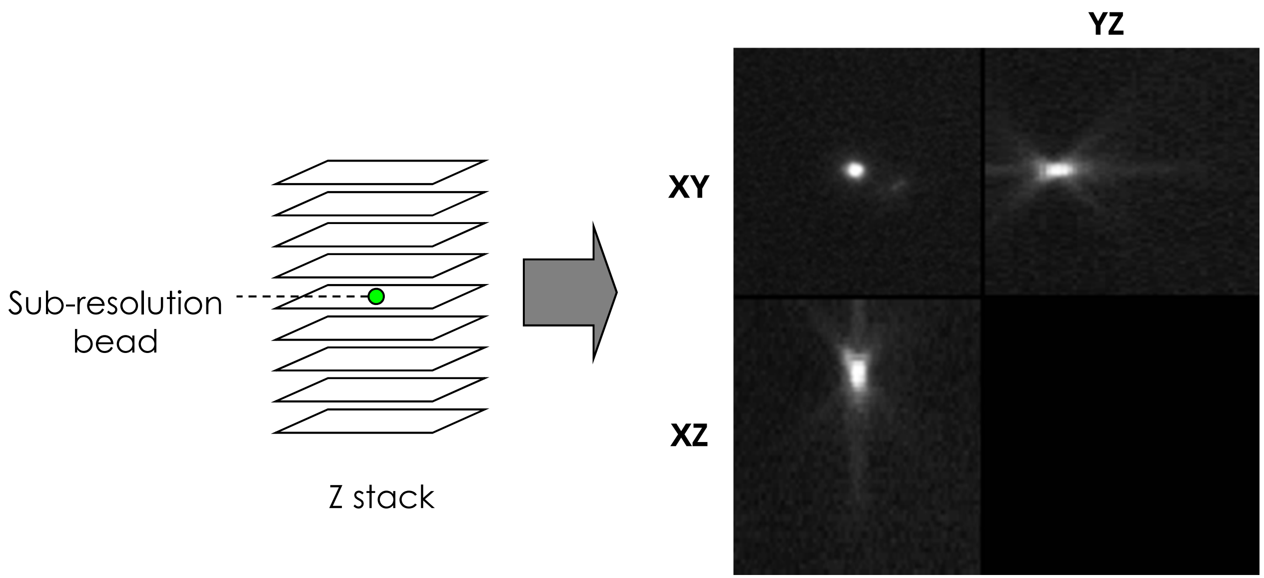
Adaptive Blind:
•iteratively reconstructs both the PSF and best image solution possible from the collected 3D dataset
•statistical techniques of Maximum Likelihood Estimation (MLE) and Constrained Iteration (CI)
•does not require a measured or PSF
•good when noise ratios and / or aberrations are challenging

Advantage:
- Precise
- All information is preserved (quantitative)
- Adaptive (can correct noise and optical aberrations)

Disadvantage:
- Can be extremely computer-intensive

Non-blind deconvolution (measured PSF)

Measuring the PSF

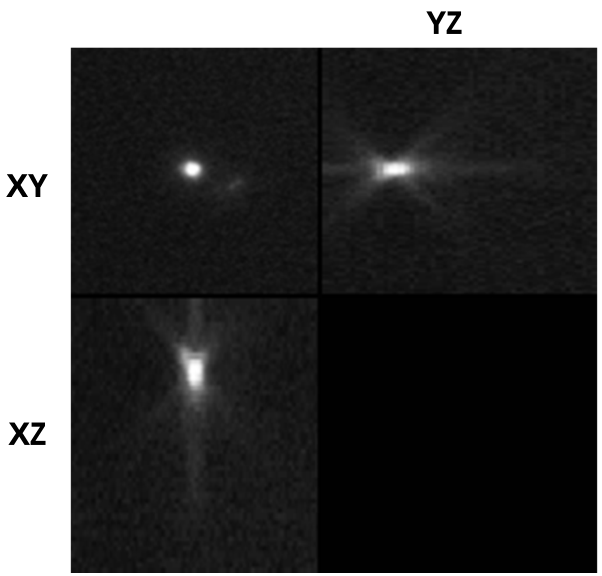


Out-of-focus light is essential for deconvolution!!

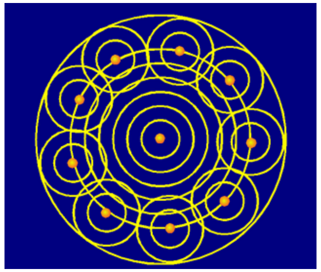
→ don't crop PSF <u>and</u> image it in X, Y, Z, intensity (saturation)

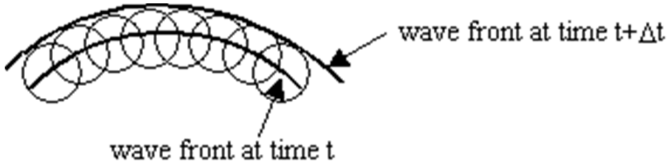
Non-blind deconvolution (measured PSF)

Measuring the PSF

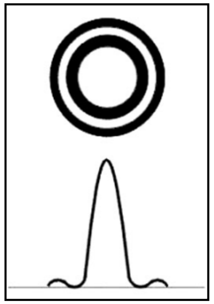


Huygens' Principle (1678; after Christiaan Huygens):
The wavefront of a propagating wave of light at any instant conforms to the envelope of spherical wavelets emanating from every point on the wavefront at the prior instant.





Two-dimensional point spread function of a point source (Airy disk)



Non-blind deconvolution (measured PSF)

Measuring the PSF

Rules for PSF and sample:

- Clean sample, high-quality coverslips
- Z spacing: ≤Nyquist rate (½ Z resolution)

  e.g. widefield, 63x 1.4NA, GFP (Em 520nm): Z = 277nm

  → see online Nyquist calculator online
- Choice of objective:

  Water: least problems with refractive index mismatch

  Oil: least affected by uneven coverslip thickness
- Immersion oil: can be adapted to temperature
- Avoid / correct imaging aberrations

  (uneven illumination / camera sensitivity, sample movement, unstable light)
- Don't crop image and out-of-focus light

  in X, Y, Z, intensity

Non-blind deconvolution (measured PSF)

Measuring the PSF

Rules for PSF :

- Bead size: ≤1/3 of XY resolution ($1.22\lambda/2NA$), usually 150nm
- Imaging conditions as close as possible to sample:
  - ideally beads added to sample
  - Single beads (PSFs not overlapping)
  - Same NA, fluorescence, objective, NA, mounting medium, temperature, …
  - Same distance to coverslip
- Optimal image quality (averaging)

→ *must be reimaged whenever any part of the imaging system changes!*

Non-blind deconvolution (measured PSF)

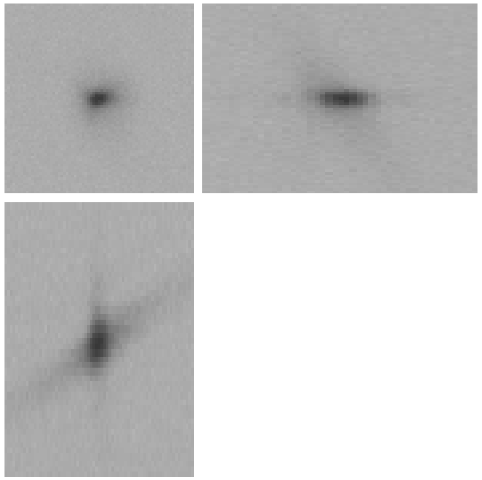
(Dis)Advantages of measured PSF

Advantage:

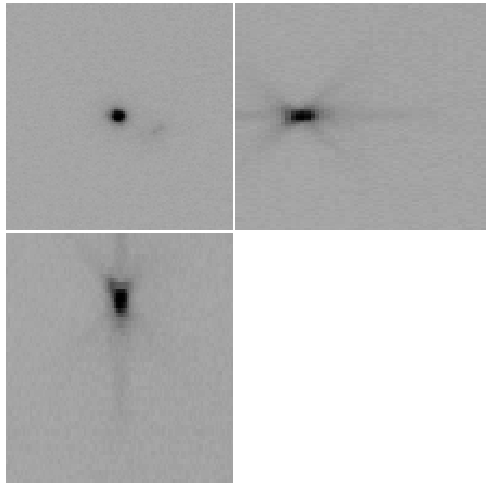
- Accounts best for any aberrations specific for the acquisition setup (individual aberration of lenses, mounting medium, ...)
- Speeds up computation (fewer iterations, 10-100)

Disadvantage:

- Acquiring a perfect PSF is virtually impossible:
  - Noise
  - Imaging conditions not identical to sample
  - Changes with distance to coverslip



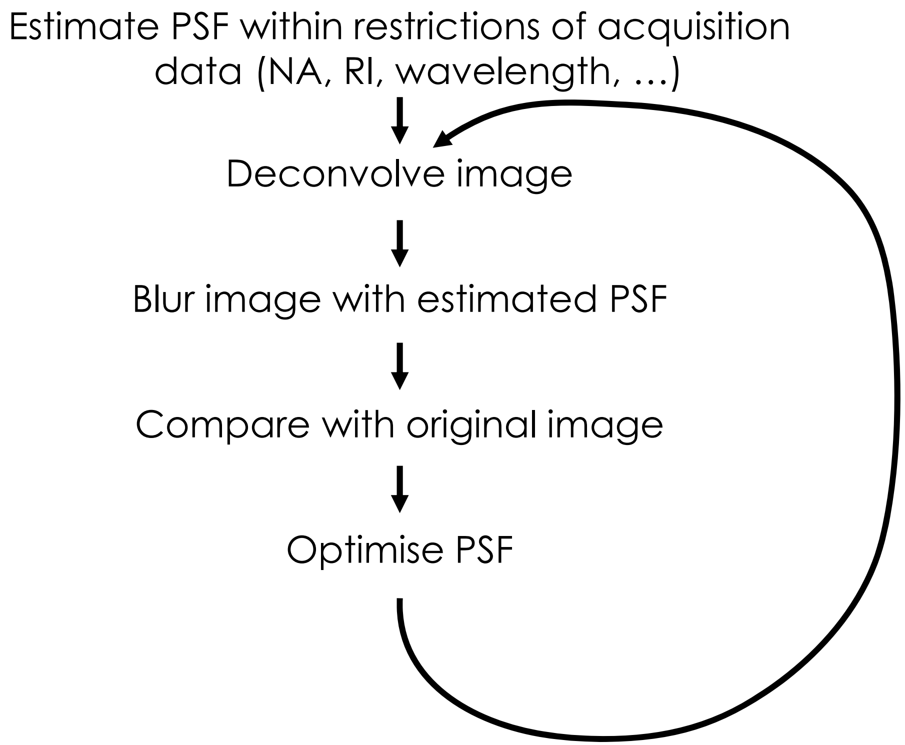
blue



red

Blind deconvolution (calculated PSF)

Tries to solve the convolution problem for both the image and the PSF from a single dataset



$$\hat{h}^k_{i+1}(x,y,z) = \left\{ \left[\frac{g(x,y,z)}{\hat{h}^k_i(x,y,z) \otimes \hat{f}^{k-1}(x,y,z)} \right] \otimes \hat{f}^{k-1}(-x,-y,-z) \right\} \hat{h}^k_i(x,y,z),$$

$$\hat{f}^k_{i+1}(x,y,z) = \left\{ \left[\frac{g(x,y,z)}{\hat{f}^k_i(x,y,z) \otimes \hat{h}^{k-1}(x,y,z)} \right] \otimes \hat{h}^{k-1}(-x,-y,-z) \right\} \hat{f}^k_i(x,y,z),$$

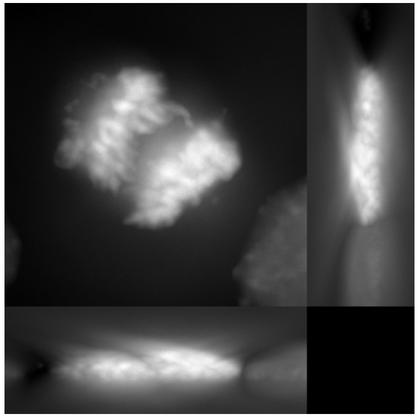
Non-blind deconvolution (measured PSF)

Needed information

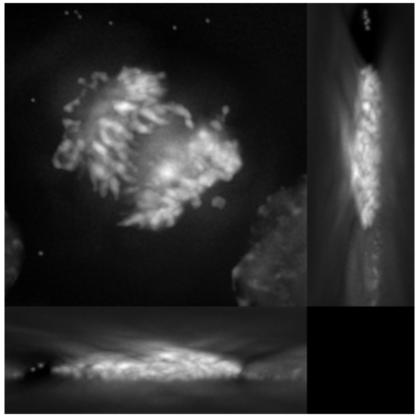
- Imaging mode (widefield, confocal, …)
- Magnification
- Numerical aperture
- Pixel dimensions (X,Y,Z)
- Refractive index immersion oil
- Refractive index mounting medium
- Thickness coverslip (water objectives)
- Emission wavelength
- Distance from coverslip

Non-blind deconvolution (measured PSF)

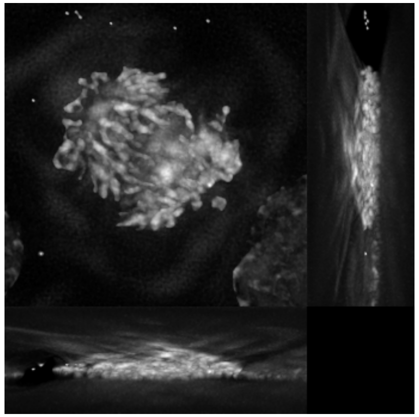
Examples



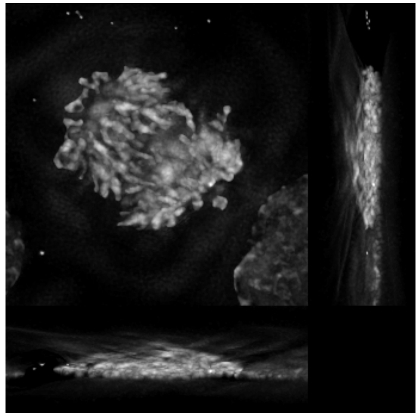
original



10



100



1000 iterations

Blind deconvolution (calculated PSF)

(Dis)Advantages of calculated PSF

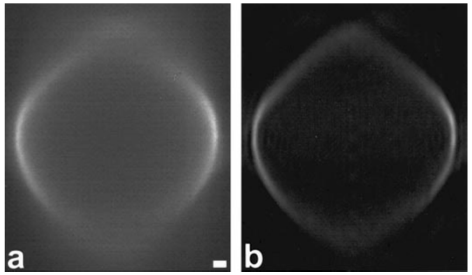
Advantage:
- Most flexible and adaptive
- Accounts for variations within an image (in XY, variable distance from coverslip)
- The object estimate converges to the most accurate solution as defined by imaging model
- Good when noise and / or aberrations are challenging

Disadvantage:
- One more unknown variable at beginning of deconvolution
- Even more computer intensive (can be >1,000 iterations)
- No PSF quality control during acquisition

Limits of deconvolution

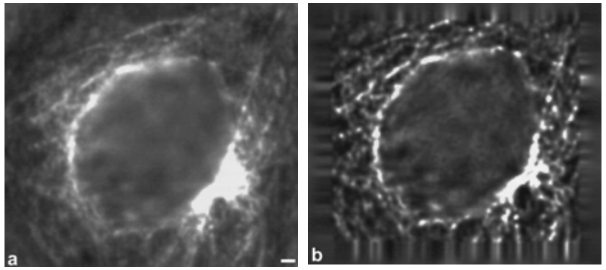
Potential pitfalls and artefacts

Z elongation



Prevention:
confocal (+ deconvolution)

Edge artefacts

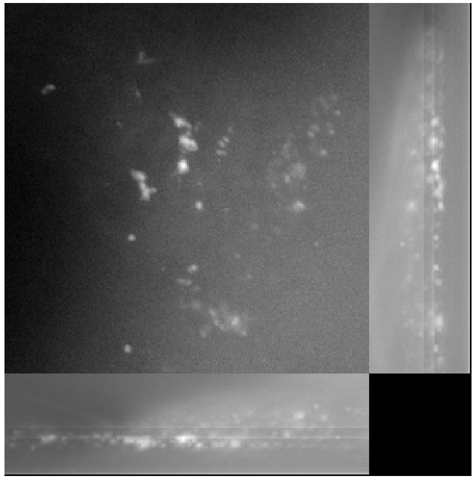


Prevention:
object central,
sufficient extra space in X, Y, Z, intensity

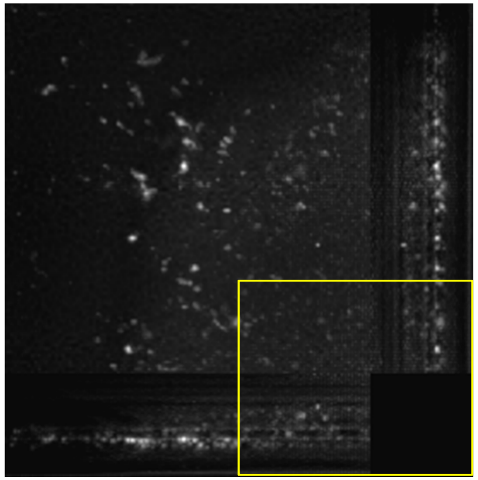
Limits of deconvolution

Potential pitfalls and artefacts

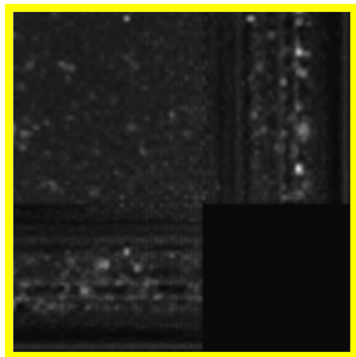
Noise artefacts

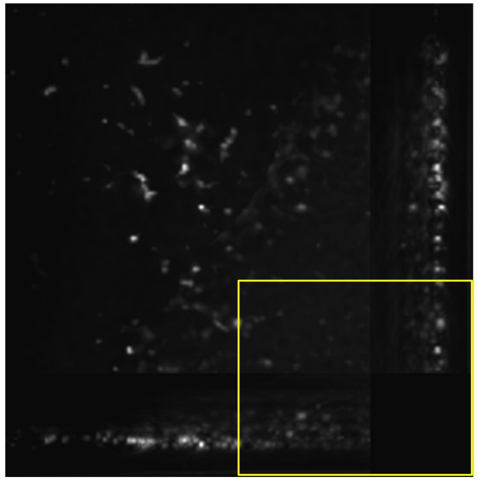


Original

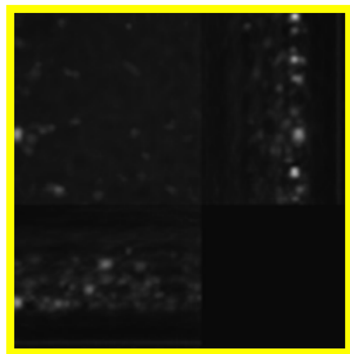


500 iterations blind,
Noise level set to ‘low’





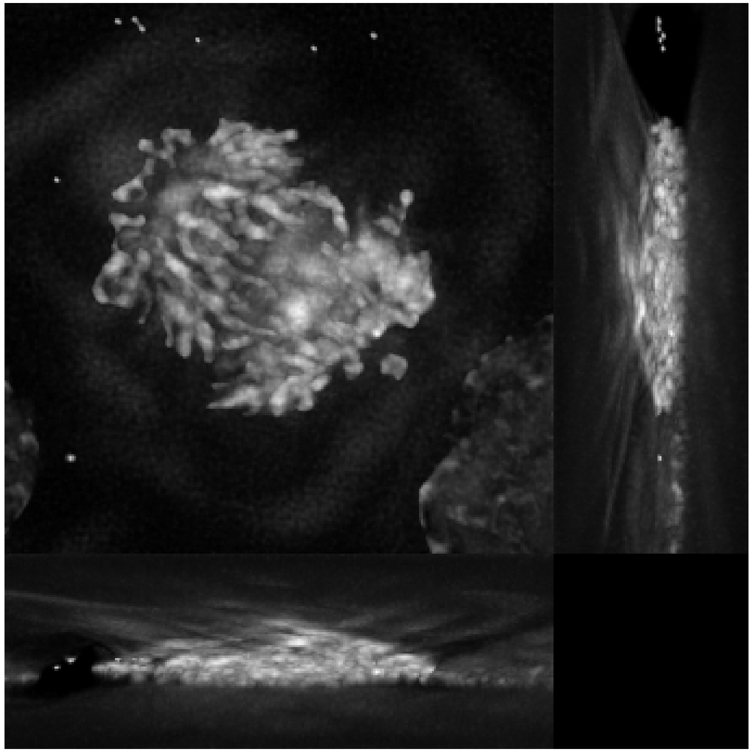
500 iterations blind,
Noise level set to ‘high’



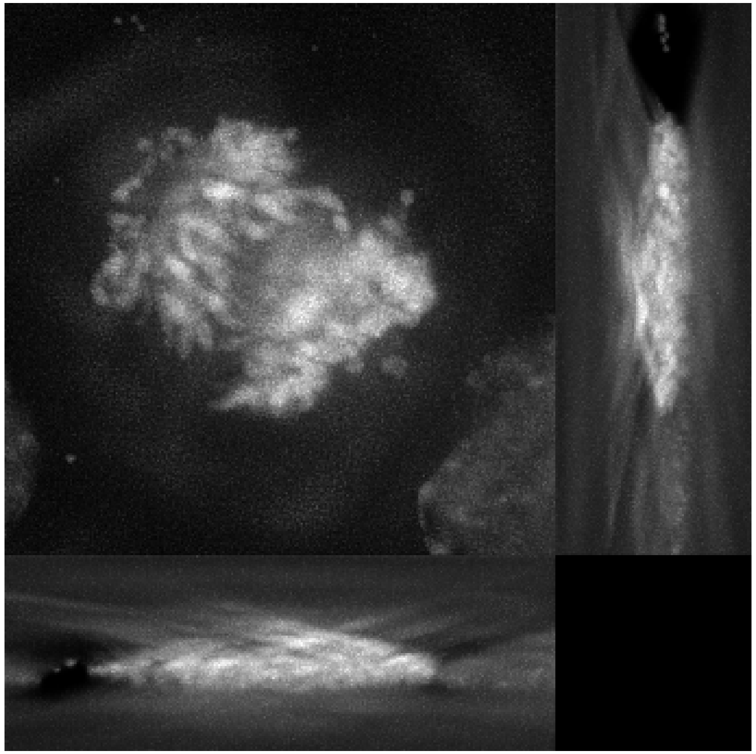
Limits of deconvolution

Potential pitfalls and artefacts

Noise artefacts



100 iterations blind,
Noise level 'medium'



100 iterations blind,
Noise level 'low'

Summary: What does deconvolution do

Golden rule of deconvolution*:
"Rubbish in, rubbish out"

- Quantitative method to improve the information content of a 3D image
- Allows to generate accurate 3D data from low-light imaging
- No 'best' method, blind and non-blind iterative methods have their advantage, of in doubt best try both
- Deconvolution limited by image quality, noise, aberrations ***(bottom line: Structures must be visible in original data)***
- Very efficient for structured images, impossible for diffuse stainings (e.g. "cytosolic")
- Can also be used to improve confocal images, especially if the pinhole has to be opened

**...and microscopy in general*