Daniel Sage & Arne Seitz

Course

3D Deconvolution Microscopy

Deconvolution

"In mathematics, deconvolution is an algorithm-based process used to enhance signals from recorded data. Where the recorded data can be modeled by a convolution"

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Wikipedia

"Deconvolution is a computational method to improve digital image quality by using knowledge of the way the microscope forms images"

***

David Agard

YouTube CH

"Deconvolution is a computationally intensive image processing technique used to improve the contrast and sharpness of images captured using a microscope"

***

Olympus

Goals

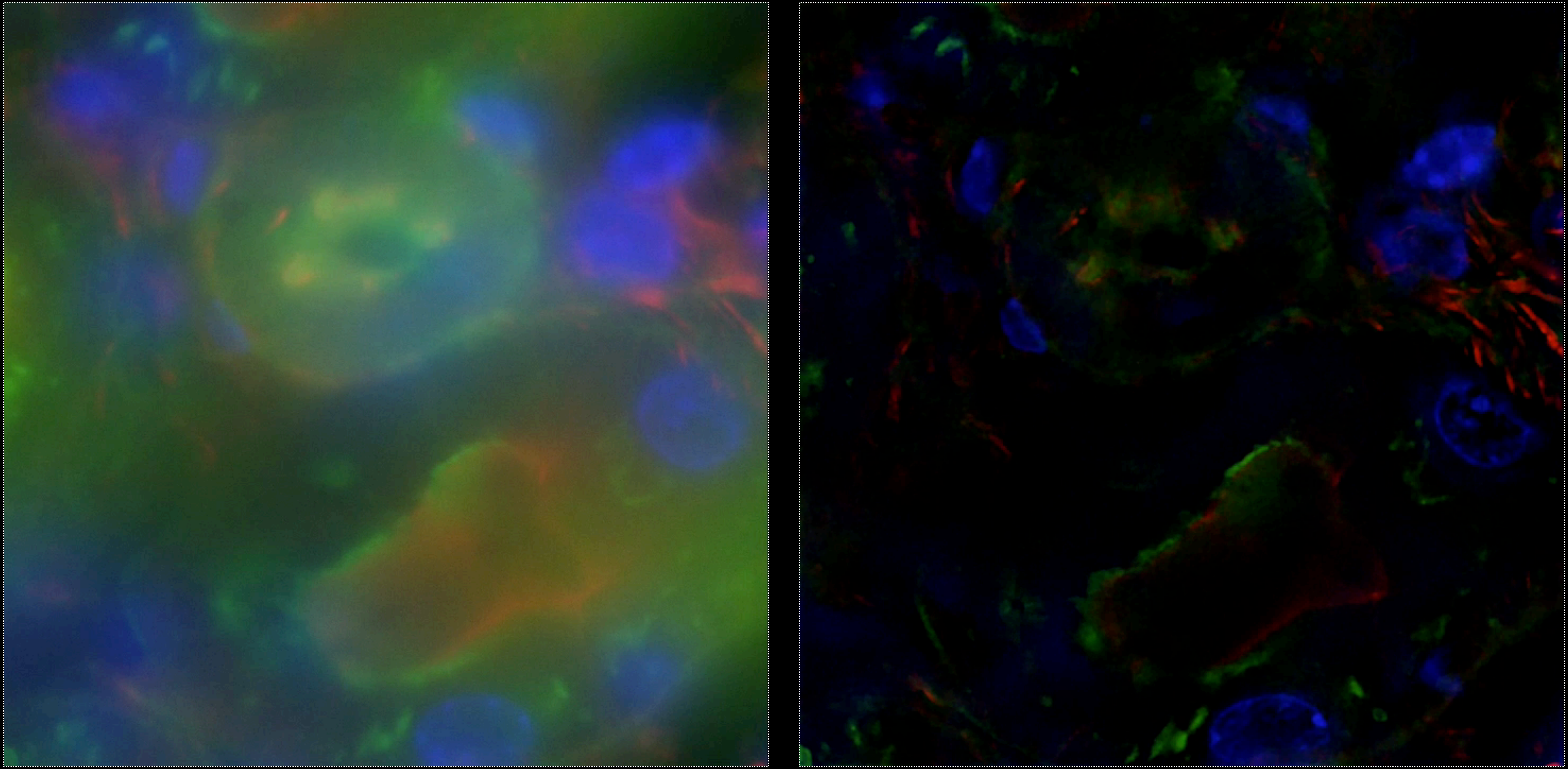
Improve quality
Enhance contrast
Improve resolution?

Image formation model

Image formation models
Using knowledge
Convolution model

Computational methods

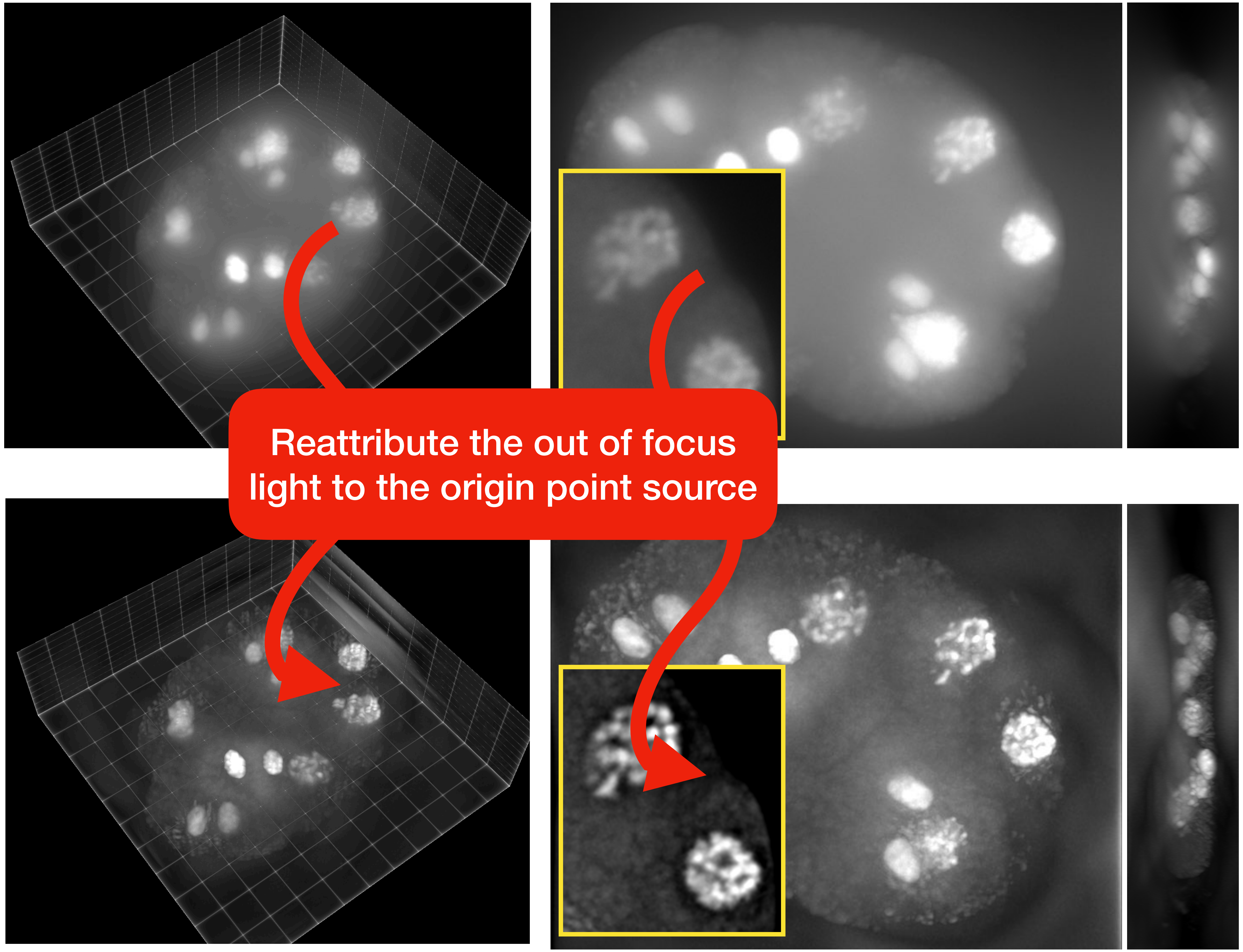
Algorithm-based process
Computational method
Intensive technique



Courtesy of Ferréol Soulez

Why Deconvolution?

c-elegans embryo. DAPI nuclei



DeconvolutionLab2 TIRF 0.0004

Core idea

Put the photons back where they belong

No signal is lost

Benefits

Increases contrast

Reduces noise

Improves resolution

Enhances small structures

Promotes the optical sectioning

Usage

Preprocessing step

Simplification for segmentation

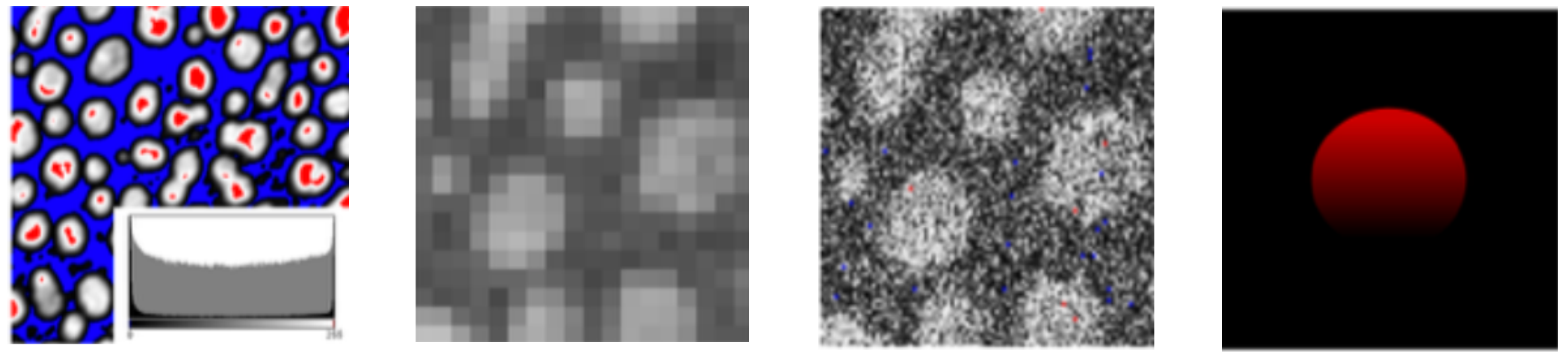
Quantification of intensity

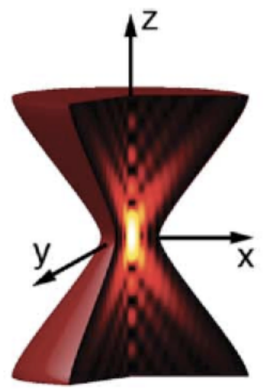
Deconvolution in Microscopy

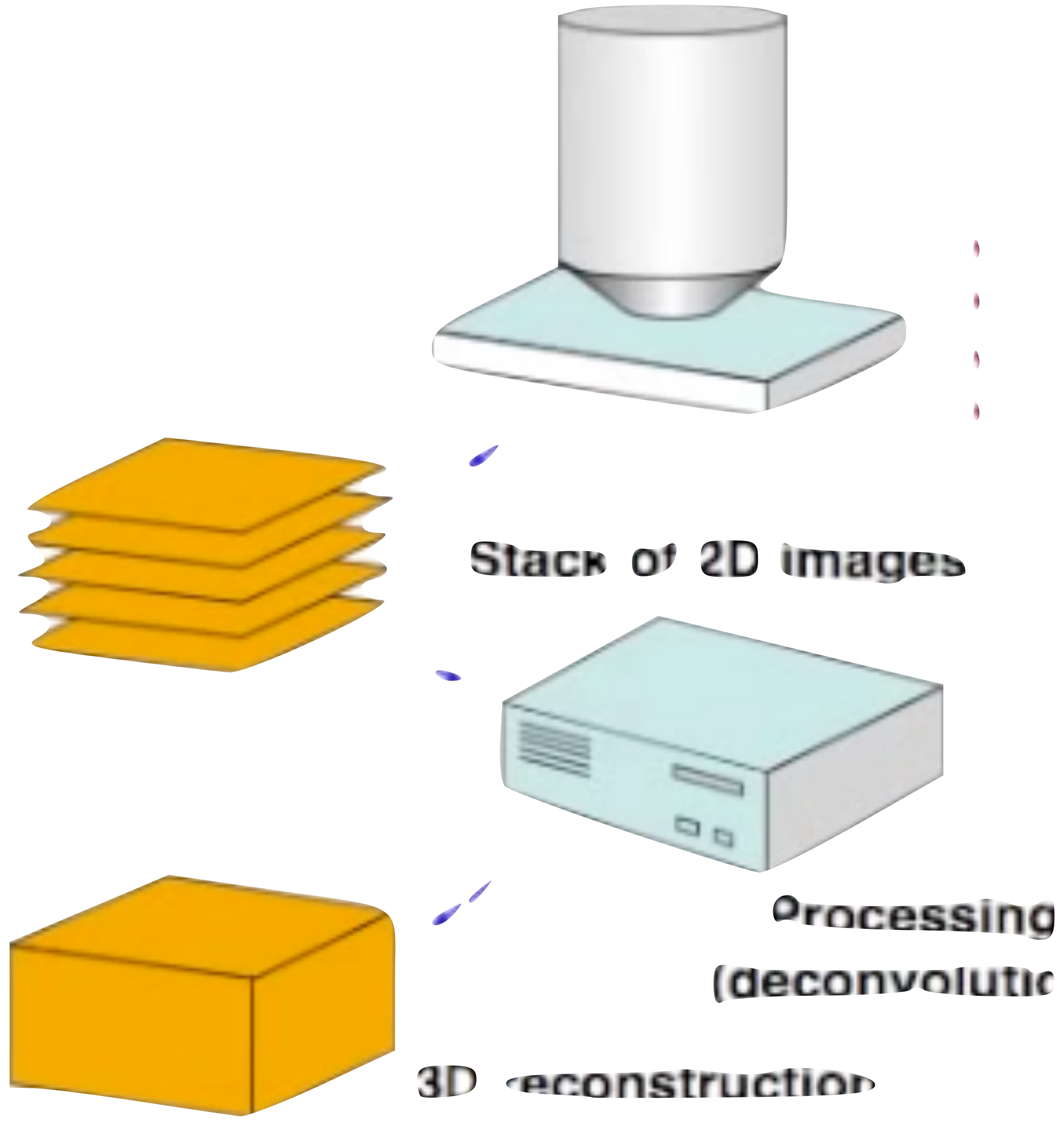
convolution	**image formation**	unknown
high dynamic preserve (bleach)	**signal**	noise, saturation high sampling
fine, detail	**structure**	smooth
known, signature	**PSF**	shift variant
3D	**out-of-focus**	2D
automatic quantitative	**image analysis**	observation qualitative

BEST PRACTICES

Acquisition and computational

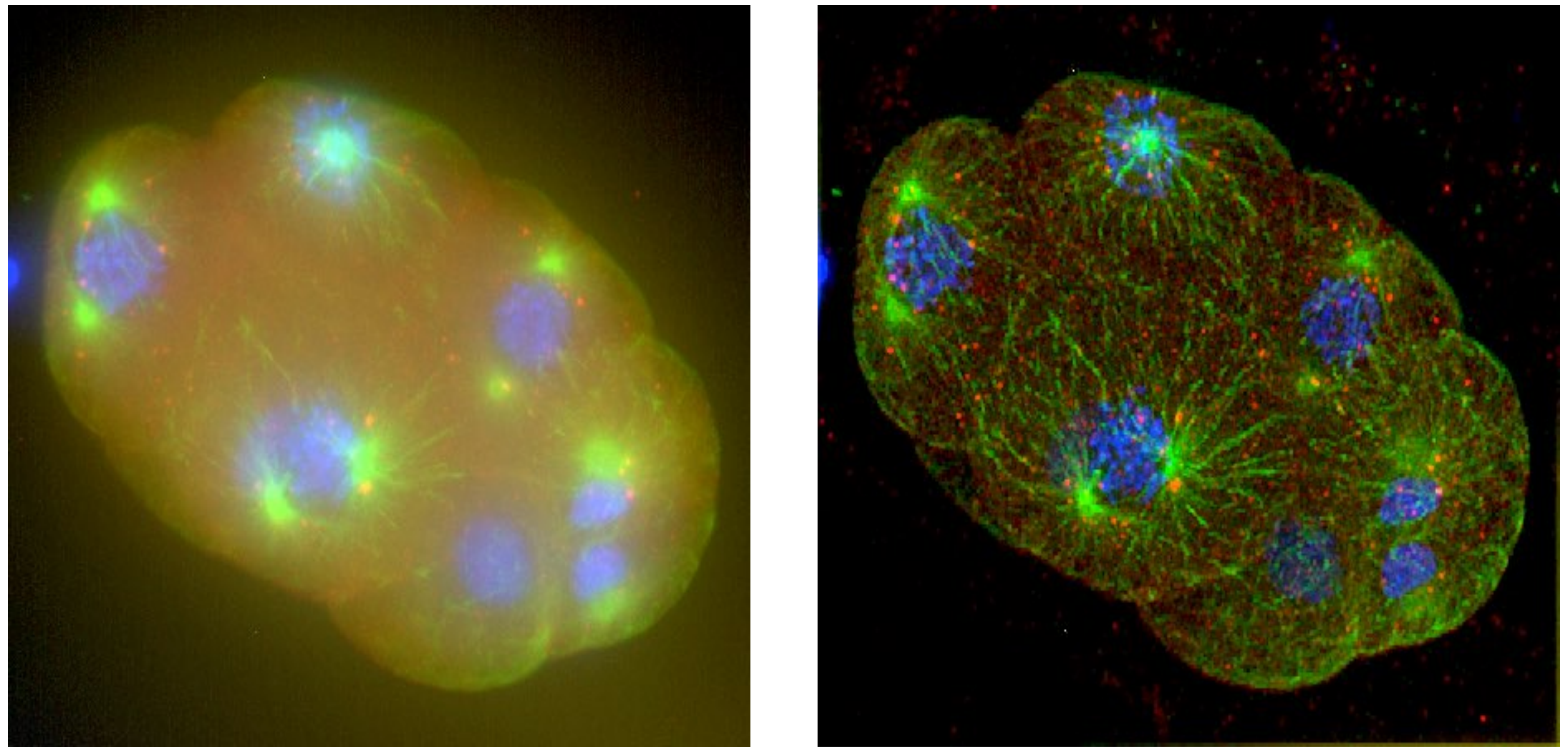






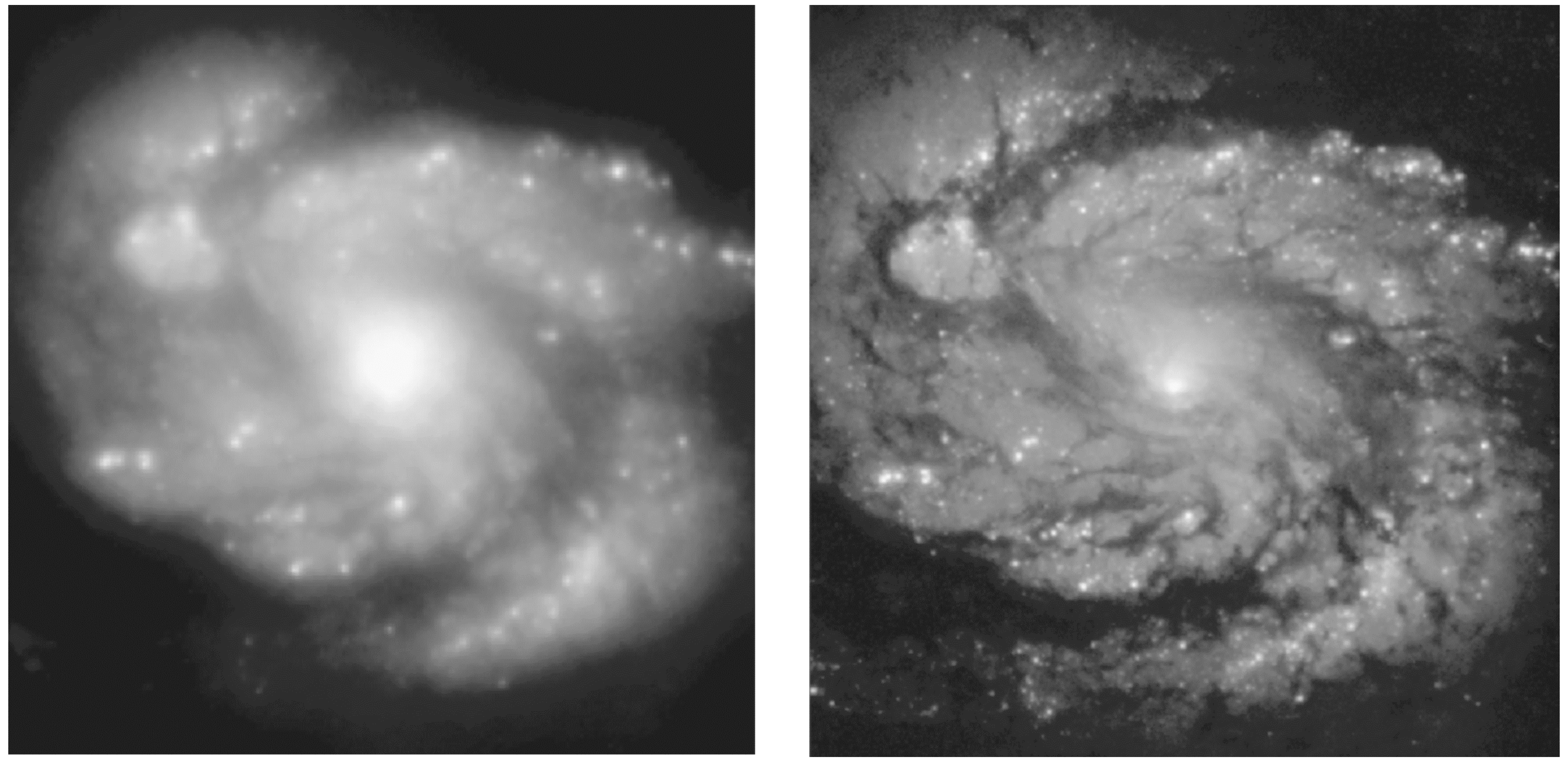
Application Cases

Light microscopy



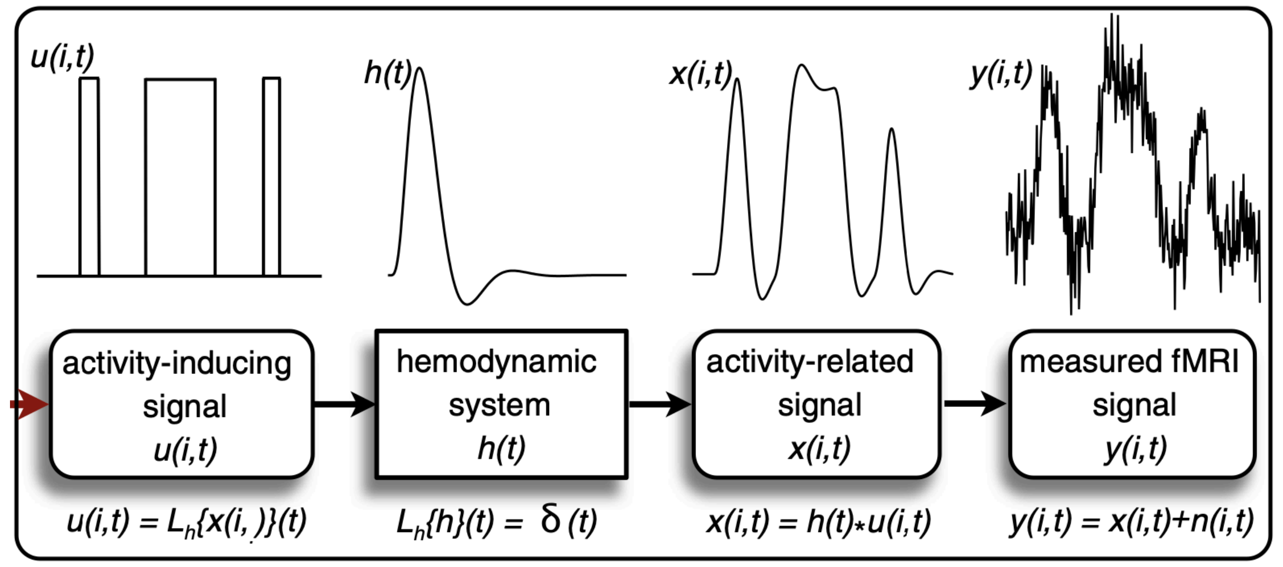
c-elegans embryo. DAPI (nuclei in blue), FITC (microtubules in green) and Cy3 (proteins in red) staining

Astronomy



J. L. Starck, 2002

Total activation in fMRI: spatio-temporal deconvolution



Işık Karahanoğlu,, NeuroImage 2013

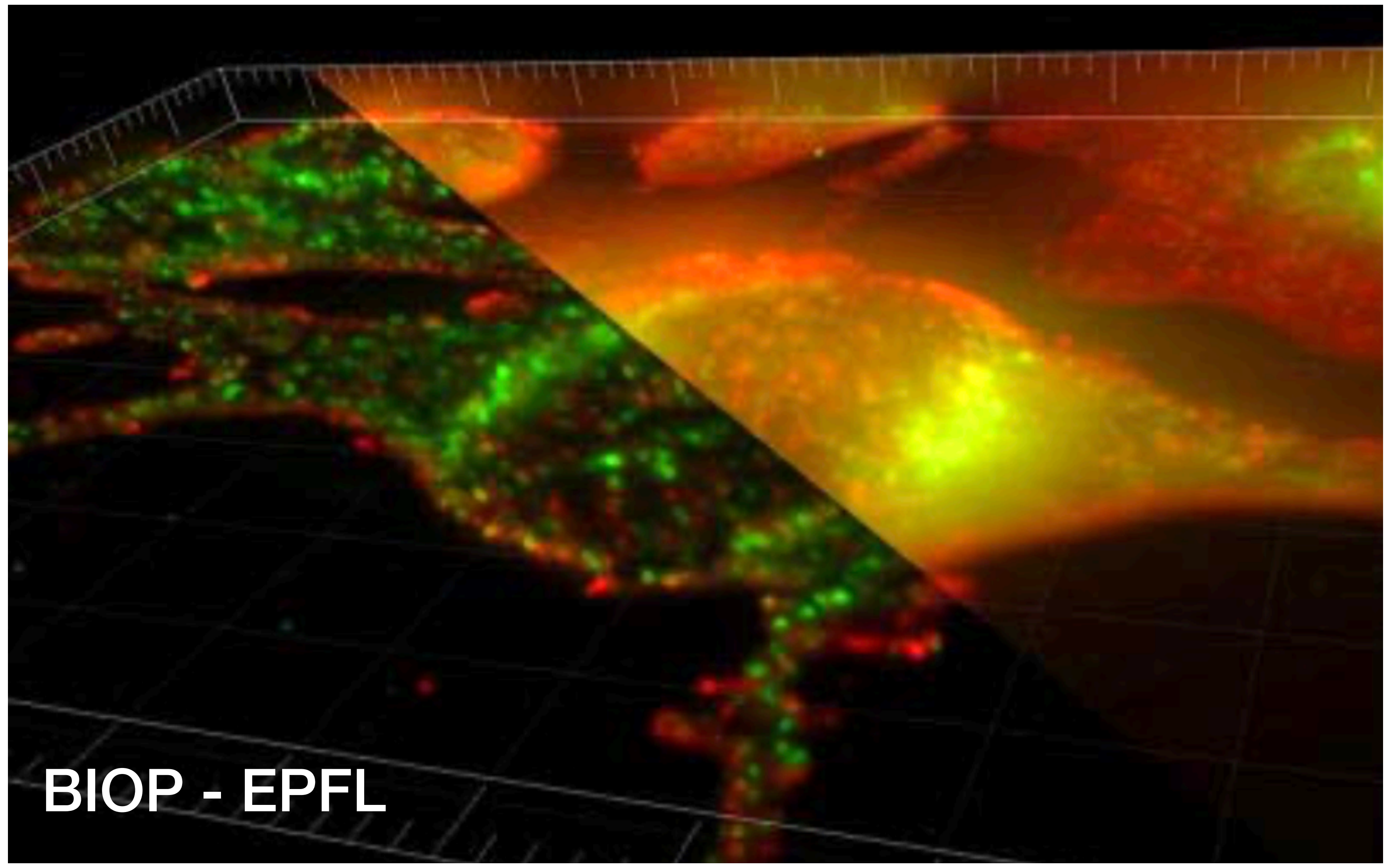
Many fields

- Seismic
- Satellite imaging
- Medical Imaging
- Ophthalmology
- Imaging reconstruction
- Scanning EM (beam)
- Communication (speech)
- Industrial vision

Deconvolution in Microscopy

Active field of research

- Algorithmic aspects: optimization, regularization
- Integration of prior knowledge
- Fast: GPU, block decomposition, vector acceleration
- Deep-learning network
    - learn the structure specimen
    - learn the physical model
- Blind deconvolution, parameter-free deconvolution



Review papers

D. Agard Optical Sectionning Microscopy: Cellular Architecture in Three Dimensions, Ann. Rev. Biophys. Bioeng. **1984**.

J. McNally et al. Three-dimensional imaging by deconvolution microscopy, Methods, **1999**.

W. Wallace et al. A Workingperson's Guide to Deconvolution in Light Microscopy, BioTechniques, **2001**.

J.-B. Sibarita, Deconvolution microscopy, Microscopy Techniques, **2005.**

P. Sarder et al. Deconvolution Methods for 3-D Fluorescence Microscopy Images, IEEE SPM, **2006.**

E. Maalouf Contribution to fluorescence microscopy 3D thick samples deconvolution, Thesis, **2010.**

D. Sage et al. DeconvolutionLab2: An open-source software for deconvolution microscopy, Methods, **2017.**

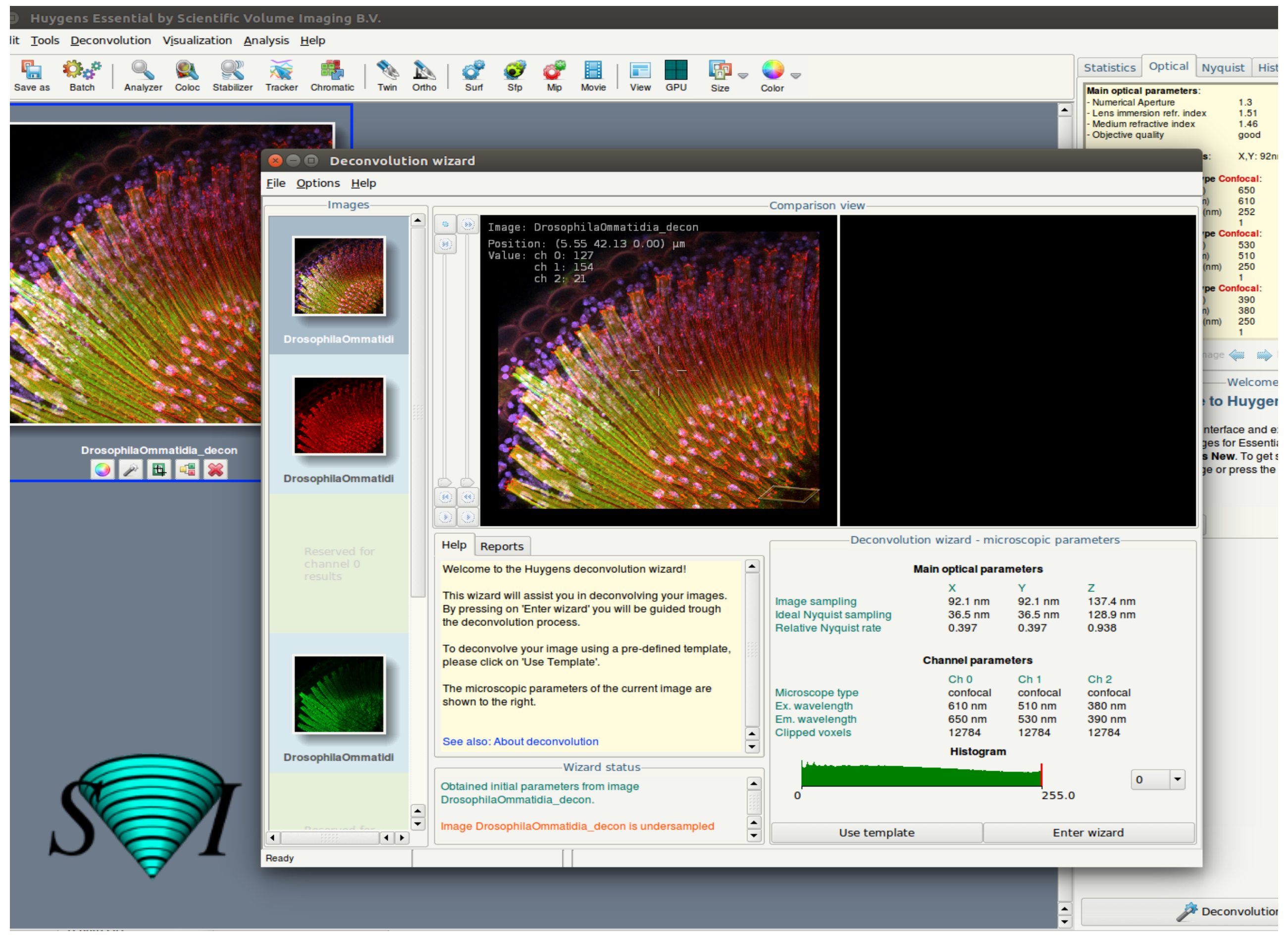
Software for Deconvolution Microscopy

Commercial Software

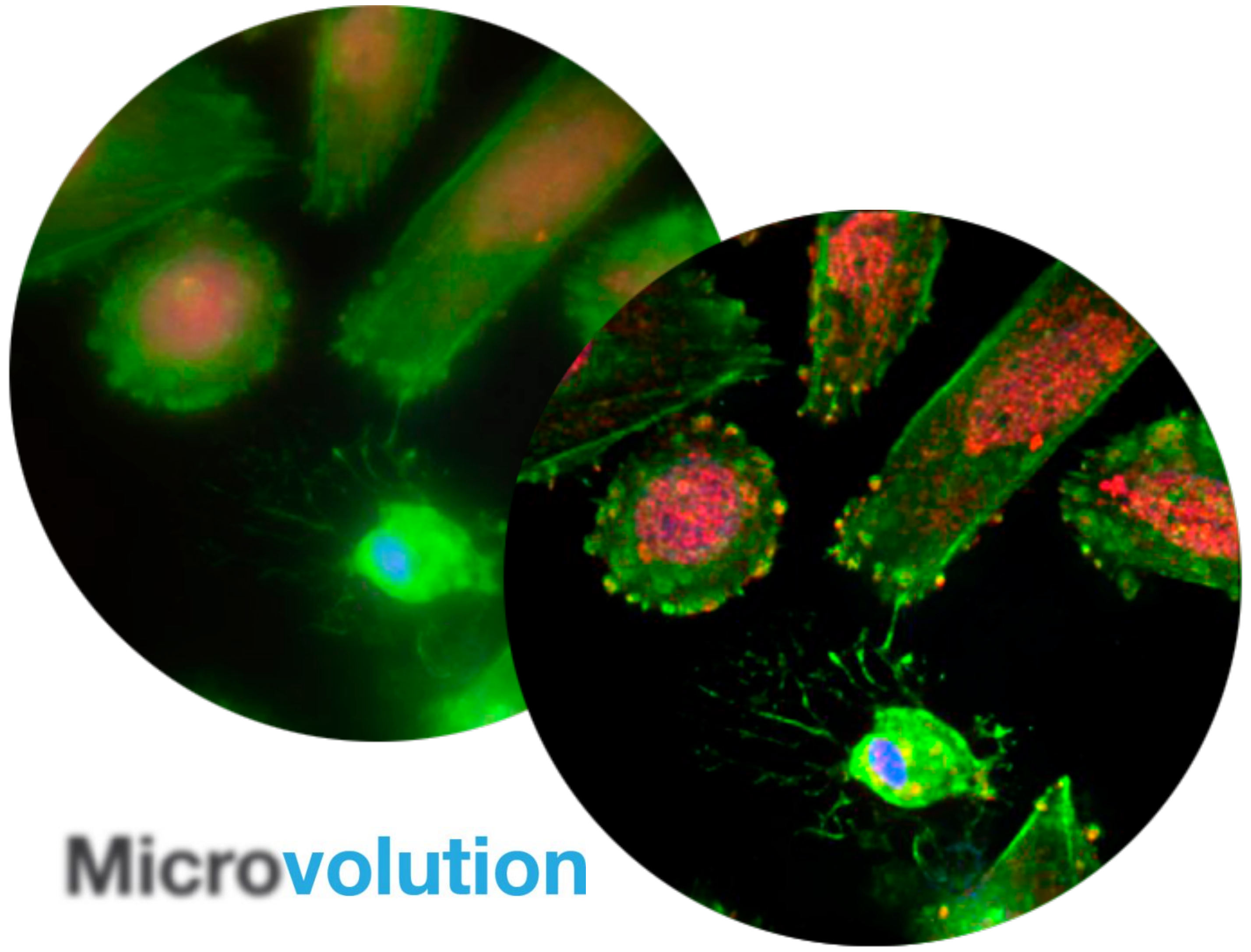
- Huygens, Scientific Volume Imaging
- Microvolution (RL, GPU)
- AutoQuant, MediaCybernetics
- DeltaVision, Applied Precision
- Modules: Zeiss, Nikon, Leica (Hyvolution), …

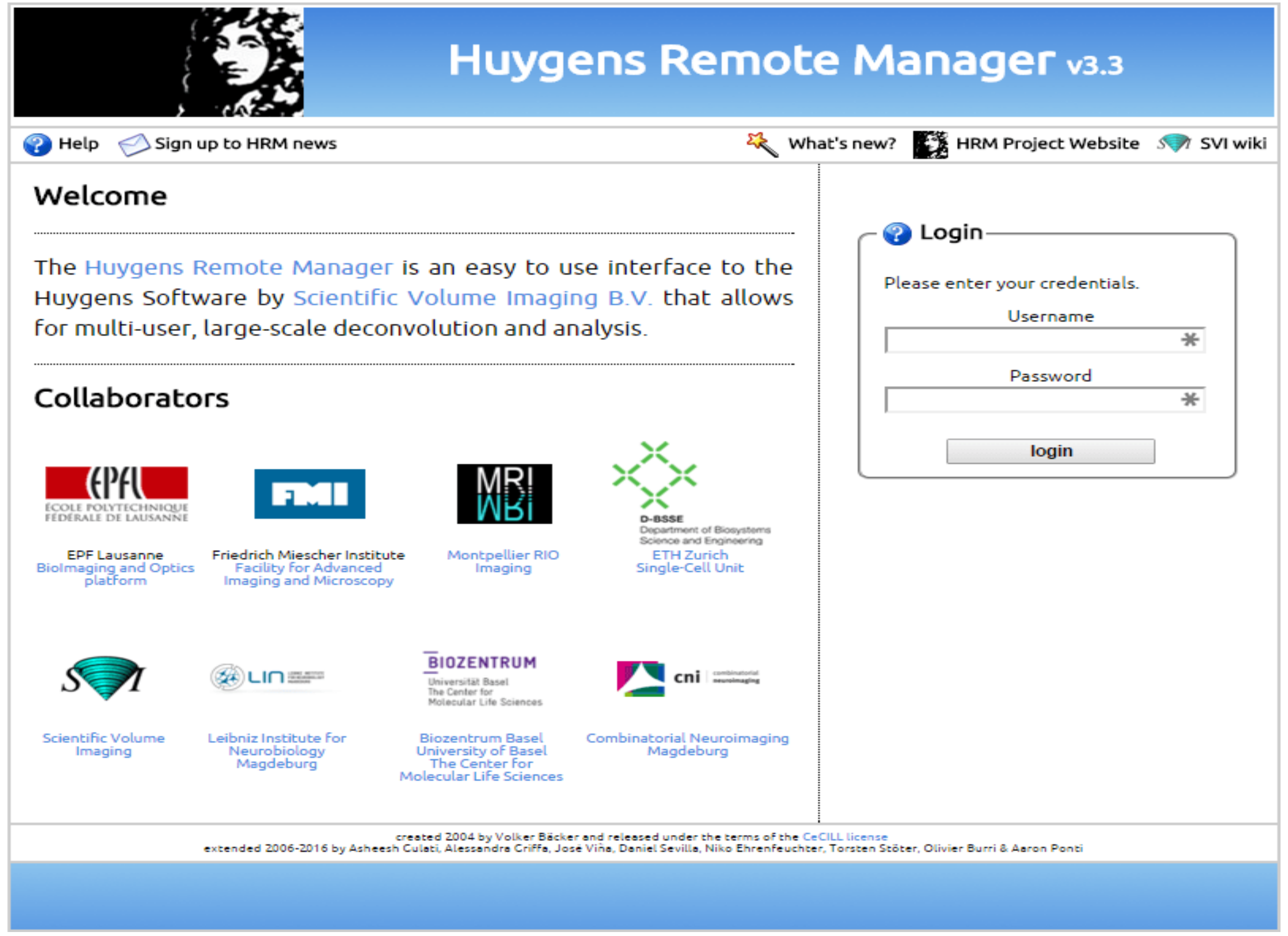
Open-source software

- RL Deconvolution on Ops ImageJ2 [Brian Northan]
- RL Deconvolution on CLIJ /GPU [Robert Haase]
- DeconvolutionLab2 [Daniel Sage]
- Parallel Iterative Deconvolution [Piotr Wendykier]
- Sdeconv on Napari [Sylvain Pringent]
- EpiDEMIC on ICY [Ferréol Soulez]



SVI Huygens





[Ponti 2007]

Mathematical Model

Computer Vision
Machine Learning
Deep Learning

Deblurring
Deconvolution
Sharpening
Upsampling
Denoising
Super-Resolution

Notation

Image	$\boldsymbol{x} = (x_1, \ldots x_d) \in \mathbb{R}^d$	Vectorial notation
Filter/Operation	$\mathbf{Hx}$	Matrix notation

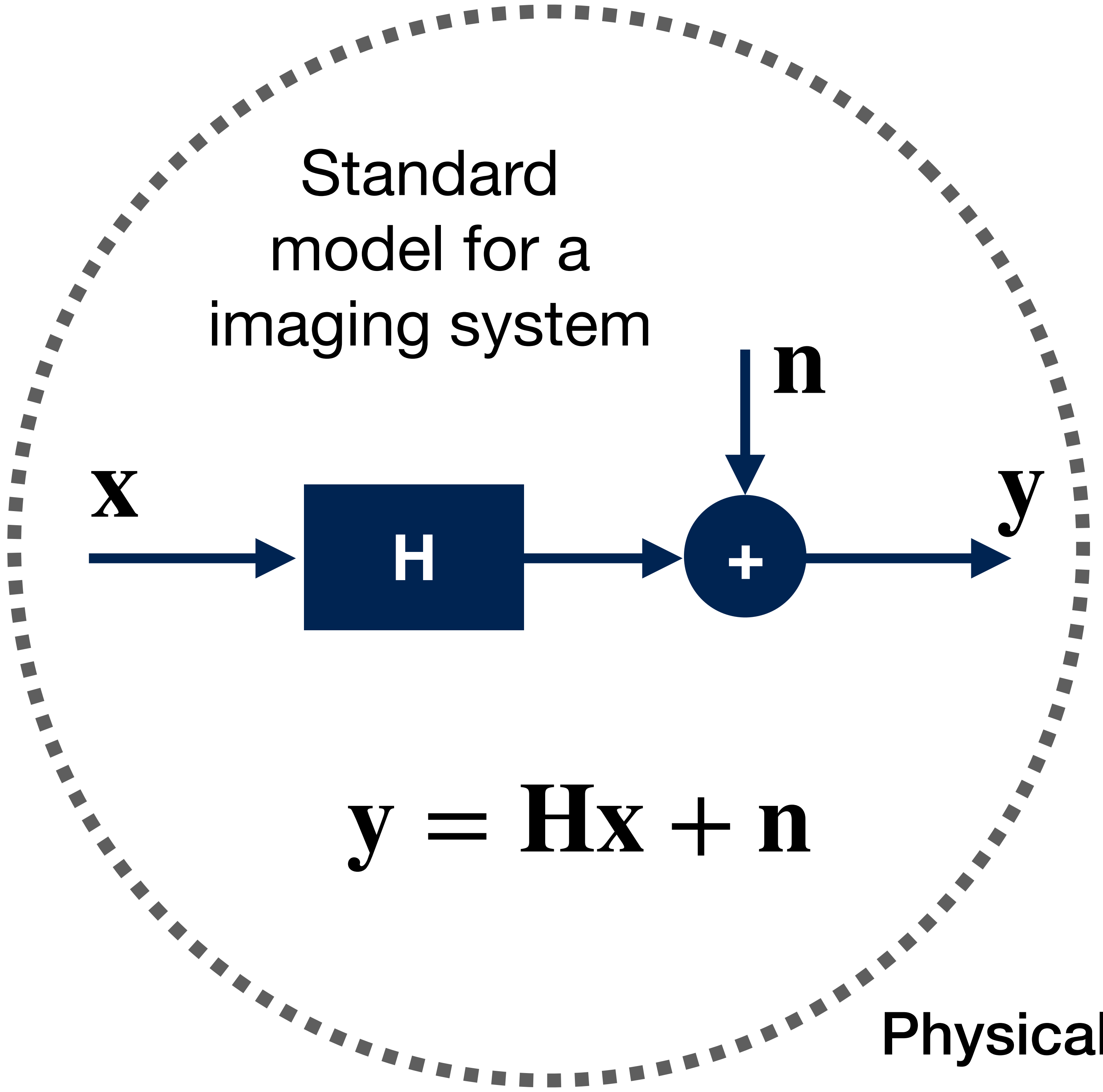


IMAGE DEGRADATION

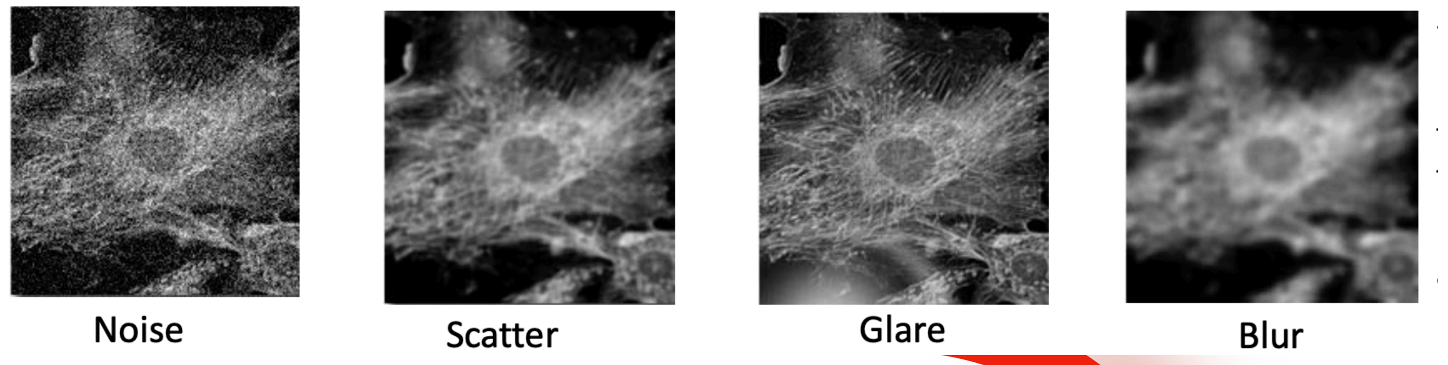
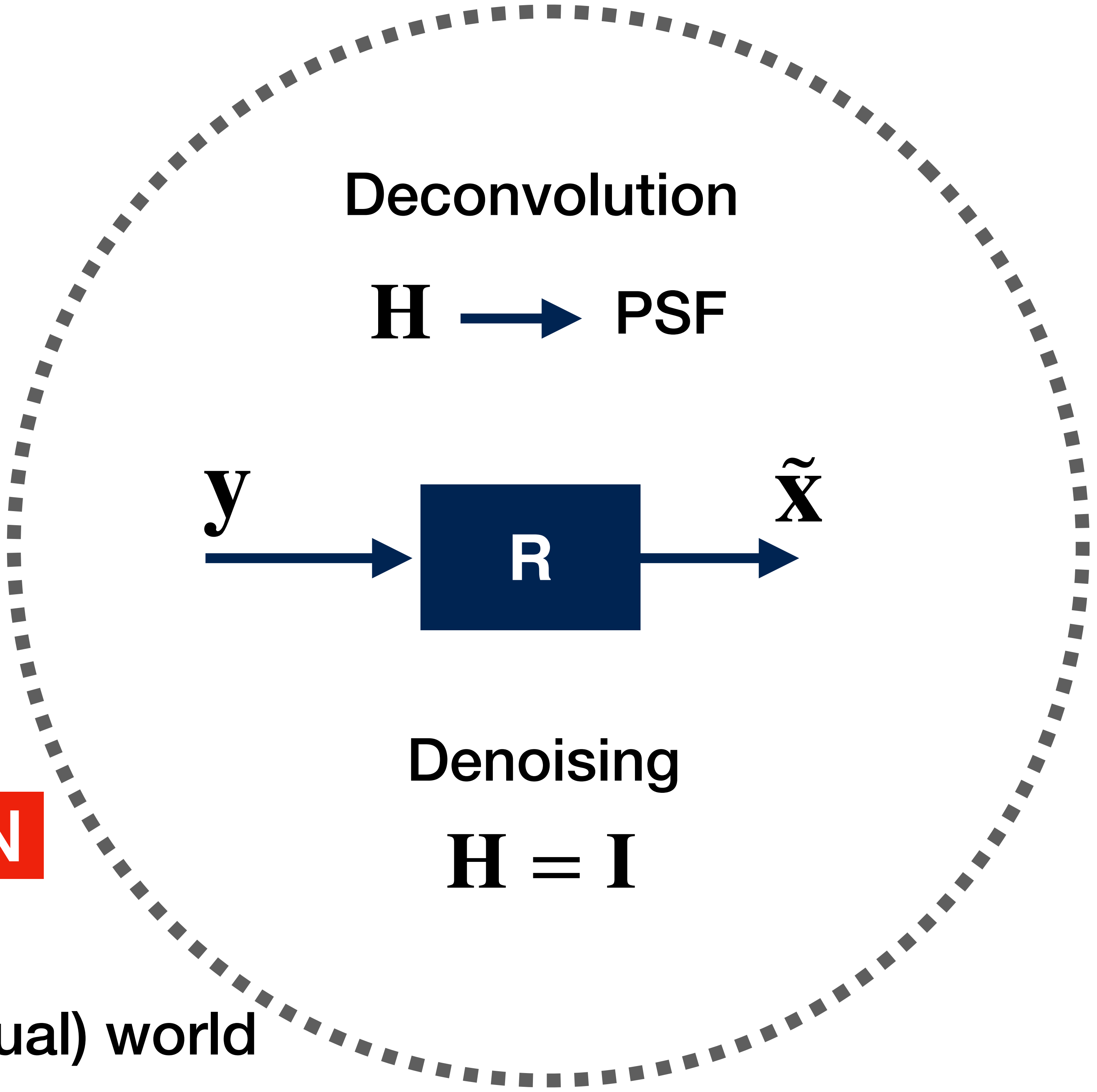


IMAGE RESTORATION

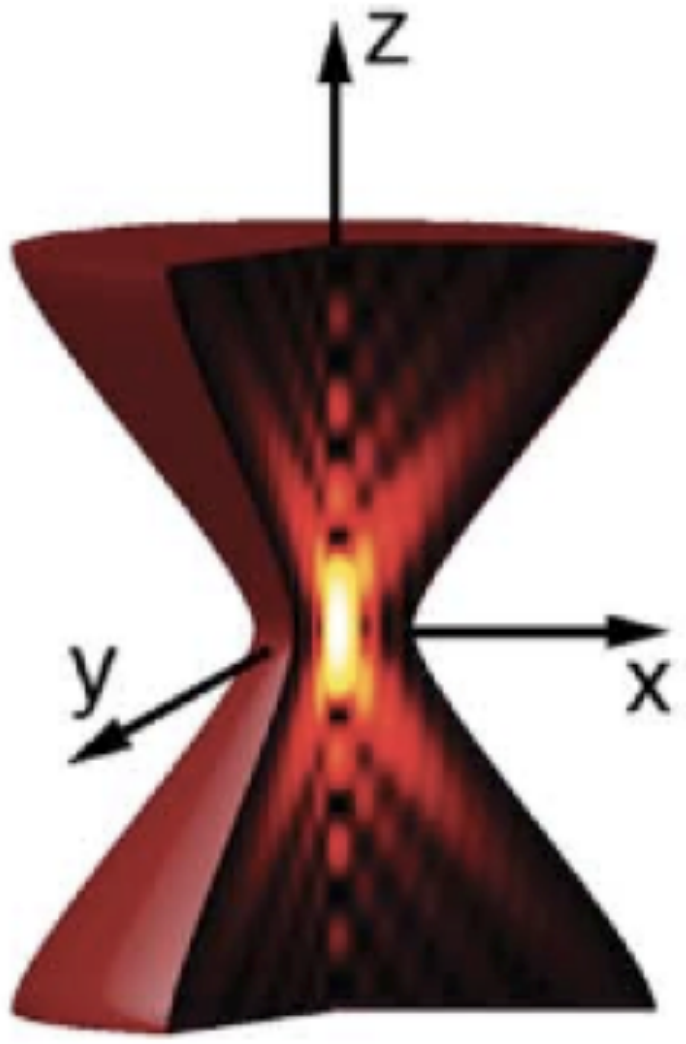


Physical (real) world

Digital (virtual) world

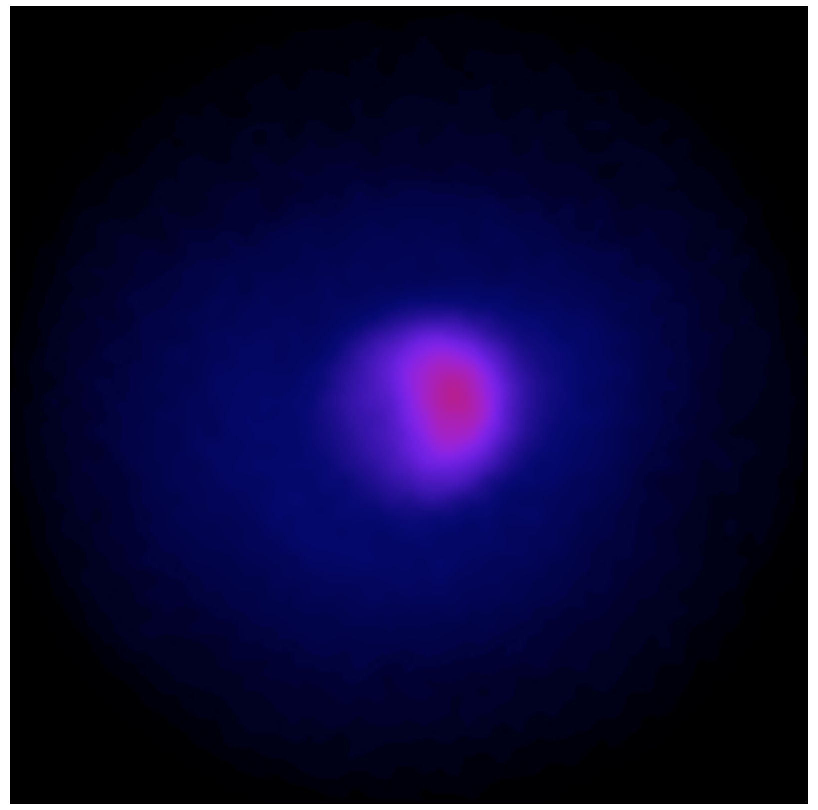
3D Deconvolution Microscopy

Forward Model

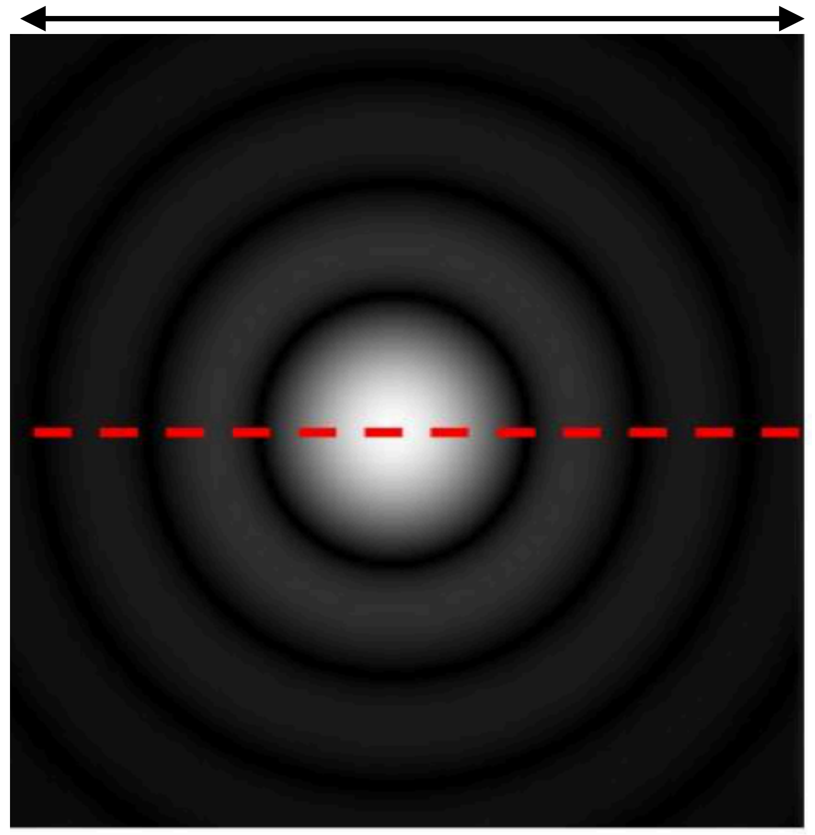


Point-Spread Function

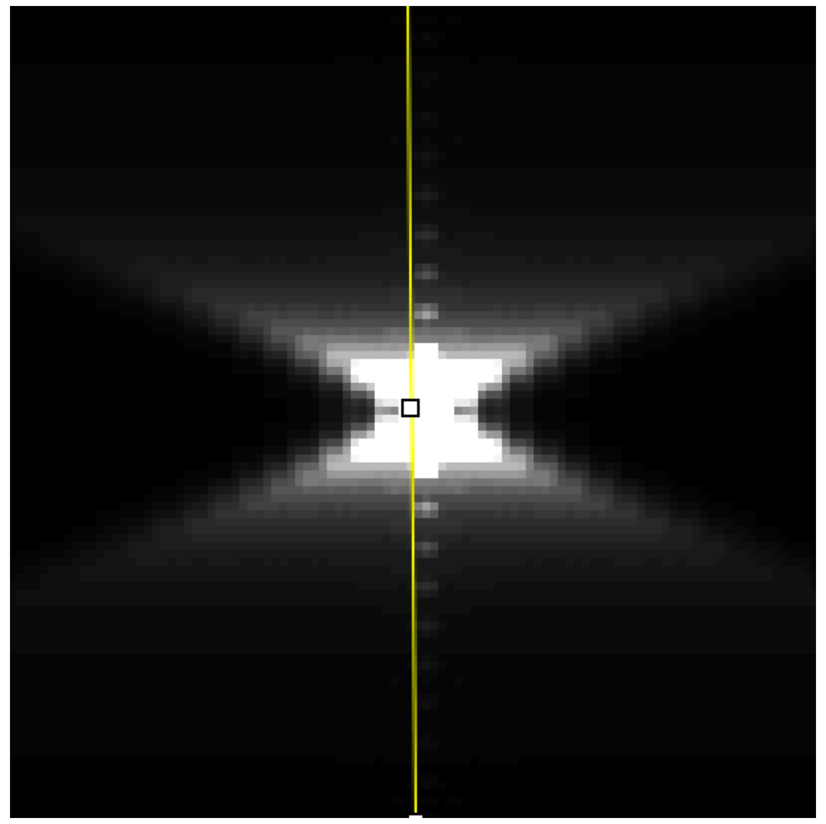
1 µm



z-stack of an experimental PSF
Fire LUT



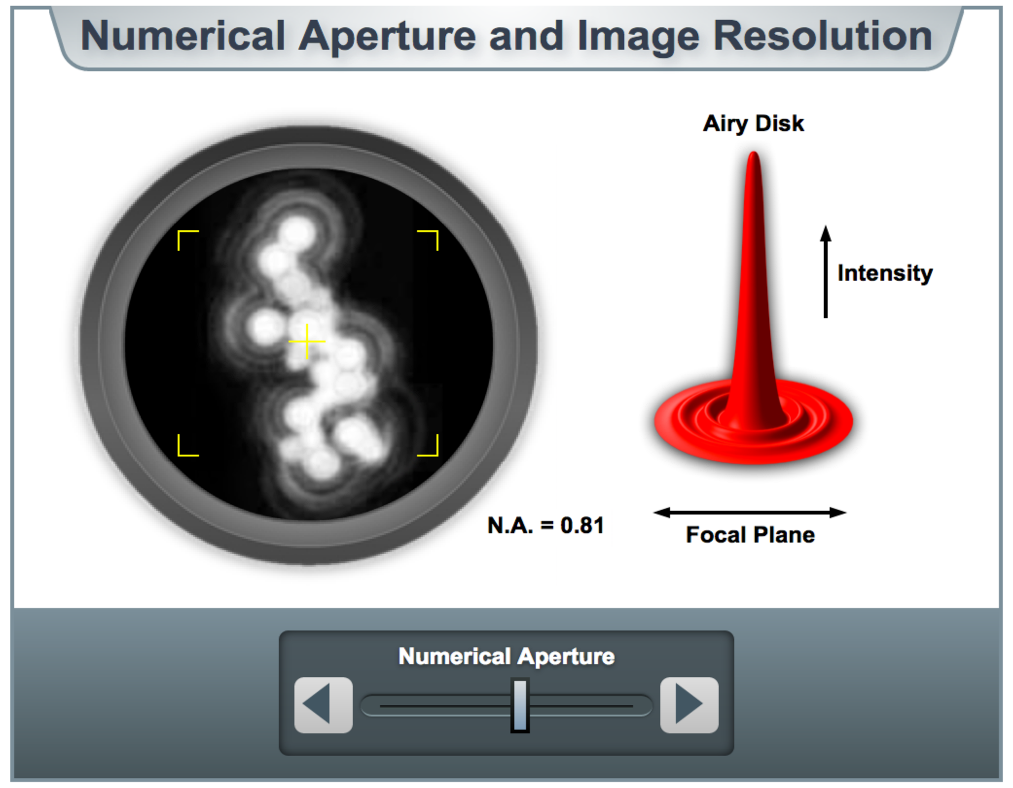
Lateral profile. Airy disk bright central region and **Airy pattern** series of concentric rings



Axial profile. Very intense in the center on the **focal plane** and very diffuse elsewhere giving **out-of-focus**

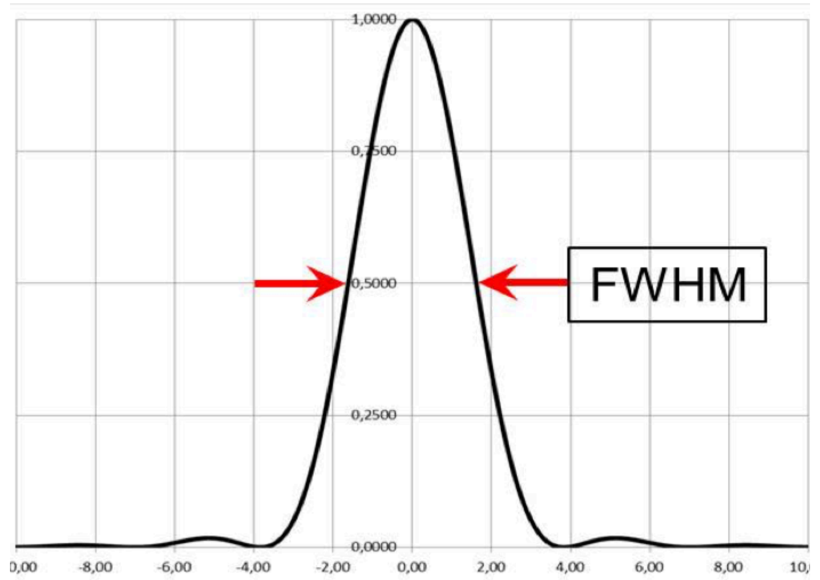
For optical engineer
Optical transfer function **OTF**

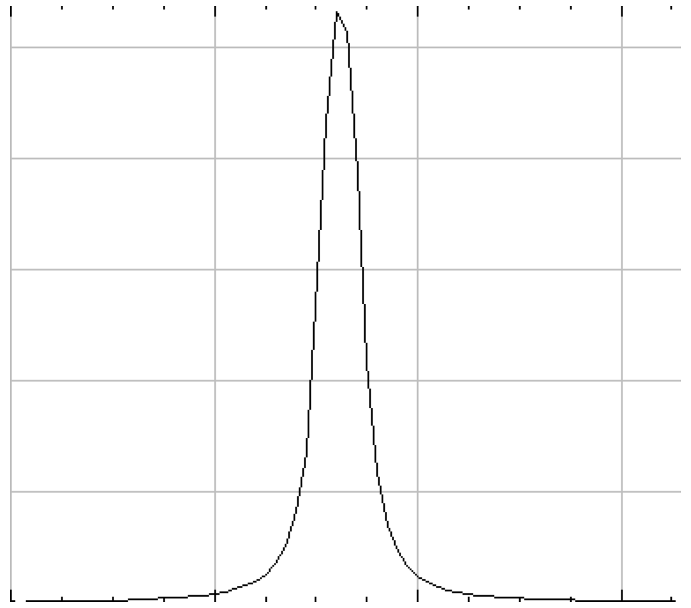
For signal-processing engineer
Impulse response **IR**



Animation from MicroscopyU
https://www.microscopyu.com/

Concentric rings resulting diffraction pattern of a uniformly illumination through circular aperture





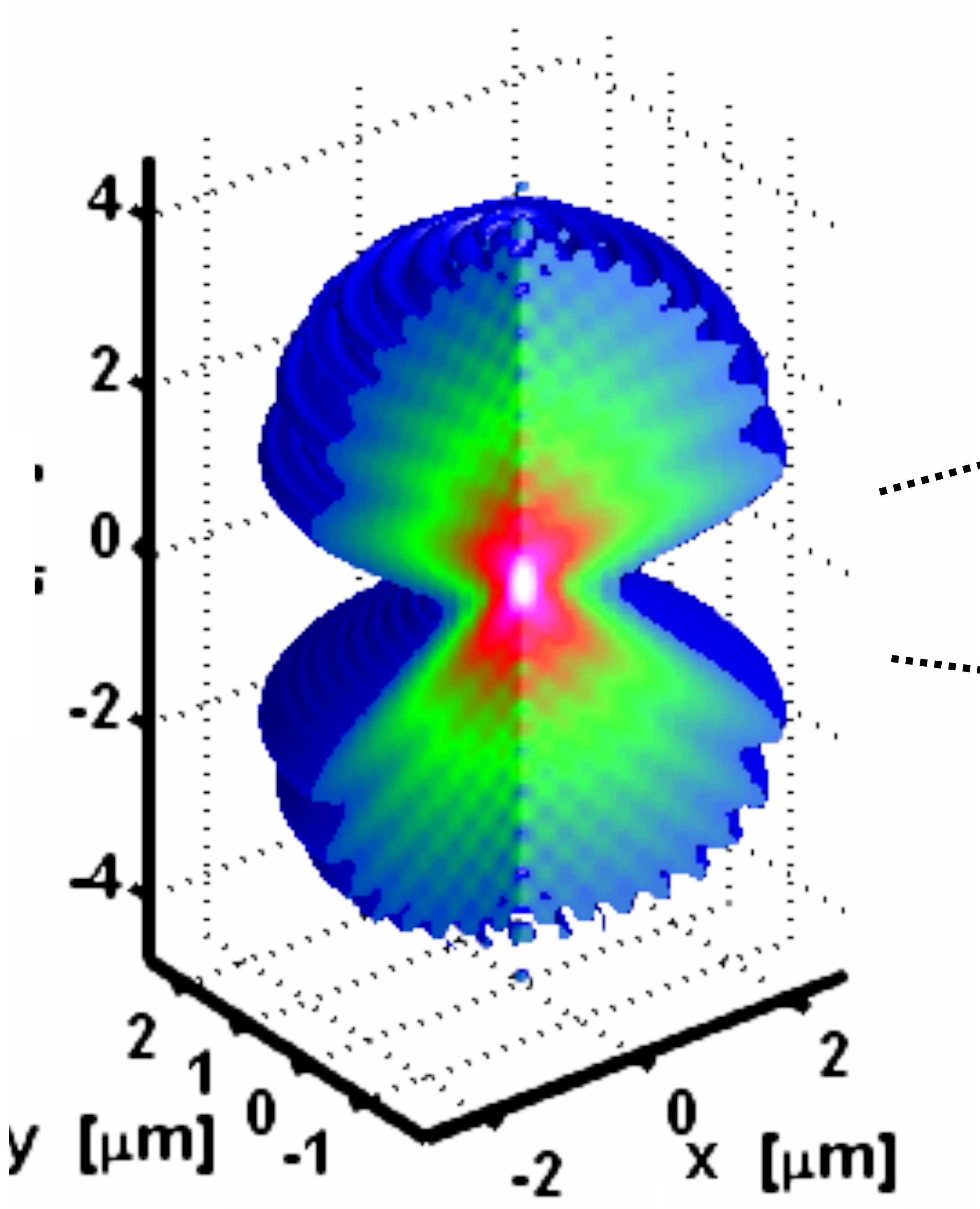
$$\Delta_x = \frac{\lambda}{2 \cdot \mathrm{NA}}$$

$$\Delta_z = \frac{2\, n_i\, \lambda}{\mathrm{NA}^2}$$

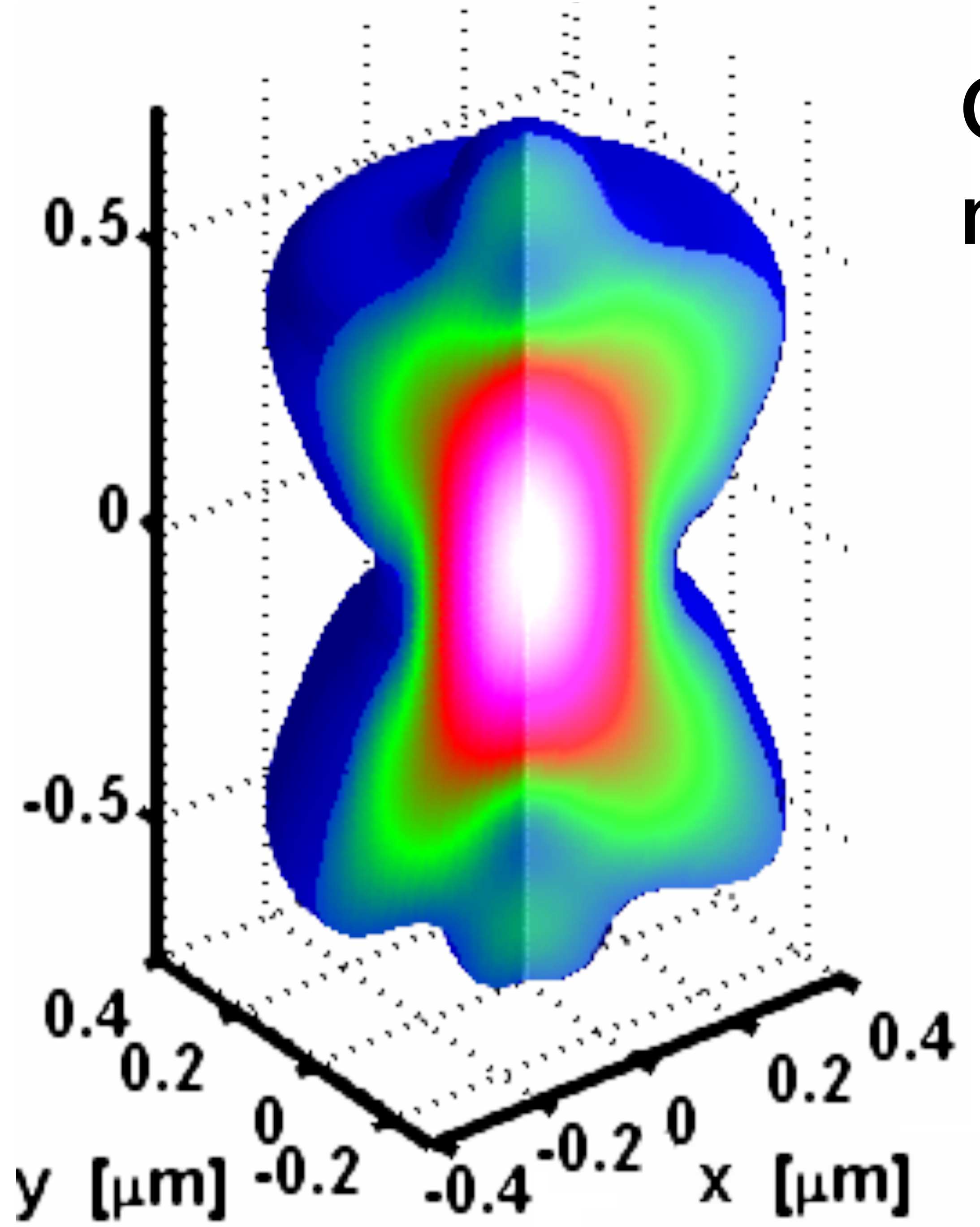
Wavelength	Lateral	Axial
400	174	531
550	240	730
650	283	862

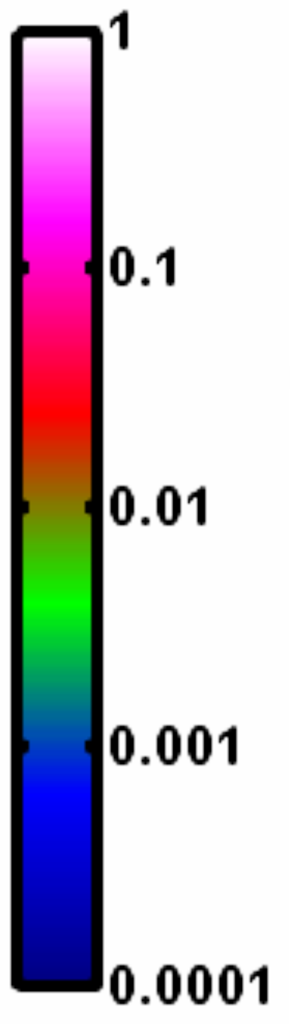
Point-Spread Function

Widefield microscope



Confocal microscope

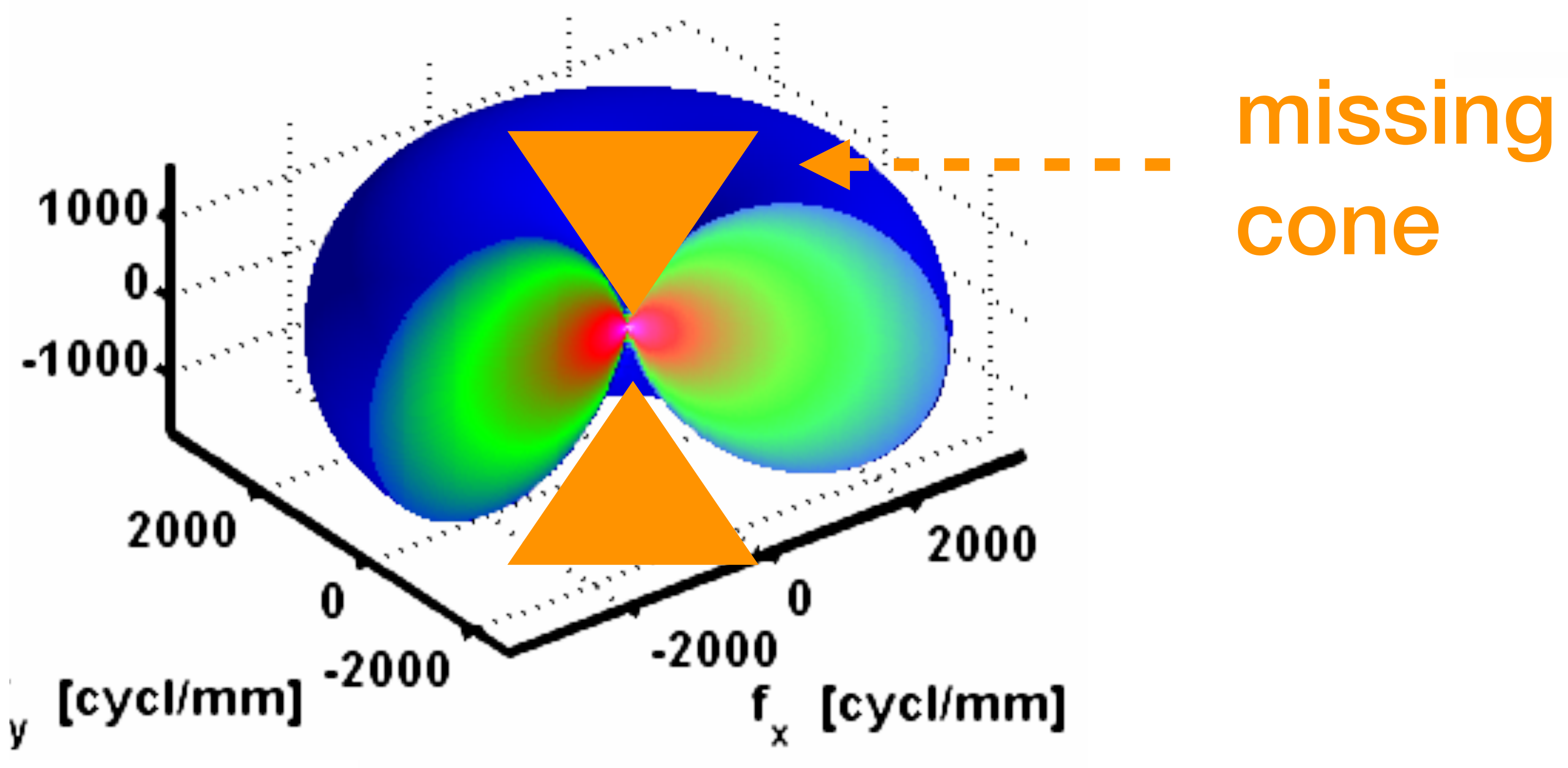




$\lambda = 600\,\mathbf{nm}$

$\mathbf{NA} = 1.49$

$n_i = 1.52$



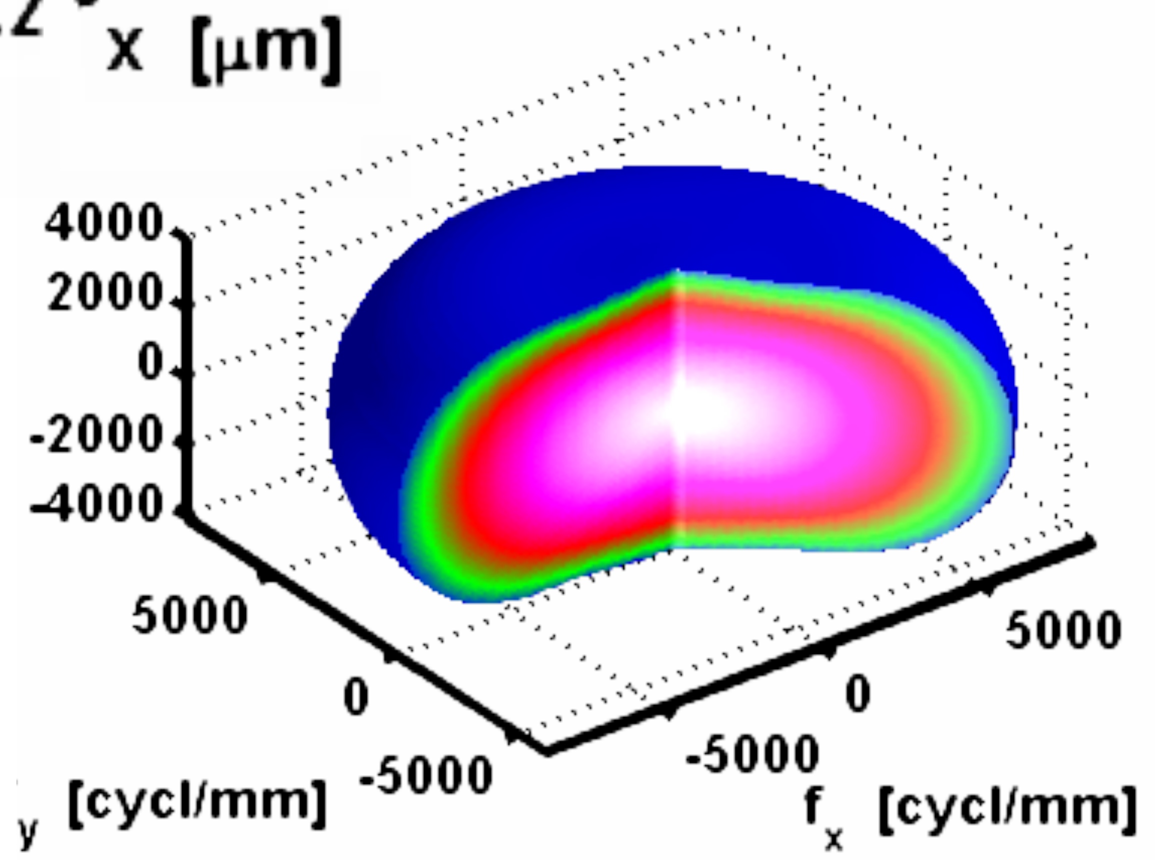


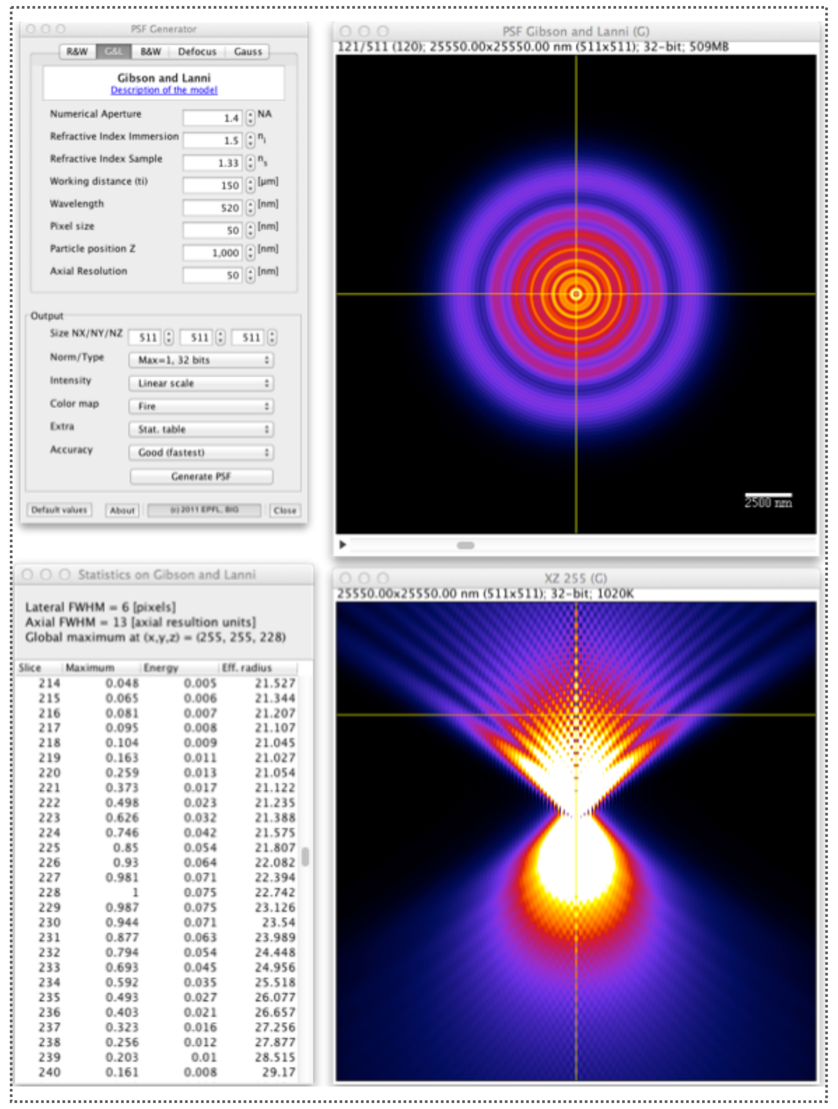
Illustration from Wikipedia

Obtaining a PSF

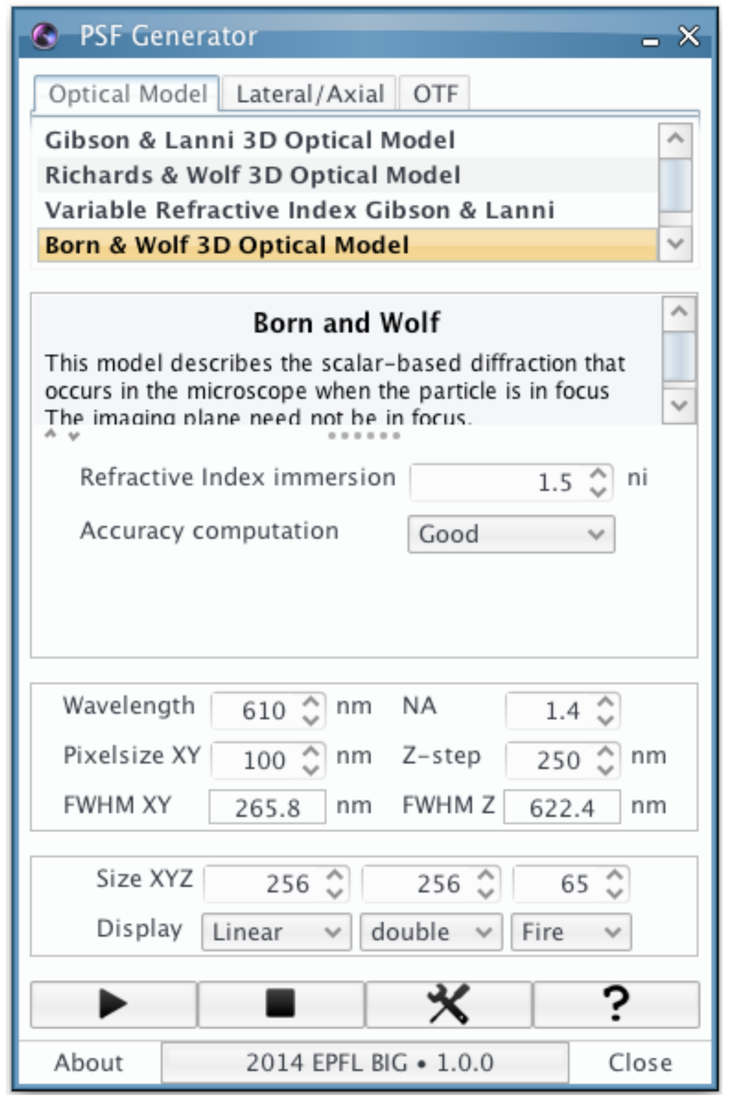
Theoretical PSF

Experimental PSF

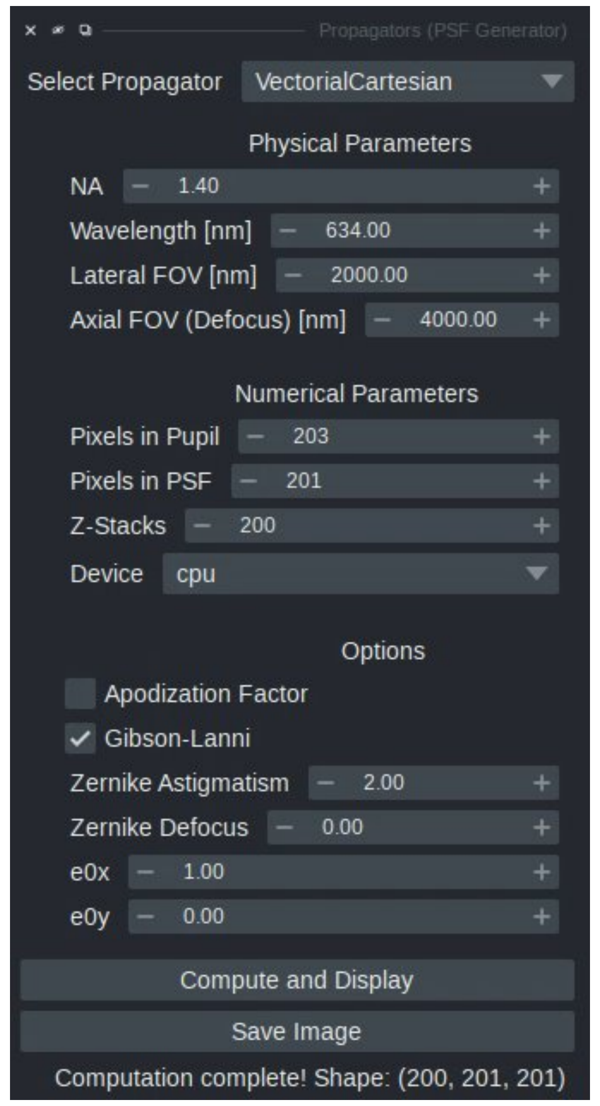
PSF Generator



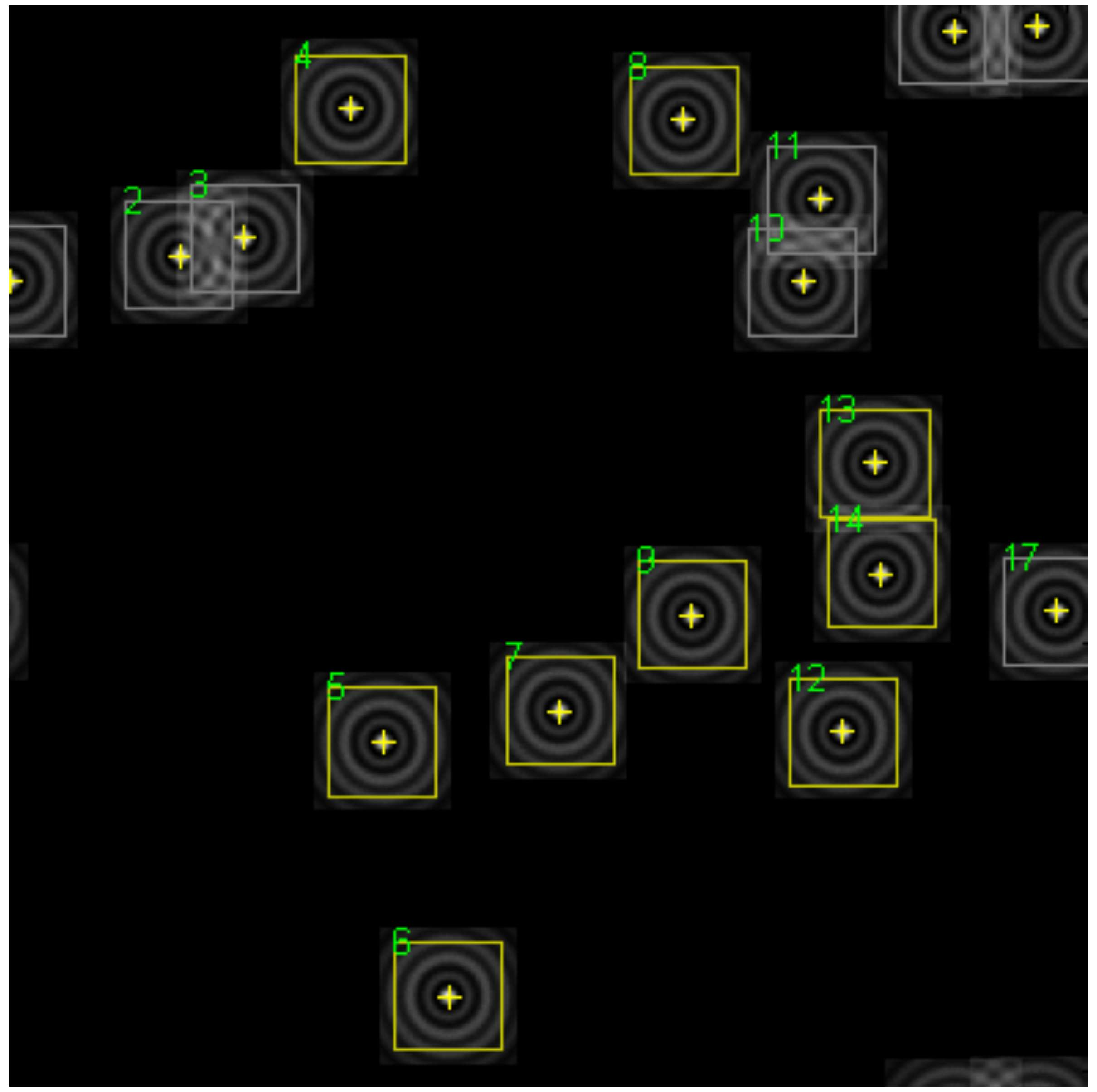
3D



[Kirshner, 2012]



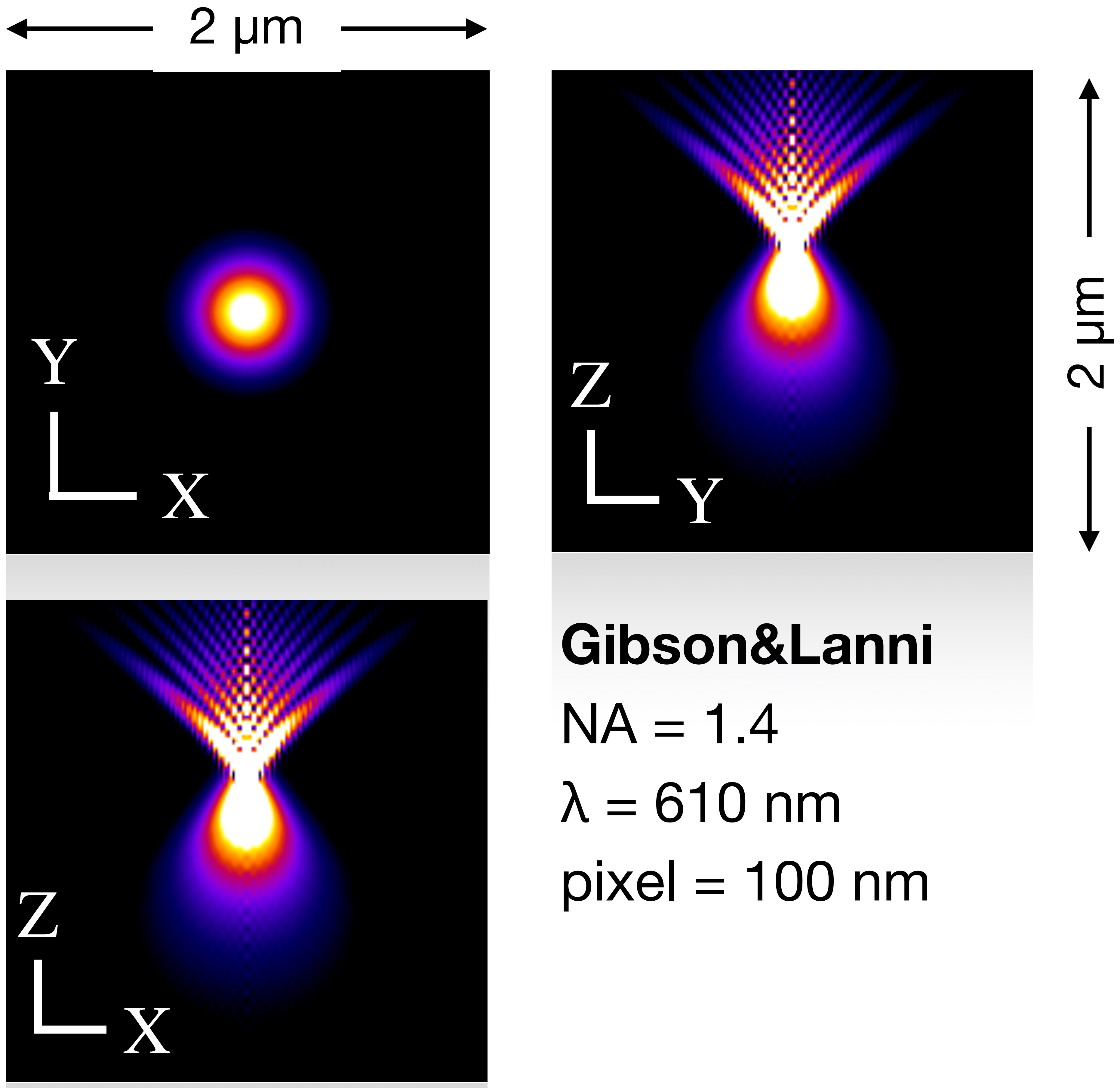
[Liu 2025]



- Microscopy parameters:
  NA, wavelength, ni, thickness, pixel size

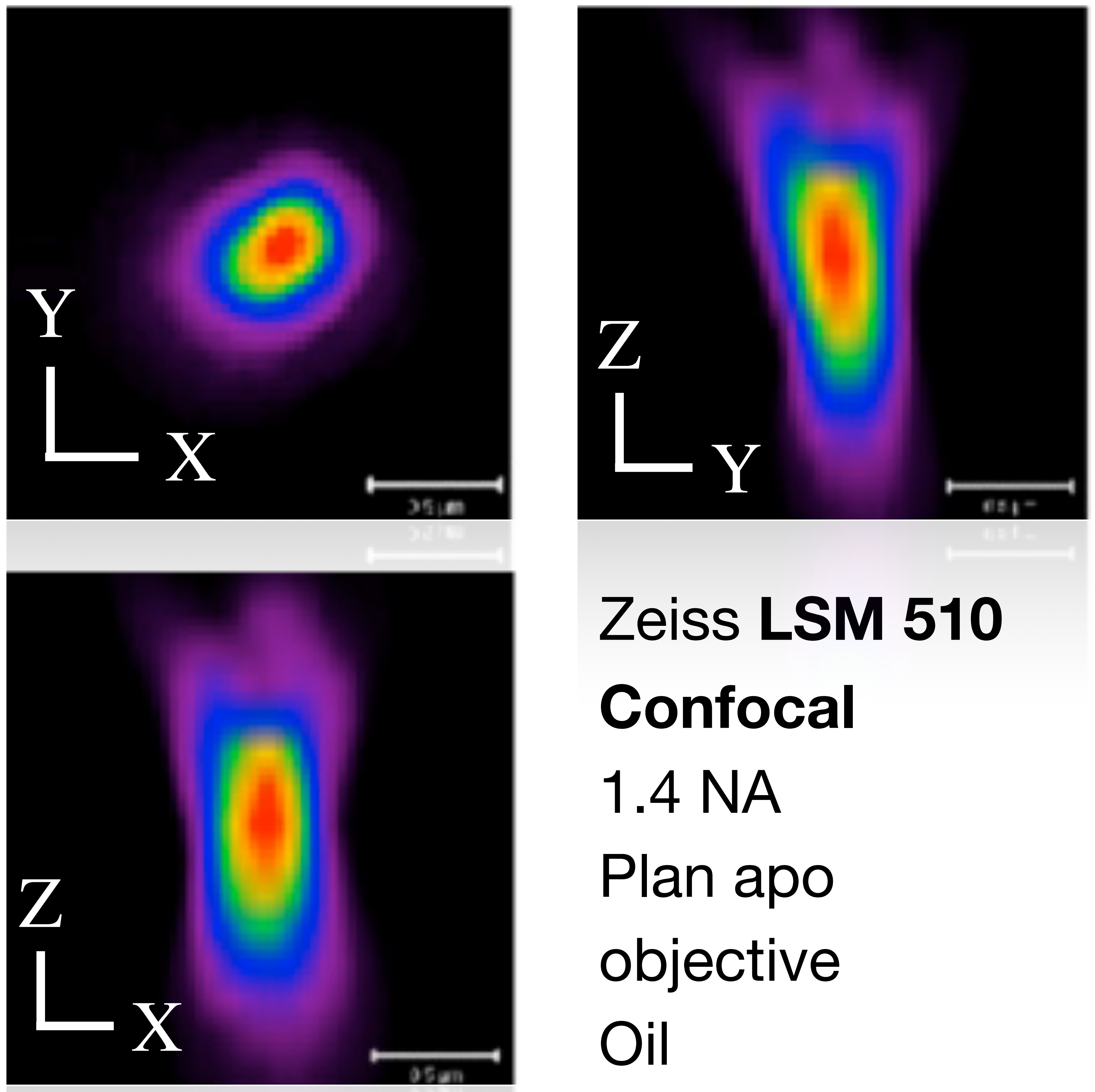
Obtaining a PSF

Theoretical PSF	Experimental PSF



Gibson&Lanni
NA = 1.4
λ = 610 nm
pixel = 100 nm

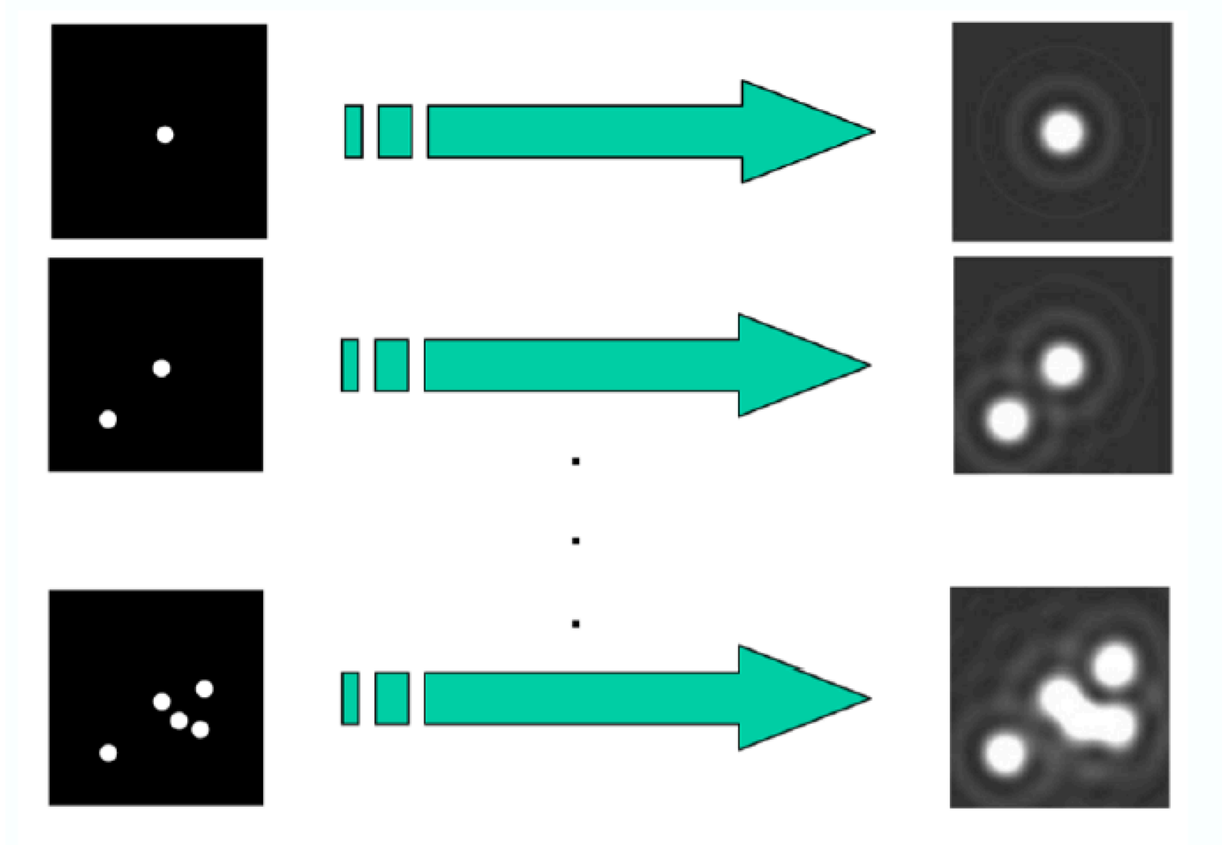
PSF Generator Open source software - EPFL

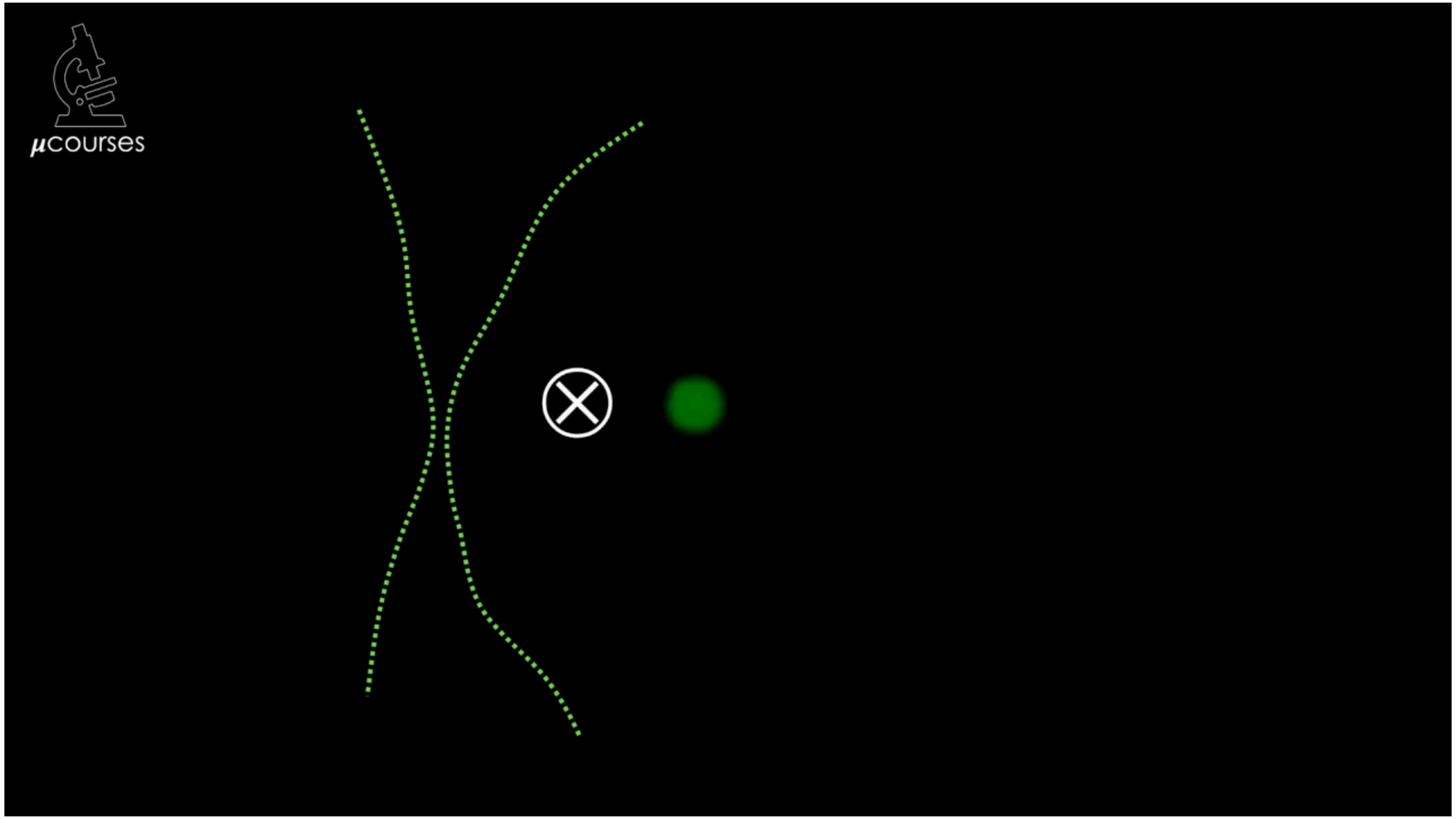


Zeiss **LSM 510**
Confocal
1.4 NA
Plan apo
objective
Oil

Illustration of SVI and Institut de Cardiologie de Montreal.

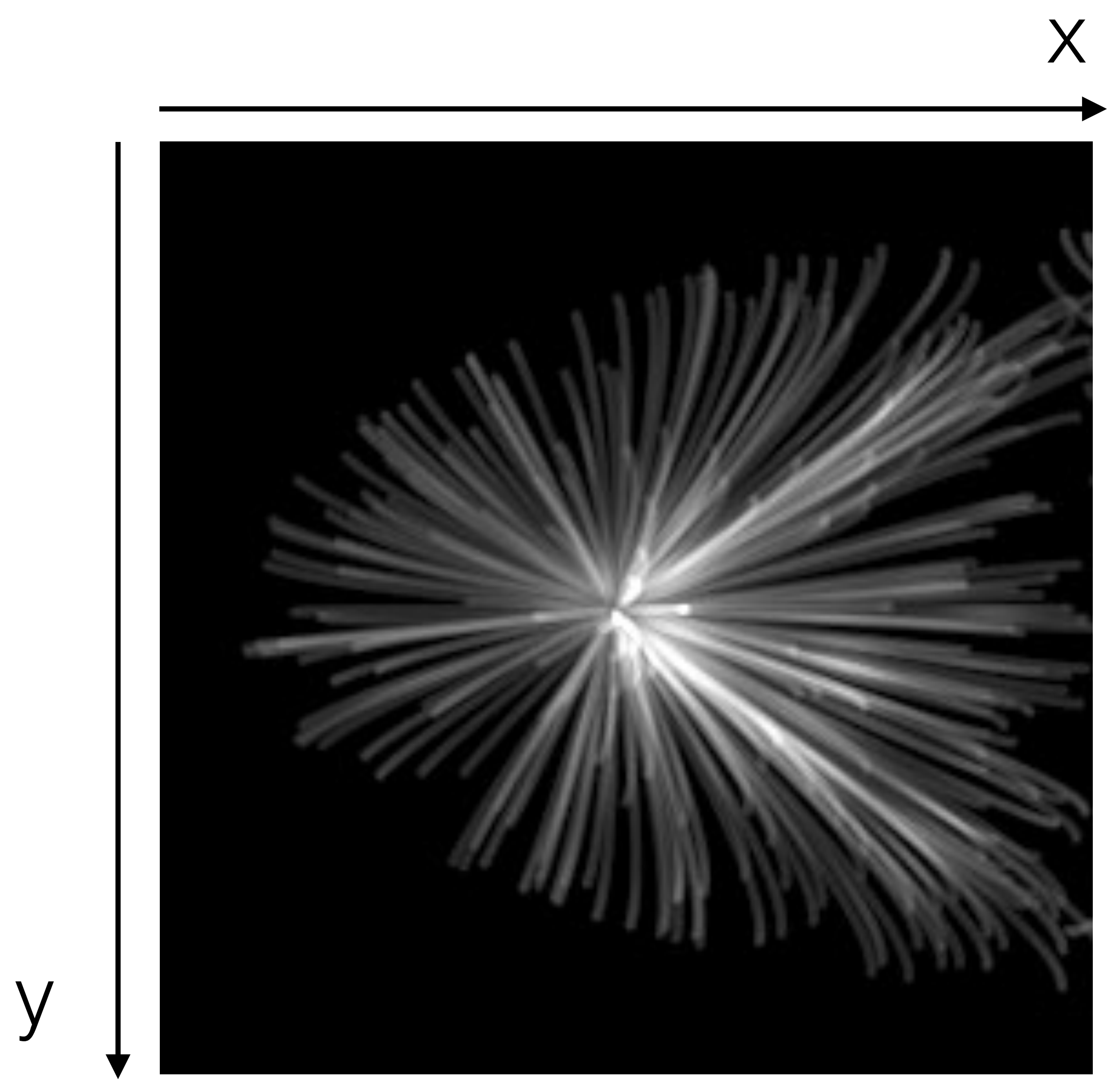
Convolution Intuition



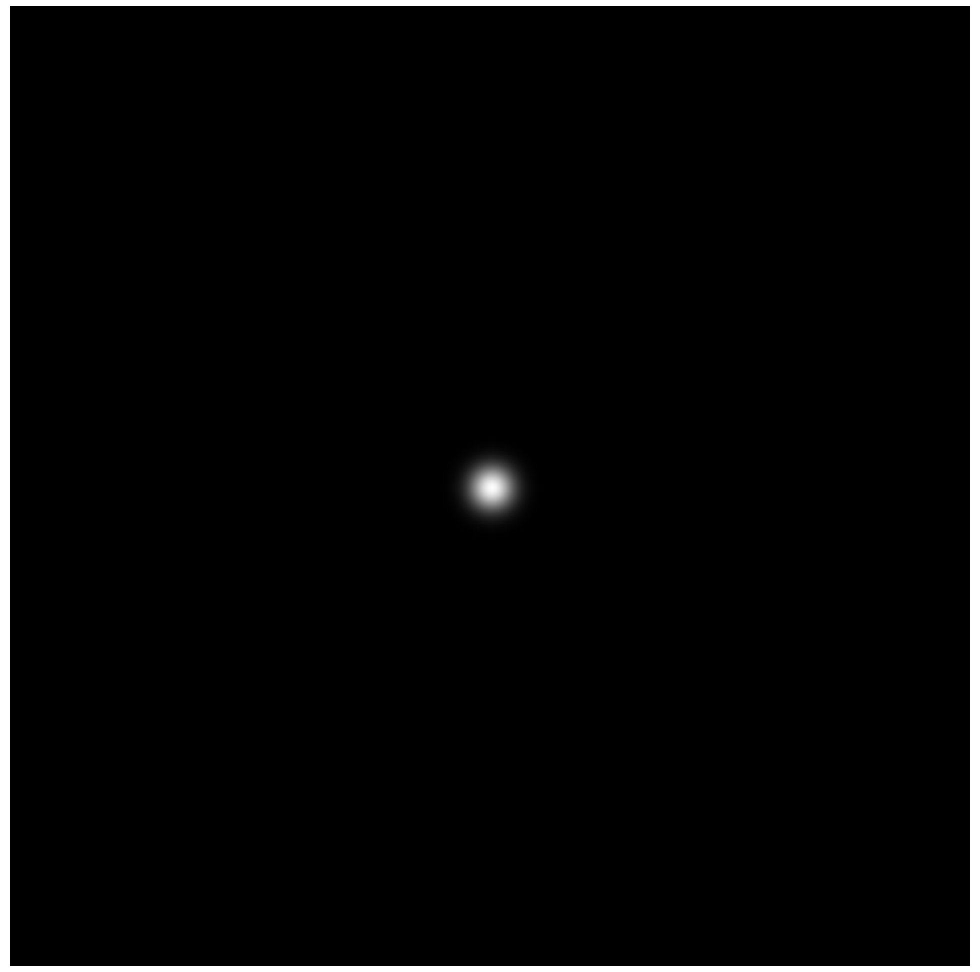


YoutubeVideo: Jennifer C. Water
Nikon Imaging Center Harvard
Medical School

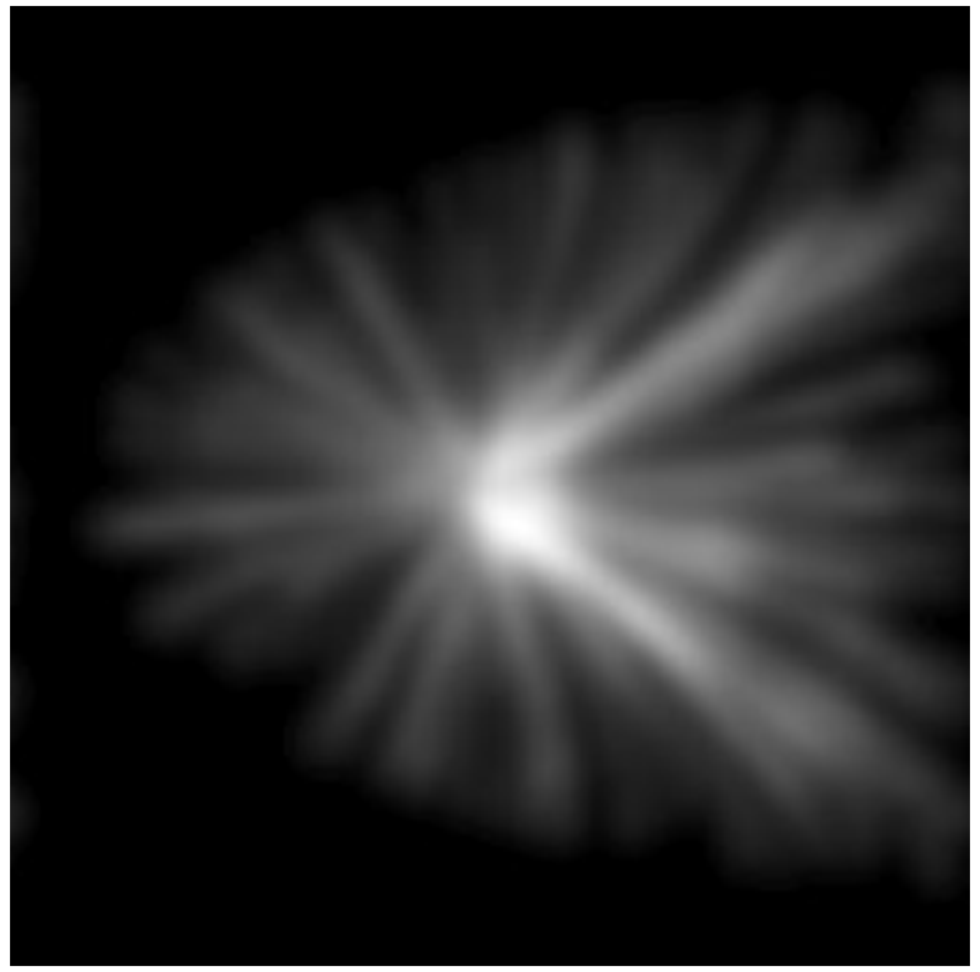
Convolution Visual Effect



Object



PSF

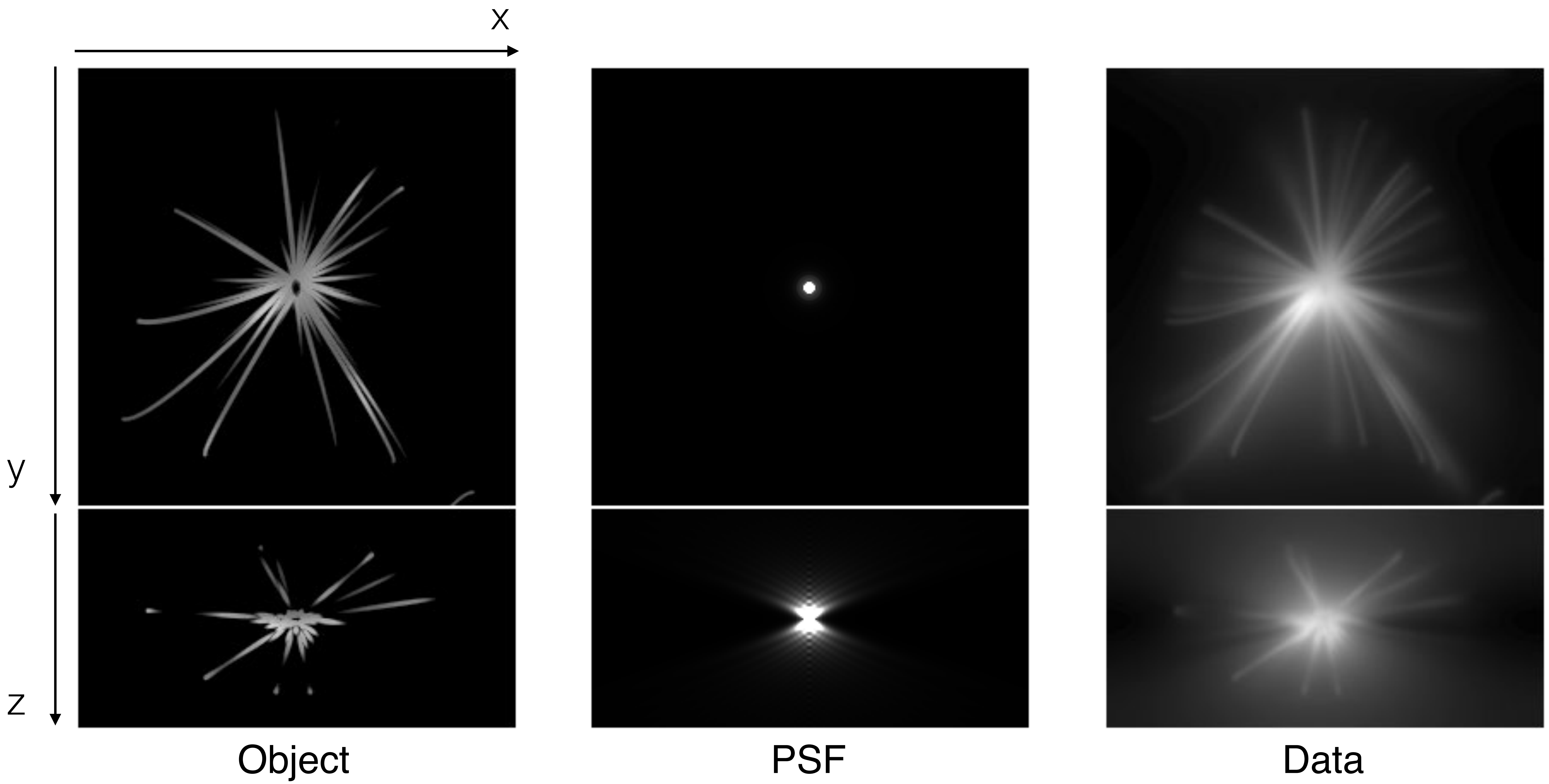


Data

maximum-intensity projection

▶ Blurring

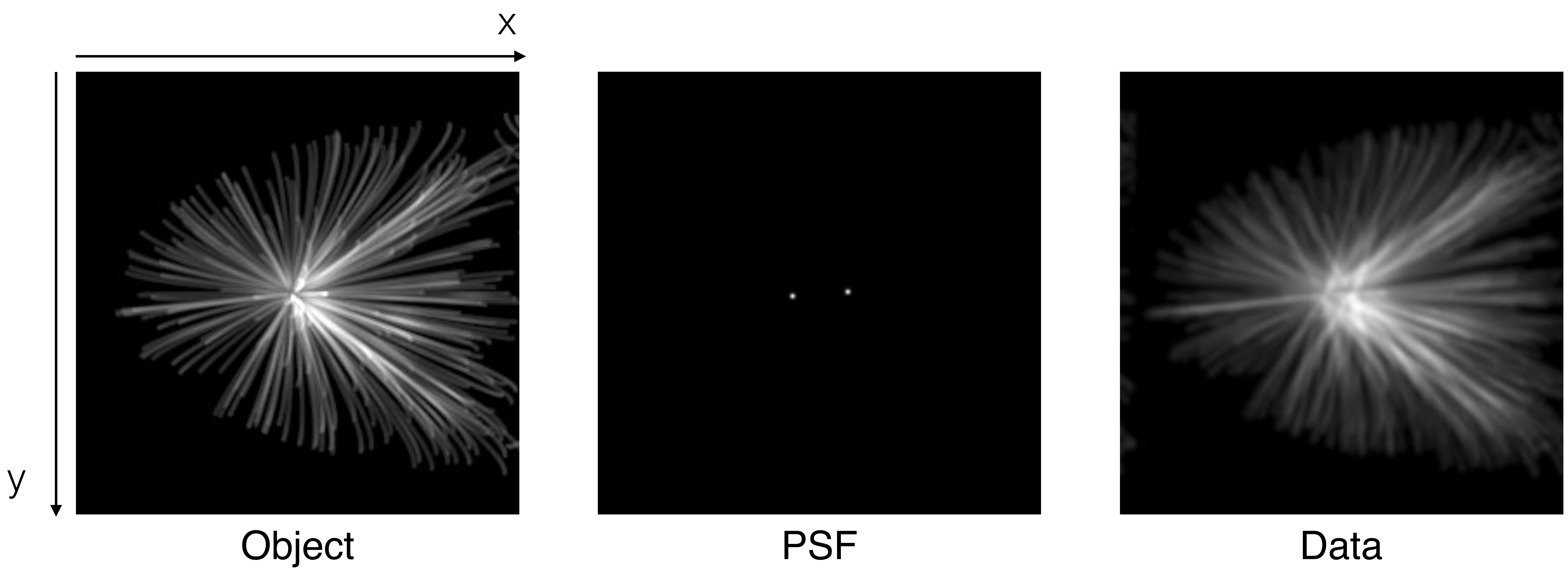
Convolution Visual Effect



orthogonal views

▶ Out-of-focus

Convolution Visual Effect



maximum-intensity projection

▶ Distorsions

Assumptions

- PSF sums up the effects of the imaging setup on the observations
- PSF preserve the light energy
- Shift invariance
- No optical dependencies from the specimen

3D Deconvolution Microscopy

Algorithms

Methods

Mathematical Approach

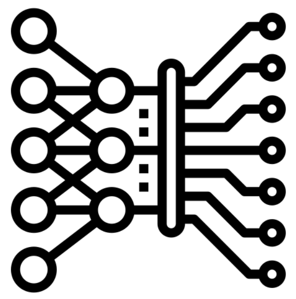
Solving the problem based on the image formation model

- **PSF-based**
  - Inverse Filter — **One shot**
  - Inverse Problems — **Iterative**
- **Blind deconvolution**

Learning Approach

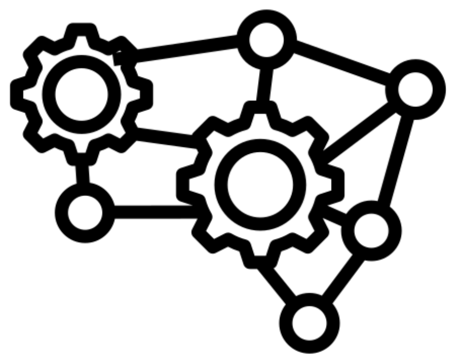
Prediction based on a trained model using the ground-truth.

- **Image-to-image**
  - Supervised Learning



  - Self-Supervised Learning
- **Physical-informed Neural Network**

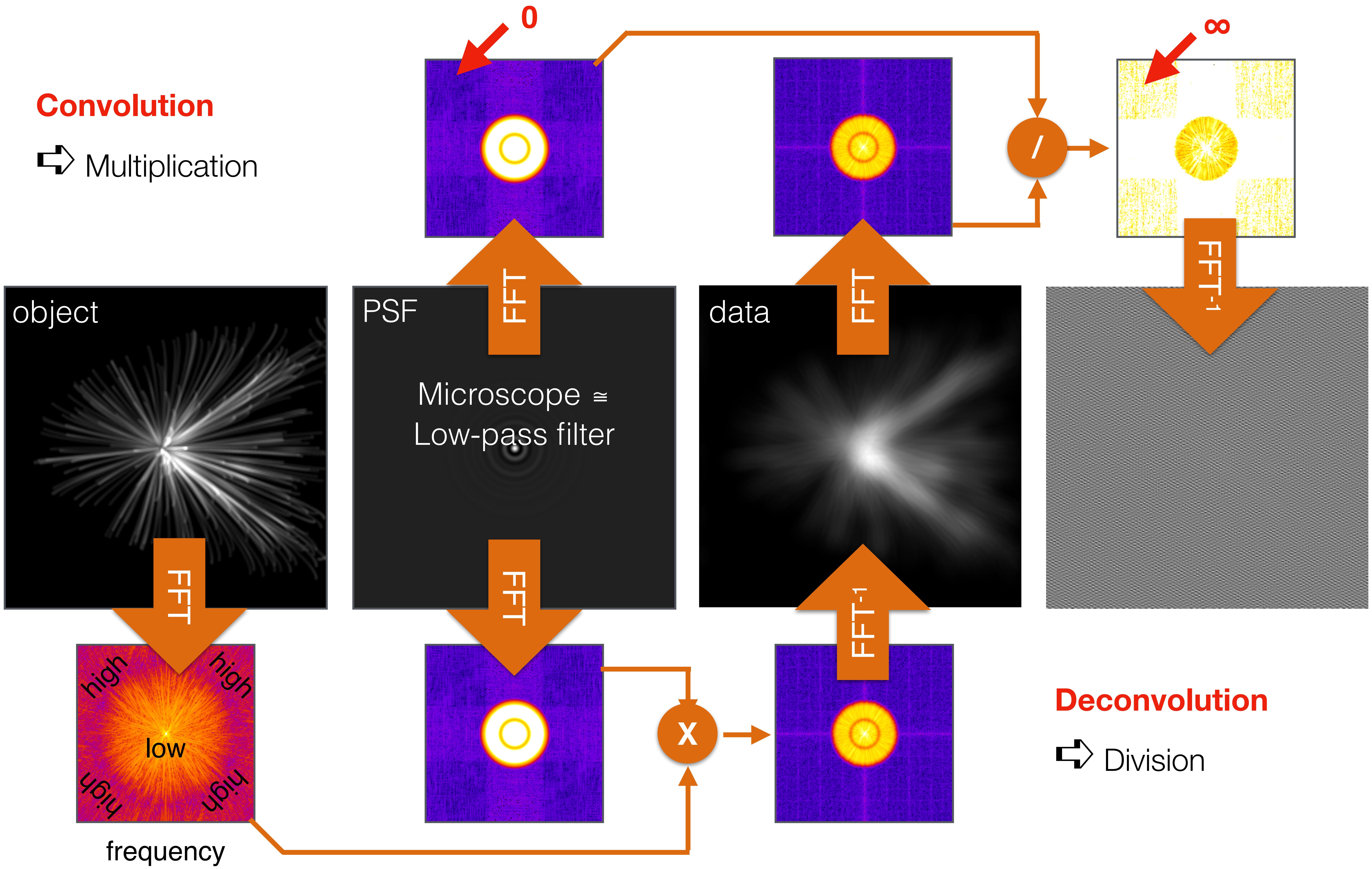
Using PSF
Using prior information on the solution



Inverse Filters

Convolution

➪ Multiplication



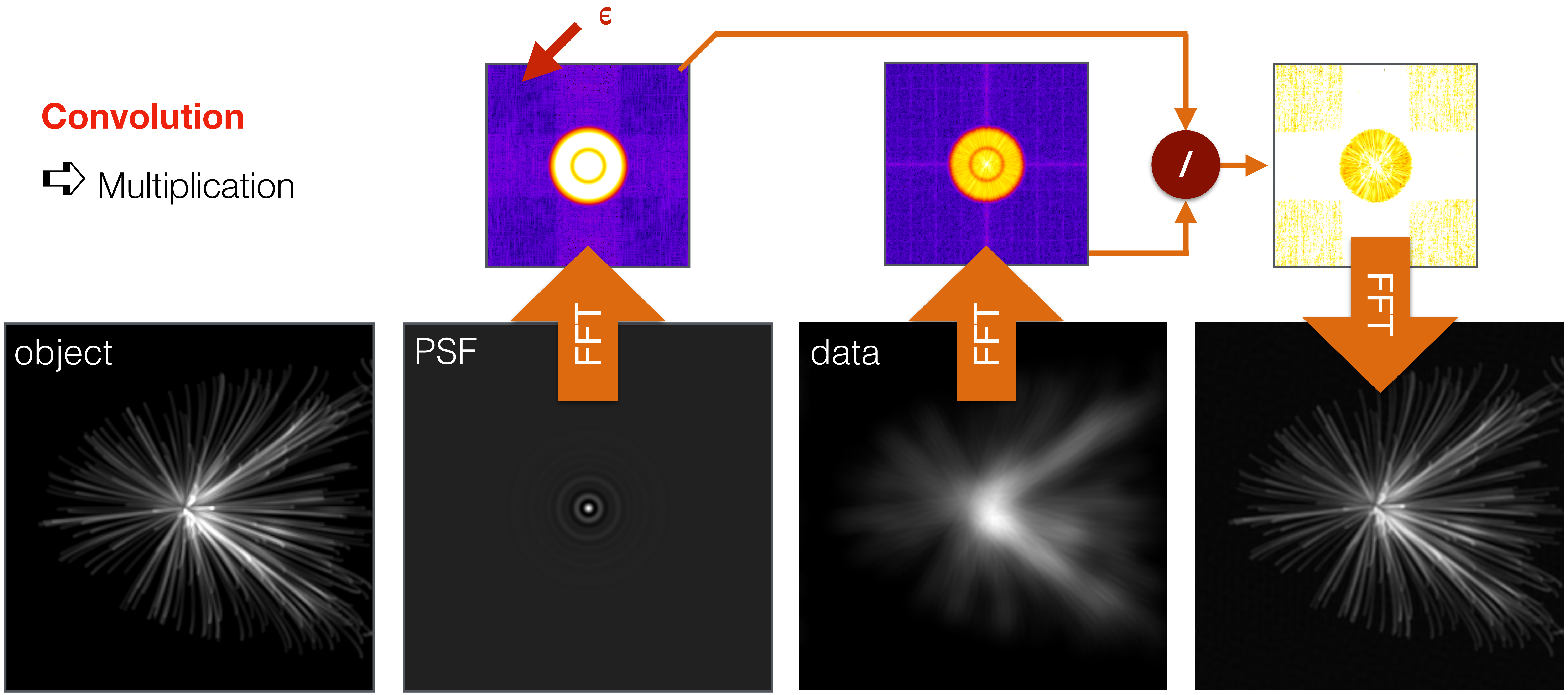
Deconvolution

➪ Division

Intuition

Convolution

⇨ Multiplication



truncates denominator

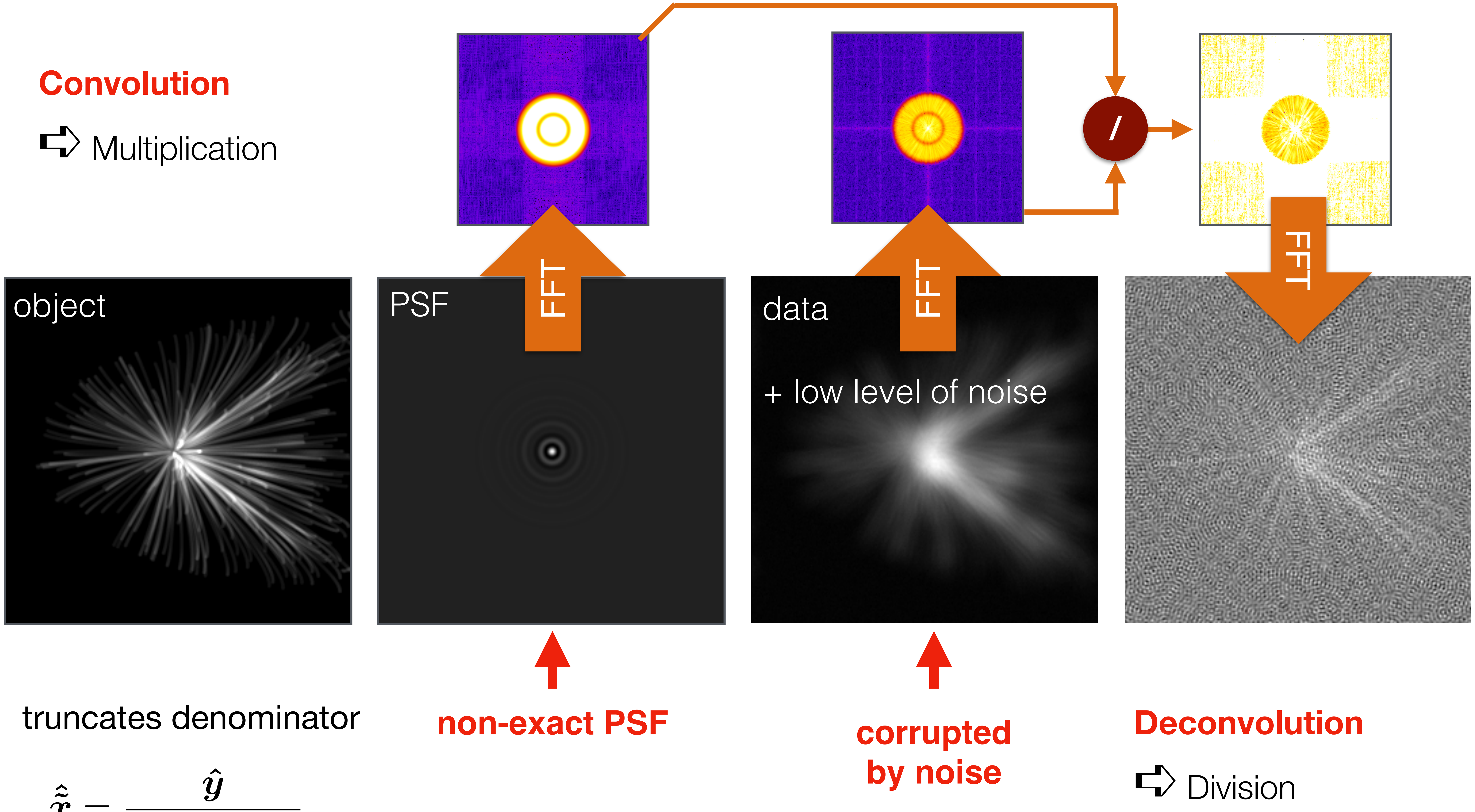
$$\hat{\tilde{\boldsymbol{x}}} = \frac{\hat{\boldsymbol{y}}}{\max\left(\hat{\boldsymbol{h}}, \epsilon\right)}$$

Inverse crime!

Deconvolution

⇨ Stabilized Division

Naive Deconvolution



truncates denominator

$$\hat{\tilde{x}} = \frac{\hat{y}}{\max\left(\hat{h}, \epsilon\right)}$$

Inverse Filters in DeconvolutionLab2

NIF **Naive Inverse Filter**

$$\hat{f}_{NIF} = \frac{1}{\max(\hat{h}, \epsilon)}$$

Never works in real life

WIF **Wiener Inverse Filter**

$$\hat{f}_{WIF} = \frac{1}{\hat{h(\omega)} + \frac{S_n(\omega)}{S_y(\omega)}}$$

WIF Requires noise of signal-to-noise ratio at each frequency

TRIF **Tikhonov Regularized Inverse Filter**

$$\mathscr{C}(\mathbf{x}) = \| \mathbf{Hx} - \mathbf{y} \|^2 + \lambda \| \mathbf{x} \|^2$$

$$\nabla \mathscr{C}(\mathbf{x}) = 0 \quad \Longrightarrow \quad 2\mathbf{H}^T(\mathbf{Hx} - \mathbf{y}) + 2\lambda \mathbf{x} = 0$$

$$\mathbf{x} = (\mathbf{H}^T\mathbf{H} + \lambda \mathbf{I})^{-1}\mathbf{H}^T\mathbf{y}$$

$$\hat{f}_{TRIF} = \frac{1}{\hat{h(\omega)} + \lambda}$$

RIF **(Laplacian) Regularized Inverse Filter**

$$\mathscr{C}(\mathbf{x}) = \| \mathbf{Hx} - \mathbf{y} \|^2 + \lambda \| \mathbf{Lx} \|^2$$

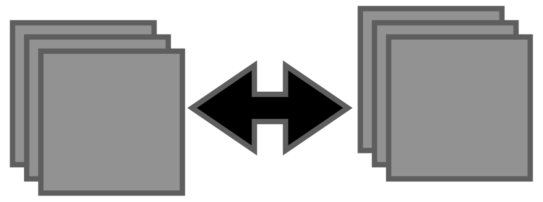
- Acts as a whitening filter
- Finer controls on most natural images

$$\mathbf{x} = (\mathbf{H}^T\mathbf{H} + \lambda \mathbf{L}^T\mathbf{L})^{-1}\mathbf{H}^T\mathbf{y}$$

$$\hat{f}_{LRIF} = \hat{f}_{RIF} = \frac{1}{\hat{h(\omega)} + \lambda\,\omega^2}$$

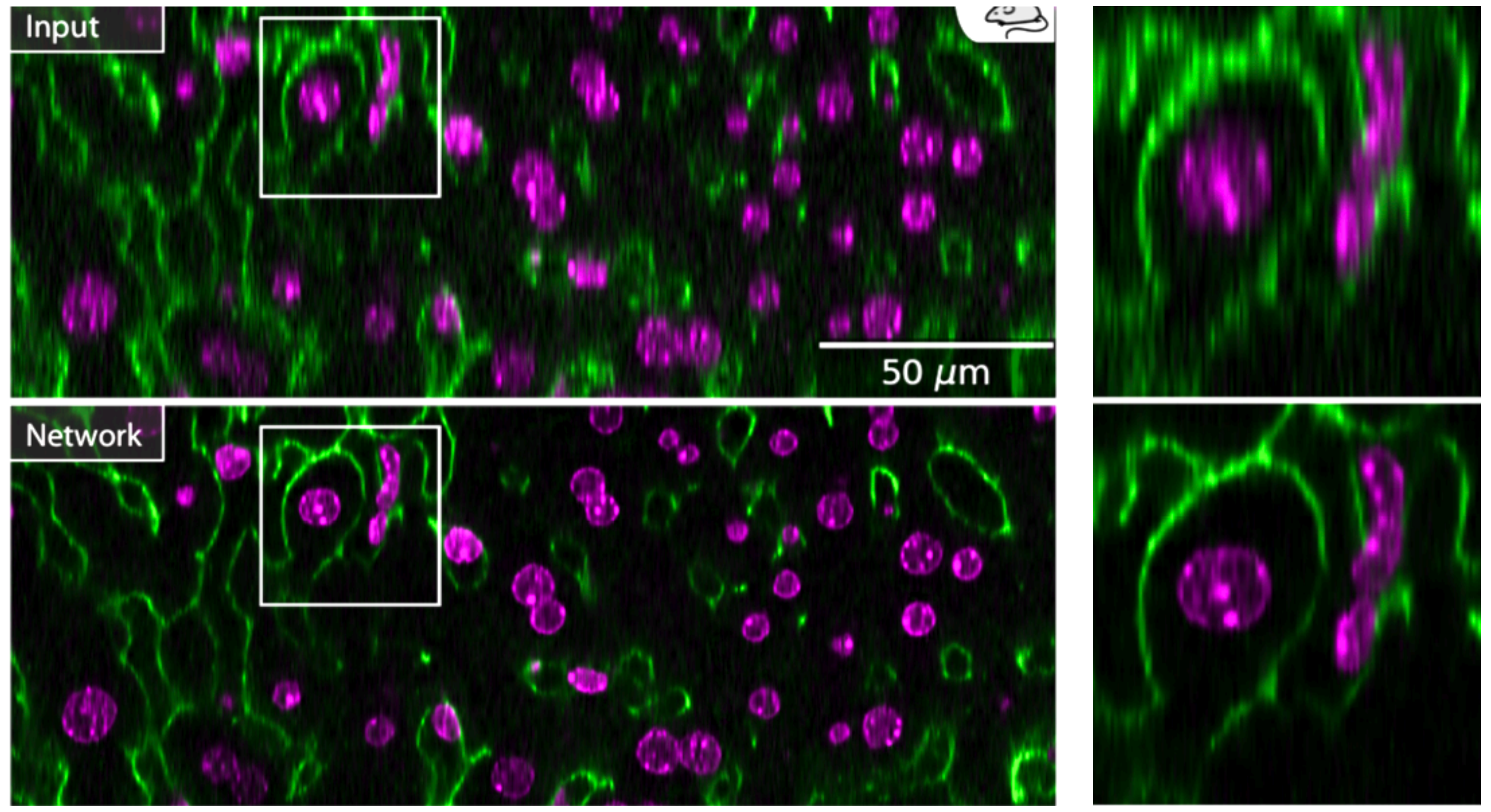
Examples DL Deconvolution

Supervised Learning
- Super-resolution **vs.** standard res.
- Axial **vs.** Lateral
- Real data data **vs.** Degraded data
- Using synthetic strategies

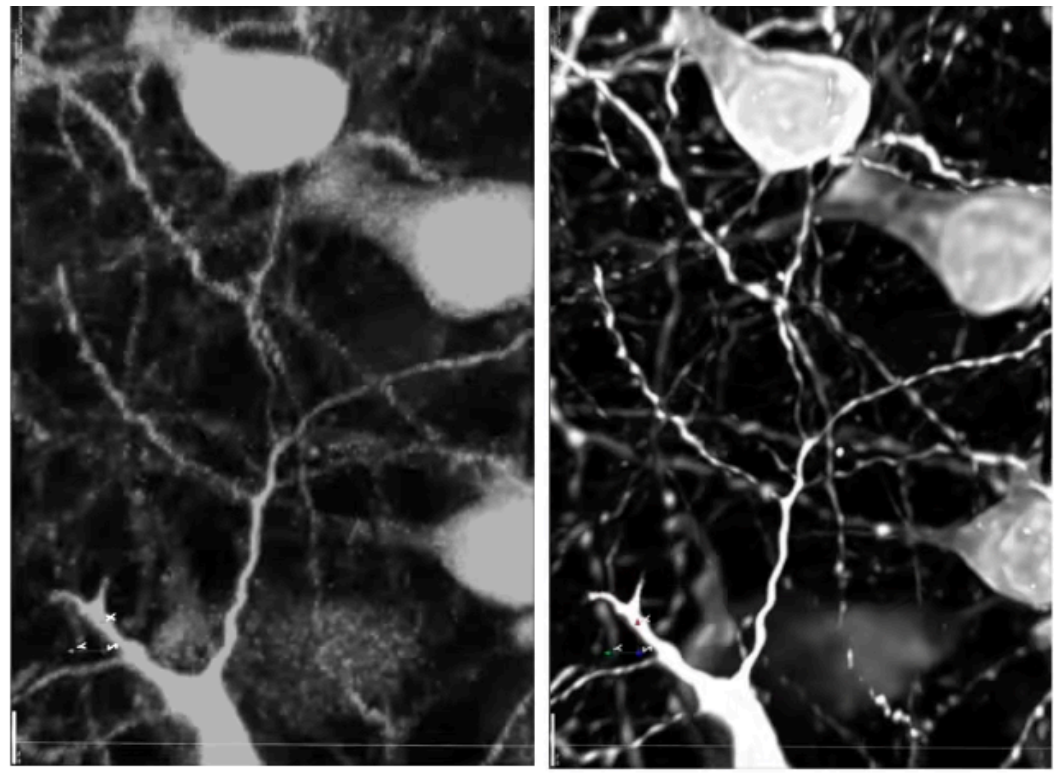


CARE Weigert, Nature Meth., 2019

Content Aware Restoration
- Axial restoration and deconvolution
- Good data ➔ **lateral plane**
- Degraded data ➔ **axial plane**



Aivia / Zeiss

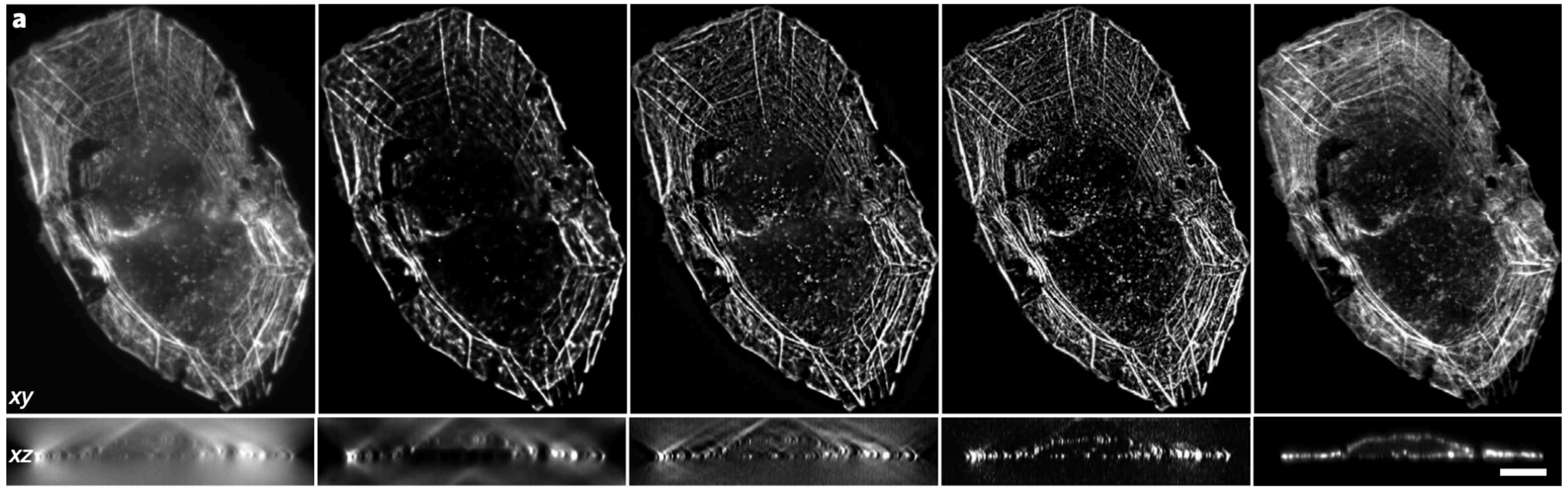


Datasets
- 10 pairs of 3D data
- 2k x 2k x 200
- Confocal microscopy
- LQ (single scan)
- HQ (64 scans)

Training
- 3 hours / 8 x GPU

RLN Richardson–Lucy Network

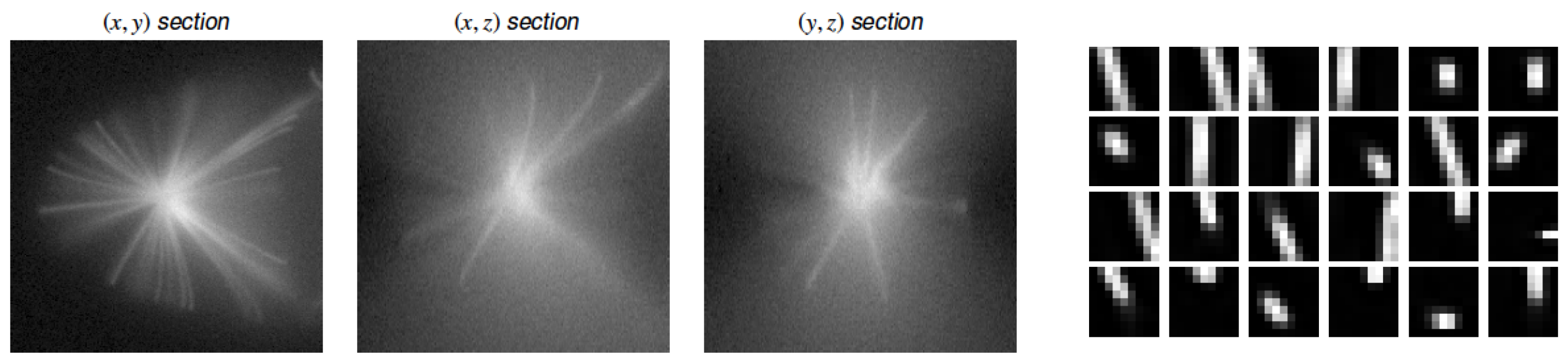
Li, Nature Meth., 2022



Widefield RL Thunder RLN Confocal

Machine Learning

Sparse Dictionary Learning — **Super Res CNN**



Widefield | SIM | Simple deconvolution | [Soulez, ISBI 2014] | [*Dong*, ECCV 2014]

Cell Image Library **CIL 36797**. Microtubules in a Drosophila S2, Alexa Fluor 488, Zeiss Elyra SIM NA = 1.4 (1024×1024×44) (40×40×110 nm)

Iterative Algorithm

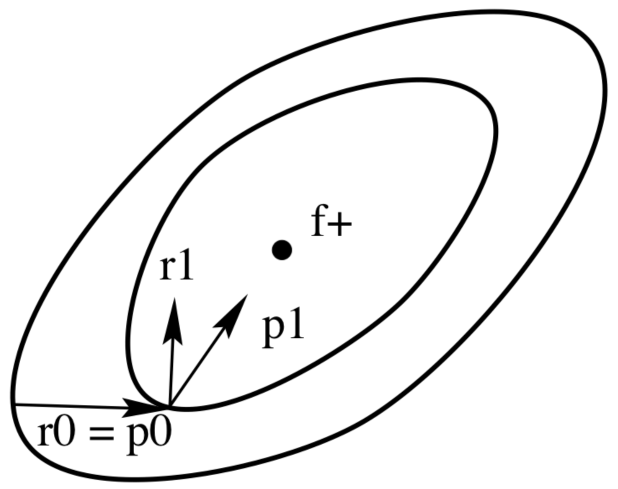
Linear Least Square (LLS)

$$\mathscr{C}(\mathbf{x}) = \|\mathbf{y} - \mathbf{H}\mathbf{x}\|^2$$

Steepest Gradient Descent

$$\mathbf{x}^{(k+1)} = \mathbf{x}^{(k)} - \gamma \nabla \mathscr{C}(\mathbf{x})$$

$$\nabla \mathscr{C}(\mathbf{x}) = -2\mathbf{H}^T\left(\mathbf{y} - \mathbf{H}\mathbf{x}\right)$$



Landweber algorithm LW

$$\mathbf{x}^{k(+1)} = \left(\mathbf{I} - \gamma \mathbf{H}^T\mathbf{H}\right)\, \mathbf{x}^{(k)} + \gamma \mathbf{H}^T\mathbf{y}$$

iterations in the Fourier domain

→ Positivity constraint

Landweber algorithm LW+

Maximum Likelihood Estimator (MLE)

$$\mathscr{C}(\mathbf{x}) = \mathbf{H}\mathbf{y} - \mathbf{y}^T\left(\log \mathbf{H}\mathbf{x}\right)$$

- Statistically interpretation
- Poisson noise
- Assumption of positive signals
- Slow

Richardson-Lucy RL

$$\mathbf{x}^{k(+1)} = \mathbf{x}^{(k)} \times \mathbf{H}^T \frac{\mathbf{y}}{\mathbf{H}\mathbf{x}^{(k)}}$$

iterations in the space domain

No parameter

Early stopping number of iterations

Iterative Algorithms in DeconvolutionLab2

LW Landweber iteration

[Landweber, 1951]

$$\mathcal{C}(\boldsymbol{x}) = \|\boldsymbol{y} - \mathbf{H}\boldsymbol{x}\|^2$$

$$\boldsymbol{x}^{k+1} = \left(\mathbf{I} - \gamma \mathbf{H}^T \mathbf{H}\right) \boldsymbol{x}^k + \gamma \mathbf{H}^T \boldsymbol{y}$$

- Least-square minimization
- Controllable step
- Dominant Gaussian noise

LW+ Landweber + positivity

$$\boldsymbol{x}^{k+1} = \mathcal{P}\left\{\left(\mathbf{I} - \gamma \mathbf{H}^T \mathbf{H}\right) \boldsymbol{x}^k + \gamma \mathbf{H}^T \boldsymbol{y}\right\}$$

- Known also NNLS
- Non-negative constraint ➪ slow down!

RL Richardson-Lucy

[Richarsdon, 1972, Lucy 1974]

$$\boldsymbol{x}^{(k+1)} = \boldsymbol{x}^{(k)} \times \mathbf{H}^T \left(\frac{\boldsymbol{y}}{\mathbf{H}\boldsymbol{x}^{(k)}}\right)$$

- Poisson noise
- Assumption of positive signals (MLE)
- Slow, iteration in the spatial domain
- One parameter to tune (iter)

TM Tikhonov-Miller

$$\mathcal{C}(\boldsymbol{x}) = \|\boldsymbol{y} - \mathbf{H}\boldsymbol{x}\|^2 + \lambda \|\mathbf{L}\boldsymbol{x}\|_2^2$$

$$\boldsymbol{x}^{(k+1)} = \boldsymbol{x}^{(k)} + \gamma \left(\mathbf{H}^T \boldsymbol{y} - \left(\mathbf{H}^T \mathbf{H} + \lambda \mathbf{L}^T \mathbf{L}\right) \boldsymbol{x}^{(k)}\right)$$

- Tikhonov regularization

ICTM Iterative Constrained T.M.

[Kempen, 1996]

$$\boldsymbol{x}^{(k+1)} = \mathcal{P}\left\{\boldsymbol{x}^{(k)} + \gamma \left(\mathbf{H}^T \boldsymbol{y} - \left(\mathbf{H}^T \mathbf{H} + \lambda \mathbf{L}^T \mathbf{L}\right) \boldsymbol{x}^{(k)}\right)\right\}$$

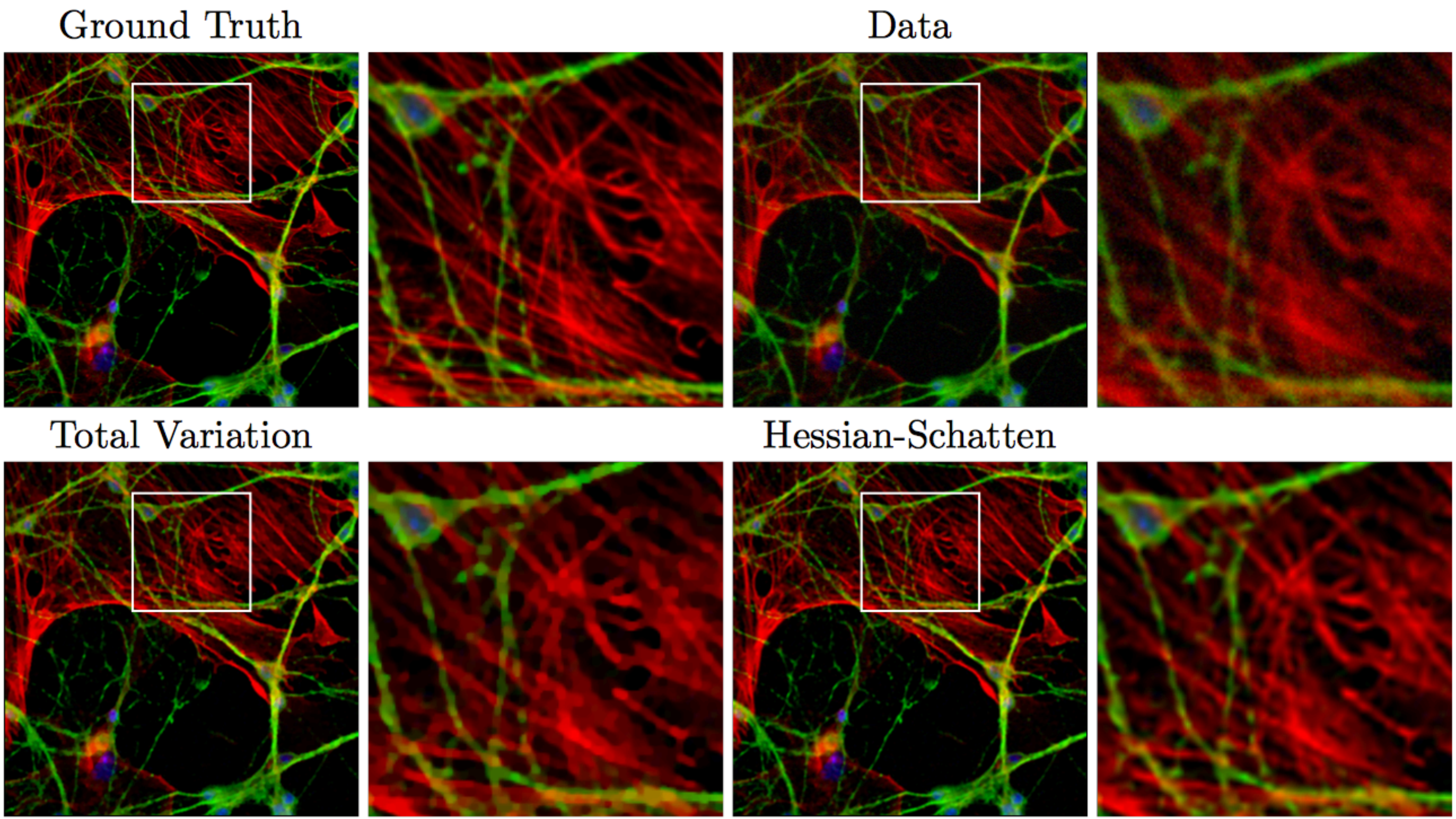
RLTV RL with Total Variation

[Dey, 2006]

$$\boldsymbol{x}^{(k+1)} = \boldsymbol{x}^{(k)} \times \mathbf{H}^T \left(\frac{\boldsymbol{y}}{\mathbf{H}\boldsymbol{x}^{(k)}}\right) + \lambda \|\mathbf{D}\boldsymbol{x}\|_1$$

- Preserve the edges

Regularization

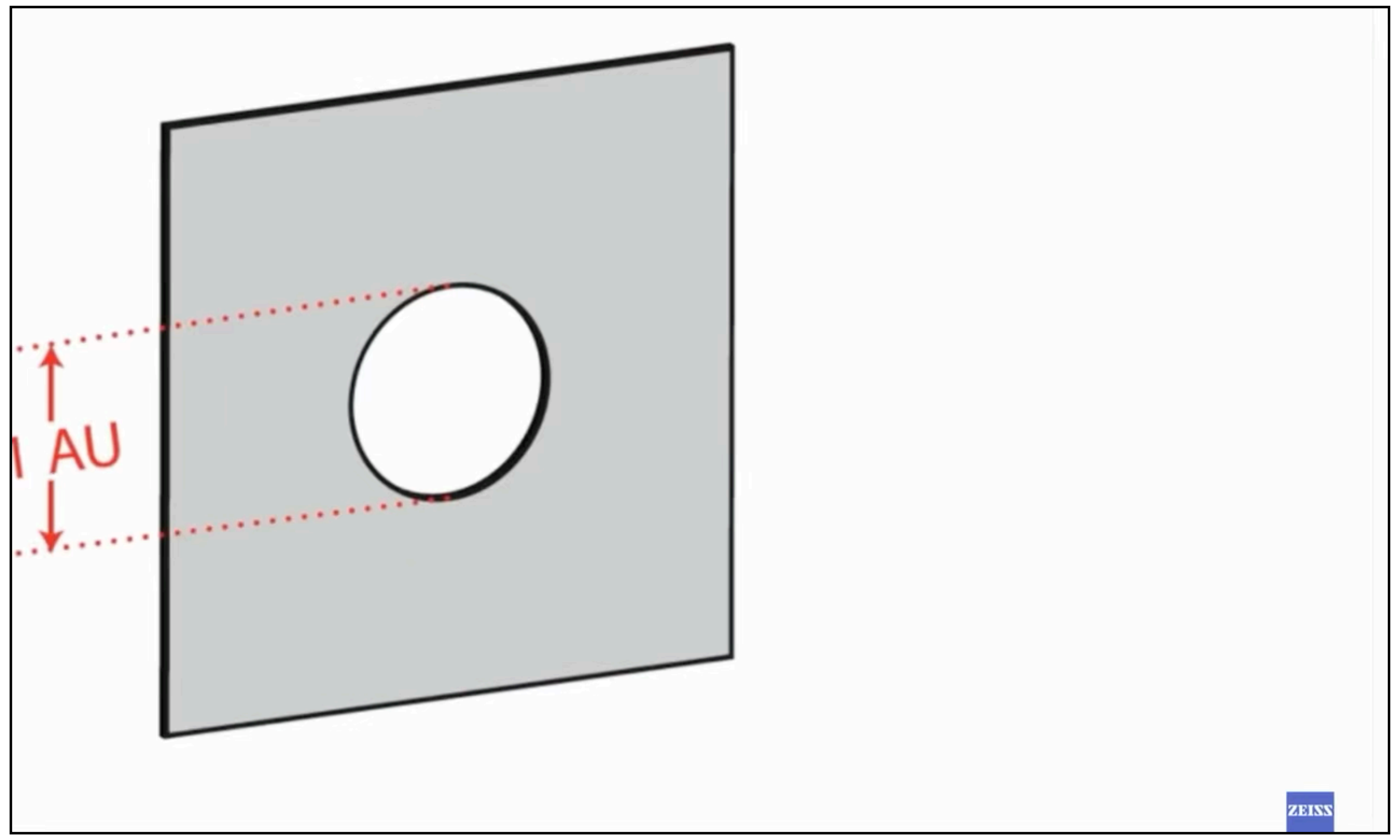


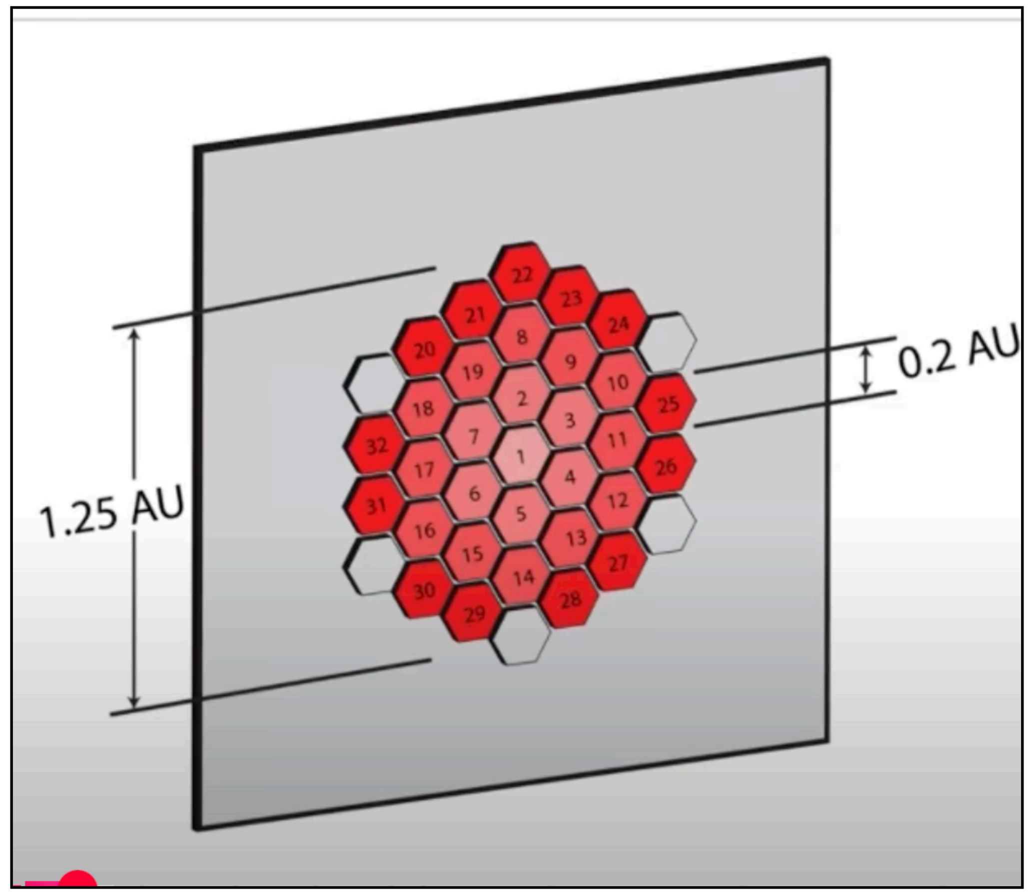
Pocket Guide to Solve Inverse Problems with GlobalBioIm
Soubies, IOP Science, 2019

Special Deconvolution

AiryScan Zeiss LSM 880

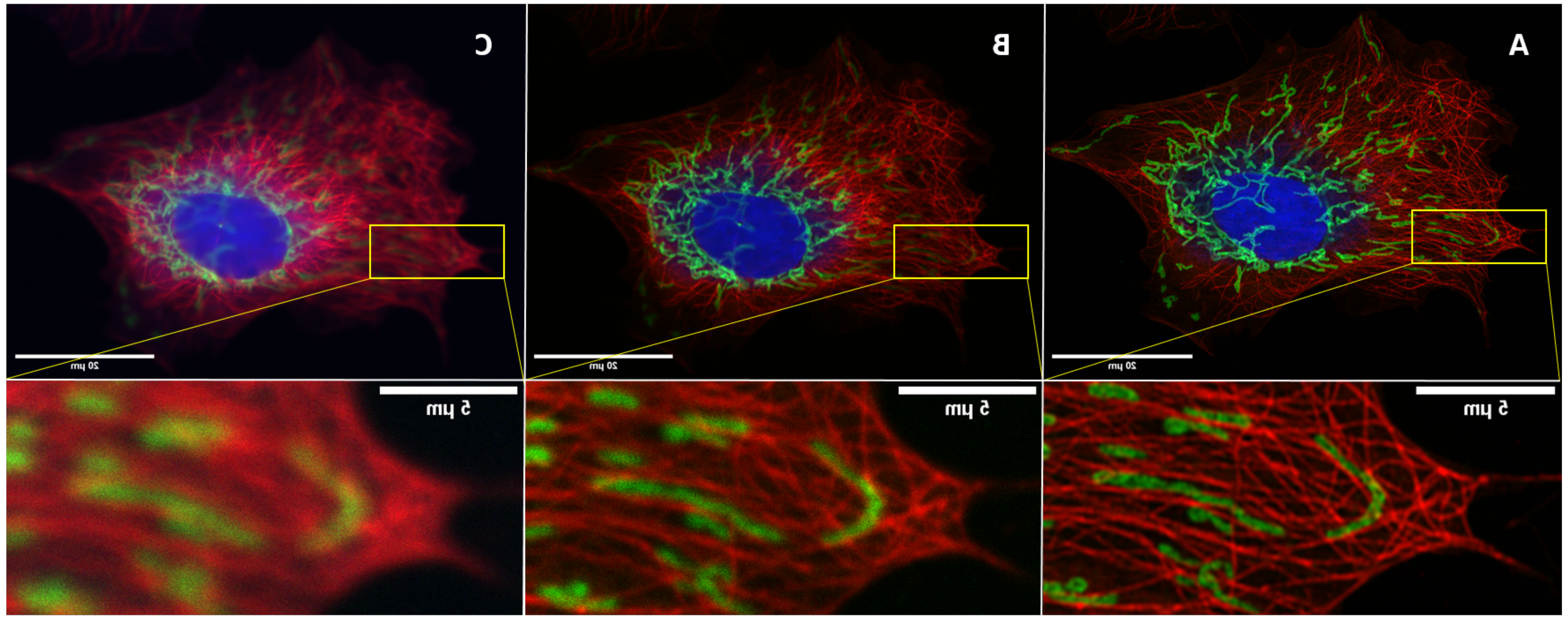
Airyscan is a 32-channel hit-sensitive photomultiplier detector that collects a pinhole-plane image at every scan position

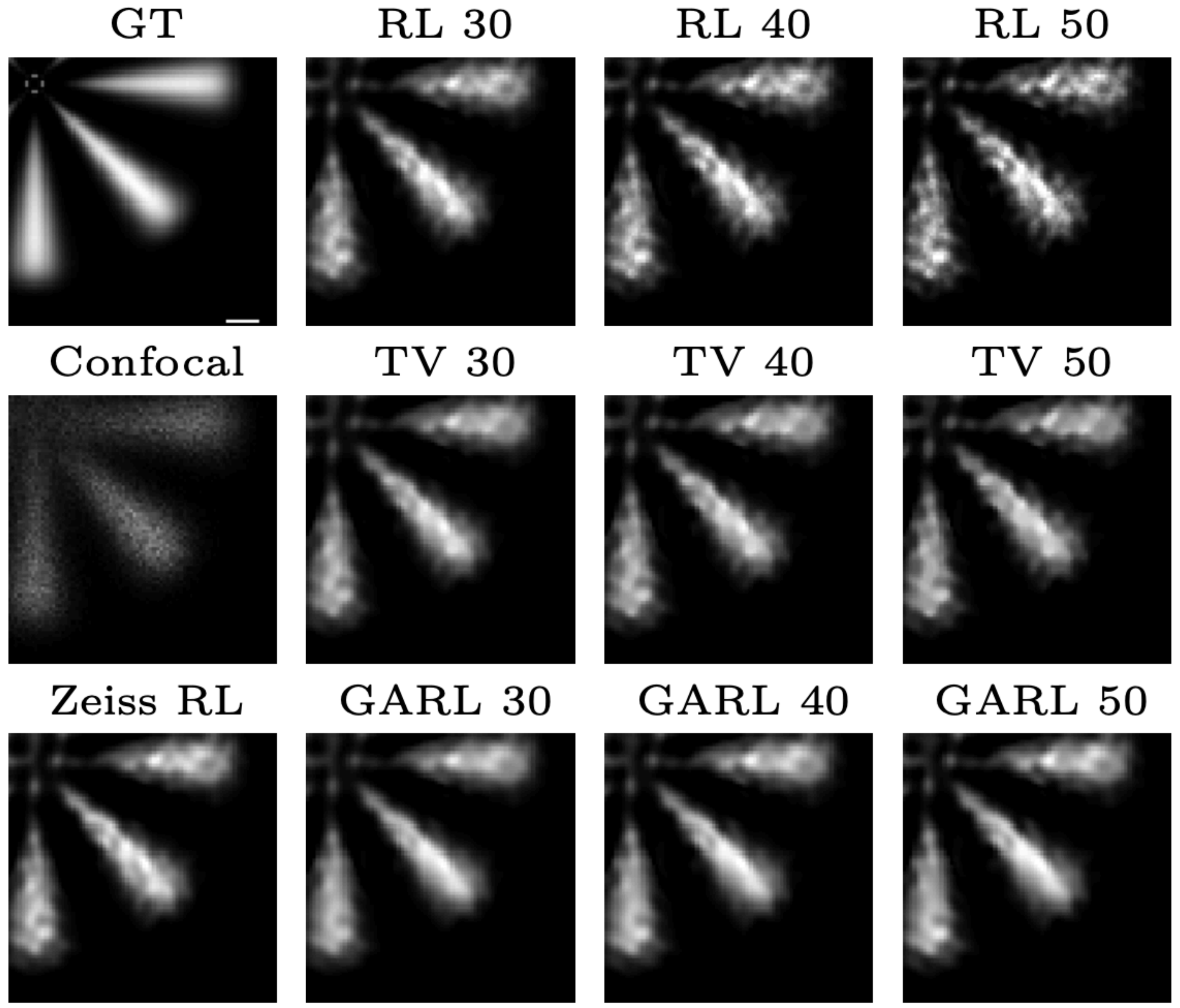




32 in focal plan of the 3D PSF

Widefield | Confocal | Airyscan

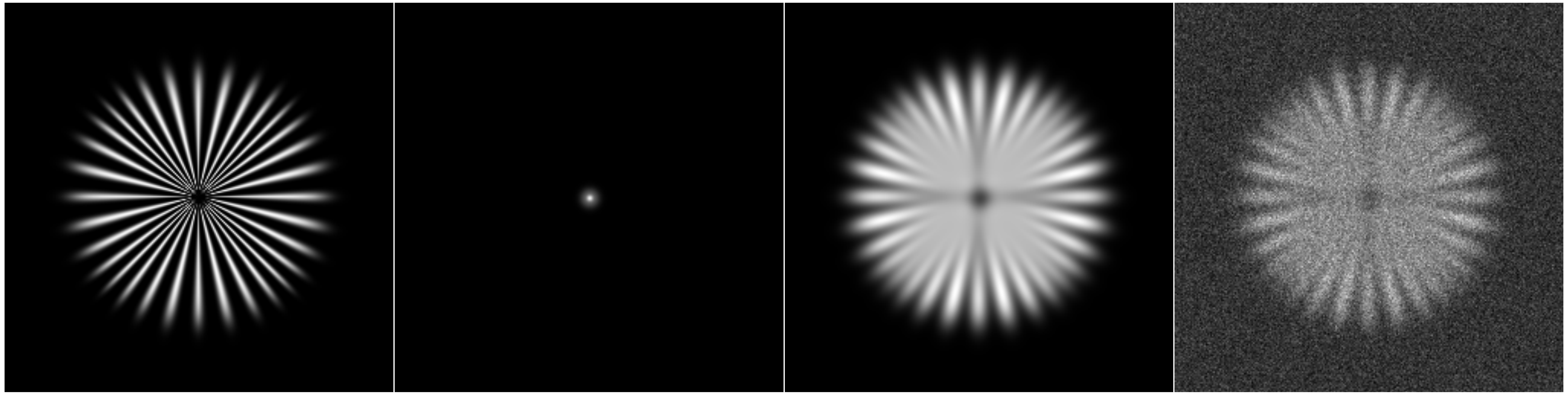




3D Deconvolution Microscopy

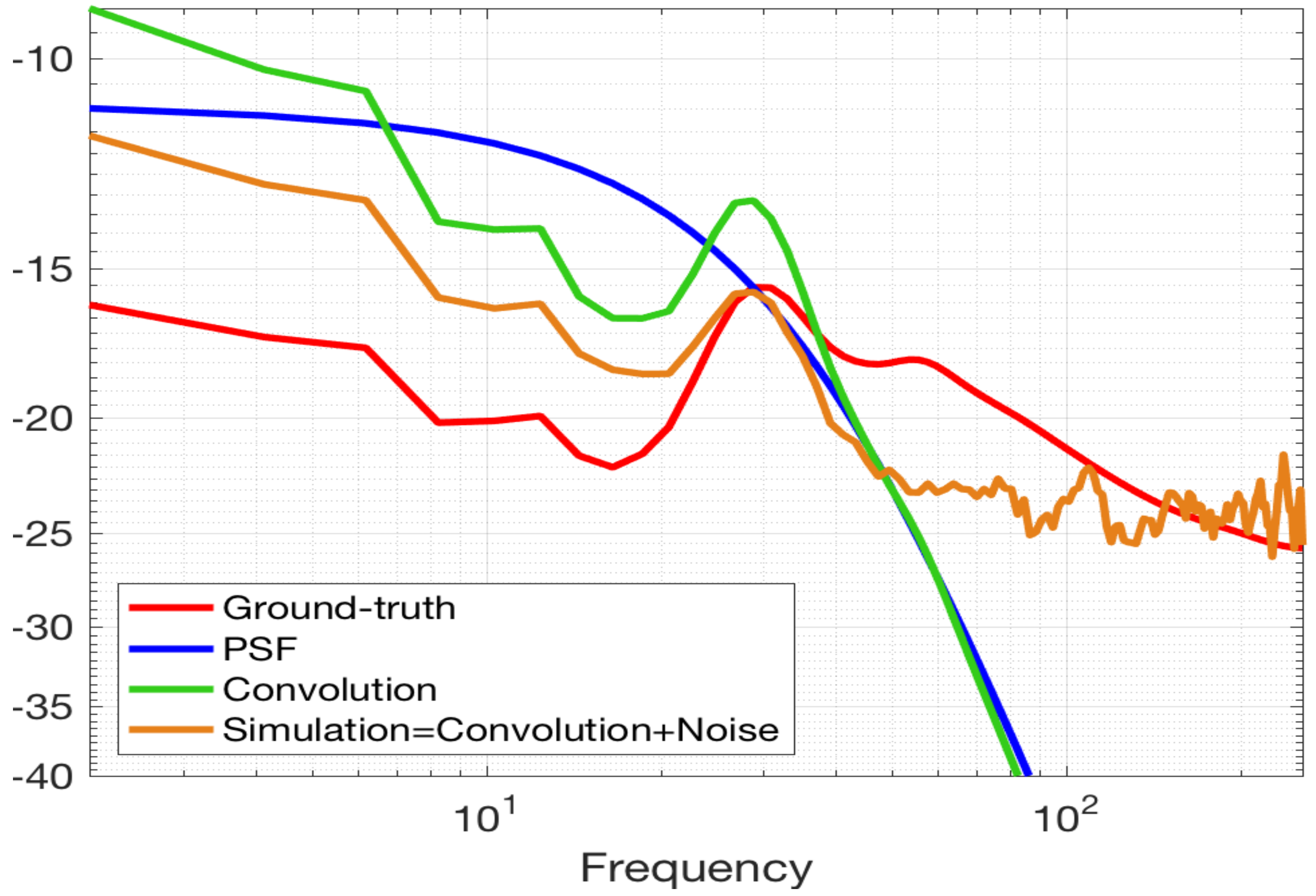
Considerations

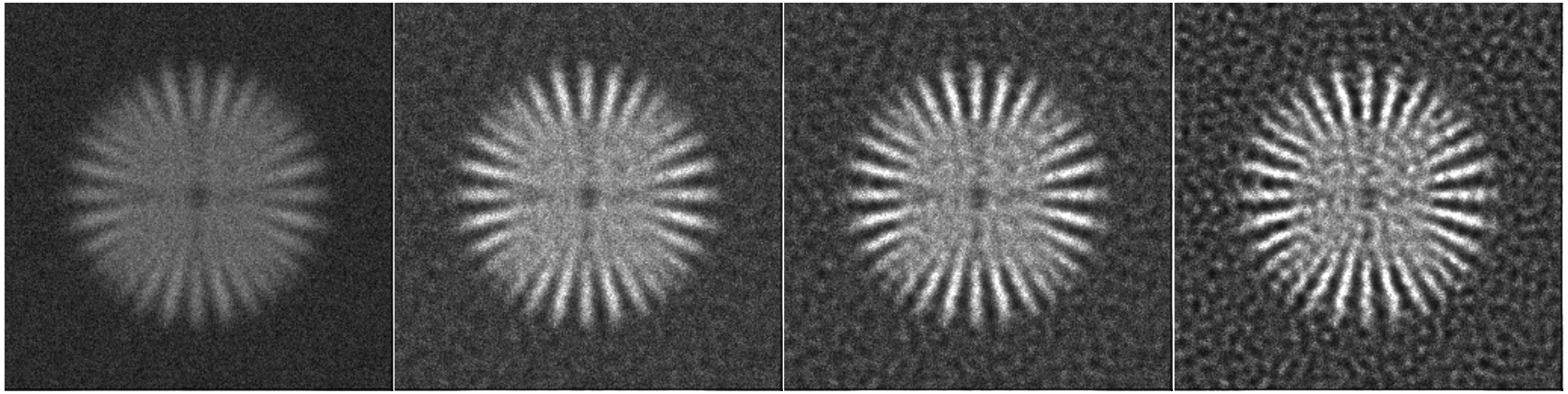
Interpretation of the Spectral Changes



Ground-truth PSF Convolution Simulation

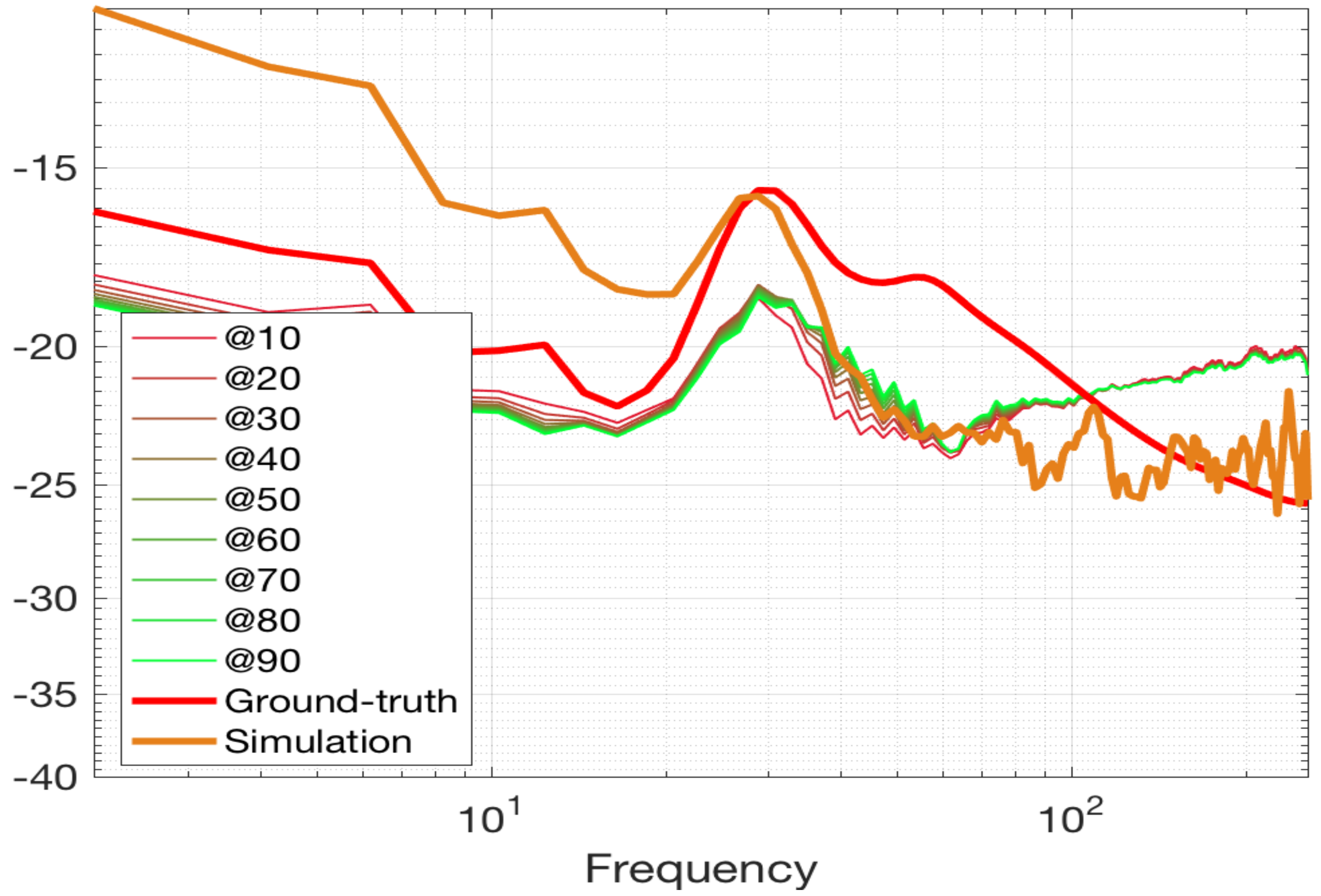
Experimental Protocol



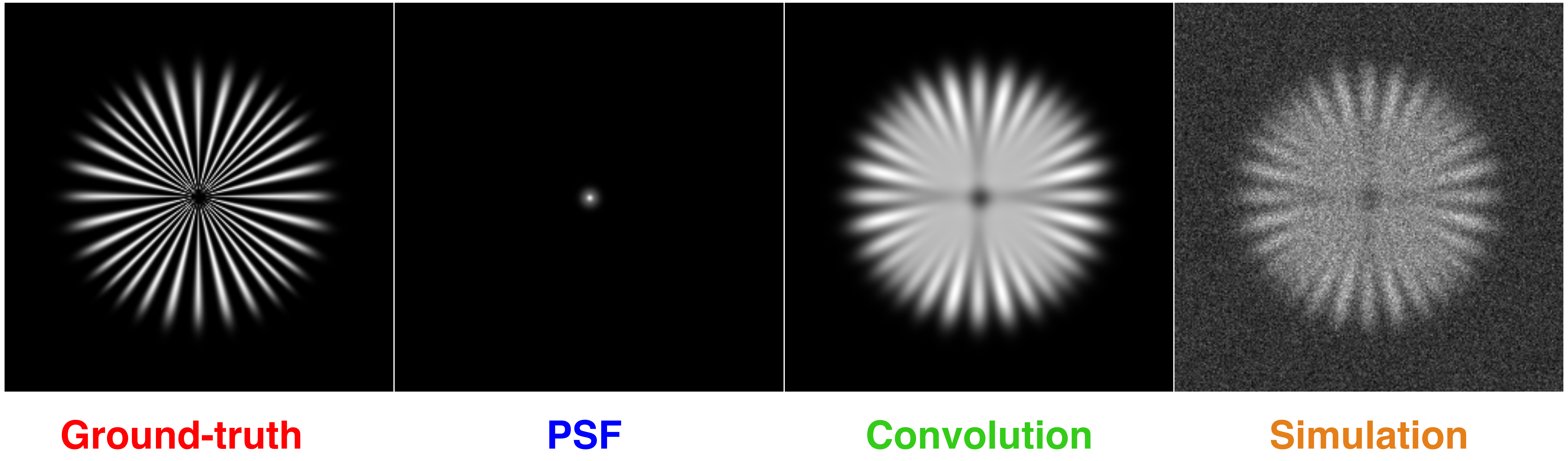


Animation RL@10 RL@30 RL@100

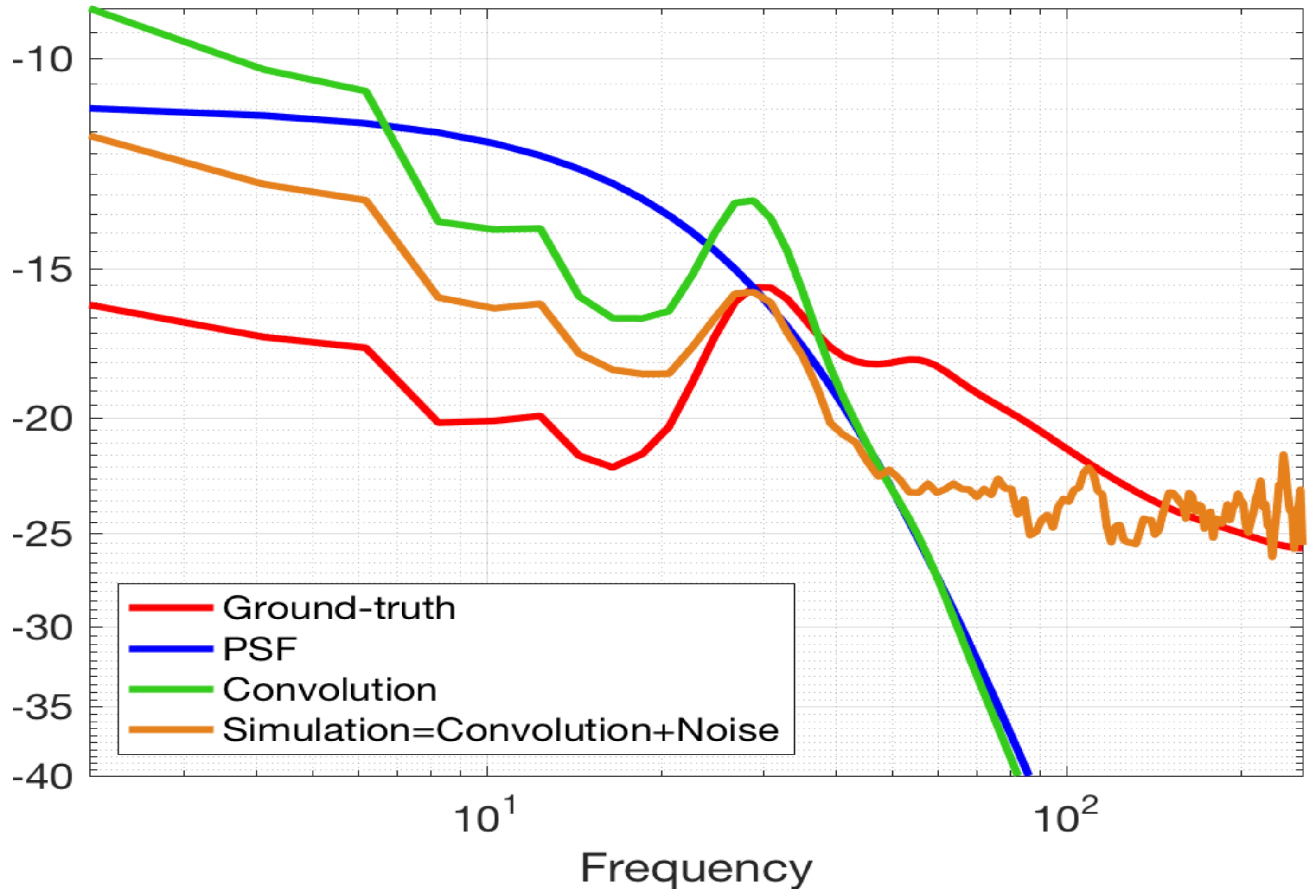
Deconvolution with Richardson-Lucy



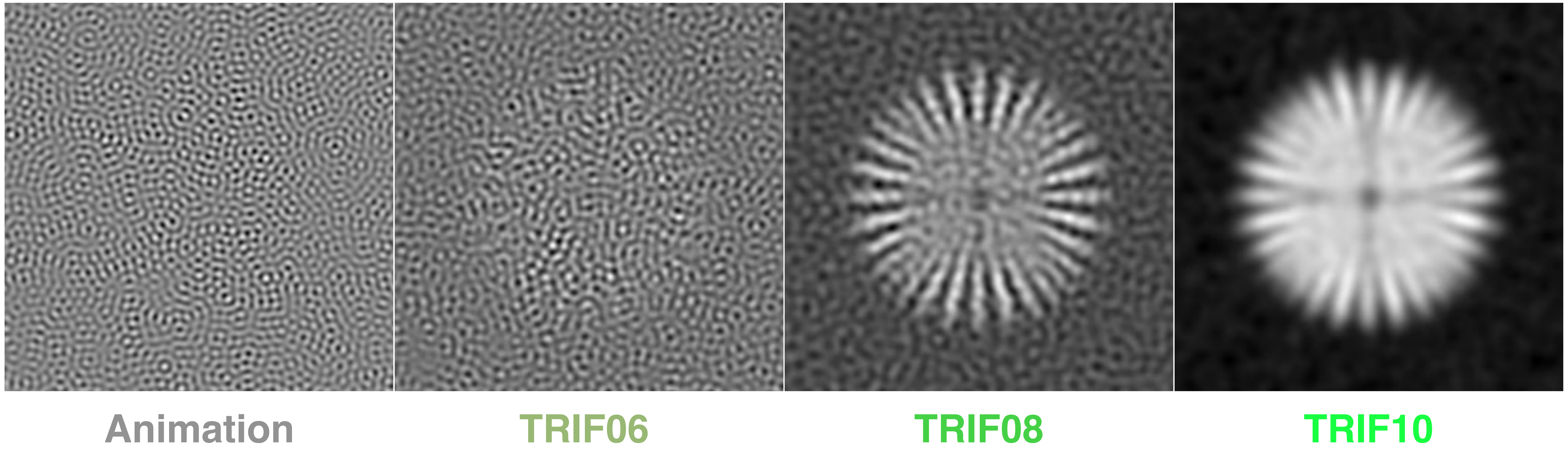
Interpretation of the Spectral Changes

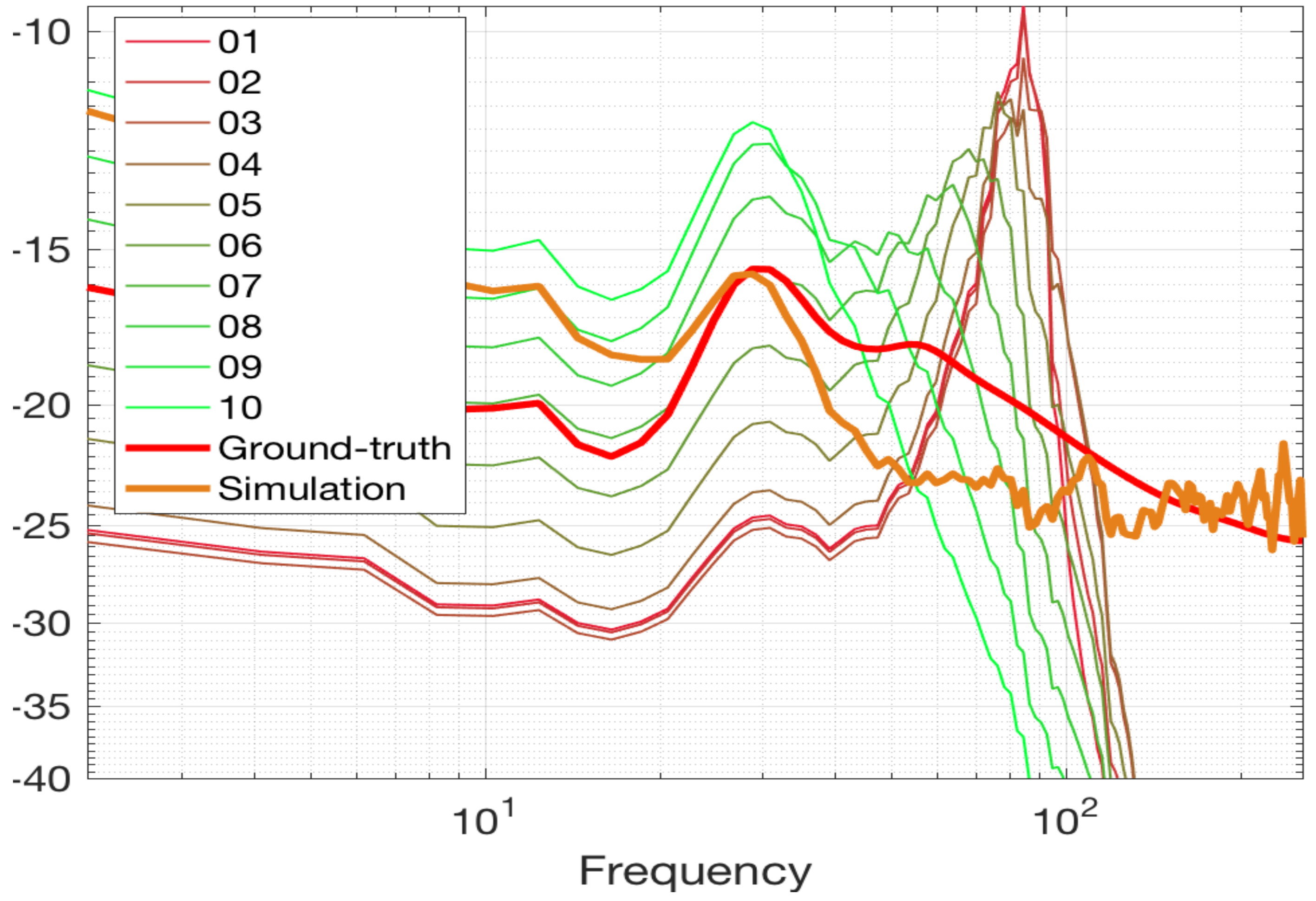


Experimental Protocol

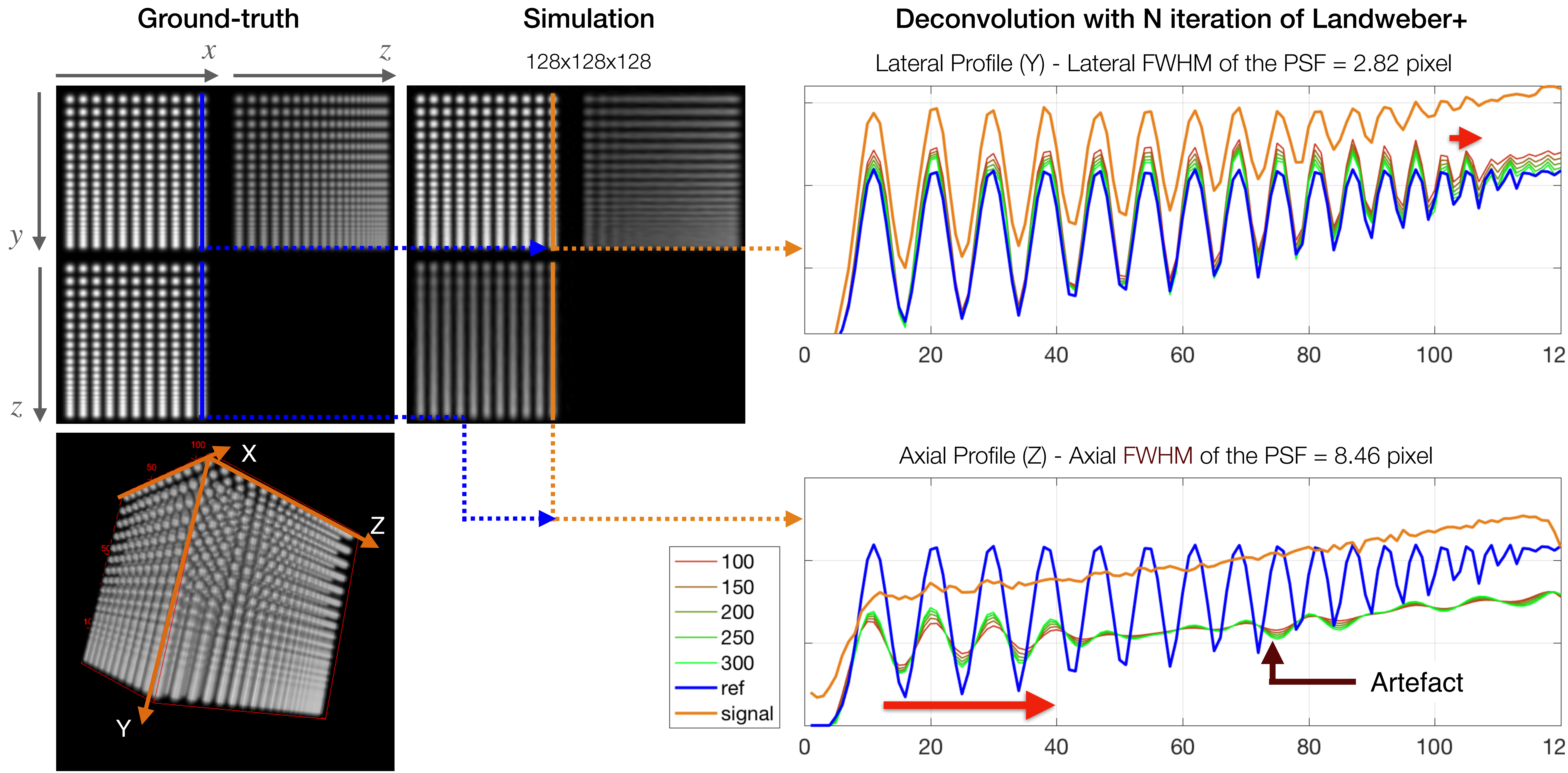


Deconvolution with Tikhonov Regularized Inverse Filter



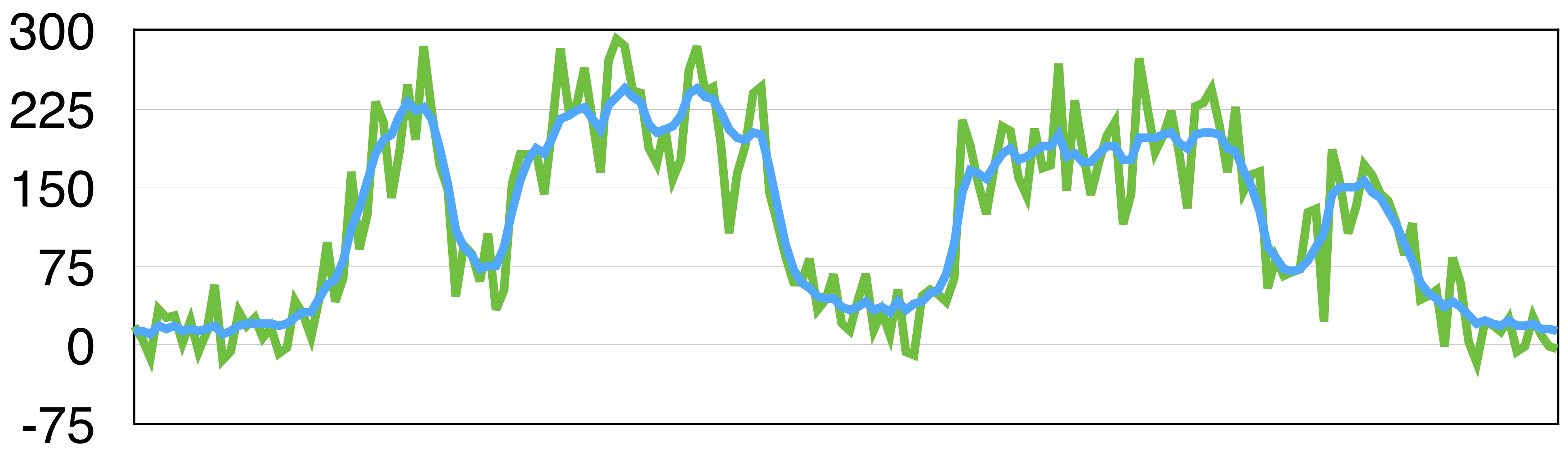


Better Resolution?

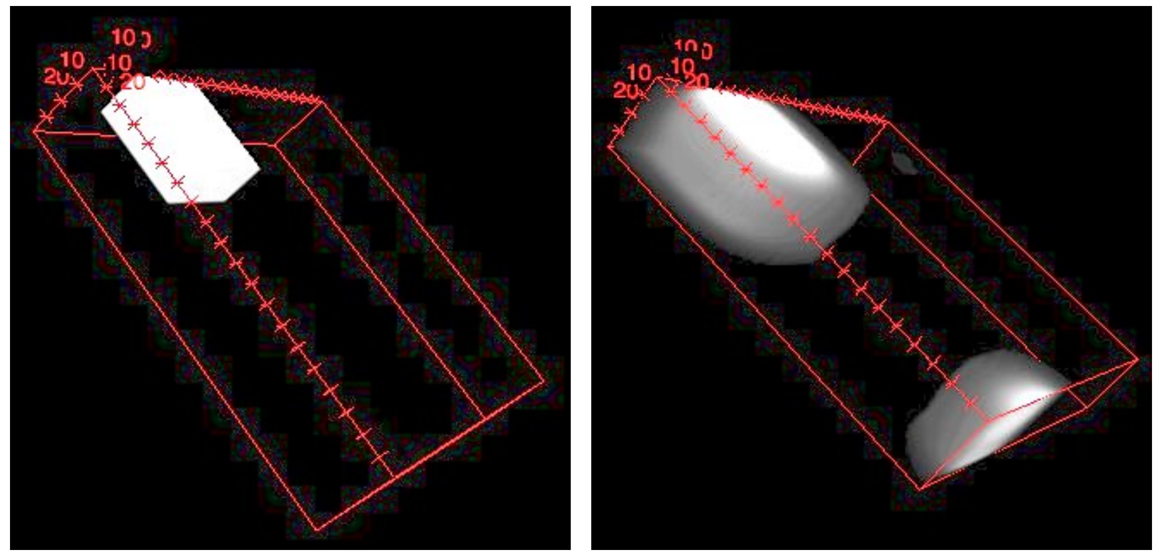


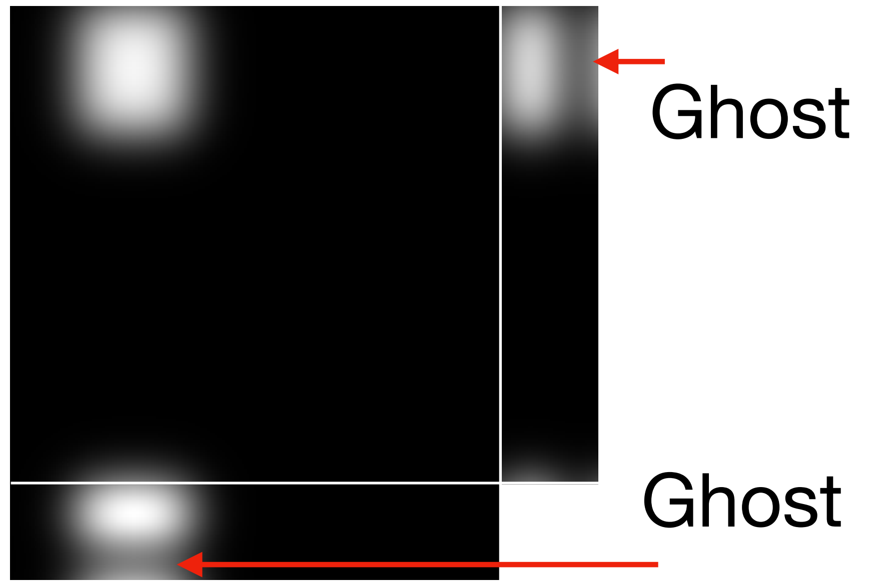
Dynamic of the Signal

Quantitative method Normalization of the PSF = 1

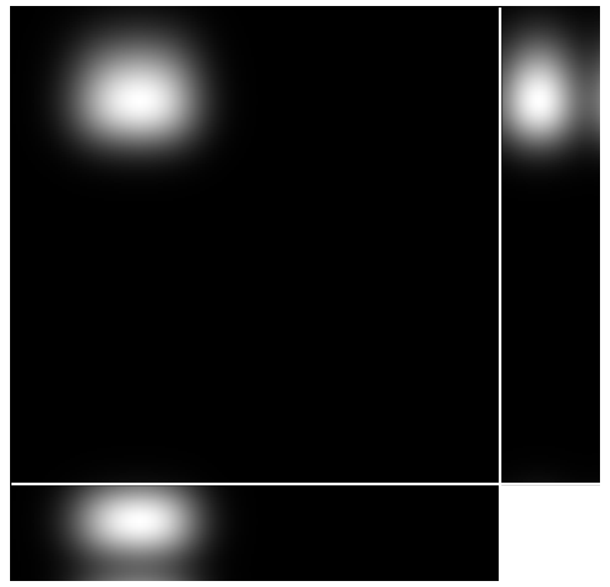


Border artefact

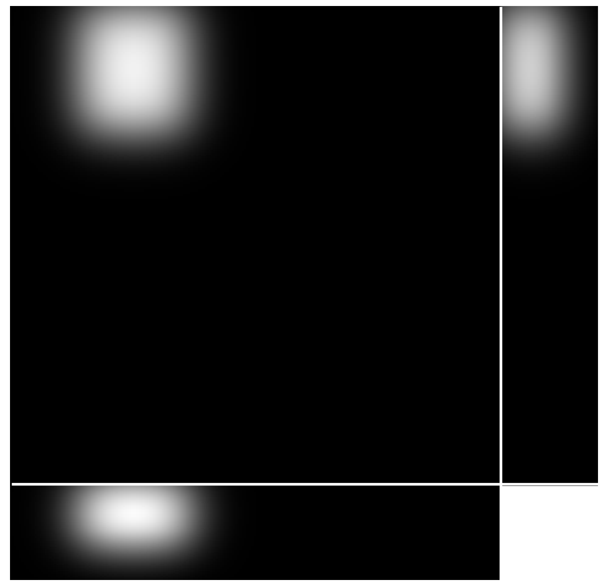




Apodization



Padding



Visualization Overshot / Undershot