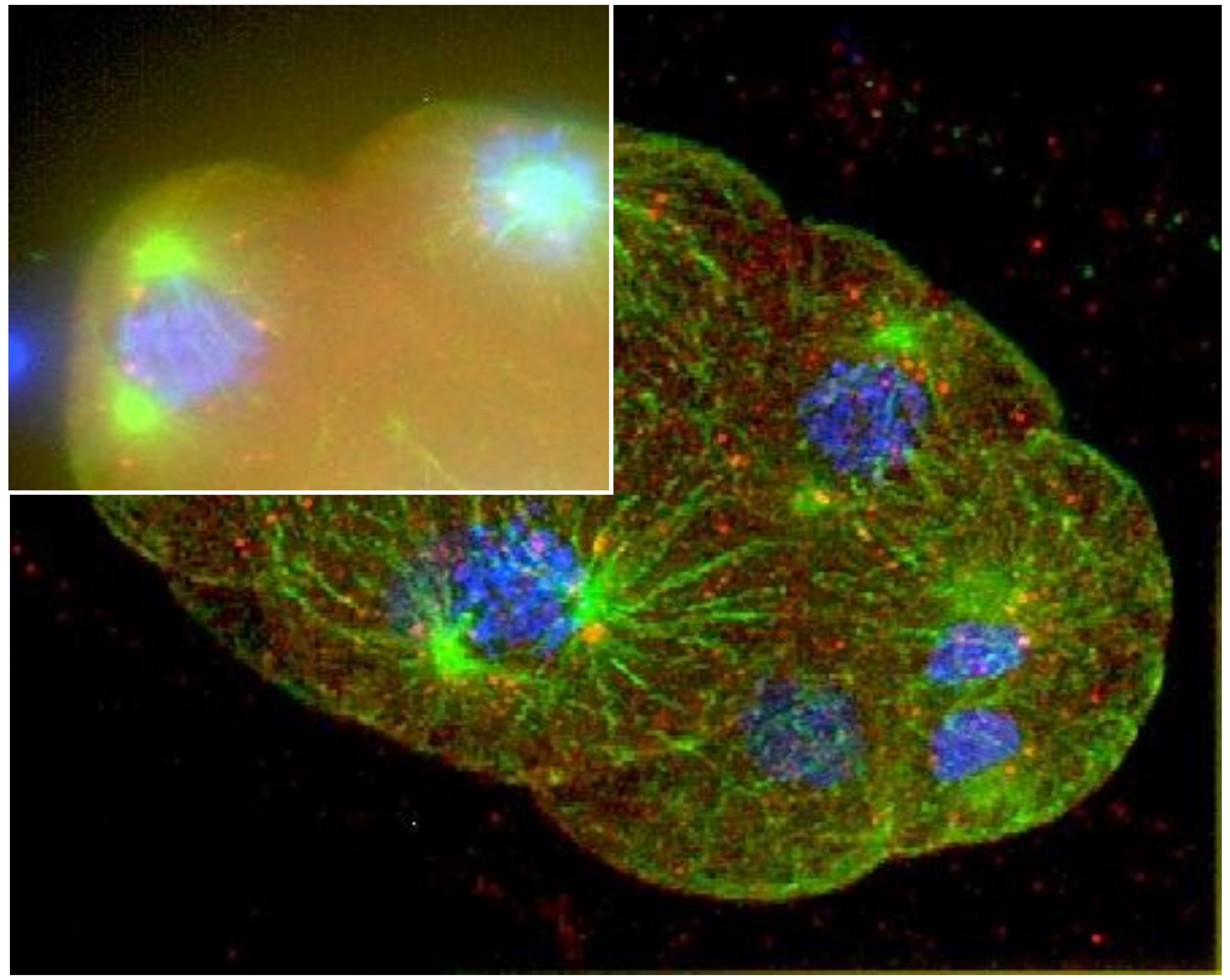
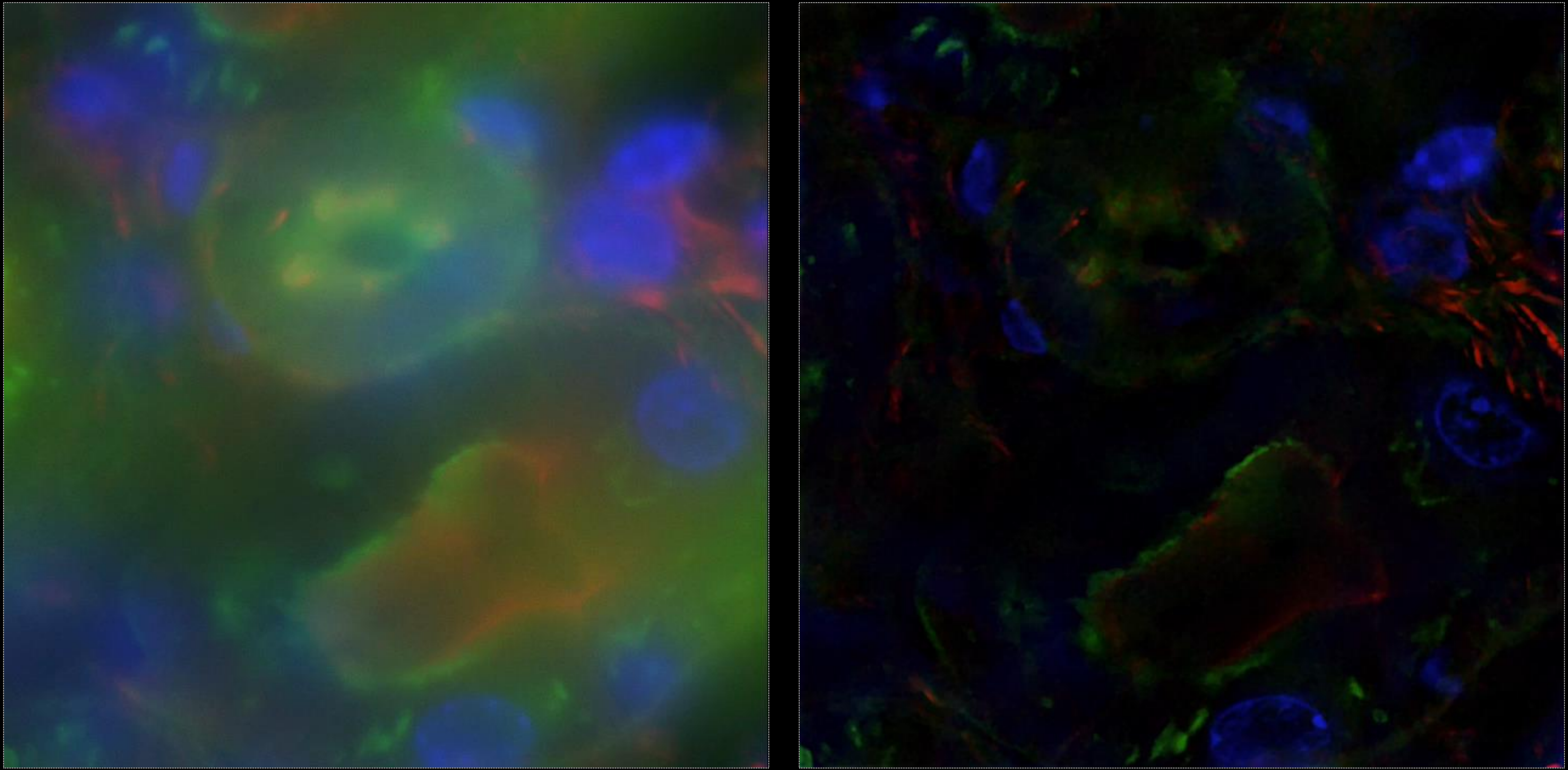
# Deconvolution: Restore Sharper Images by Inverting Acquisition



Daniel Sage and Vasiliki Stergiopoulou

EPFL Center for Imaging
Ecole Polytechnique Fédérale de Lausanne



Courtesy of Ferréol Soulez

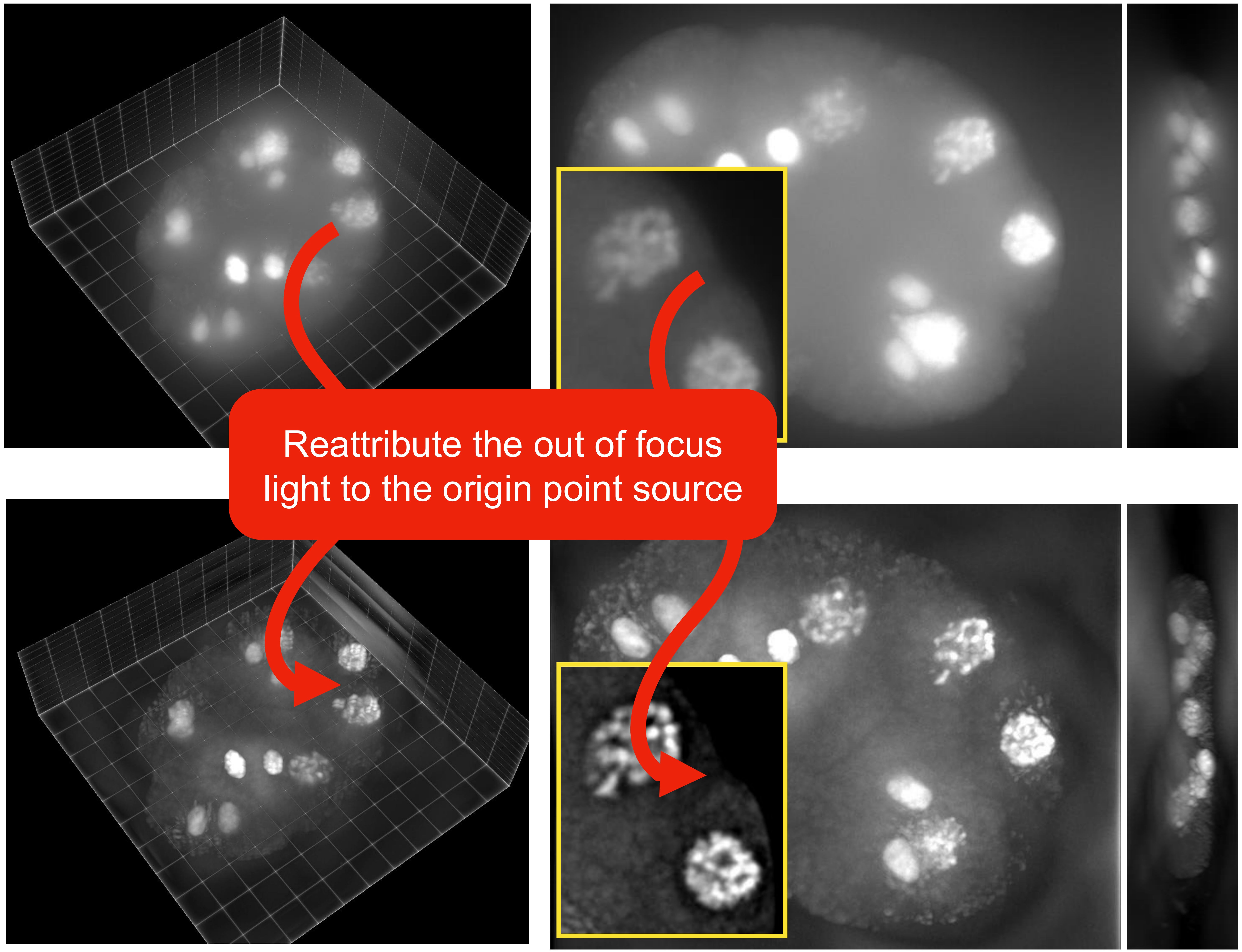
# Presentation’s Overview

- ❖Introduction and Context
- ❖Image Formation
- ❖Methods
  - ❖Direct Inversion
  - ❖Optimization-based (Variational)
  - ❖Physics-inspired Deep-learning
- ❖**Practice**: Image Formation with Deconvolution Lab 2

# Introduction and Context

# Why Deconvolution?

c-elegans embryo. DAPI (nuclei in blue)



Reattribute the out of focus light to the origin point source

## Idea of deconvolution

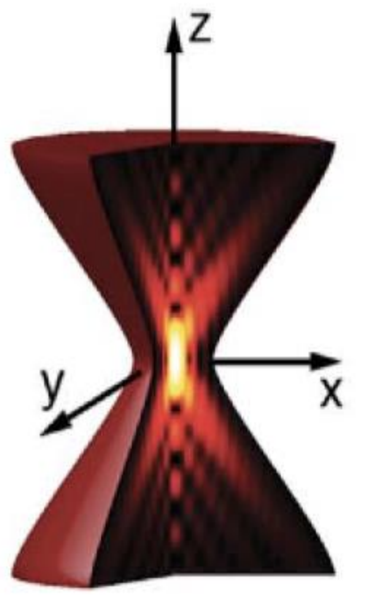
"Undo the blurring — reconstruct the original signal by reversing the effects of convolution.”

- Goal: Recover the original signal from the observed (degraded) one

- Increase contrast
- Suppress noise and artifacts
- Improve resolution
- Enhance fine details and edges
- Recover meaningful features for analysis or interpretation

# Image Deconvolution

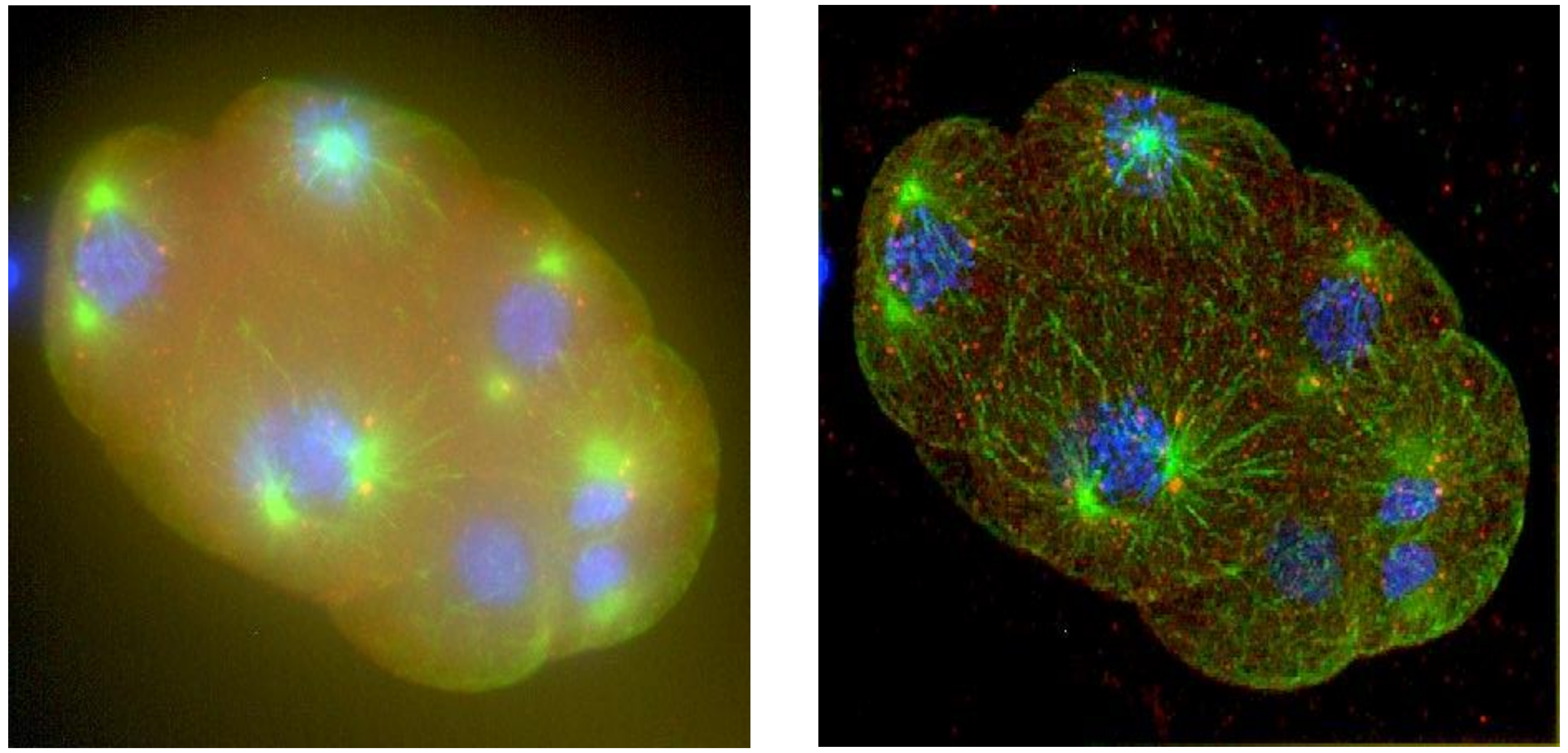
| | | | | |
|---|---|---|---|---|
| 👍 | convolution | **image formation** | unknown | 👎 |
| 👍 | high dynamic<br>preserve | **signal** | noise, saturation<br>high sampling | 👎 |
| 👍 | fine, detail | **structure** | smooth | 🤔 |
| 👍 | known, signature | **PSF** | shift variant | 🤔 |
| 👍 | automatic<br>quantitative | **image analysis** | observation<br>qualitative | 🤔 |
| 👍 | 3D | **out-of-focus** | 2D | 🤔 |



Typical Widefield Microscopy PSF

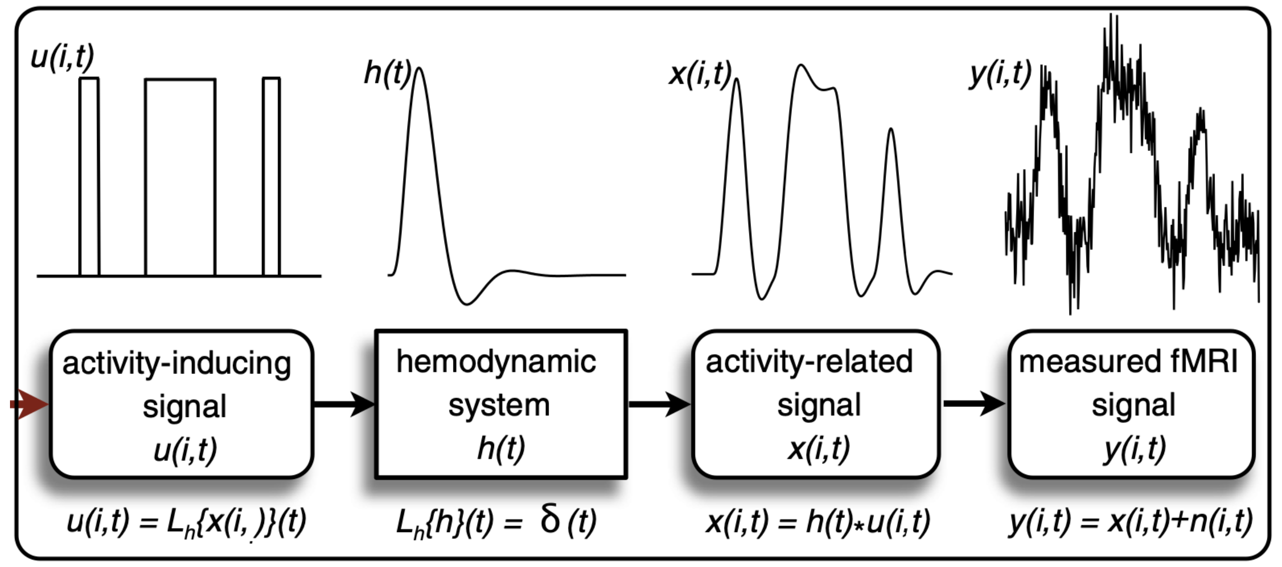
# Application Cases

## Light microscopy



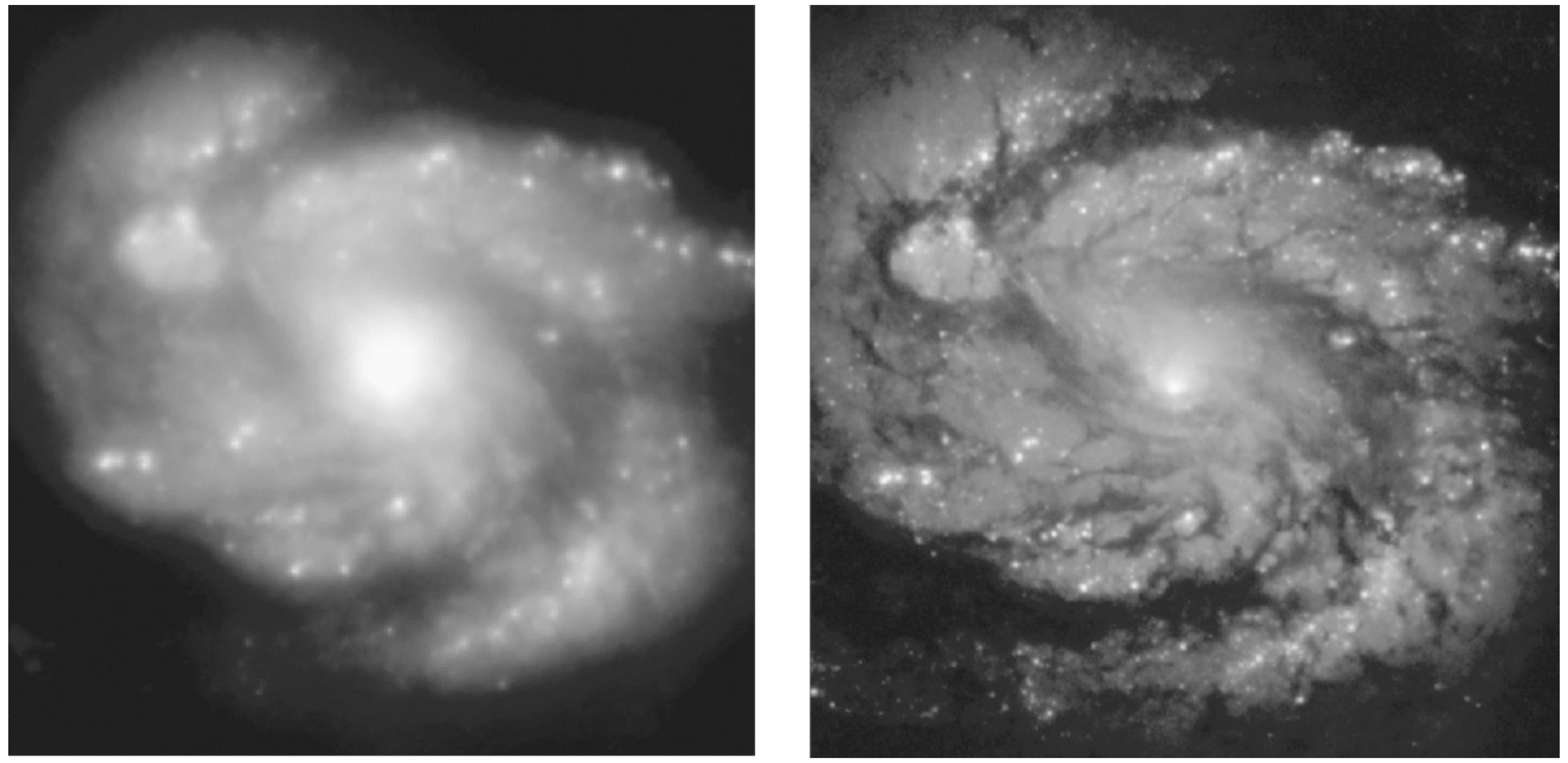
*c-elegans* embryo. DAPI (nuclei in blue), FITC (microtubules in green) and Cy3 (proteins in red) staining

Total activation in fMRI: spatio-temporal deconvolution



*Işık Karahanoğlu,, NeuroImage 2013*

## Astronomy



J. L. Starck, 2002

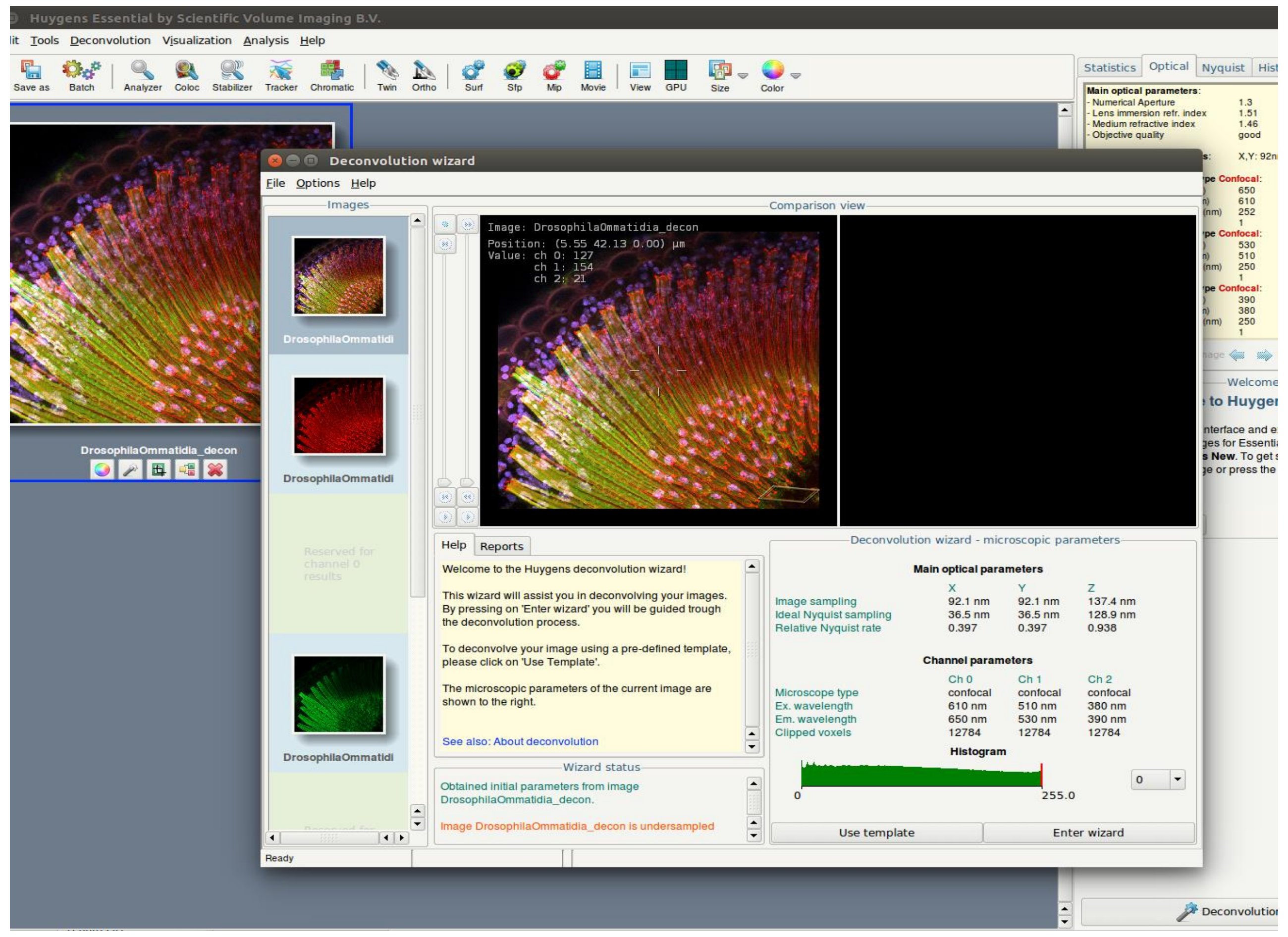
Many fields

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

# Software for Deconvolution

## Commercial Software

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



Main optical parameters:
- Numerical Aperture 1.3
- Lens immersion refr. index 1.51
- Medium refractive index 1.46
- Objective quality good

```
Image: DrosophilaOmmatidia_decon
Position: (5.55 42.13 0.00) µm
Value: ch 0: 127
       ch 1: 154
       ch 2: 21
```

Welcome to the Huygens deconvolution wizard!

This wizard will assist you in deconvolving your images. By pressing on 'Enter wizard' you will be guided trough the deconvolution process.

To deconvolve your image using a pre-defined template, please click on 'Use Template'.

The microscopic parameters of the current image are shown to the right.

See also: About deconvolution

Deconvolution wizard - microscopic parameters

Main optical parameters

| | X | Y | Z |
|---|---|---|---|
| Image sampling | 92.1 nm | 92.1 nm | 137.4 nm |
| Ideal Nyquist sampling | 36.5 nm | 36.5 nm | 128.9 nm |
| Relative Nyquist rate | 0.397 | 0.397 | 0.938 |

Channel parameters

| | Ch 0 | Ch 1 | Ch 2 |
|---|---|---|---|
| Microscope type | confocal | confocal | confocal |
| Ex. wavelength | 610 nm | 510 nm | 380 nm |
| Em. wavelength | 650 nm | 530 nm | 390 nm |
| Clipped voxels | 12784 | 12784 | 12784 |

Histogram

0 255.0

Obtained initial parameters from image DrosophilaOmmatidia_decon.

Image DrosophilaOmmatidia_decon is undersampled

Huygens

## Open-source software

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

- SDeconv on Napari [Sylvain Pringent]
- Pyxu [Sepand Kashani – EPFL Center for Imaging]
- DeepInv [Julian Tachella and co-dev]
- Scikit-Image

- GlobalBioIm [Emanuel Soubies – BIG EPFL]
- MATLAB Image Processing Toolbox

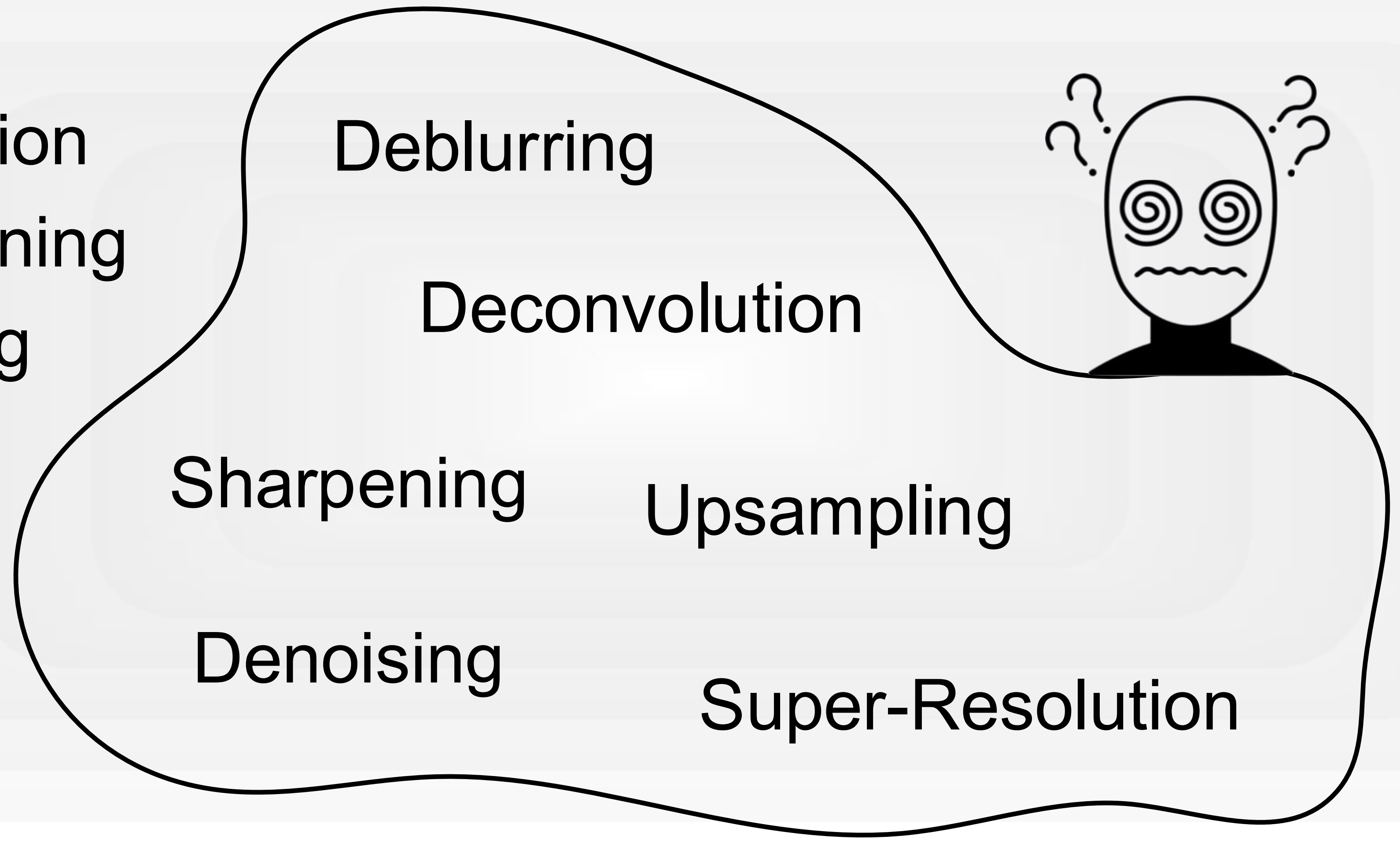
# Image Formation

Point-Spread Function, Noise, Convolution

# Mathematical Model

Notation

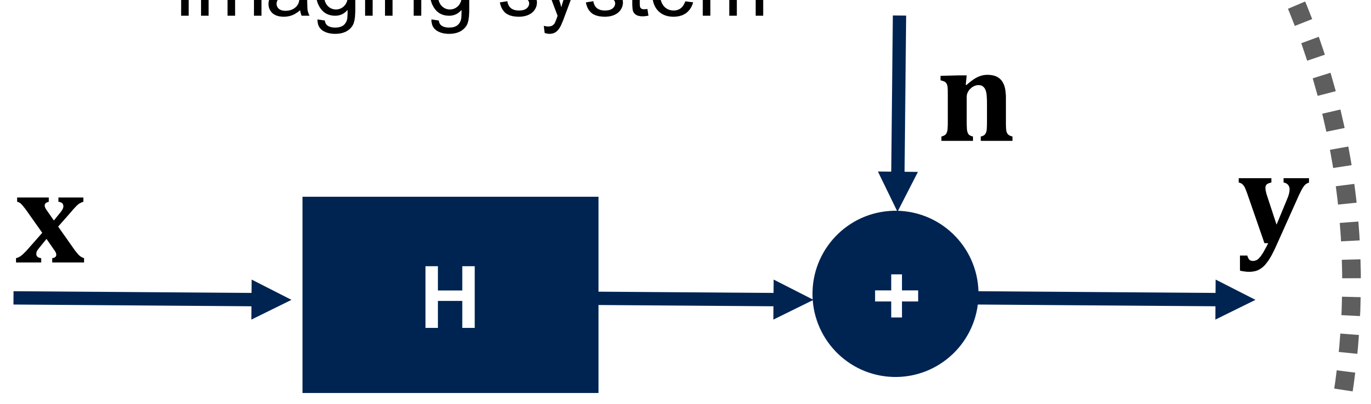
| | | |
|---|---|---|
| Image | $\boldsymbol{x} = (x_1, \dots x_d) \in \mathbb{R}^d$ | Vectorial notation |
| Filter/Operation | $\mathbf{Hx}$<br>$(\mathbf{h} * \mathbf{x})$ | Matrix notation |



Computer Vision
Machine Learning
Deep Learning

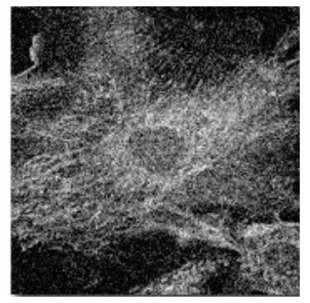
Deblurring
Deconvolution
Sharpening
Upsampling
Denoising
Super-Resolution

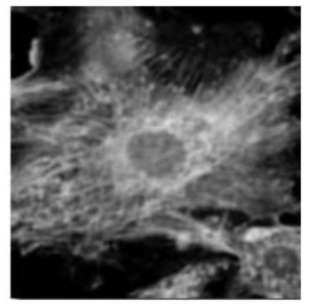
Standard model for an imaging system

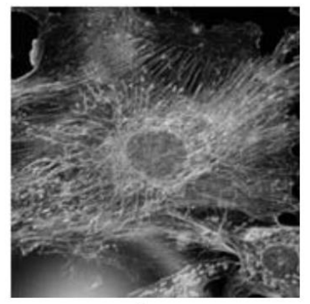


$$\mathbf{y} = \mathbf{Hx} + \mathbf{n}$$

IMAGE DEGRADATION


Noise


Scatter


Glare

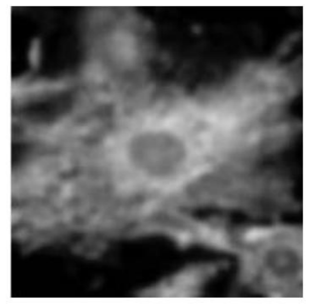

Blur

IMAGE RESTORATION

Deconvolution

$\mathbf{H} \rightarrow$ PSF

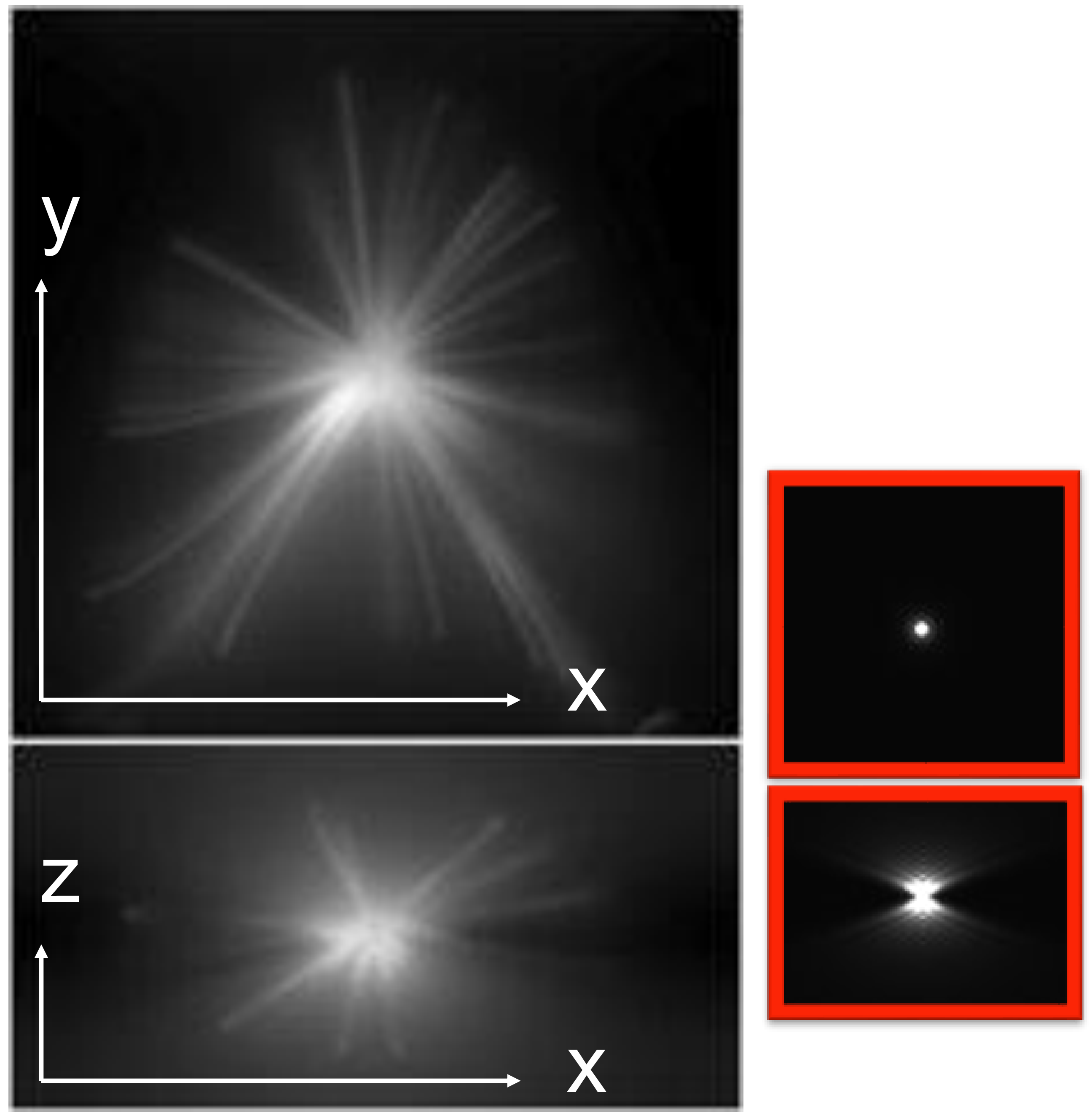
Denoising

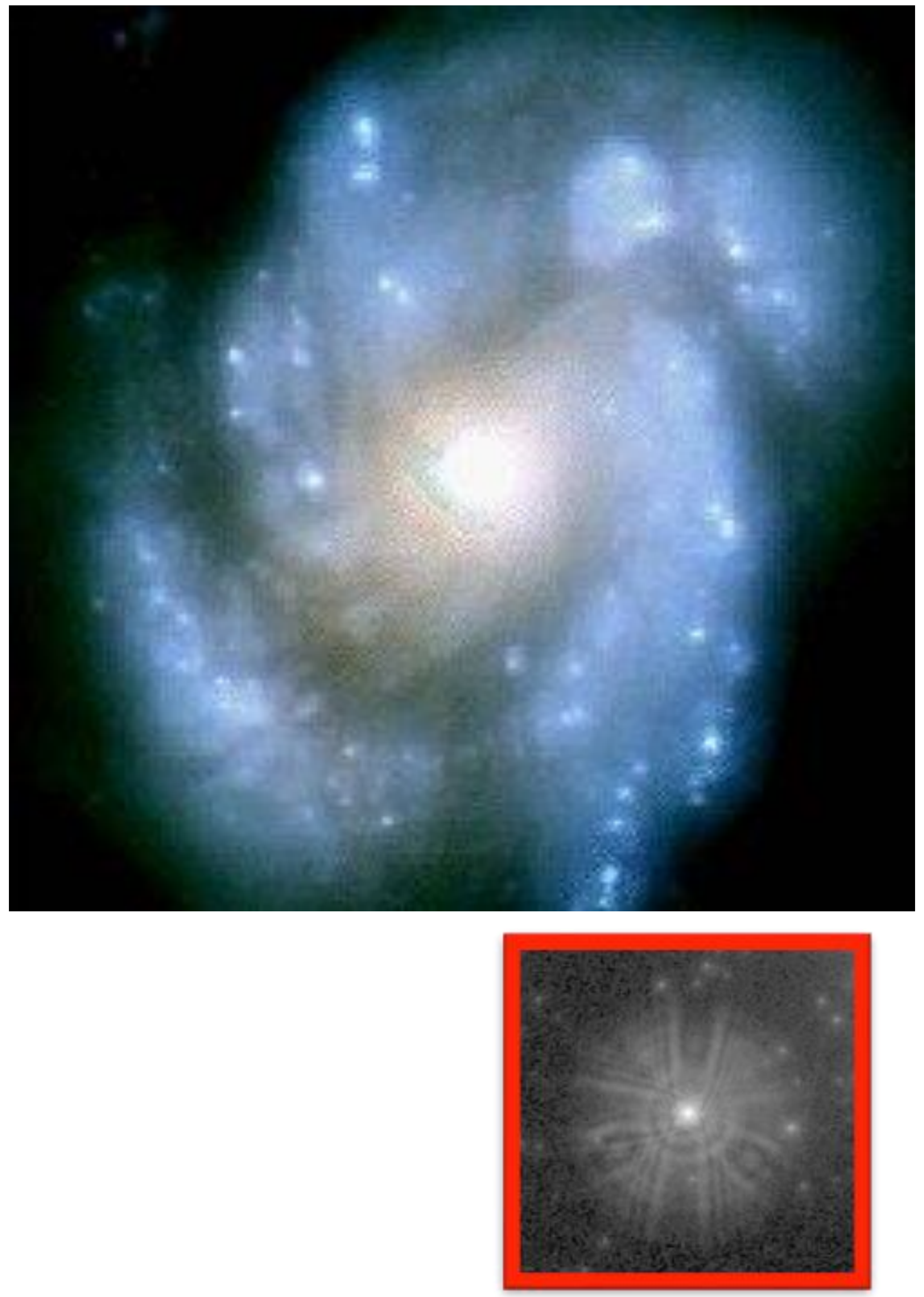
$$\mathbf{H} = \mathbf{I}$$

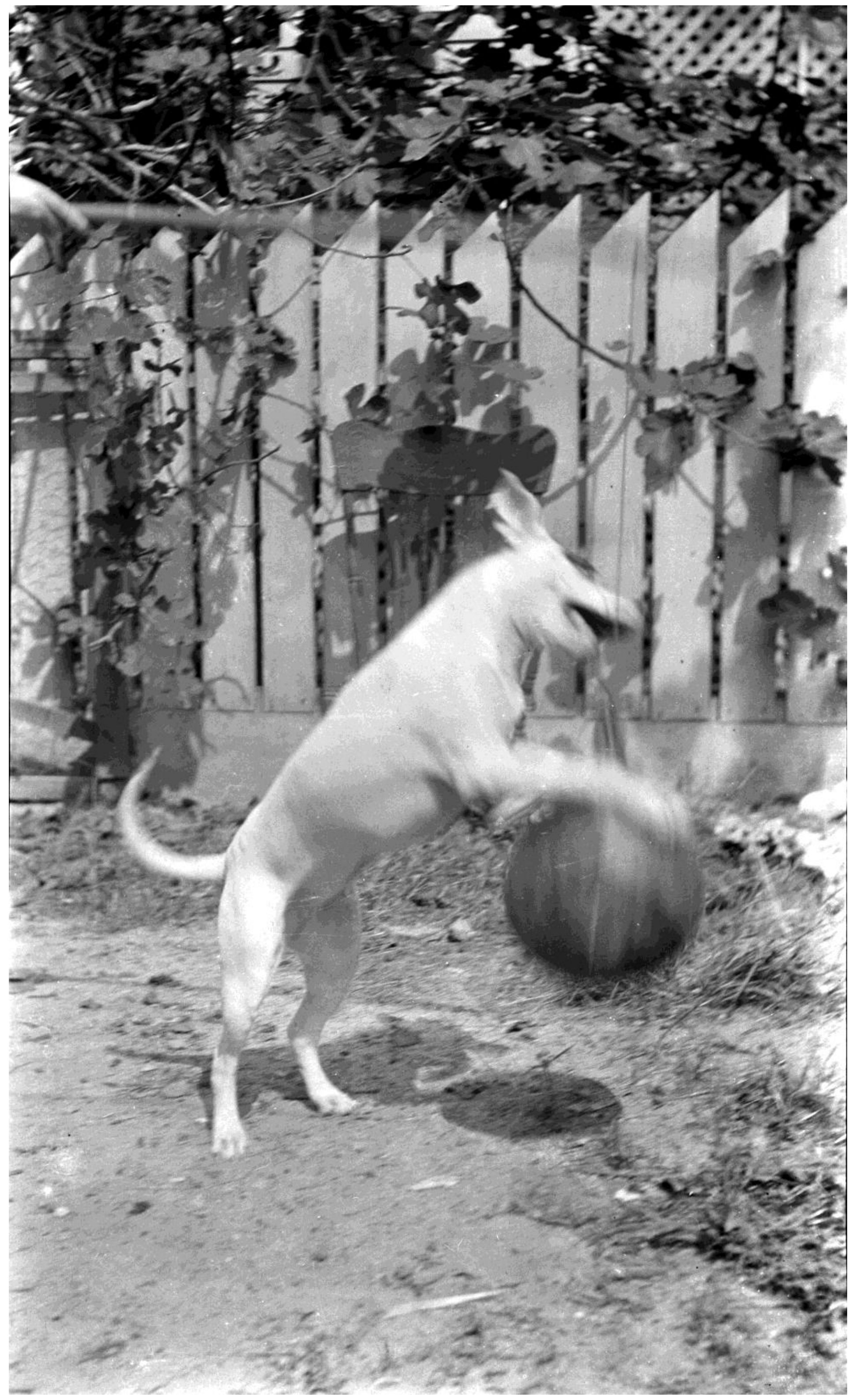
Physical (real) world

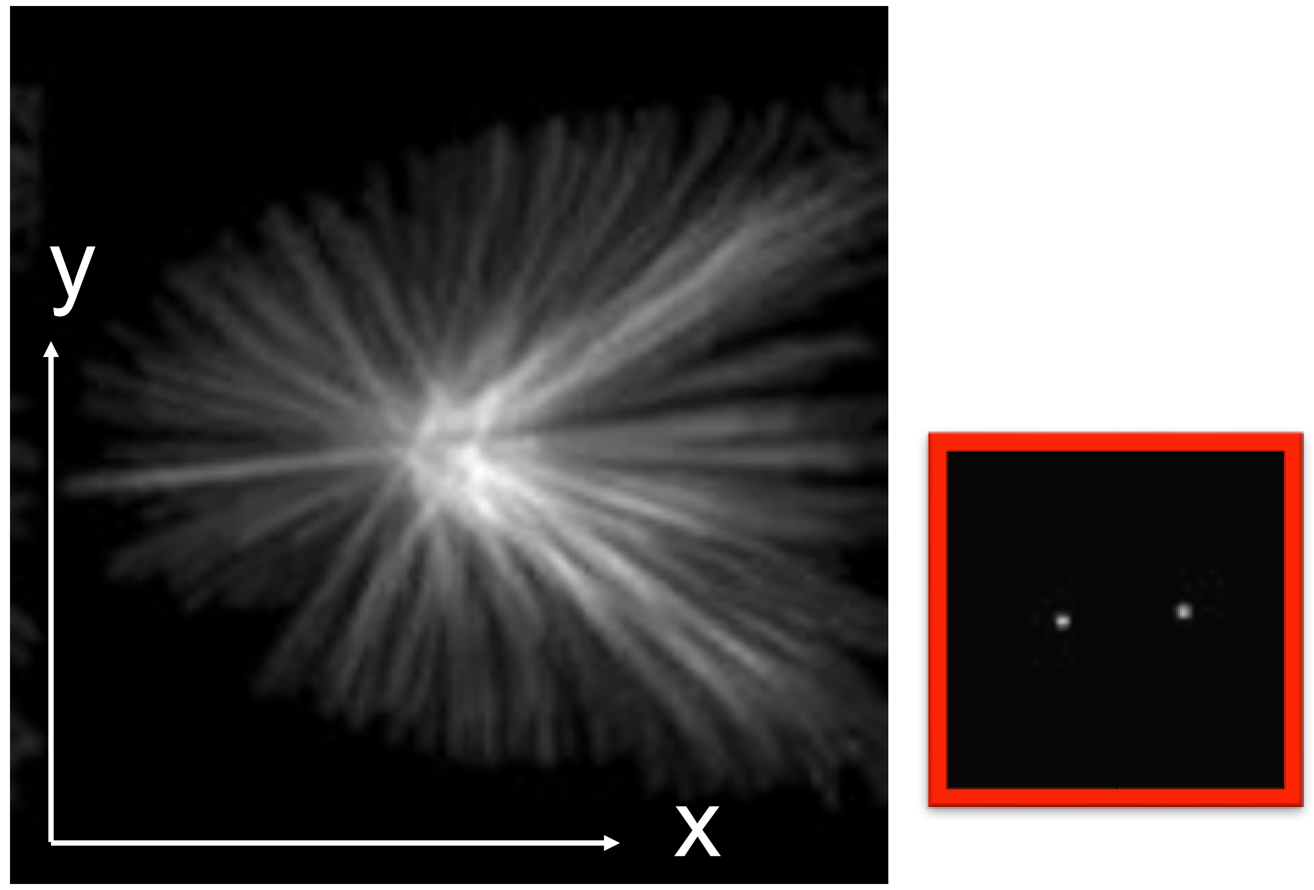
Digital (virtual) world

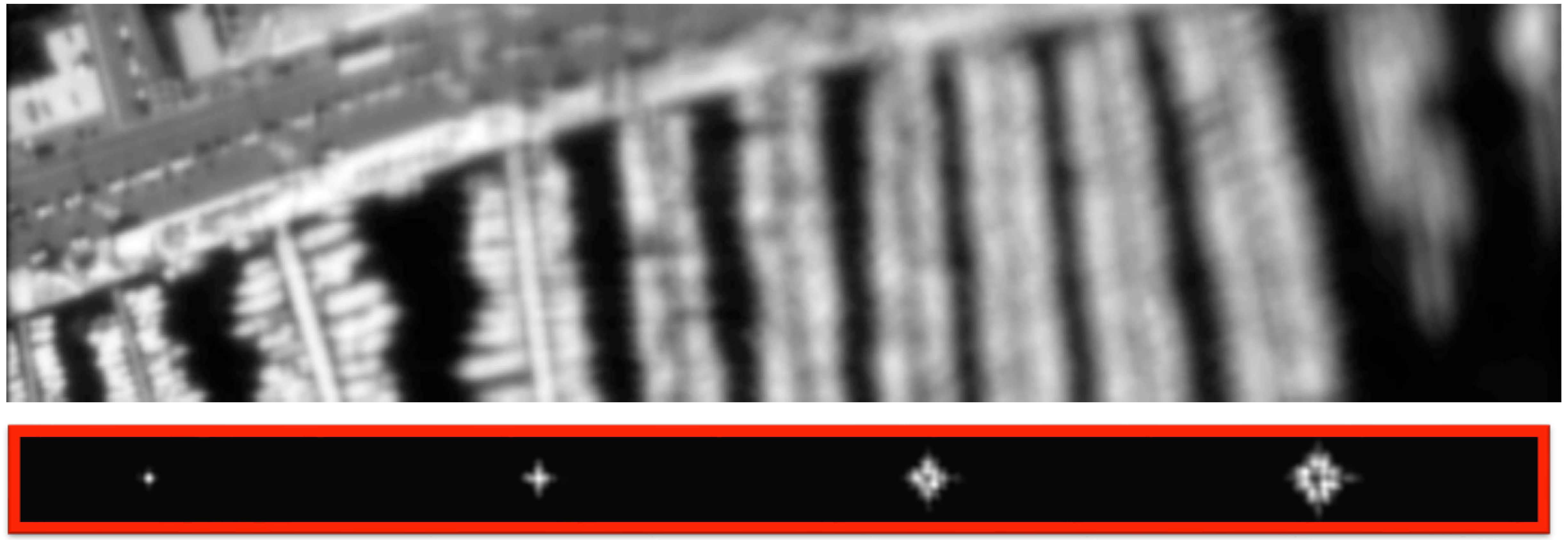
# Point-Spread Functions



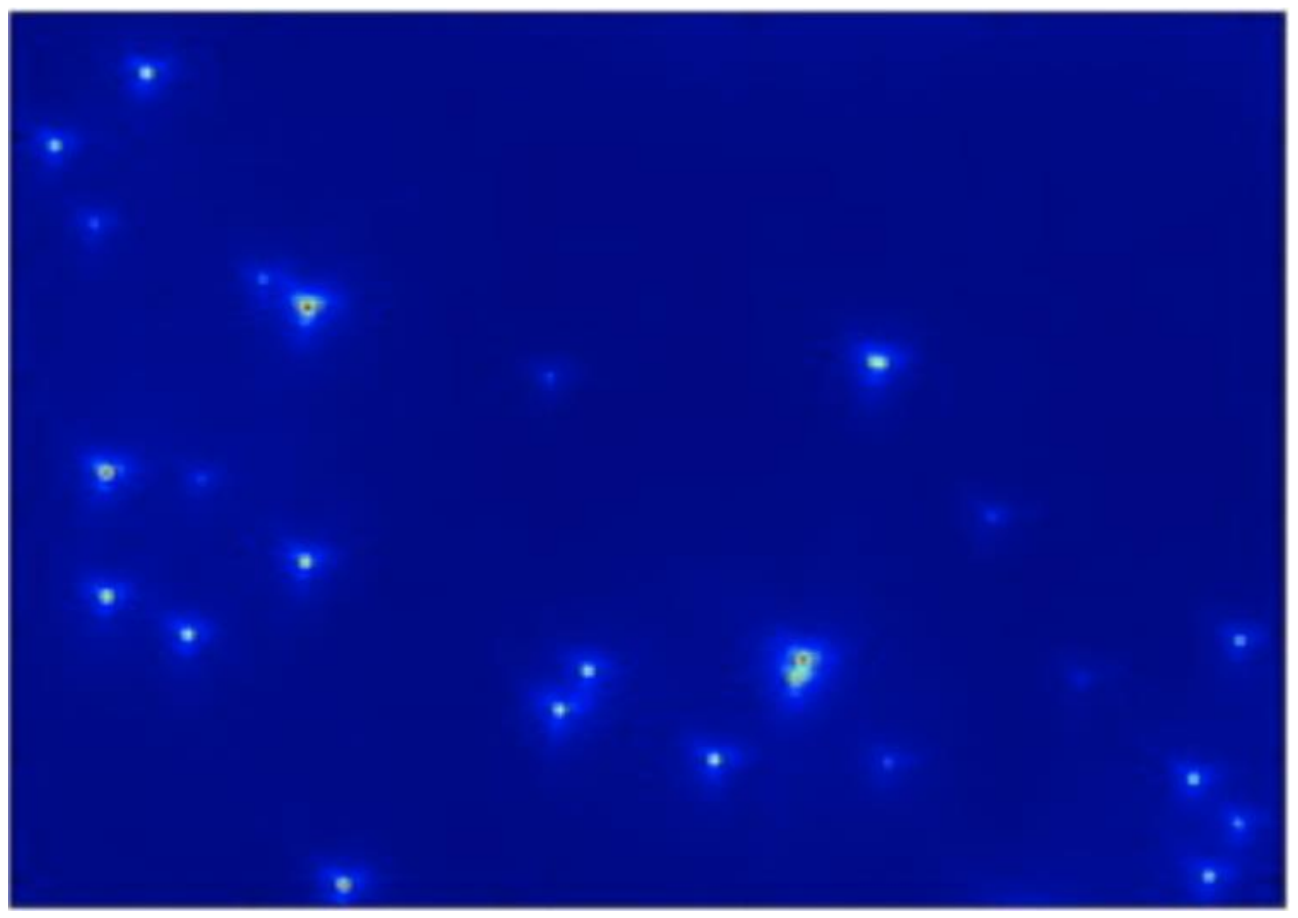


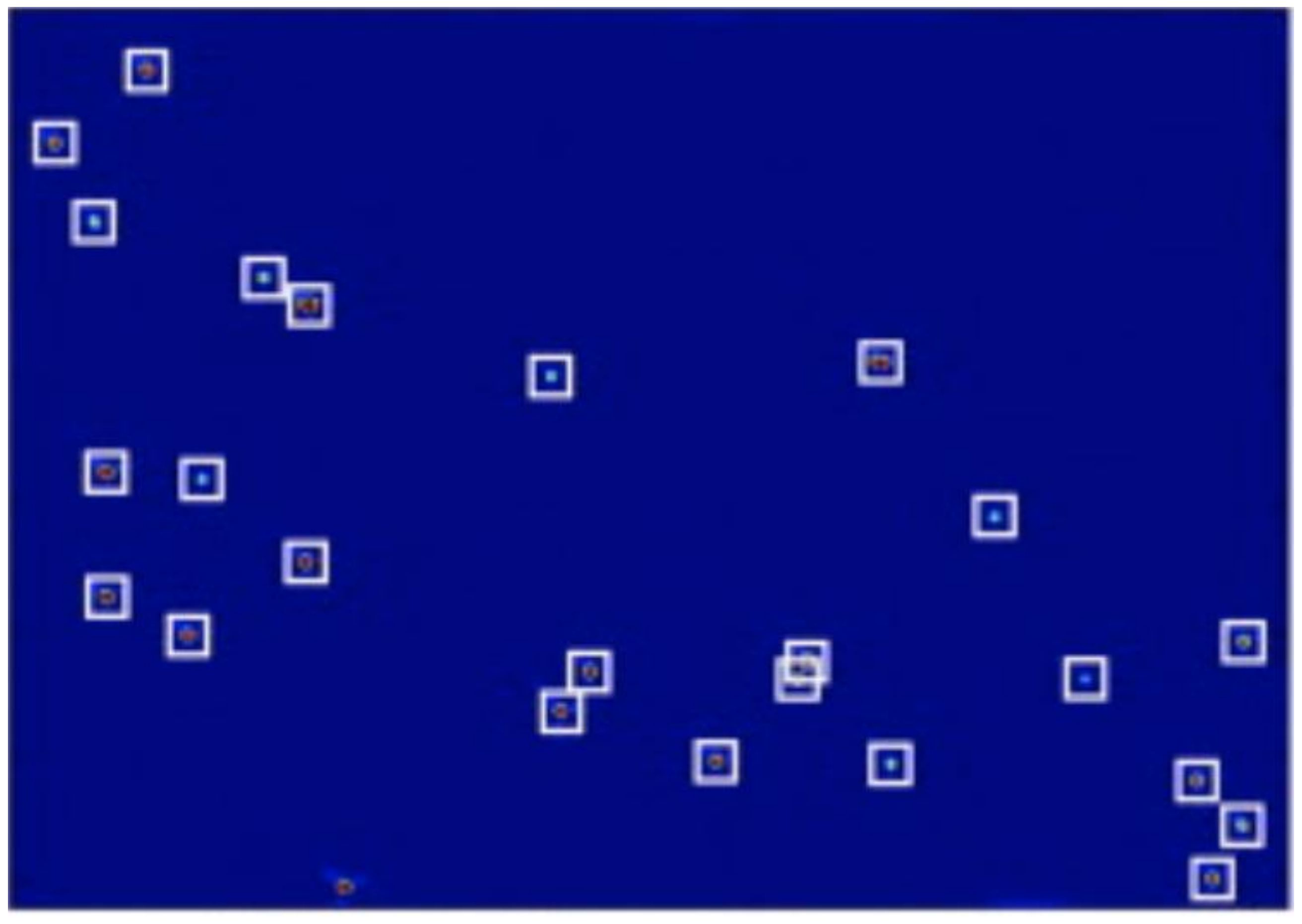






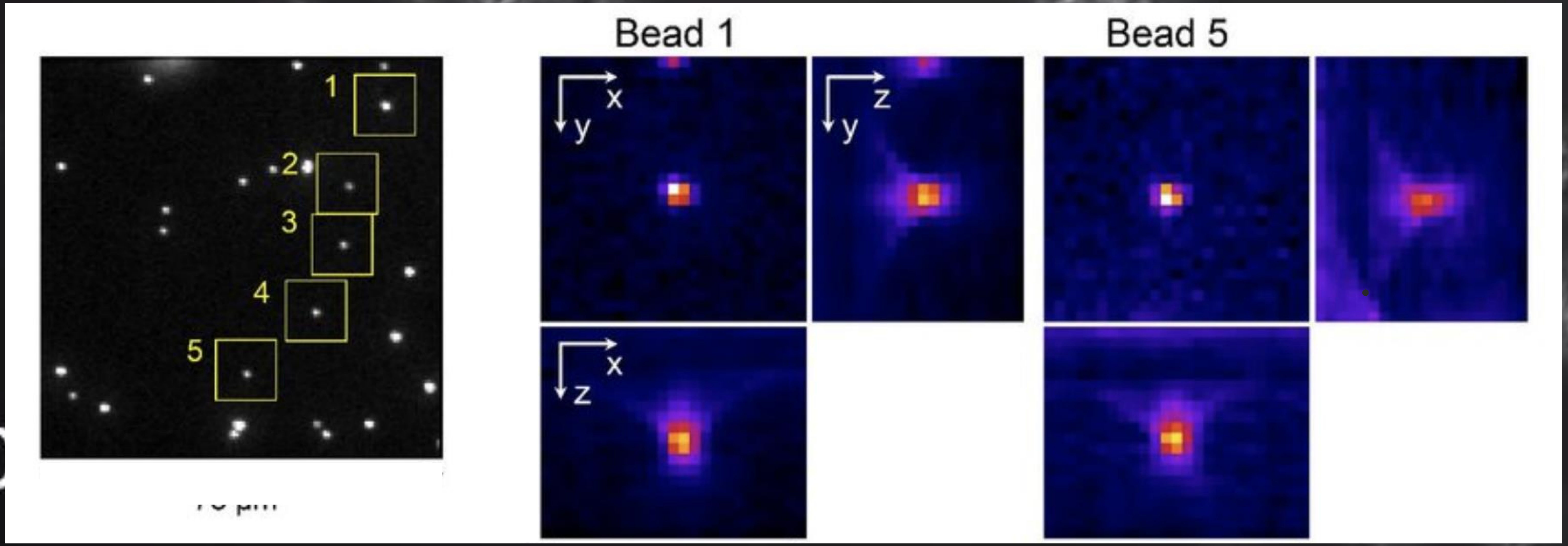
# Experimental Point-Spread Function





**Extracted from beads**

- **Z Position**
- **Size: 40 nm to 100 nm**
- **Selection of beads**
- **Smaller bead, less signal**



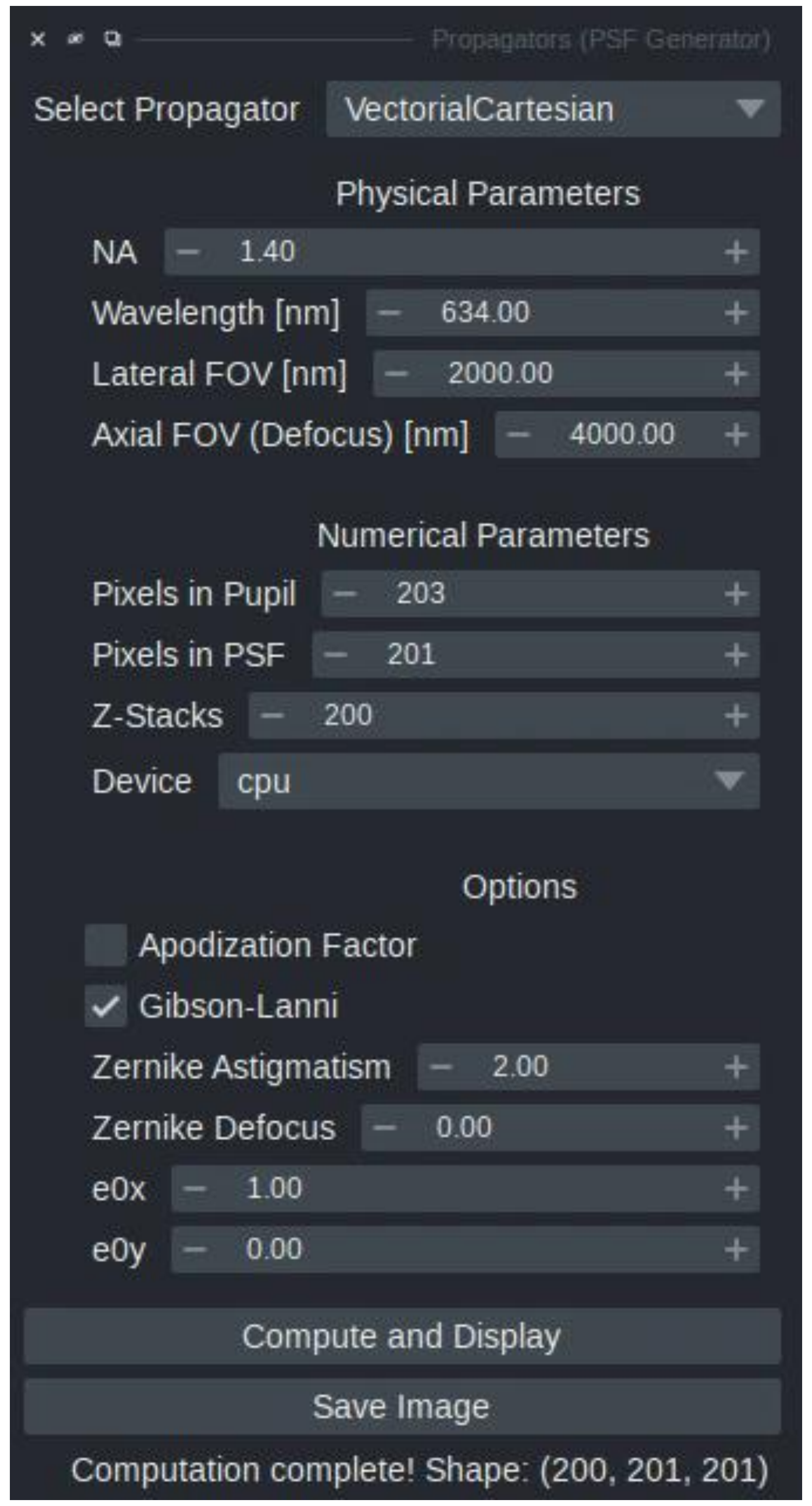
-750

1um x 1um

# Theoretical Point-Spread Function: **PSF Generator**

- ❖ Developed in **Napari/PyTorch**
- ❖ Generates 3D theoretical PSFs
- ❖ Integrated into a unified framework:
  - Multiple models
  - Full optical parameter control
  - Models all possible aberrations
- ❖ [Liu, Stergiopoulou, Sage & Dong, 2025]

- ❖ Developed in **ImageJ/Icy/Matlab**
- ❖ Generates 3D theoretical PSFs
- ❖ Supports: several models, common aberrations and all optical parameters
- ❖ [Kirshner & Sage, Journal of Microscopy, 2012]



Select Propagator: VectorialCartesian

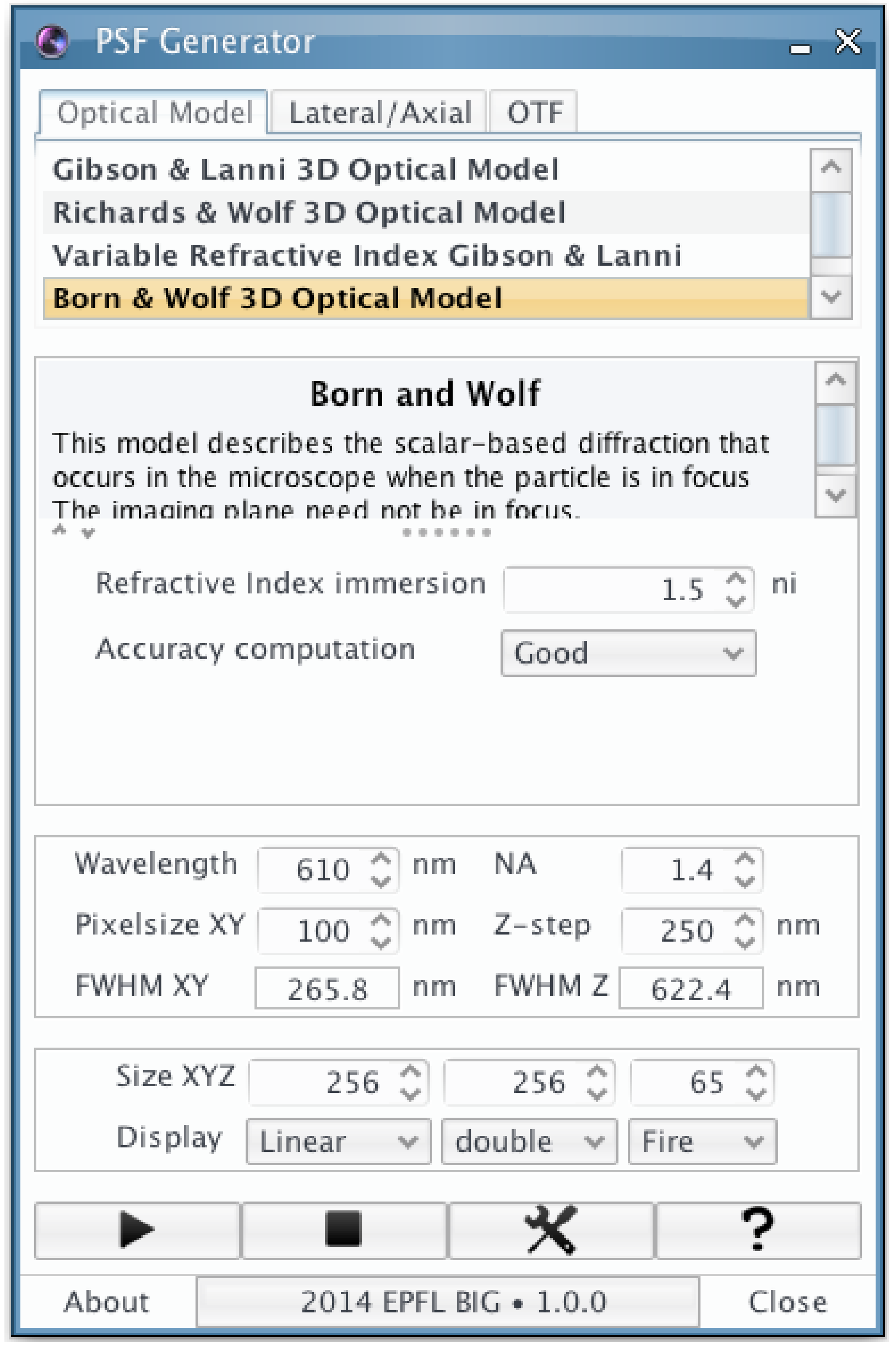
NA: 1.40
Wavelength [nm]: 634.00
Lateral FOV [nm]: 2000.00
Axial FOV (Defocus) [nm]: 4000.00

Pixels in Pupil: 203
Pixels in PSF: 201
Z-Stacks: 200
Device: cpu

☐ Apodization Factor
☑ Gibson-Lanni
Zernike Astigmatism: 2.00
Zernike Defocus: 0.00
e0x: 1.00
e0y: 0.00

Computation complete! Shape: (200, 201, 201)

Napari plugin



**Born and Wolf**

This model describes the scalar-based diffraction that occurs in the microscope when the particle is in focus The imaging plane need not be in focus.

Refractive Index immersion: 1.5 ni
Accuracy computation: Good

Wavelength: 610 nm
NA: 1.4
Pixelsize XY: 100 nm
Z-step: 250 nm
FWHM XY: 265.8 nm
FWHM Z: 622.4 nm

Size XYZ: 256 256 65
Display: Linear double Fire

2014 EPFL BIG • 1.0.0

ImageJ plugin

# Point-Spread Function

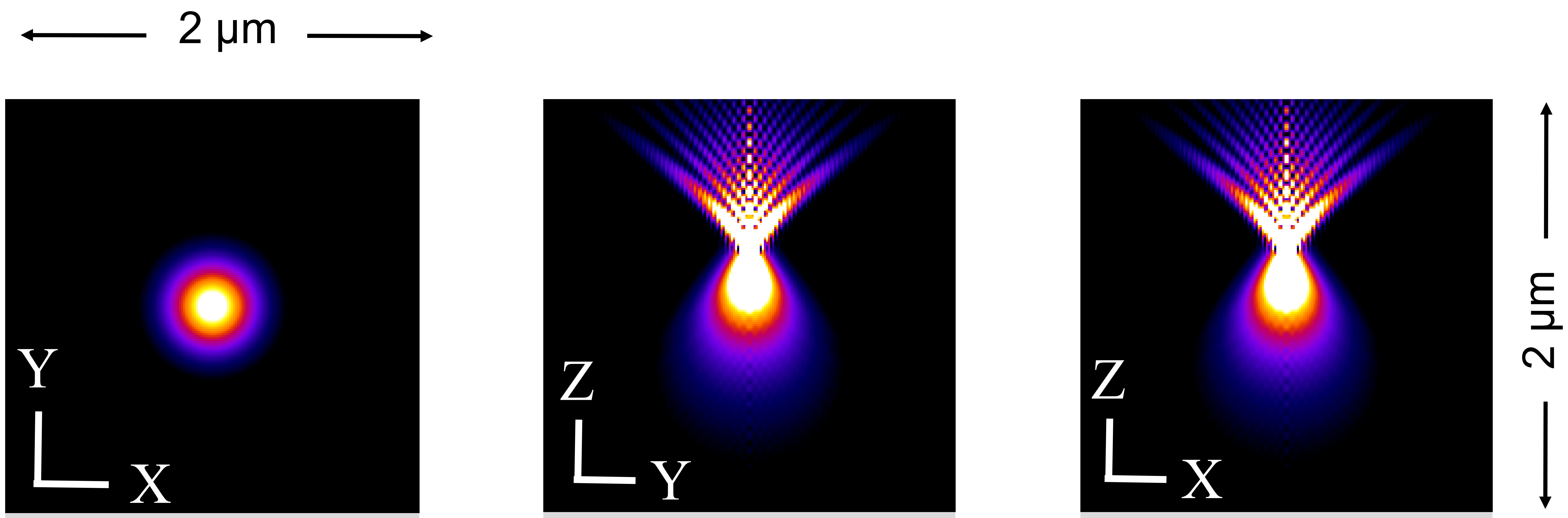
## Theoretical PSF (ImageJ Plugin)

**Gibson&Lanni**

NA = 1.4

λ = 610 nm

pixelsize = 100 nm



PSF Generator - Open source software - EPFL

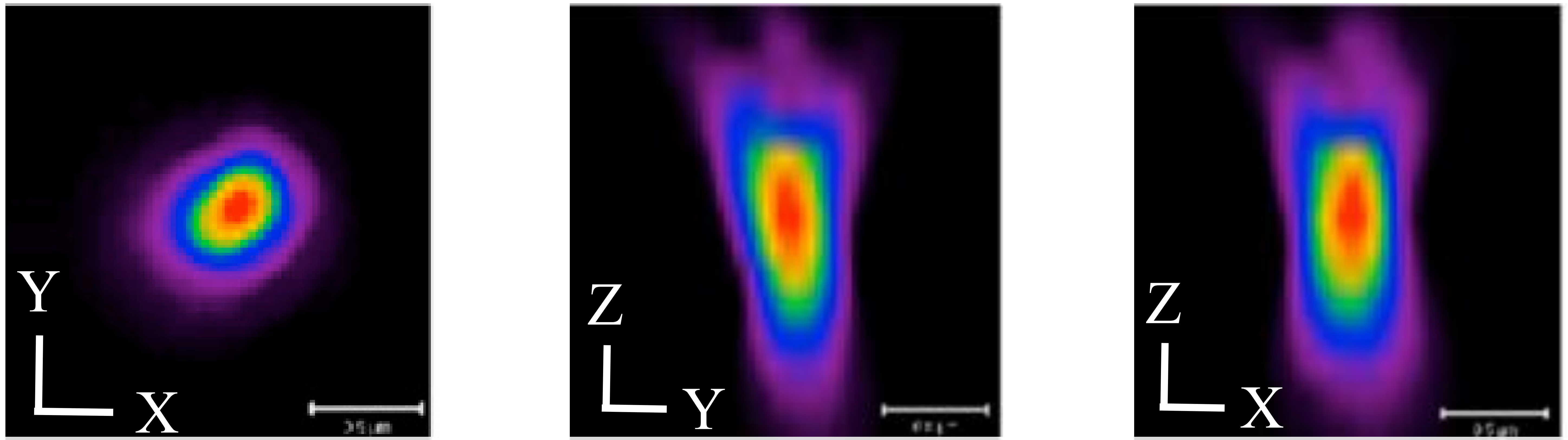
## Experimental PSF

**LSM 510 Confocal**

1.4 NA

Plan apo objective

Oil

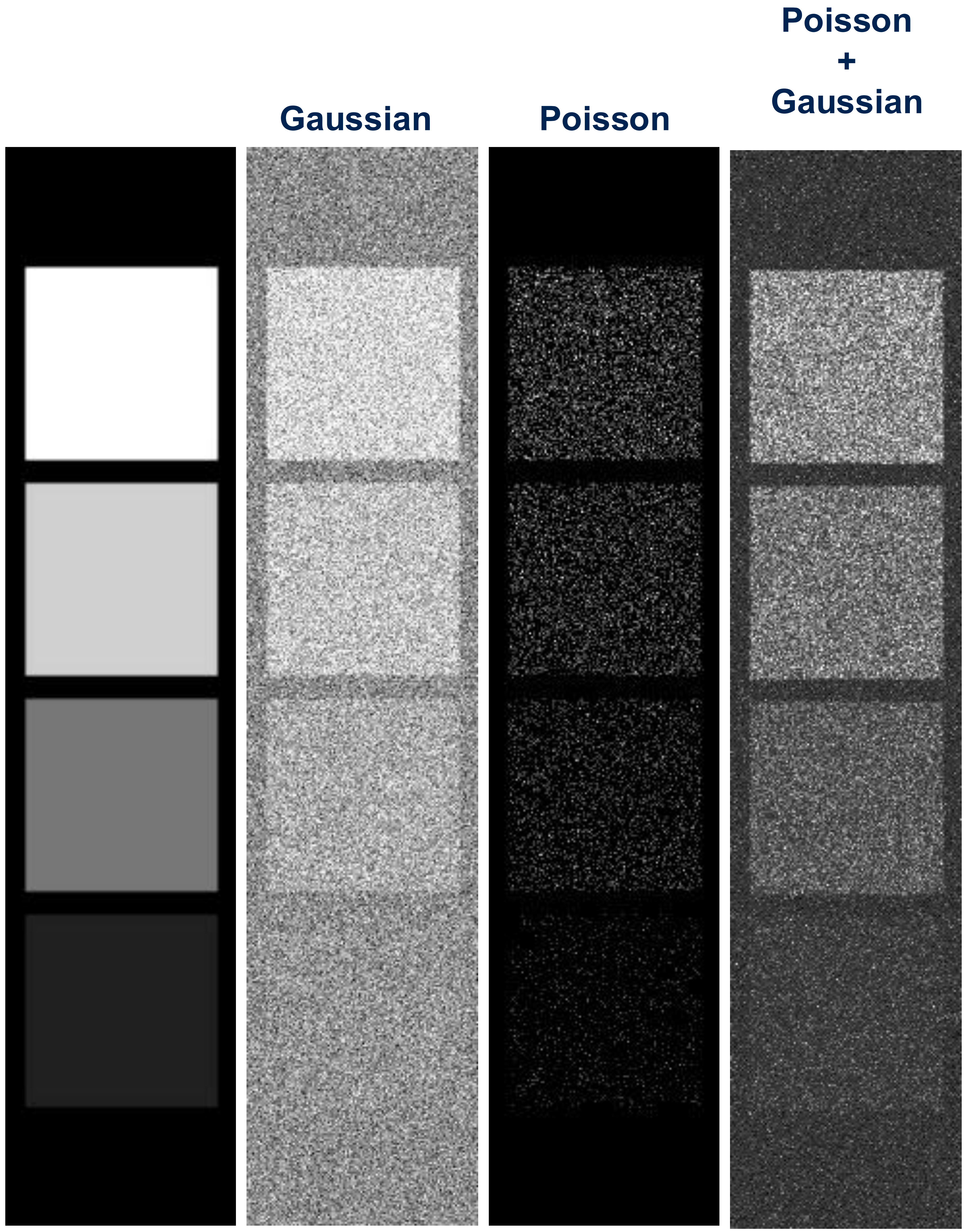


Courtesy of SVI and Institut de Cardiologie de Montreal.

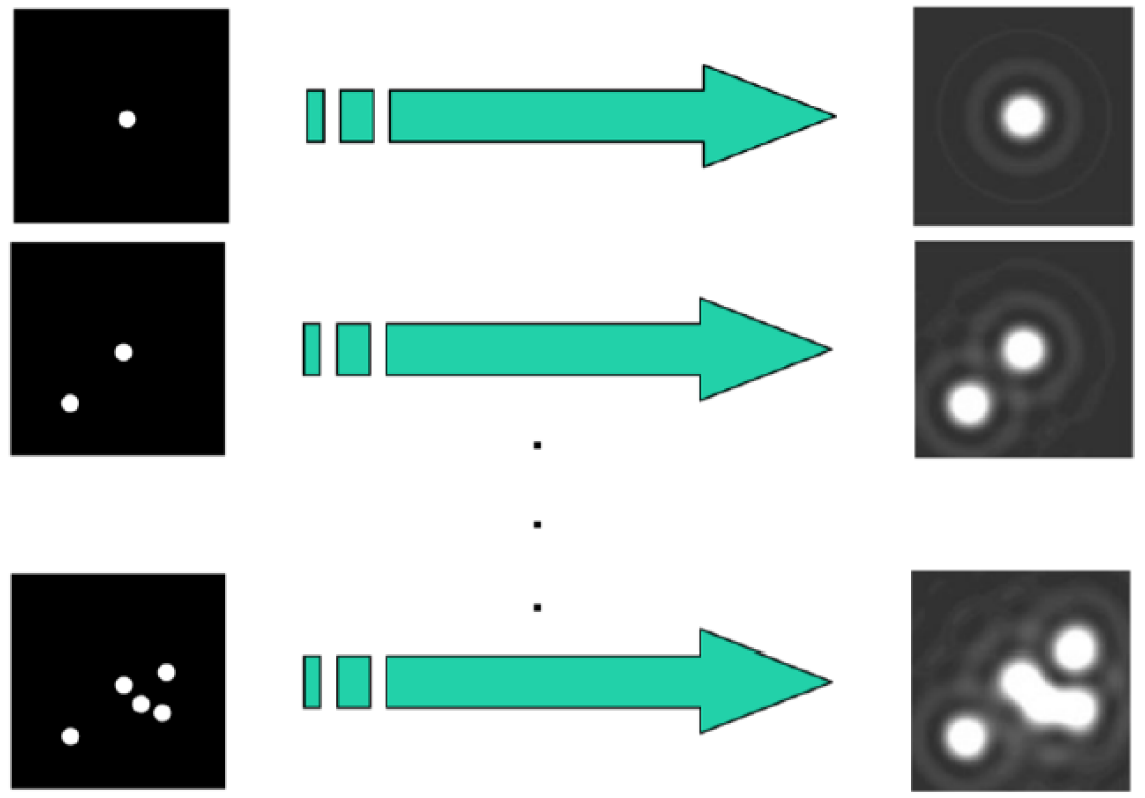
# Noise Models

Source of noise

- Photon (Shot) noise
- Thermal (Dark Current) Noise
- Electronic (Readout) Noise
- Sampling & Discretization Noise
- Quantization Noise
- Sensor Non-uniformities
- Environmental Interference
- Quantization noise

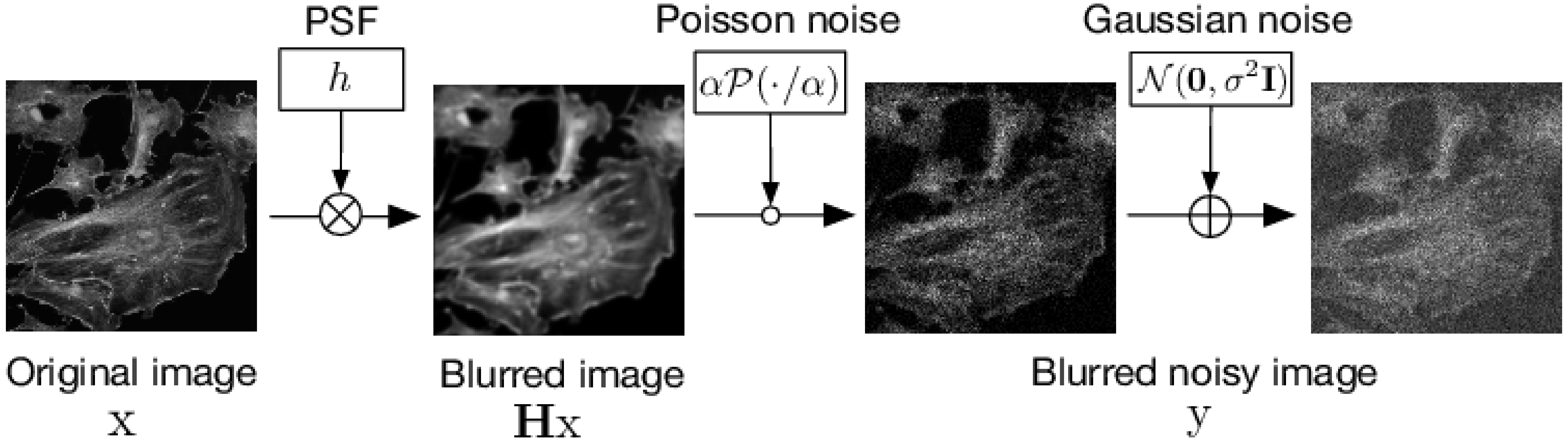


# Image Formation



Convolution is distributive

Microscopy:



Source: Li, et al., TIP, 2018

# Methods

Inverse Filters, Inverse Problems, Model-based DL methods

# Methods

## Mathematical Approach

Solving the problem based on the **image formation model**

### Inverse Filters

**One Shot**

### Inverse Problems

**Iterative**

Optimization Algorithms
e.g.  Richardson-Lucy,
GD/SGD, ISTA/FISTA,
ADMM, ...

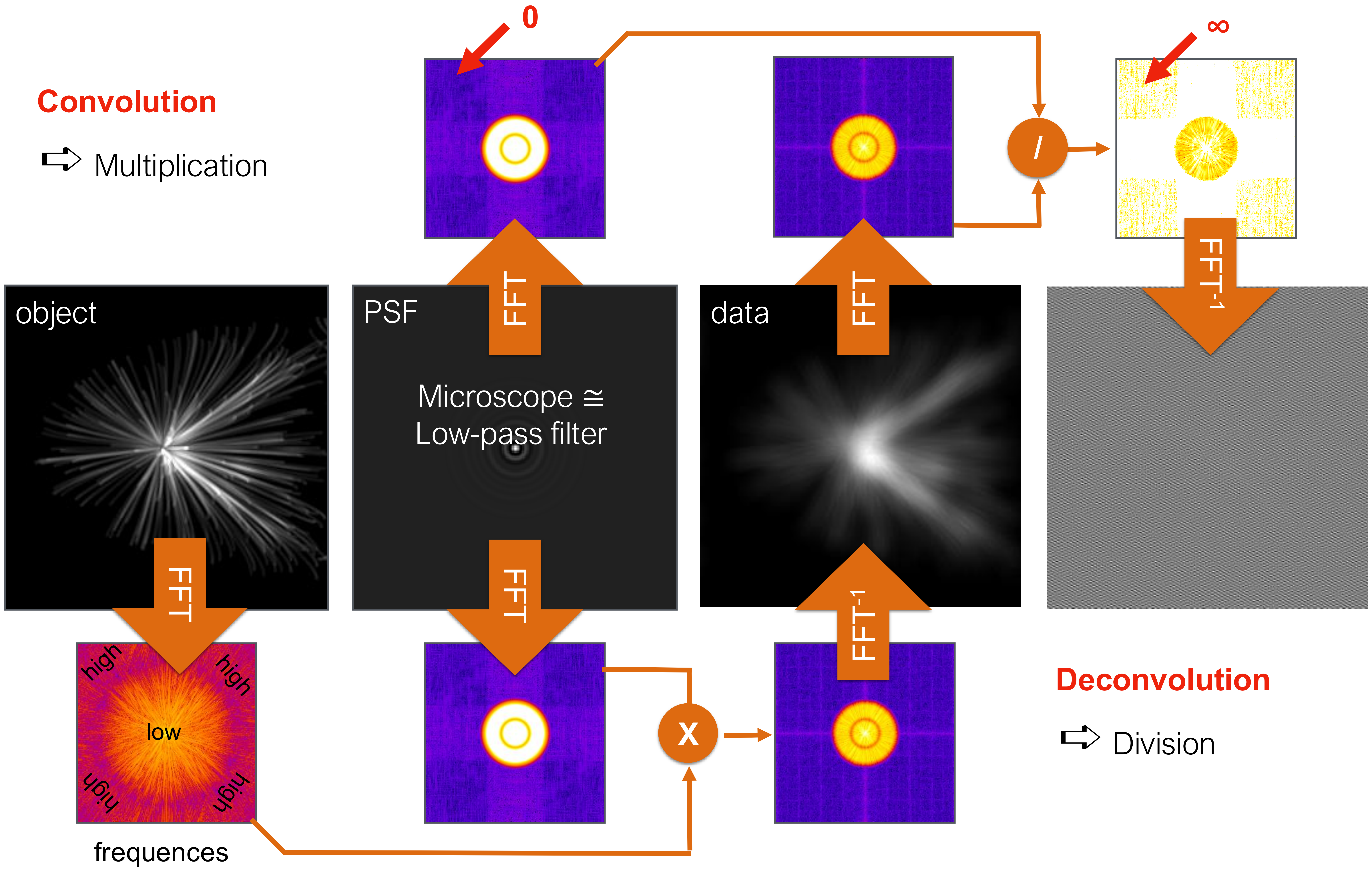
## Learning Approach

Part of the reconstruction process is **learned from data**

# Intuition

**Convolution**

⇨ Multiplication



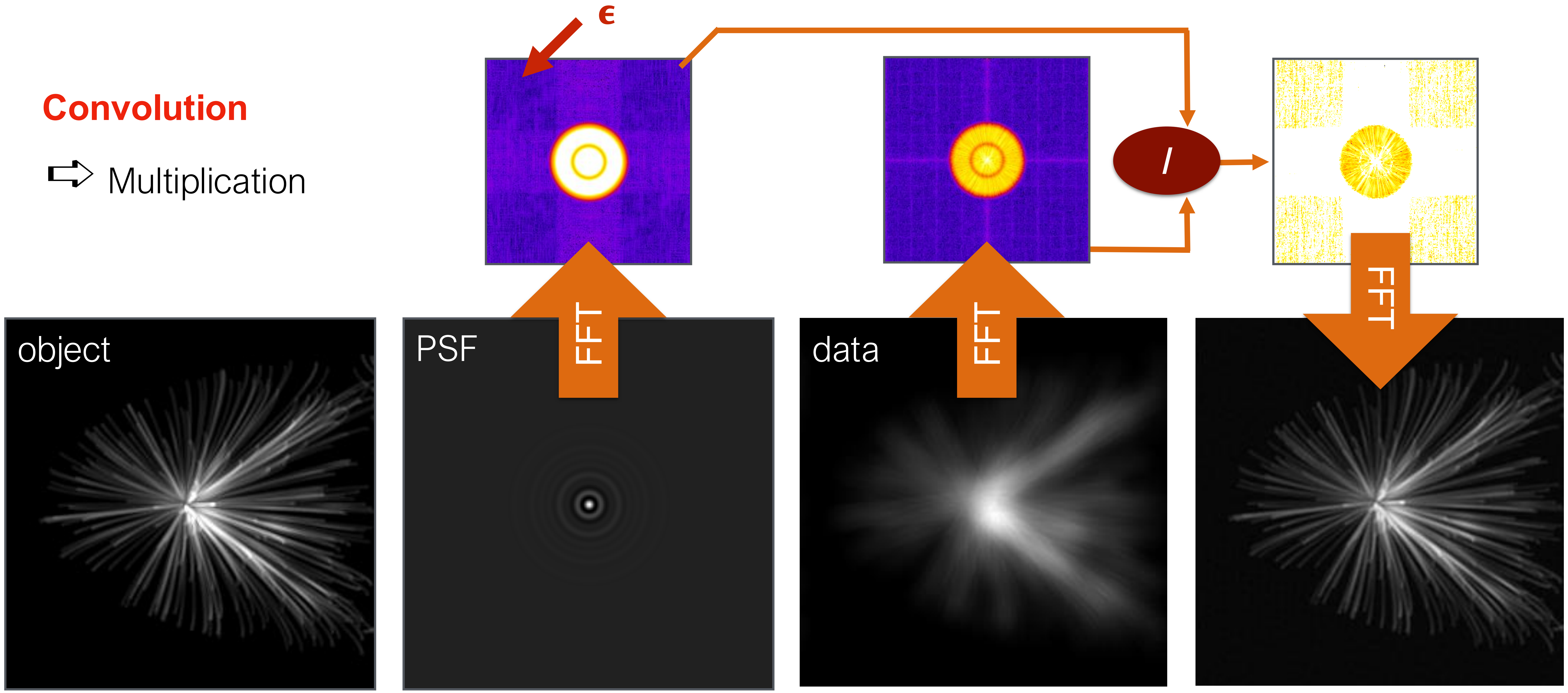
**Deconvolution**

⇨ Division

# Intuition

**Convolution**

⇨ Multiplication

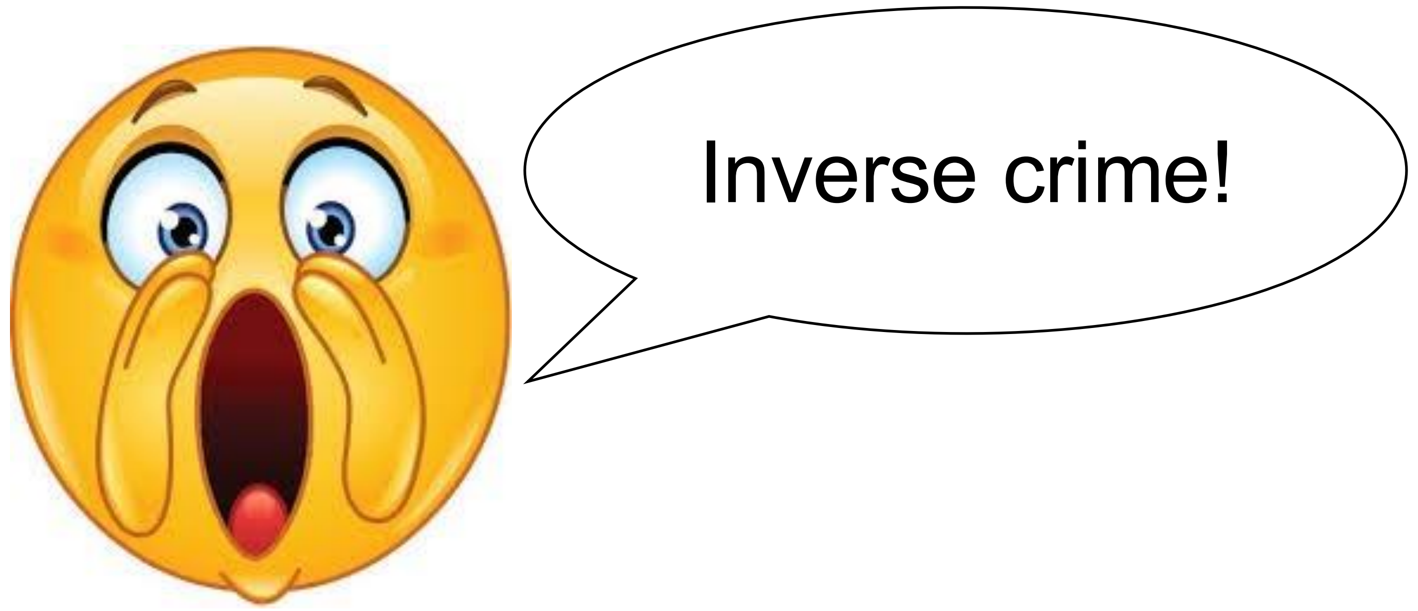


**Deconvolution**

⇨ Stabilized Division

truncates denominator

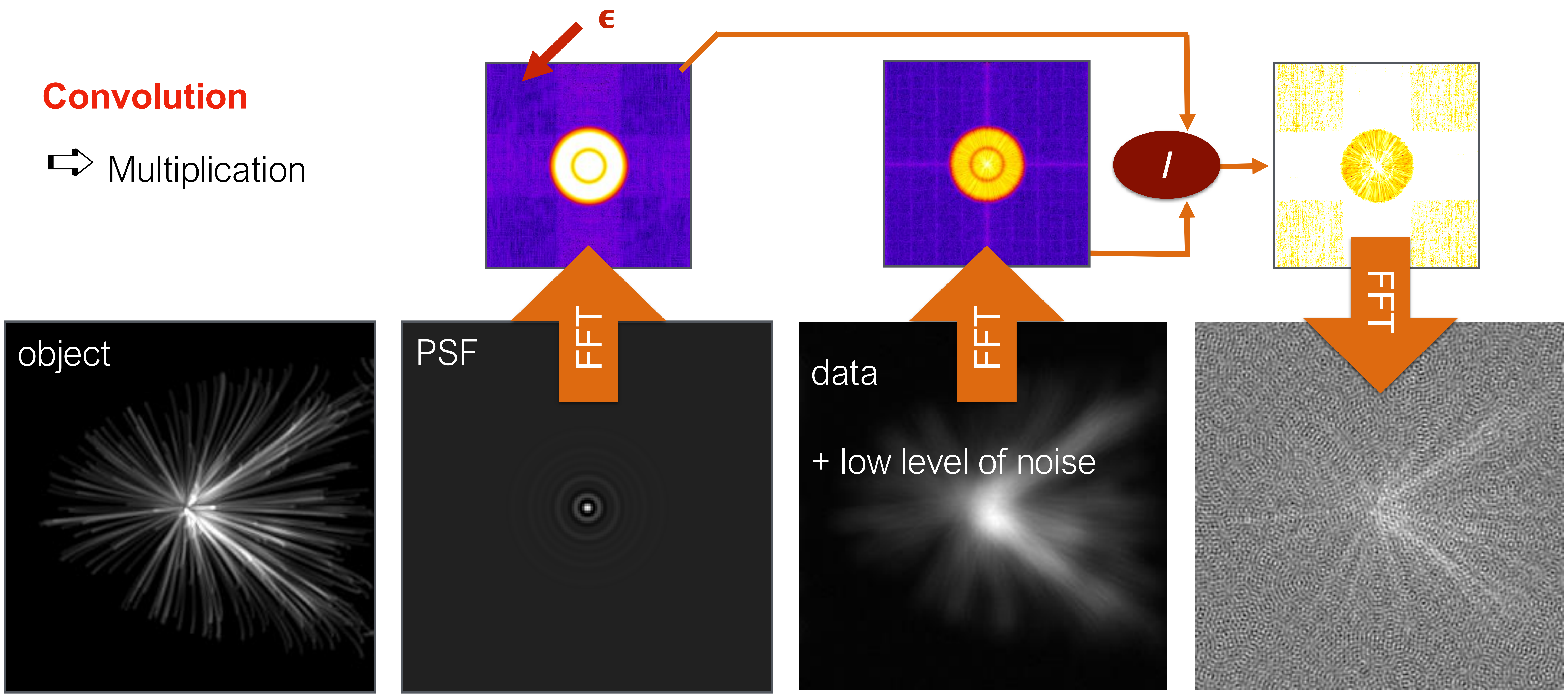
$$\hat{\hat{\boldsymbol{x}}} = \frac{\hat{\boldsymbol{y}}}{\max\left(\hat{\boldsymbol{h}}, \epsilon\right)}$$



# Naive Deconvolution

**Convolution**

⇨ Multiplication



**non-exact PSF**

**corrupted by noise**

truncates denominator

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

# Inverse Filters

### NIF Naive Inverse Filter

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

Never works
in real life

### TRIF Tikhonov Regularized Inverse Filter

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

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

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

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

### 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

### RIF (Laplacian) Regularized Inverse Filter

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

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

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

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

# Deconvolution as an Inverse Problem

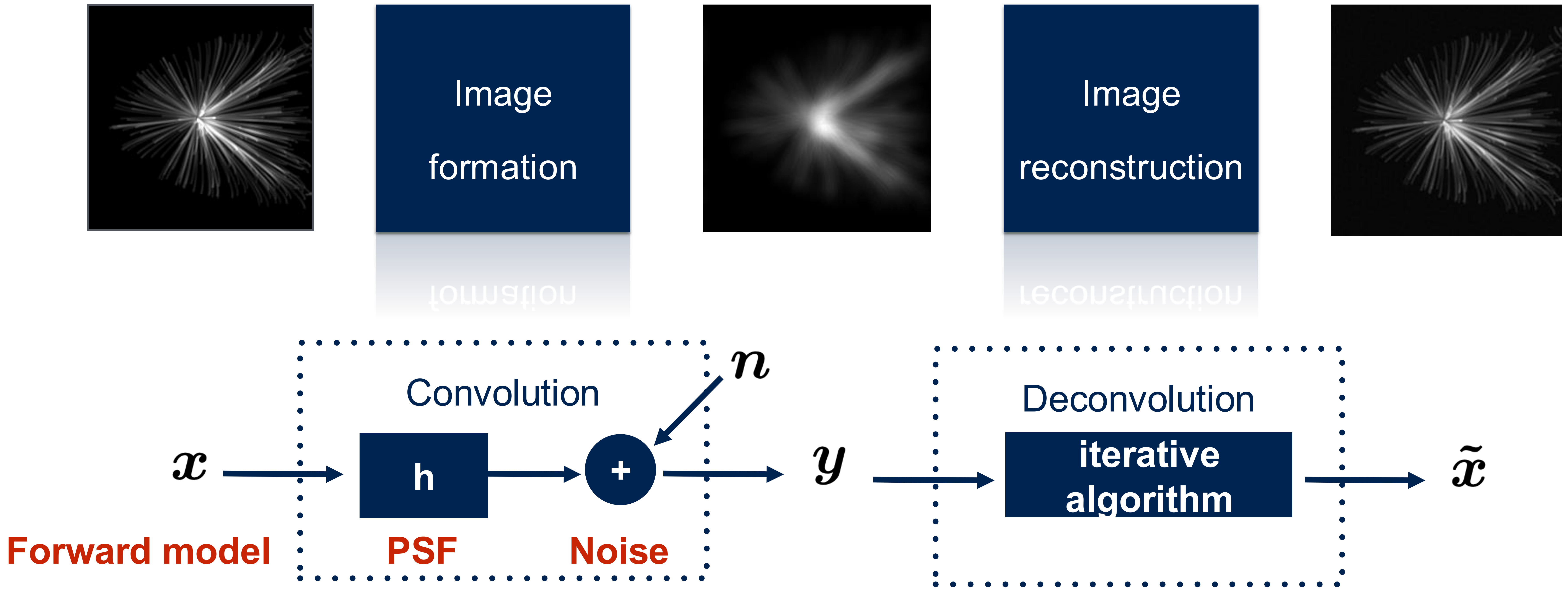


Image formation

Image reconstruction

Convolution

$n$

Deconvolution

$x$

h

+

$y$

iterative algorithm

$\tilde{x}$

**Forward model**

**PSF**

**Noise**

$$y = \mathbf{H}x + n$$

Real life

Ill-posed problem

◆

Too many unknowns
No unique solution
Partial data
Approximative physic

◆

# Optimization with Prior Knowledge

Objective function

$$\tilde{\mathbf{x}} = \underset{\mathbf{x}}{\operatorname{argmin}}\{\mathcal{D}(\mathbf{Hx},\mathbf{y}) + \lambda\mathcal{R}(\mathbf{x})\}$$

Data fidelity term

Regularization term

Forward model

$\mathcal{D}$

discrepancy between the forward model and the measures

$\lambda$

hyperparameter: balance between data term and constraint consistency.

how to tune?

Prior on solution

$\mathcal{R}$

regularity constraints on the solution (e.g. smoothness, non-negativity)

# Steepest Gradient Descent

### LW Landweber iteration

[Landweber, 1951]

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

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

- LWID, Landweber iterative deconv.
- Least-square minimization
- Controllable steepest ($\gamma < 2$)
- Dominant Gaussian noise

### Landweber + positivity LW+

Projected solution

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

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

### 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

### Iterative Constrained Tikhonov-Miller ICTM

[Kempen, 1996]

Projected solution

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

# Richardson-Lucy

**RL** Richardson-Lucy

[Richarsdon, 1972, Lucy 1974]

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

- Statistically interpretation
- Poisson noise
- Assumption of positive signals
- Maximum likelihood estimator (MLE)
- Slow, iteration in the spatial domain
- One parameter to tune (number of iterations)

**RLTV** Richardson-Lucy 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
- How to balance the TV and the deconvolution

# Promote Sparsity

| | |
|---|---|
| **ISTA** | Iterative Soft Threshold Algorithm |
| **FISTA** | Fast Iterative Soft Threshold Algorithm |

[Beck 2009]

$$\mathcal{C}(\boldsymbol{x}) = \|\boldsymbol{y} - \mathbf{H}\boldsymbol{x}\|^2 + \lambda\|\mathbf{W}\boldsymbol{x}\|_1$$

- Fast convergence
- Preserve the edges
- Preserve discontinuities

$$\begin{aligned}
\boldsymbol{z}^{(k+1)} &= \boldsymbol{s}^{(k)} - \gamma\mathbf{H}^T(\mathbf{H}\boldsymbol{s}^{(k)} - \boldsymbol{y}) \\
\boldsymbol{x}^{(k+1)} &= \mathbf{W}^T\mathcal{T}(\mathbf{W}\boldsymbol{z}^{(k+1)}, \gamma\lambda) \\
p^{(k+1)} &= \frac{1}{2}\left(1 + \sqrt{1 + 4{p^{(k)}}^2}\right) \\
\boldsymbol{s}^{(k+1)} &= \boldsymbol{x}^{(k+1)} + \frac{p^{(k)} - 1}{p_{(k+1)}}(\boldsymbol{x}^{(k+1)} - \boldsymbol{x}^{(k)})
\end{aligned}$$

- Soft-threshold in the wavelet domain
- Haar wavelets, Spline wavelets 1, 3, 5

# Methods

## Mathematical Approach

Solving the problem based on the **image formation model**

**Inverse Filters**

**One Shot**

**Inverse Problems**

**Iterative**

Optimization Algorithms
e.g. Richardson-Lucy,
GD/SGD, ISTA/FISTA,
ADMM, ...

## Learning Approach

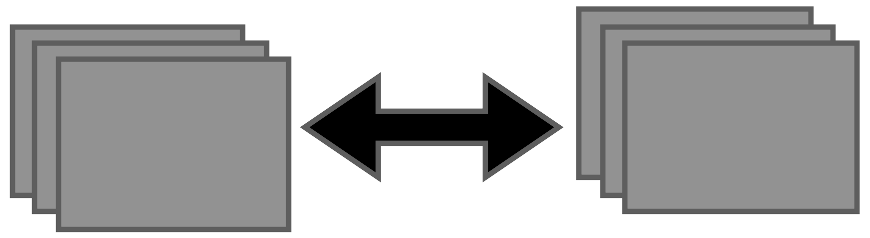
Part of the reconstruction process is **learned from data**

**Physics inspired**

No physics involved

Supervised Learning

Self-supervised Learning



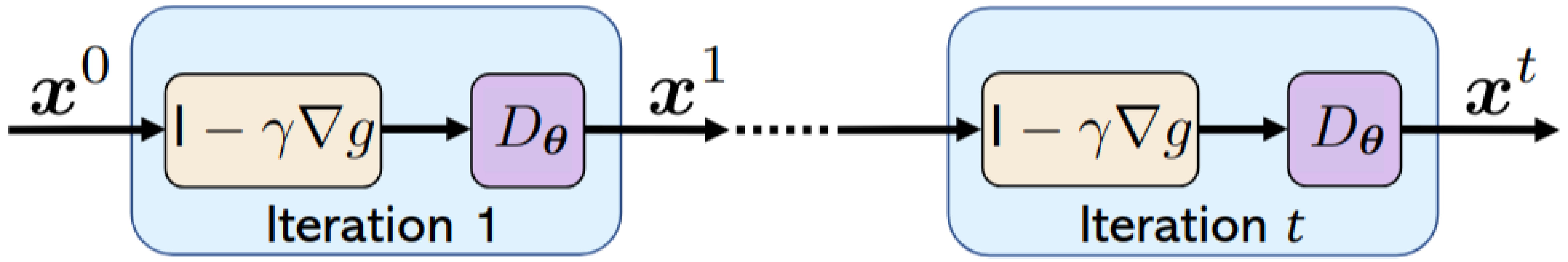
How to get pairs for training?

- High **vs.** low resolution
- Lateral **vs.** axial
- Using synthetic or data augmentation strategies

# Physics-Inspired Learning Approaches

## Plug-and-Play Priors (PnP)

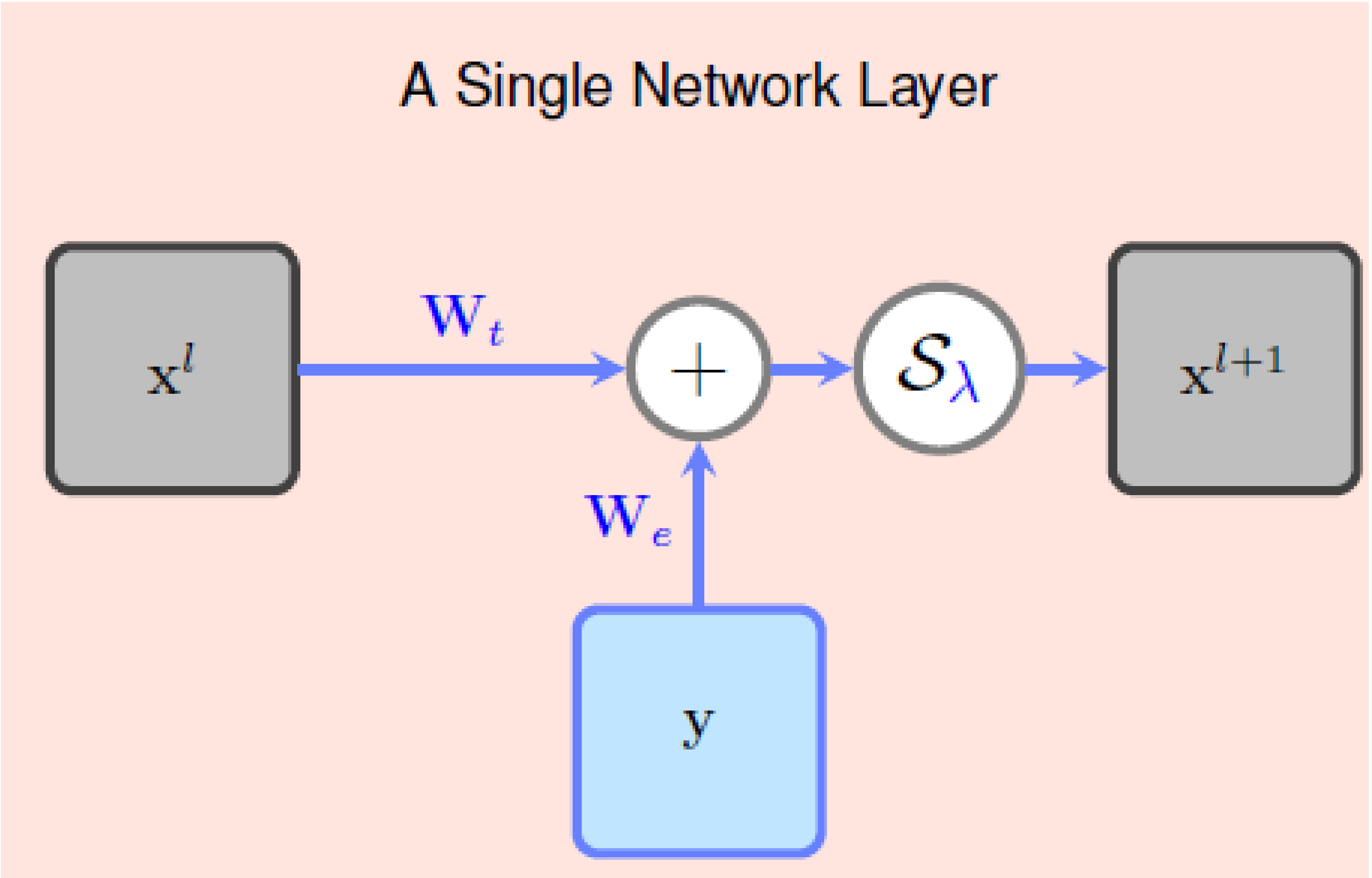
- "Plug in" a **learned denoiser** into a classical iterative optimization algorithm (e.g., ISTA, ADMM).
- **Denoiser**: mostly **CNN-based** (e.g., DnCNN, DRUNet)
- Venkatakrishnan et al. 2013 (original PnP), Romano et al. 2017 (RED), Hurault et al. 2022, Goujon et al. 2024.



PnP-ISTA

## Deep Unrolling / Unfolding

- **Unroll** an **iterative deconvolution algorithm** (e.g., ISTA, FISTA) into a neural network, with each layer corresponding to an iteration
- Learn: regularizer (or proximal operator), step size, hyper-parameter
- Gregor & LeCun 2010 (LISTA), Monga et al. 2021 (review).

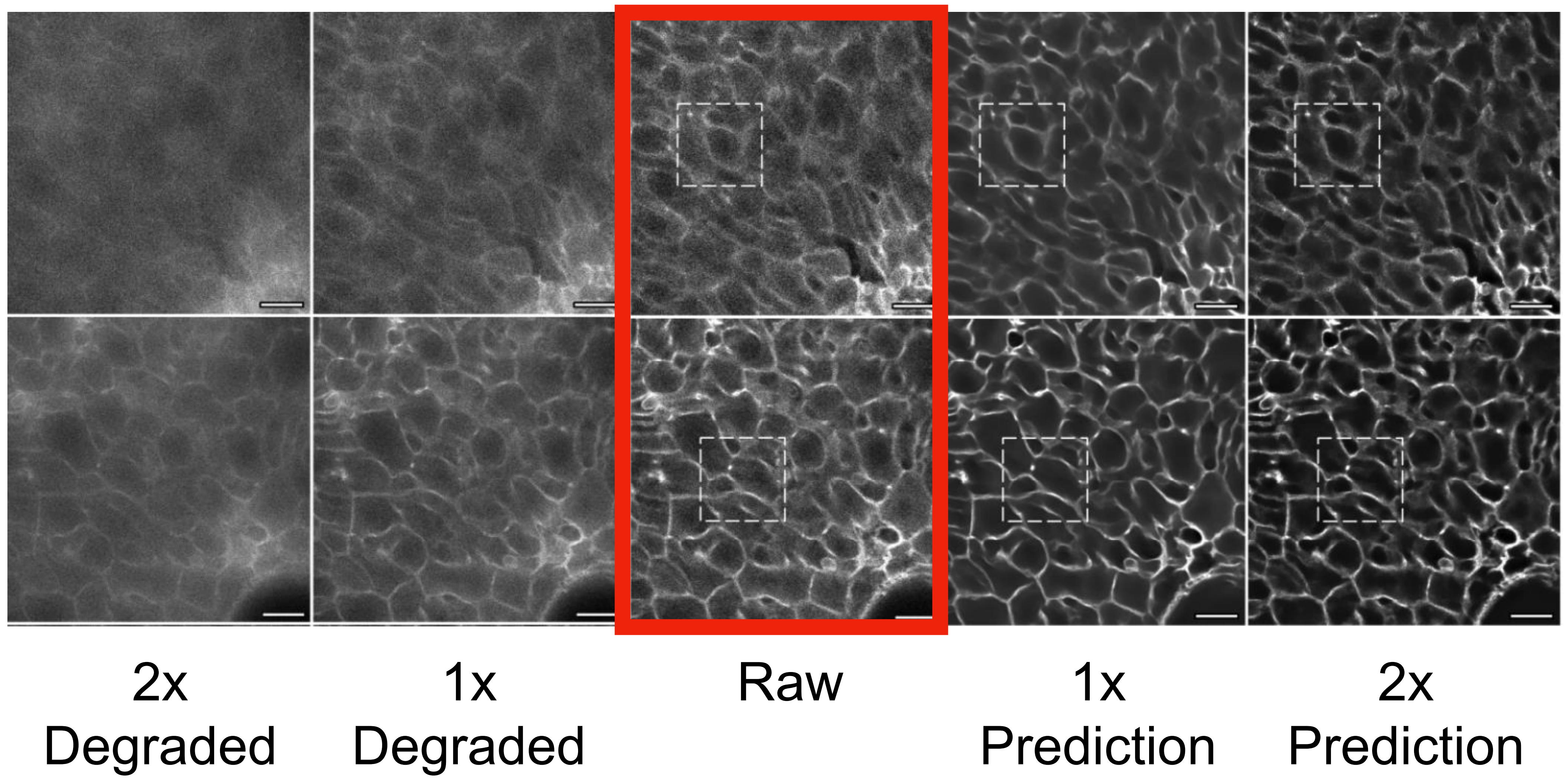


LISTA

# Physics-Inspired Learning Approaches

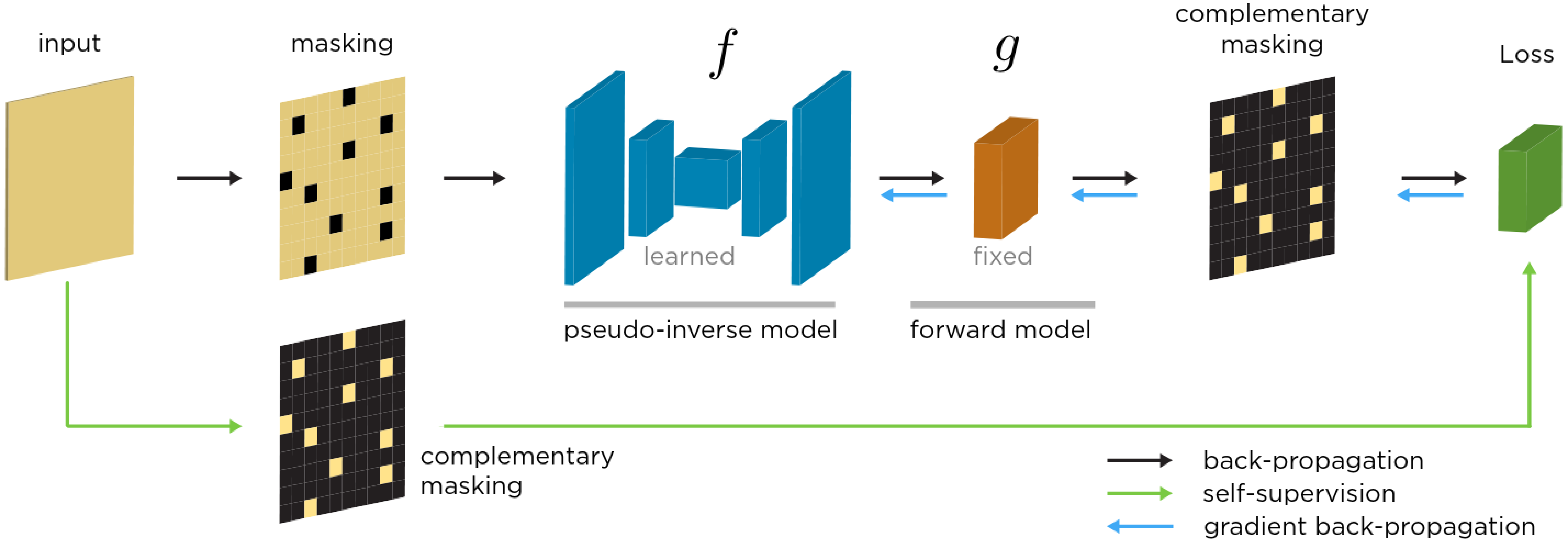
## DeepContrast

- Learn from degradation
- Iteration degradation
- Iterative prediction
- Martins et al. 2024



## Self-Superived Inversion

- Known forward model
- Assumes statistically independent noise
- Kobayashi et al. 2020 (arxiv)

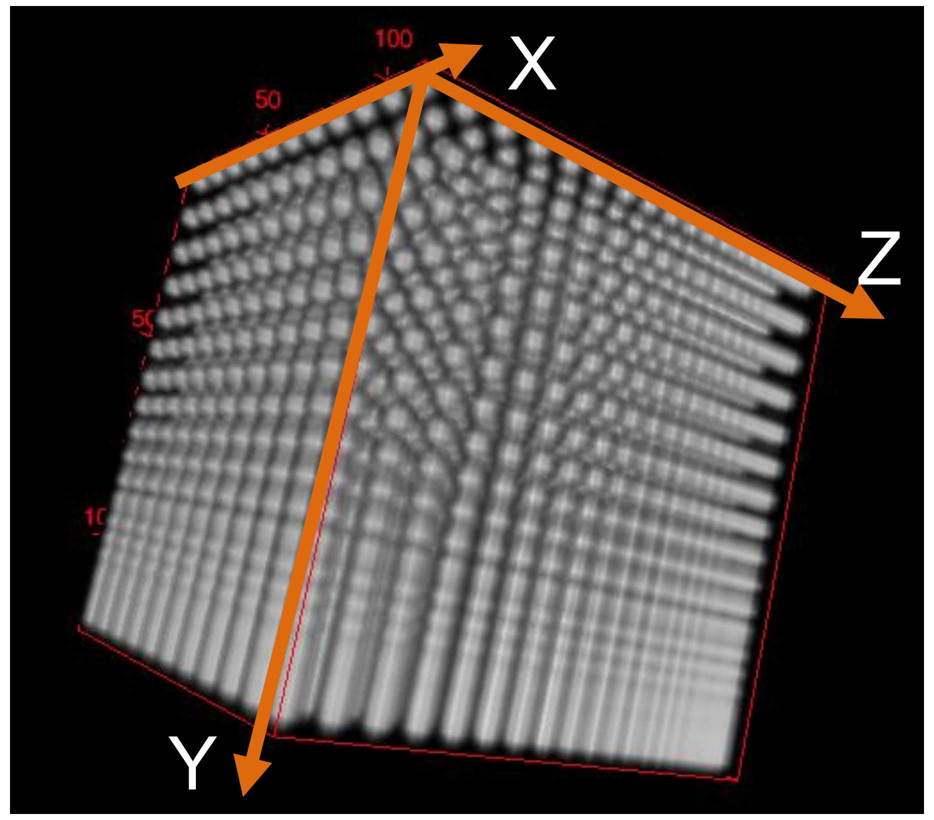


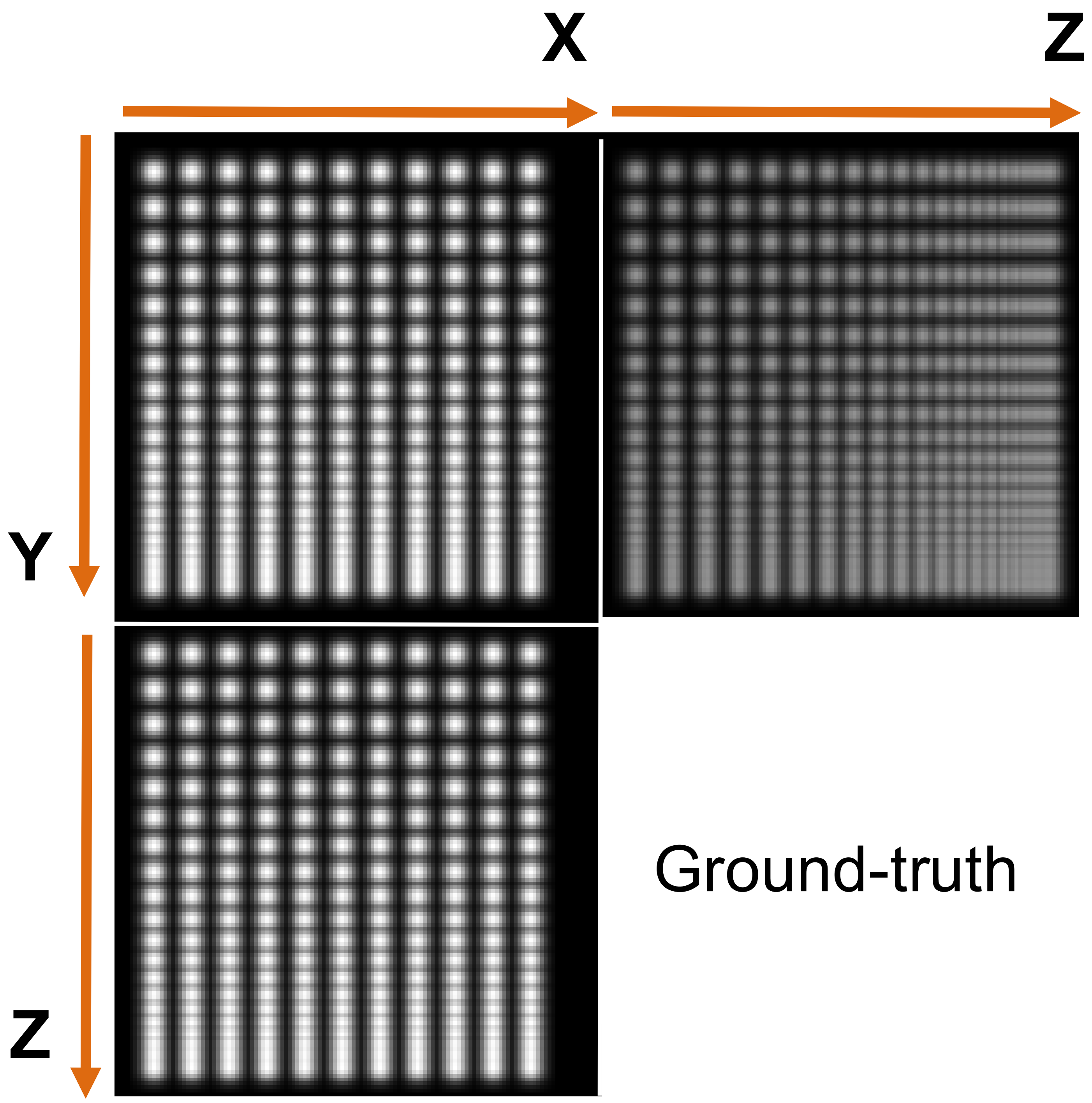
# Considerations

# Better Resolution?

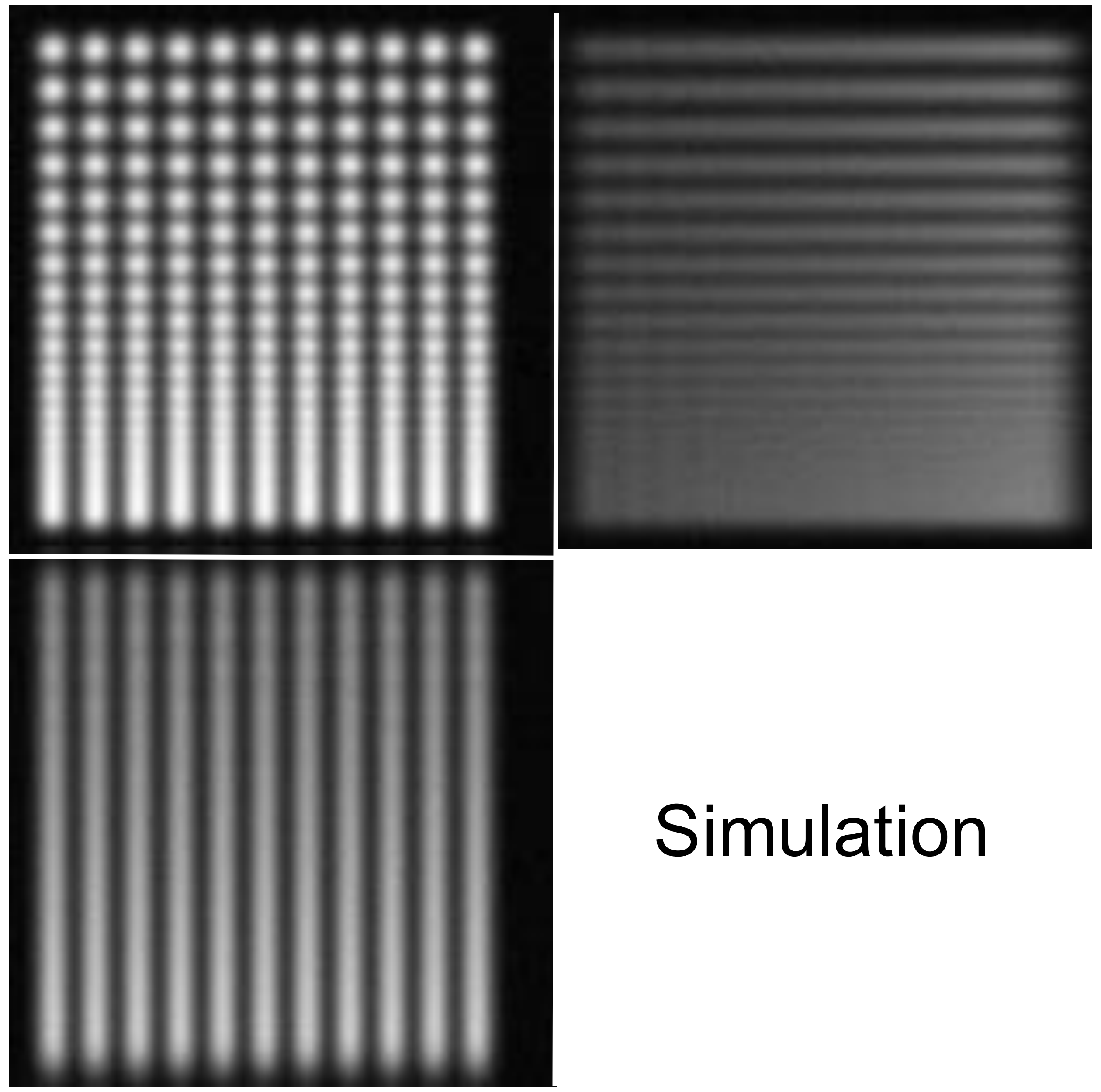
**Experimental protocol**

- Structure: beads 128x128x128 pixels
- Synthetic PSF
    - FWHMxy = 2.82 pixels
    - FWHMz = 8.46 pixels
- Plot intensity profiles f(16, y, 16) and f((16, 16, z)

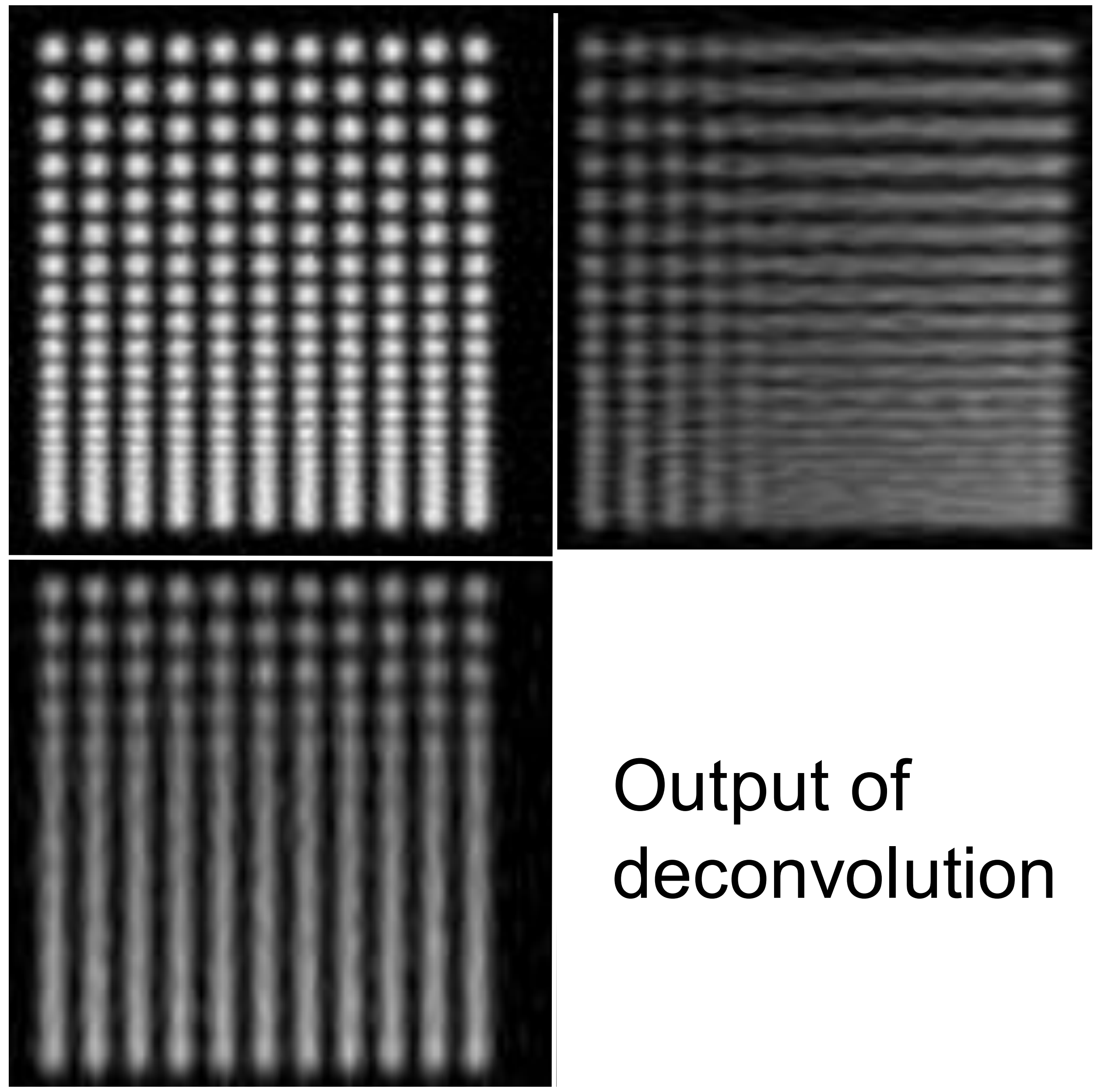




Ground-truth

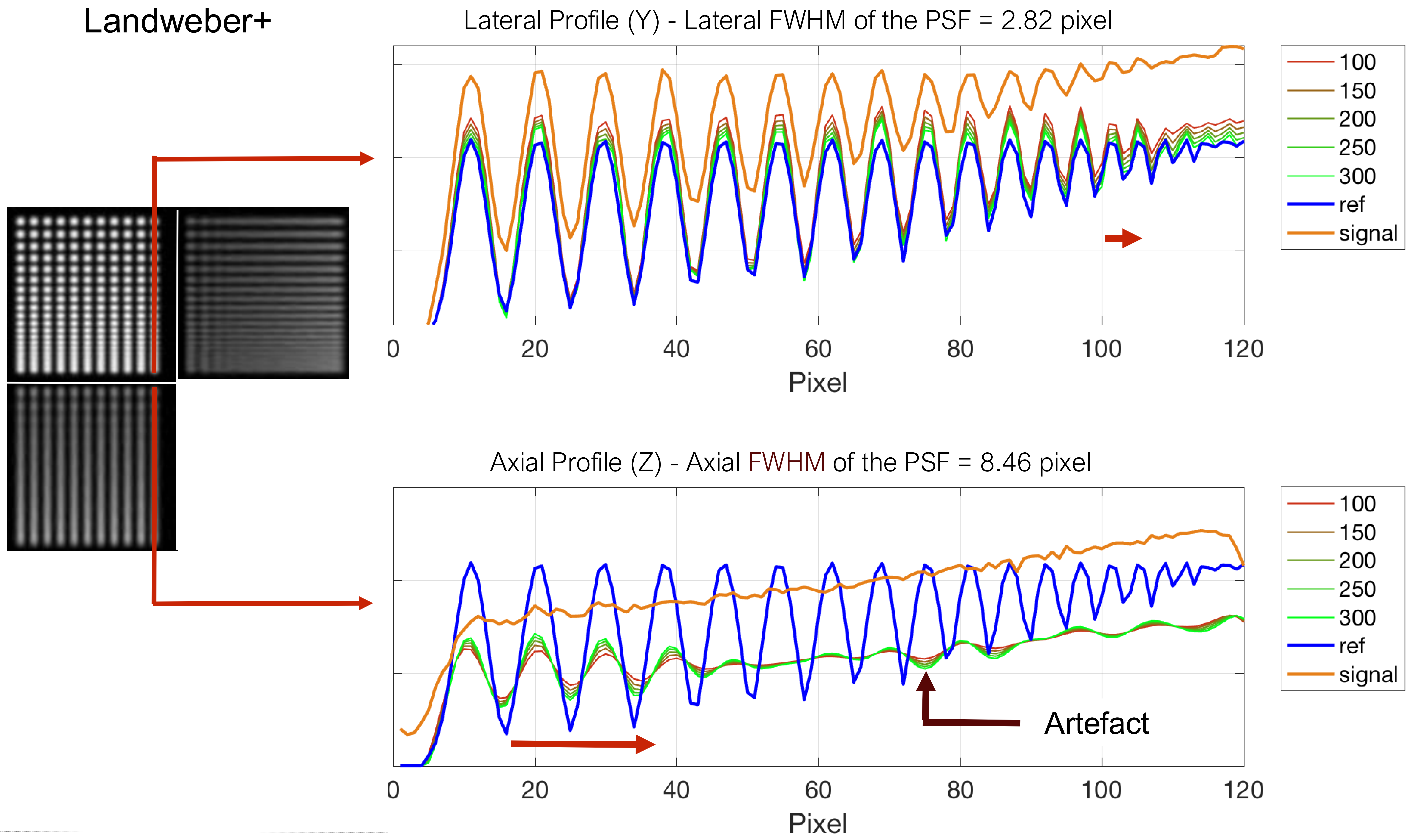


Simulation

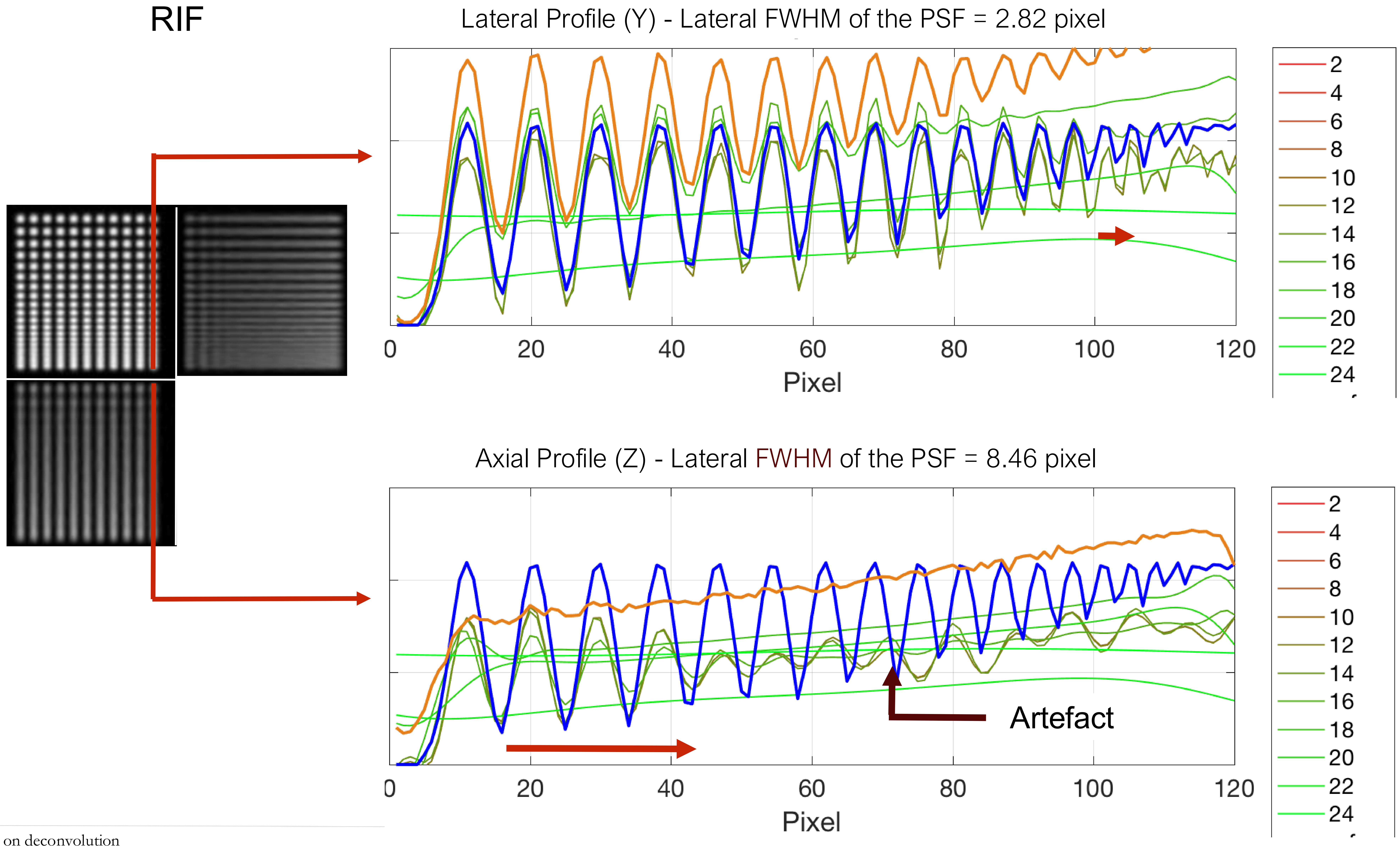


Output of deconvolution

# Better Resolution?



# Better Resolution?



RIF

Lateral Profile (Y) - Lateral FWHM of the PSF = 2.82 pixel

Axial Profile (Z) - Lateral FWHM of the PSF = 8.46 pixel

Artefact

# Assessment of Deconvolution

**Sources of problems**

- Moving too fast
- Too depth
- Too much scattering

Garbage In, Garbage Out

- Optical misalignment
- Light source (flickering lamps, lasers)
- None-Nyquist sampling
- Detector artifact (dead pixel)

- Normalization of the PSF = 1
- Variant PSF
- Overprocessing
- Edges of images

Common artefacts

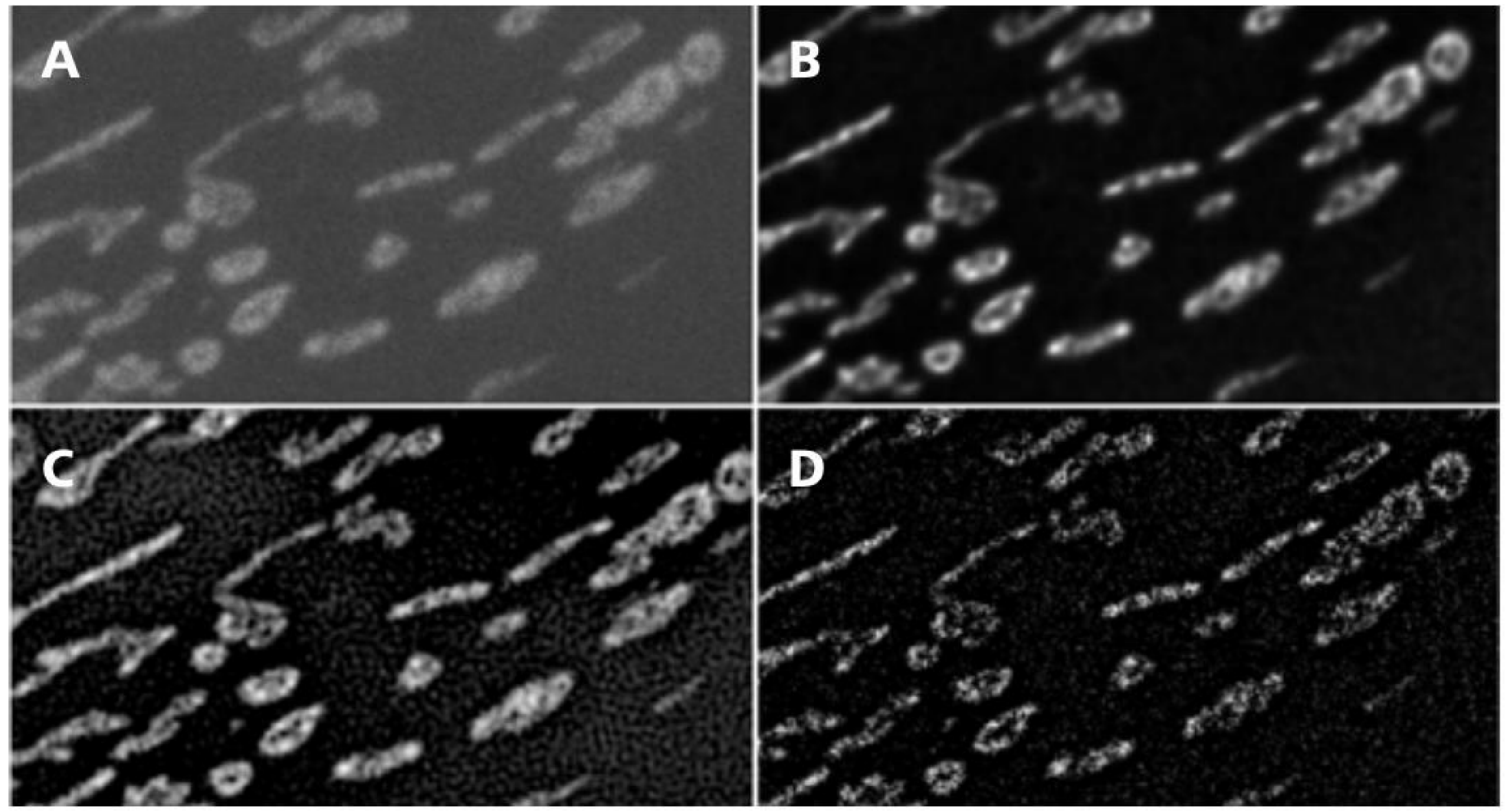
Ringing artifact

- One or multiple ripple patterns
- Around bright structures

Disappearing of small structures

- Around poor dynamic range
- Around high background noise

Source Good



Wrong background Lost Structure