Daniel Sage & Arne Seitz

Course

Denoising of Microscopy Images

Noise in Bioimage

Pushing the limits of imaging

- Low-light condition, low excitation
- High spatial resolution
- Long sequence: bleaching

Degrading in multiple dimension

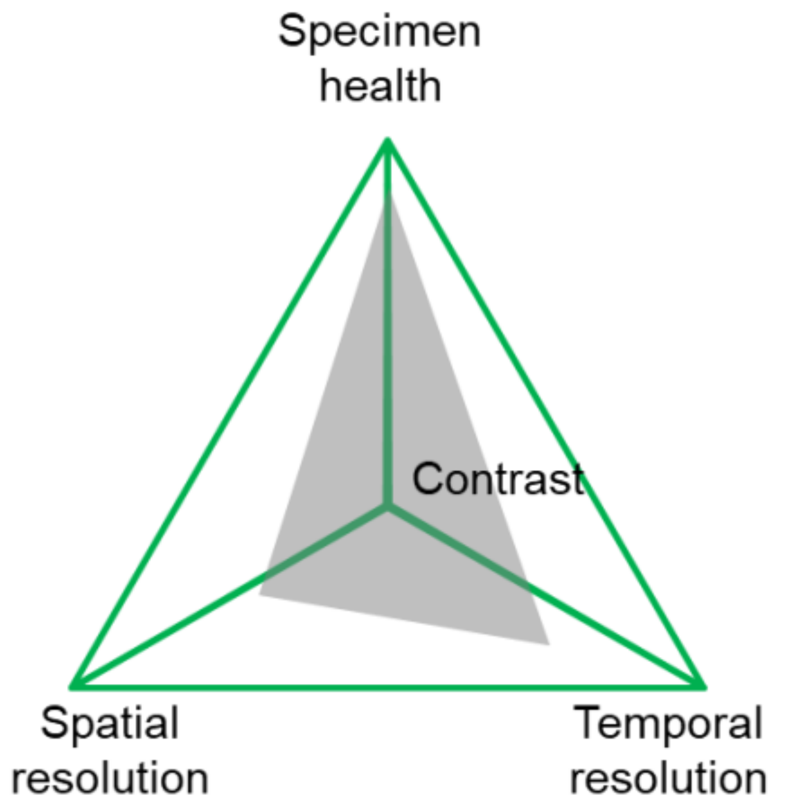
- Time: short exposure times
- Channel: bleed-through
- Depth: Scattering, less excitation

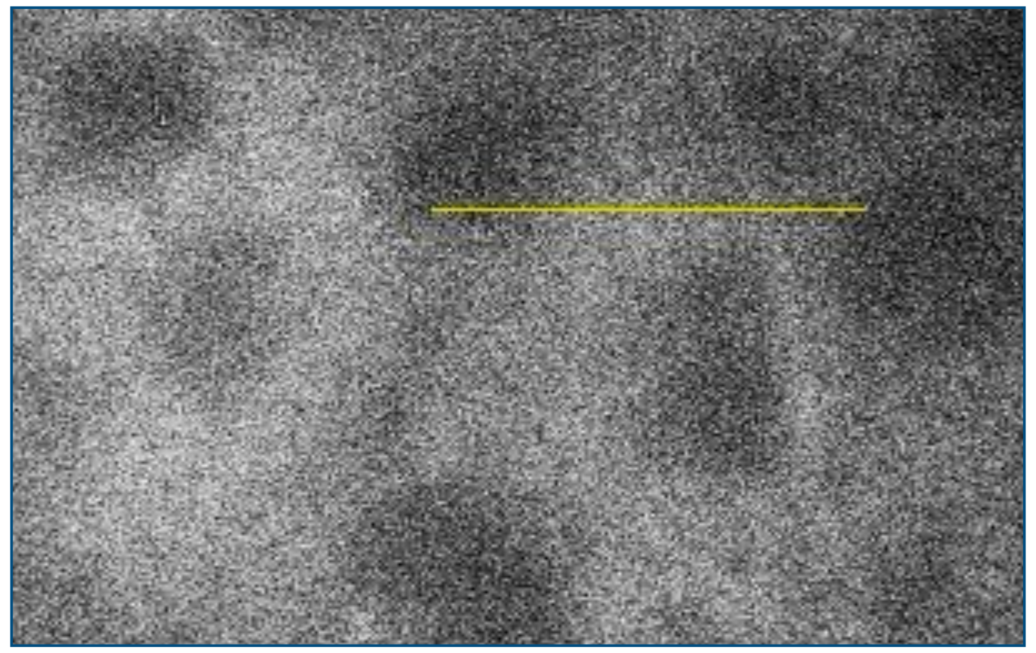
Source of noise

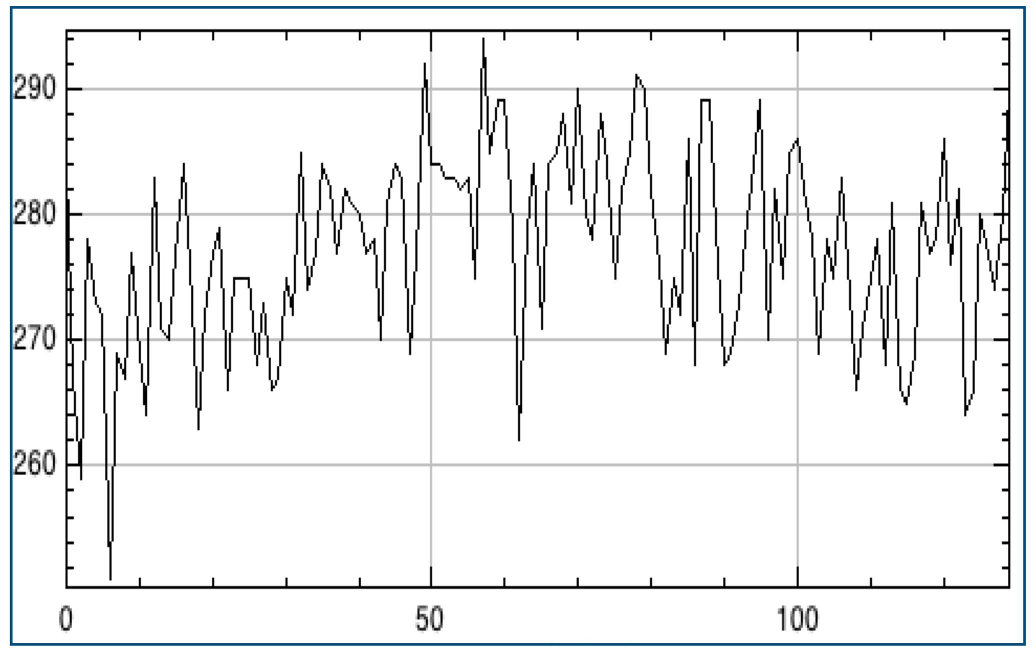
- From the acquisition device
- From the camera/detector
- From the structure
- From the background

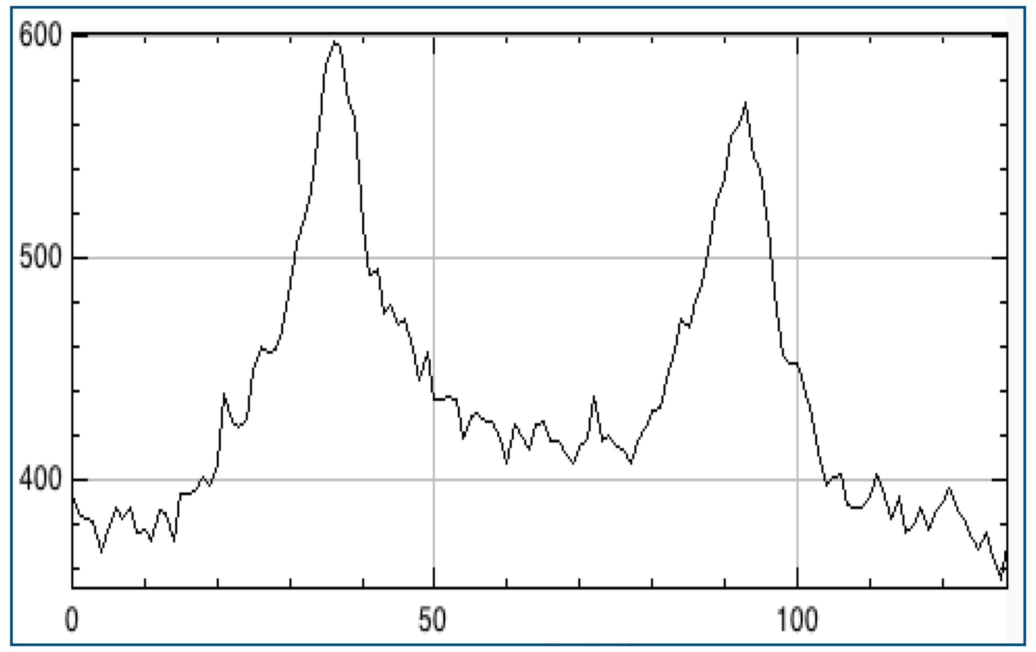
Noise in microscopy

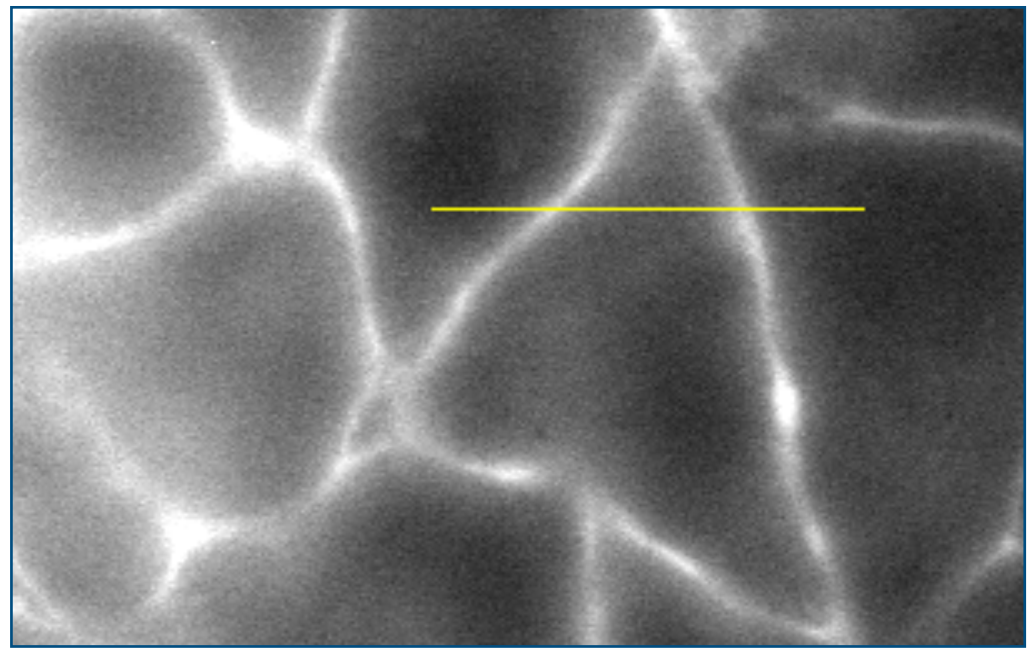
- Shot noise
- Dark current
- Read-out noise
- Spurious charge
- Thermal noise
- Quantization noise
- Dead pixels
- Stuck pixels
- Blocking JPEG







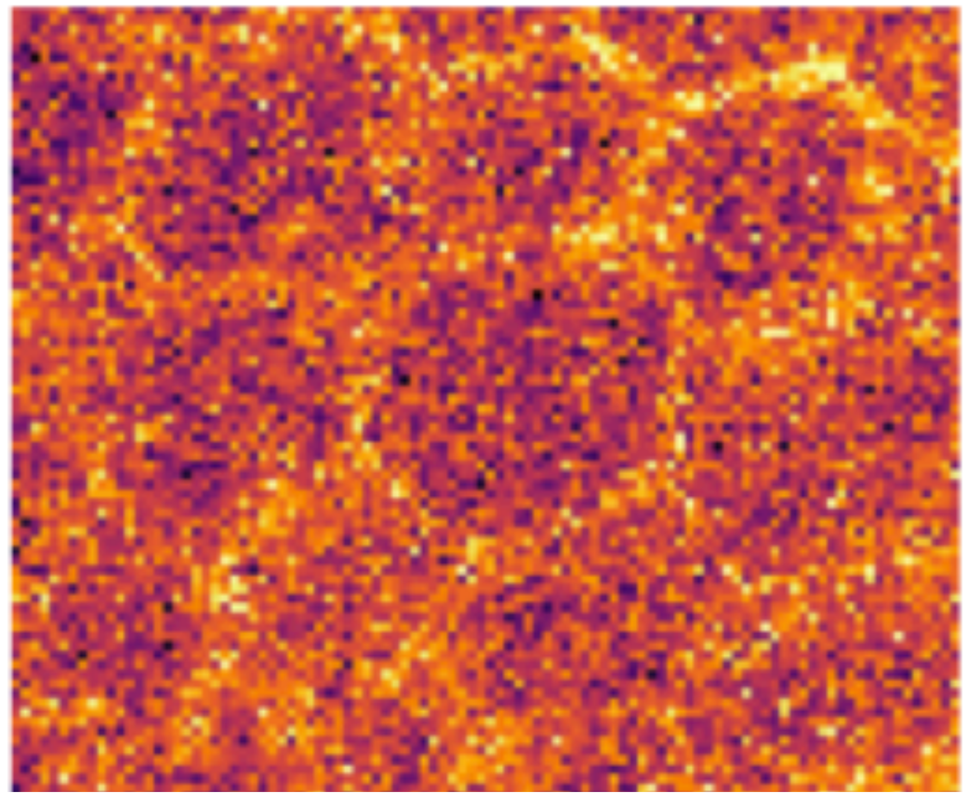




Human are good to guess structures in noisy image

Noise Structure

Pixelwise Noise



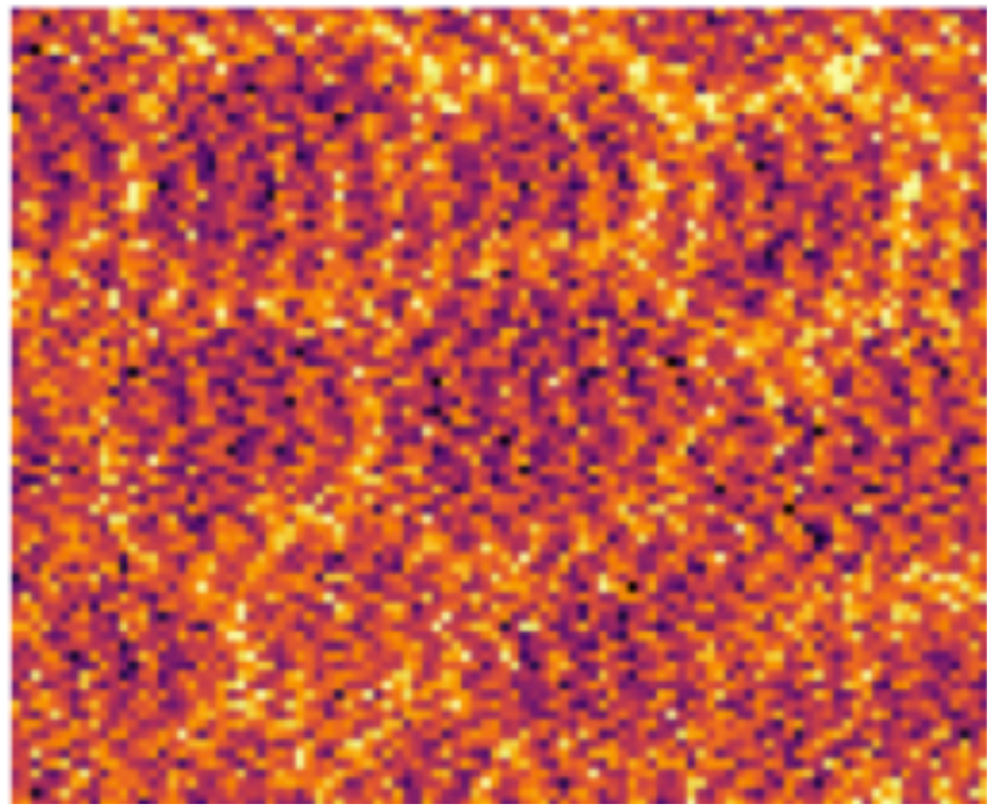
$$y[\boldsymbol{k}] \;=\; x[\boldsymbol{k}] \;+\; n[\boldsymbol{k}]$$

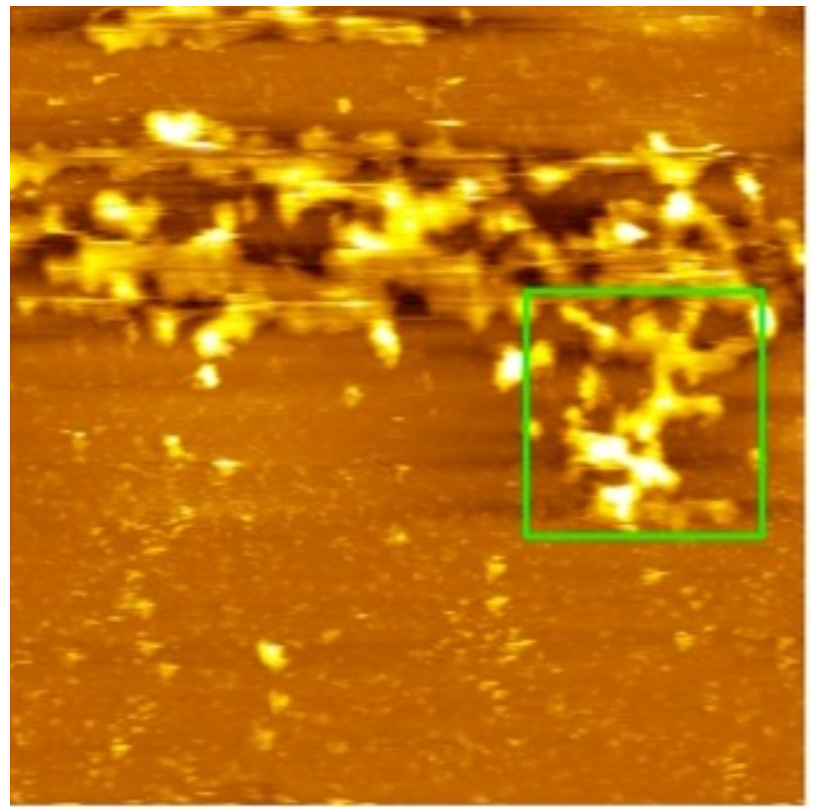
Signal prior
Correlation among pixels
Self-similarity
Patterns
Sparsity
Low frequency

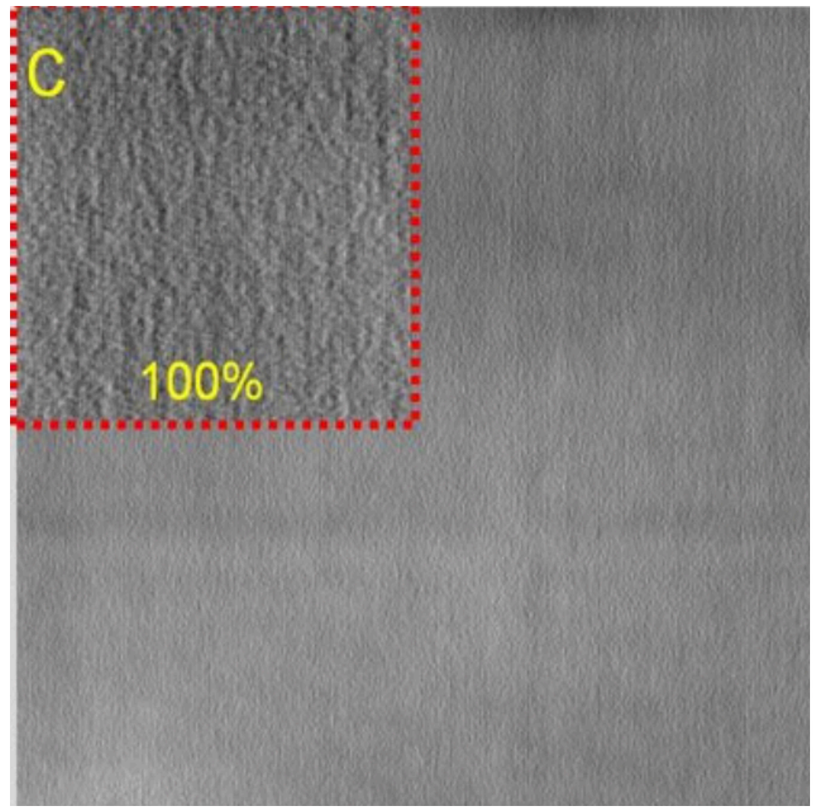
+

Noise model
Random variables
No spatial structure
Independent realizations
Statistical distribution
High frequency

Structural Noise



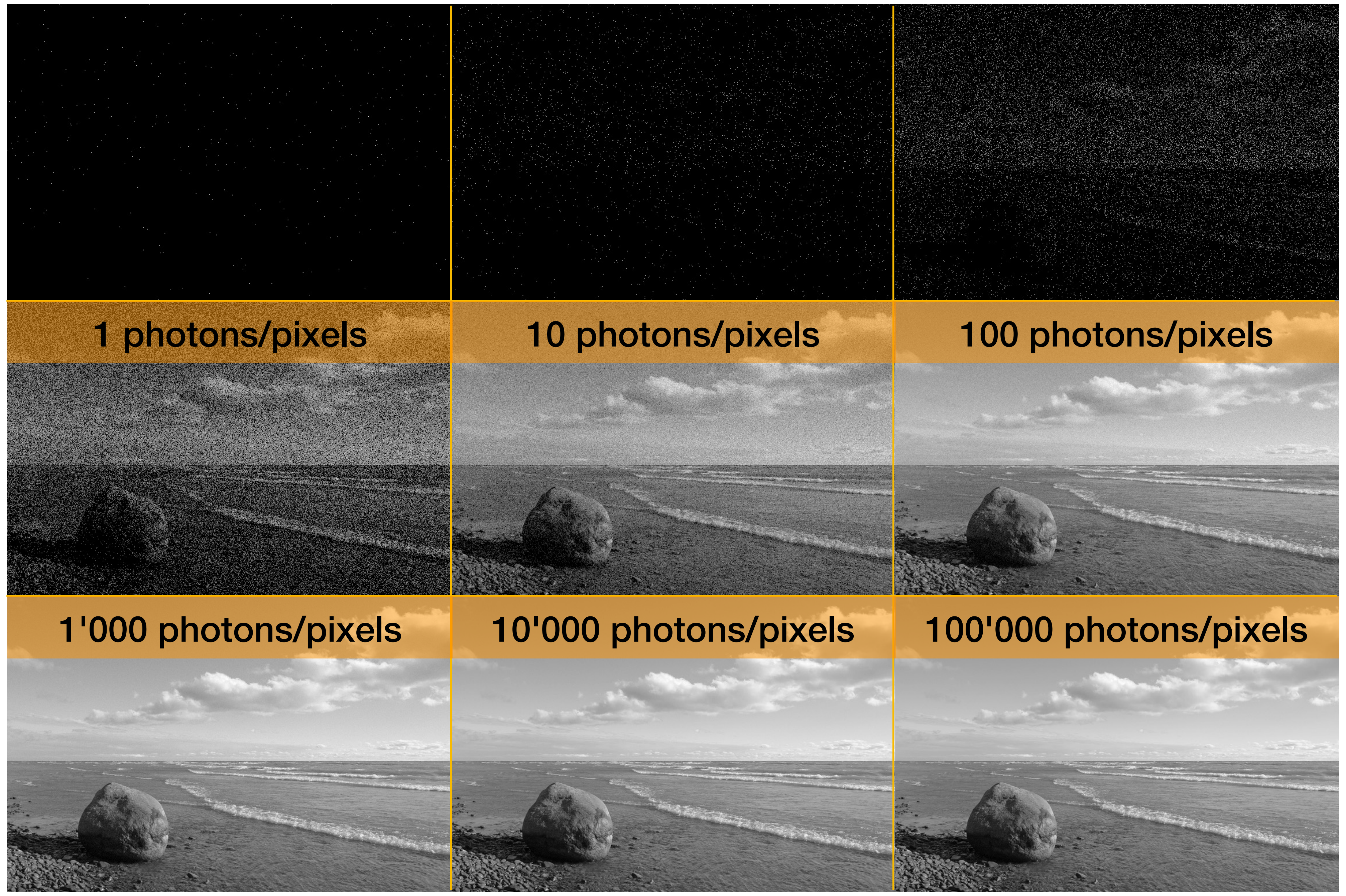




Noise Quantity

Signal-to-Noise Ratio SNR

$$SNR = \frac{\text{mean}}{\text{stdev}} \qquad SNR_{dB} = 10\log_{10}(SNR)$$

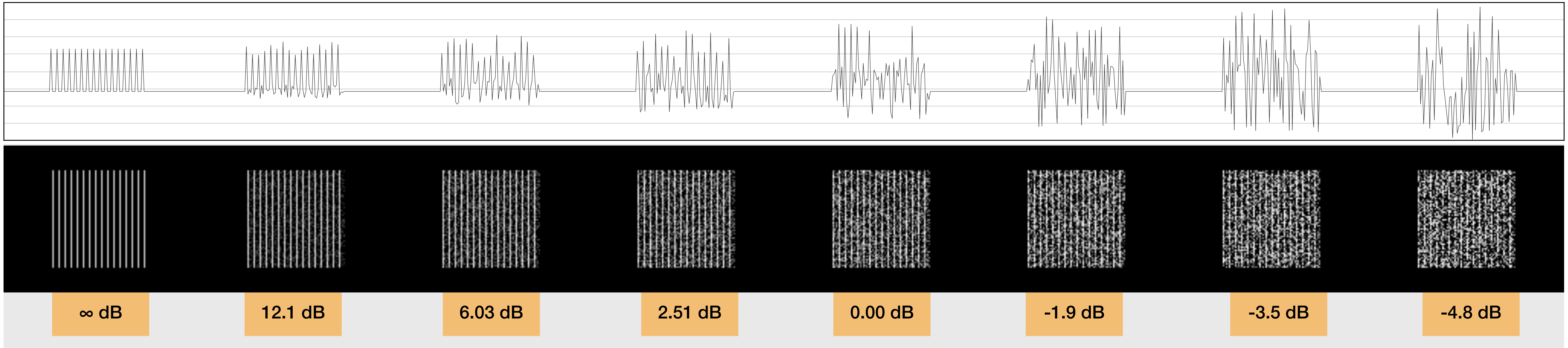


Peak Signal-to-Noise Ratio PSNR

$$PSNR = \frac{\text{dynamic range}}{\text{stdev}}$$

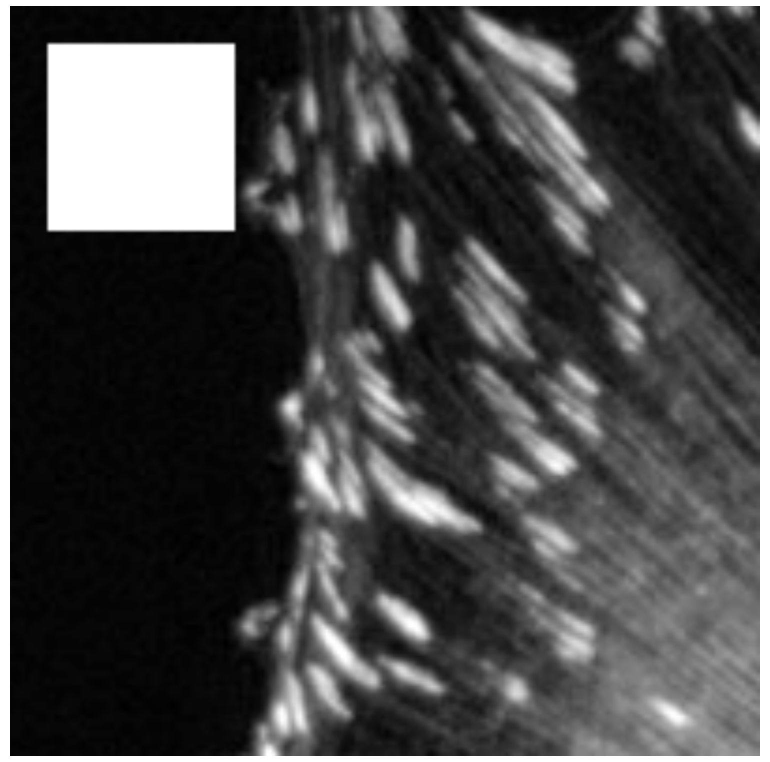
Contrast-to-Noise Ratio CNR

$$CNR = \frac{\mu_{sig} - \mu_{bg}}{\sigma}$$



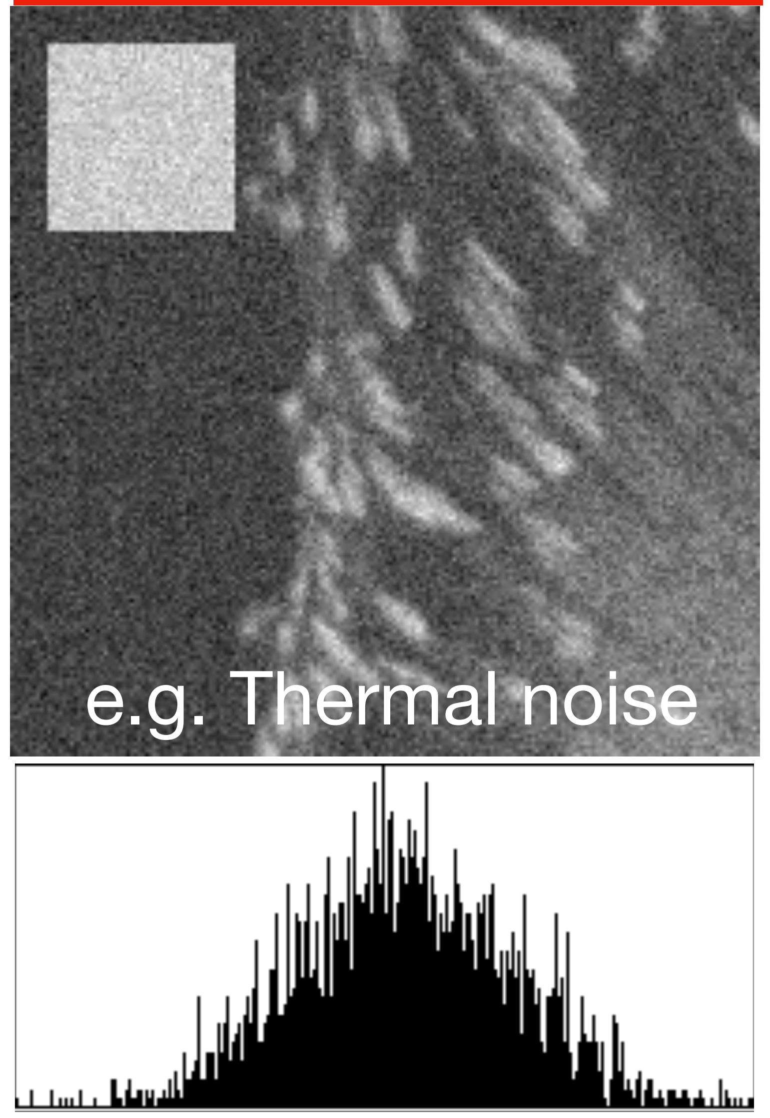
Noise Distribution Models

Test image
Noiseless



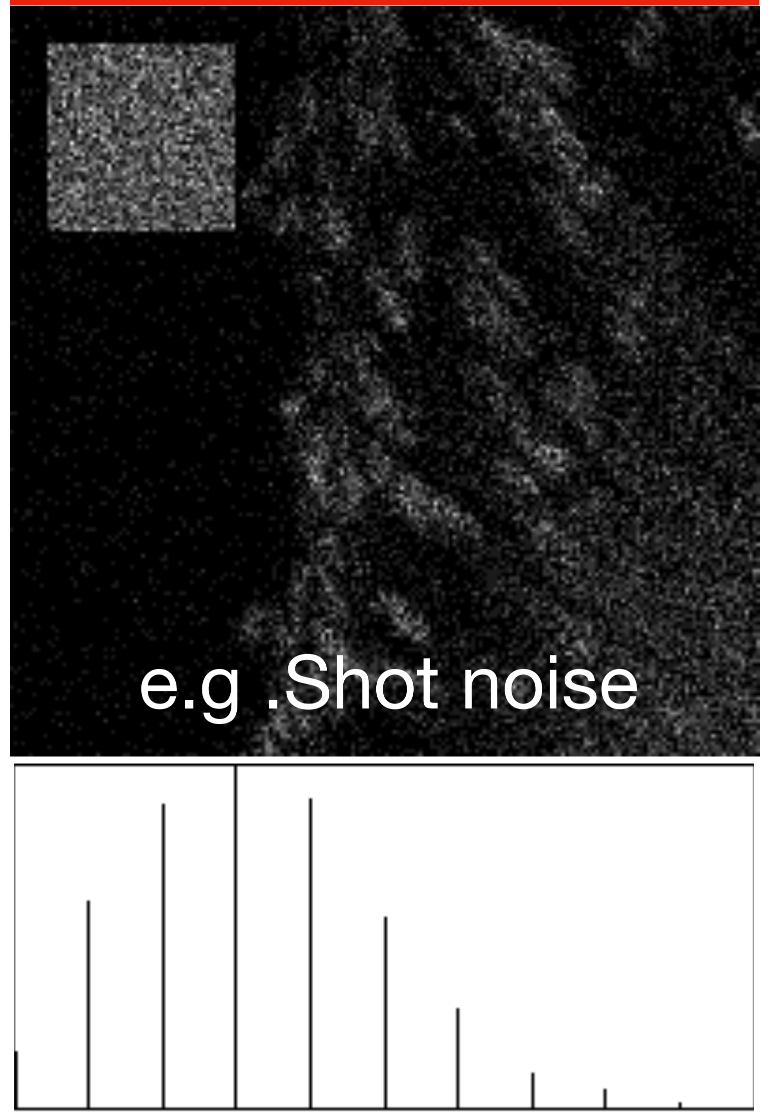
Source: CIL38985

Gaussian



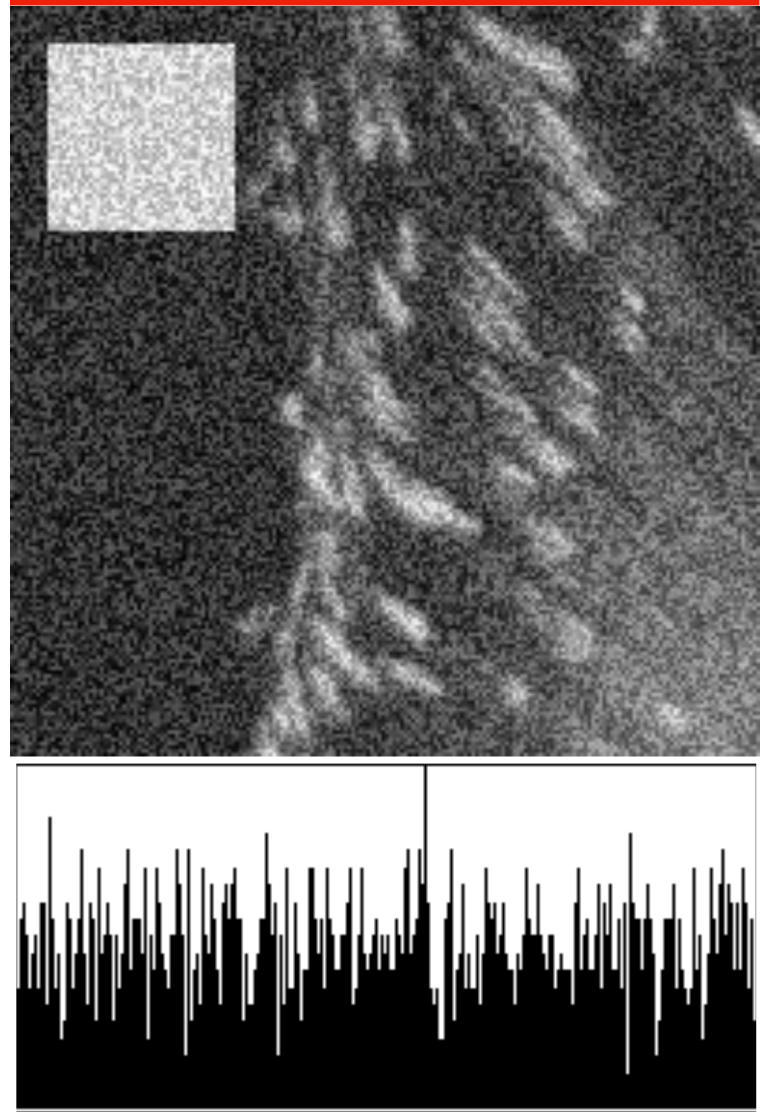
mean μ
standard deviation σ

Poisson



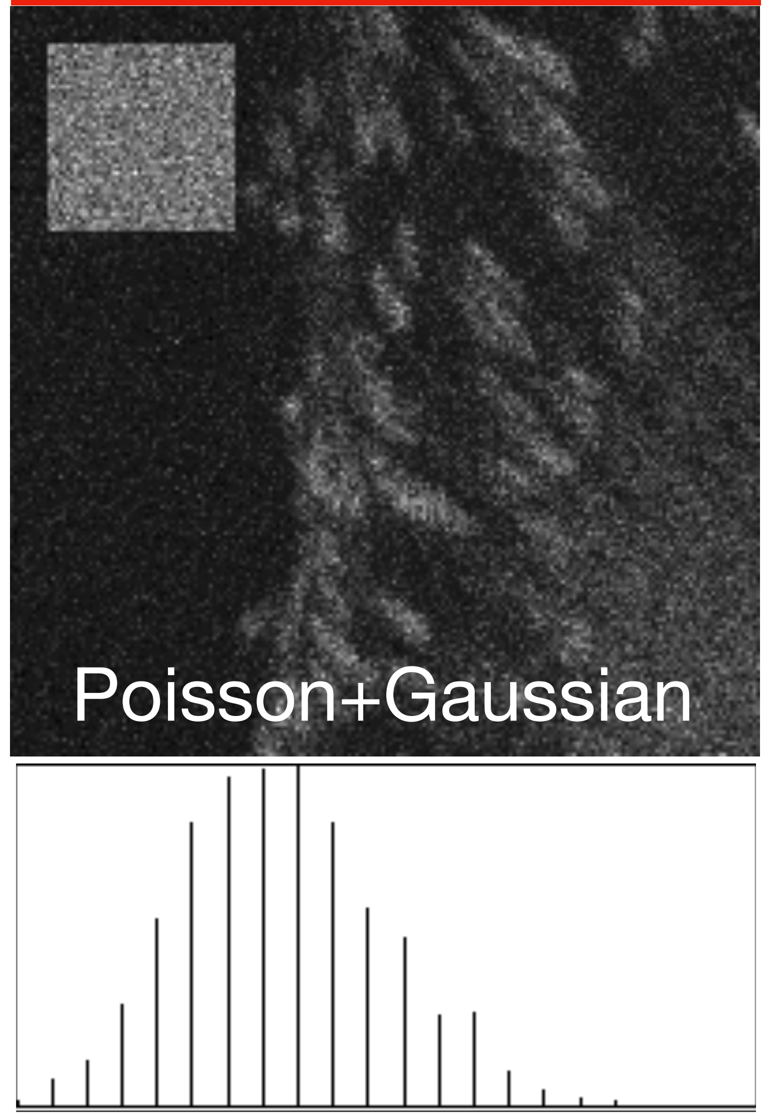
mean $\mu = \lambda$
variance $\sigma^2 = \lambda$

Uniform

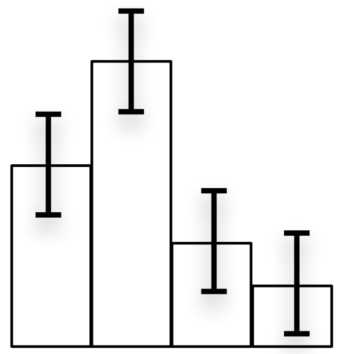


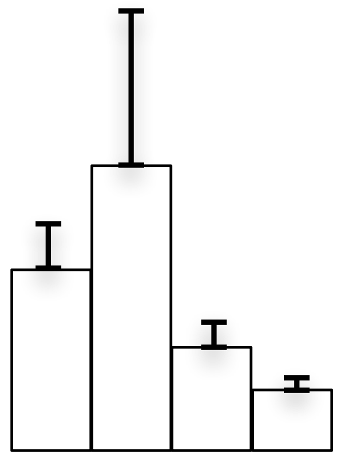
[min, max]
mean $\mu = (max+min)/2$

Mixed P. G.



Demonstration
RandomJ on Fiji





Mixed Poisson-Gaussian

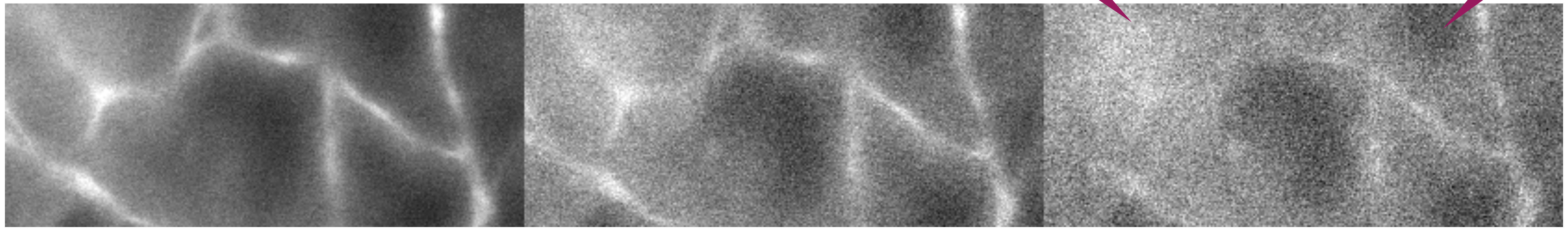
- Realistic model in fluorescence
- Poisson (shot and dark noise)
- Gaussian (readout noise)

Basic Methods

High frequency

Speckle noise

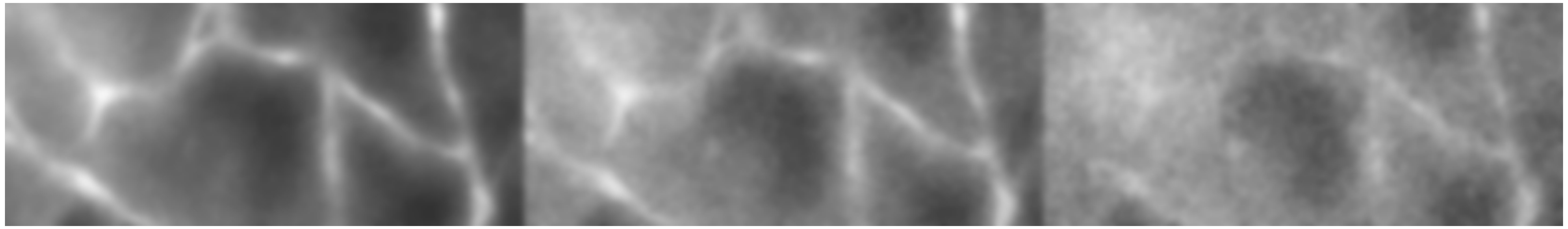
Noisy data
Frames at 3 time-points.
Signal is decreasing
(bleaching)



Source: D. Wüstner, University of Odense, Denmark

Gaussian Filter

Low-pass filter
Spatial blurring
Reduction of high
frequency noise

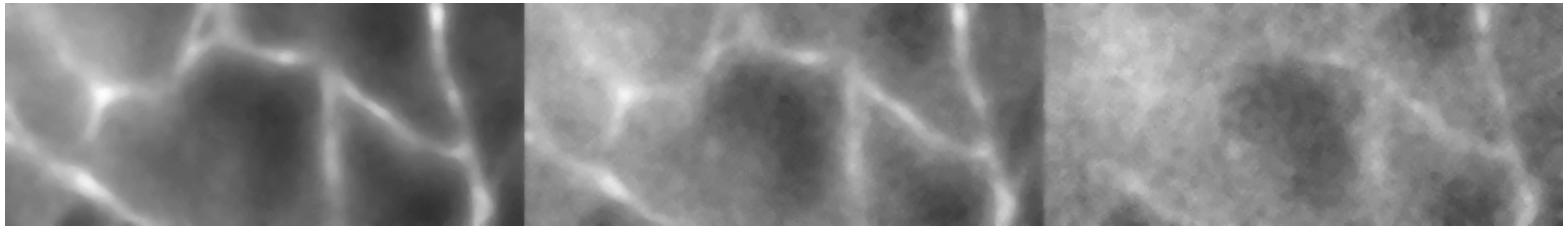


Gaussian Filter of ImageJ, sigma=2

Smoothing
Kill the HF signal
? imaging setup

Median Filter

Speckle noise
Preserve edges
Reduce shot noise
Cartoon effect



Median Filter of ImageJ, radius=3

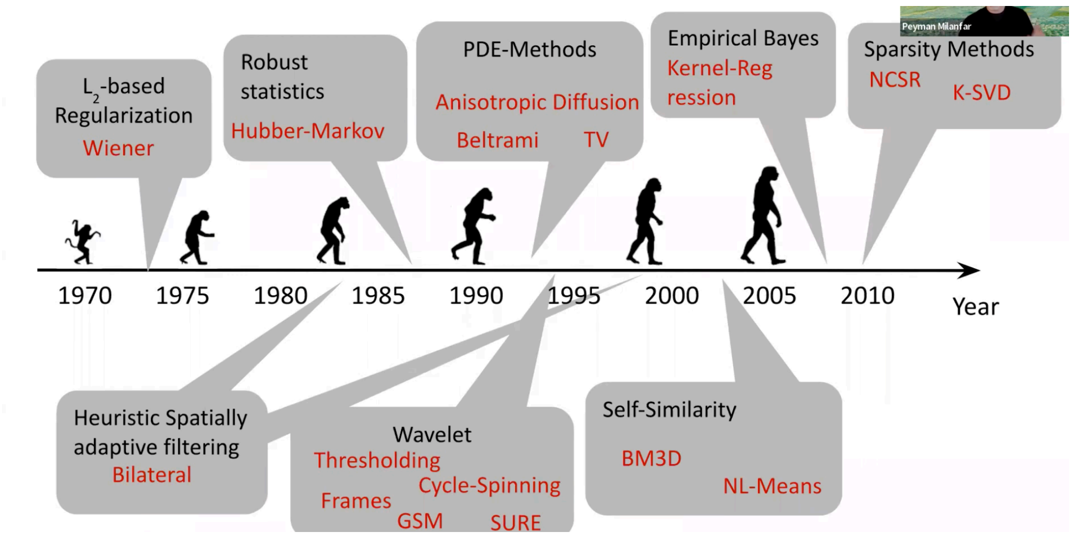
No fine tuning
Heuristic control

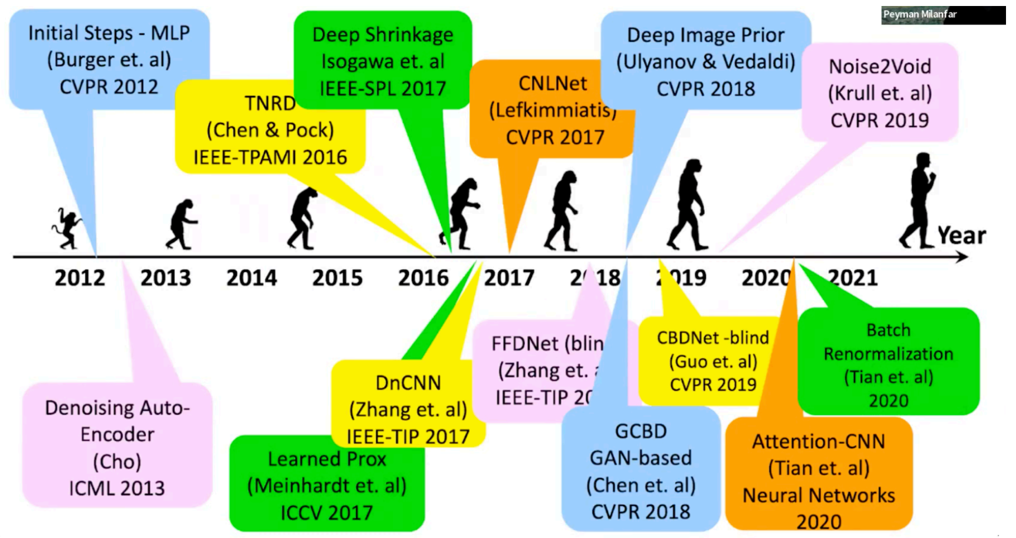
Methods for Image Denoising

Goal: Image simplification

- ✓ Preprocess before image segmentation
- ✓ Enhance the structure of interest
- ✓ Take advantages of the multidimensional data

Video tutorial of Peyman Milanfar





Remove high frequency

- Digital linear filtering (Gaussian)

Edge-preserving smoothing

- Non-linear filtering (Median)
- Anisotropic diffusion [Perona-Malik, 1990]
- Bilateral filter [Tomasi, 1998]
- Mean-shift [Comaniciu, 2002]

Sparsity prior

- Total variation [Rudin-Osher-Fatemi, 1992]
- Wavelet shrinkage [Donoho 1998]
- PureLET [Luisier 2010]

Self-similarity prior

- Non-local mean [Buades, 2005]
- Block matching [Dabov 2006]

Meiniel et al, Denoising of microscopy images : a review of the state-of-the-art, *IEEE* 2018.

Supervised Learning

- DnCNN [Zhang 2017]
- CARE [Weigert 2018]

Self-Supervised Learning

- Noise2Noise [Lehtinen 2018]
- Deep Image Prior [Ulynov 2018]
- Noise2Void [Krull 2019]

Other strategies of learning

- PnP Denosing [Ulugbek 2022]
- Diffusion Generative Model [Song 2020]

Deep-learning approaches

Laine et al., Imaging in focus : An introduction to denoising bioimages in the era of deep learning, 2021.

"Classic" Image Simplification

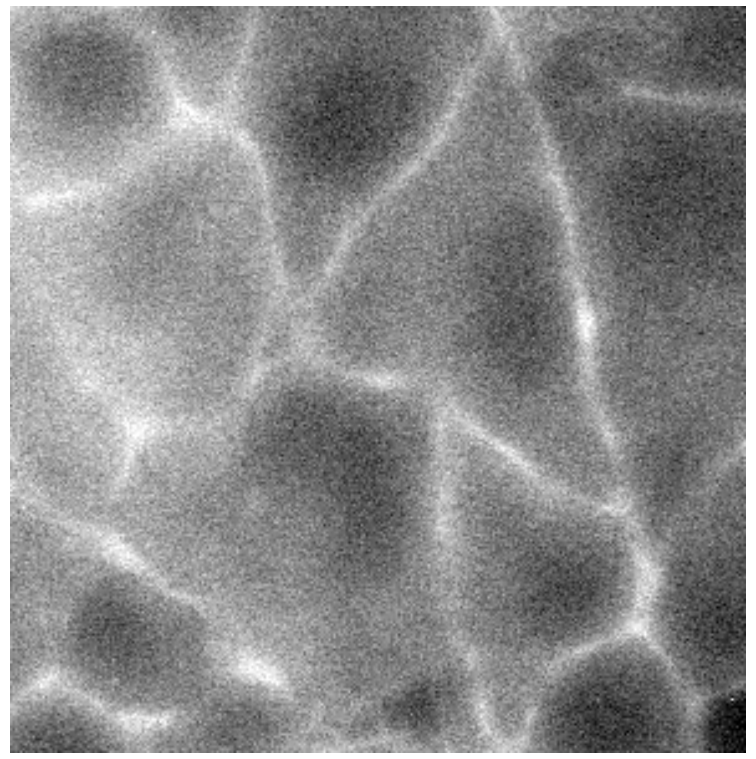
ImageJ plugin

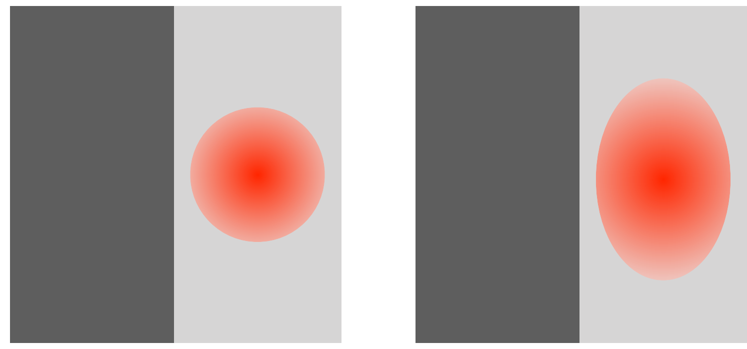
Anisotropic Diffusion

[Perona-Malik, 1990]

Iterative diffusion
Many parameters

$$\frac{\partial f}{\partial t} = \mathrm{div}(c(\|\nabla f\|)\,\nabla f)$$





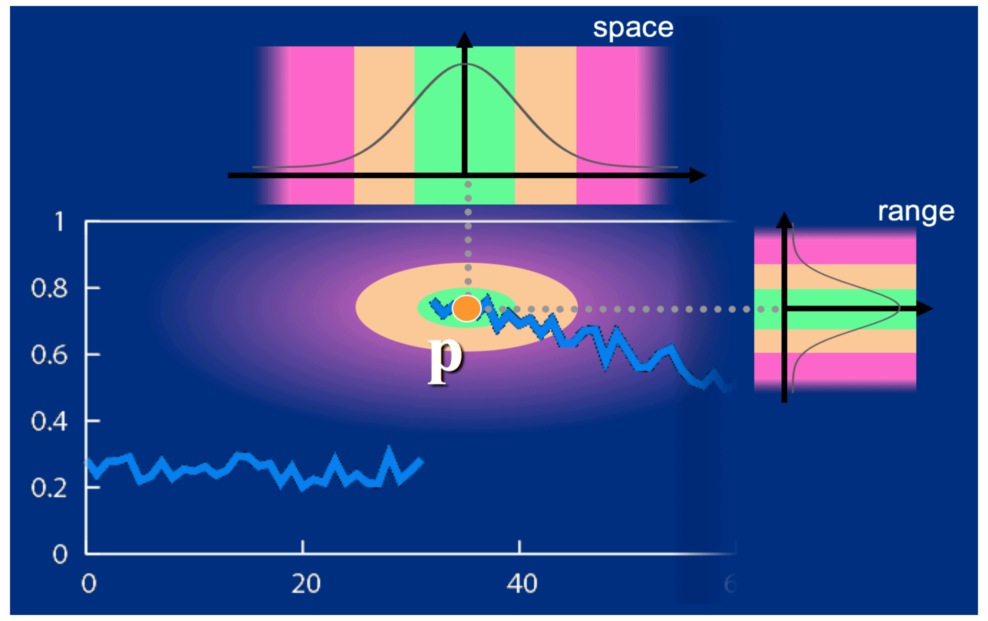
To preserve edges

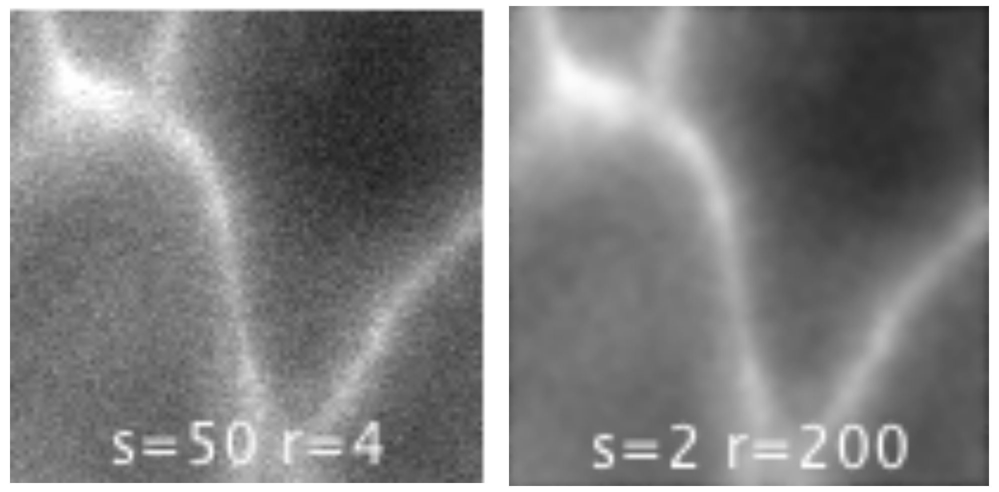
Bilateral Filter

[Tomasi, 1998]

Local adaptive blur
Preserve edges

$$h(\mathbf{x}) = \frac{1}{W}\int_{k\in\Omega} f(\mathbf{k})\, w(\mathbf{x}-\mathbf{k})\, \phi\left(f(\mathbf{x})-f(\mathbf{k})\right) \mathbf{dk}$$



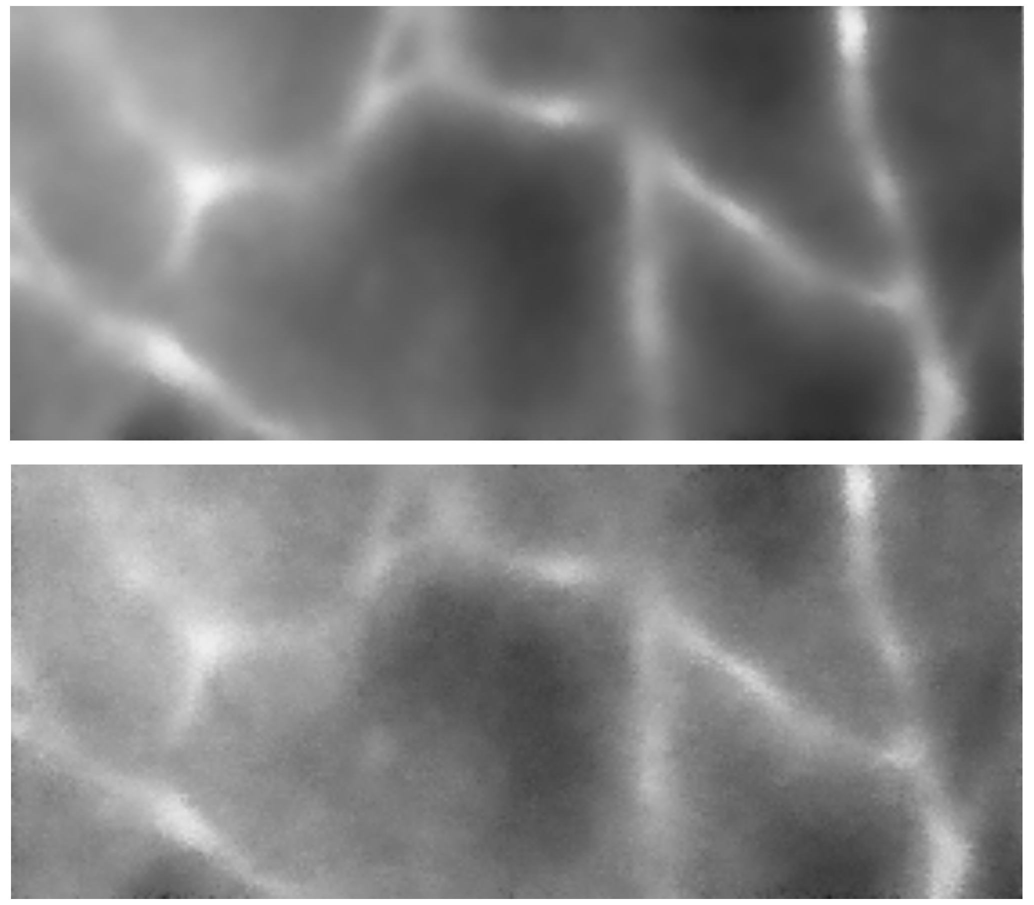


Mean-Shift

[Comaniciu, 2002]

Data clustering algorithm
Cartoon effect, extensible in nD

For every pixel
1. Calculate the center of mass on local window: **x** and **u**
2. Shift to **x**
3. Repeat step 1 until convergence

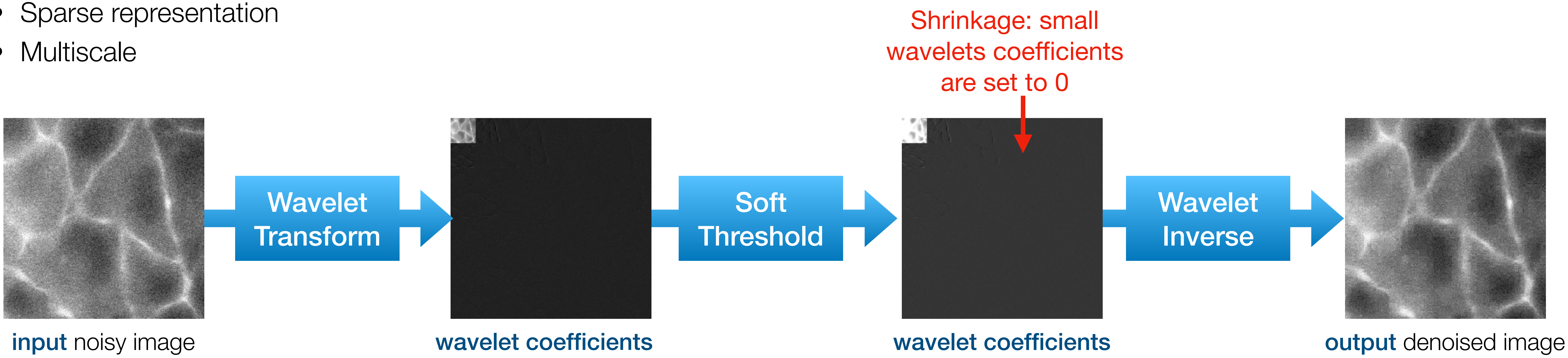


Wavelet Shrinkage

In the wavelet domain

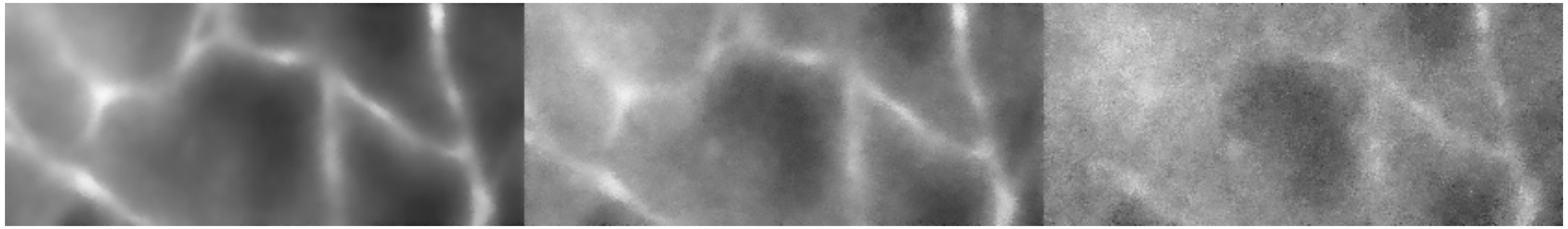
[Donoho, 1998]

- Decorrelation ability
- Sparse representation
- Multiscale



PureDenoise [Luisier, 2010]

Linear expansion of threshold using a unbiased criteria



PureDenoise, plugin of ImageJ, EPFL

Self-Similarity-Based Denoising

Non-local mean

[Buades-Coll-Morel, 2005]

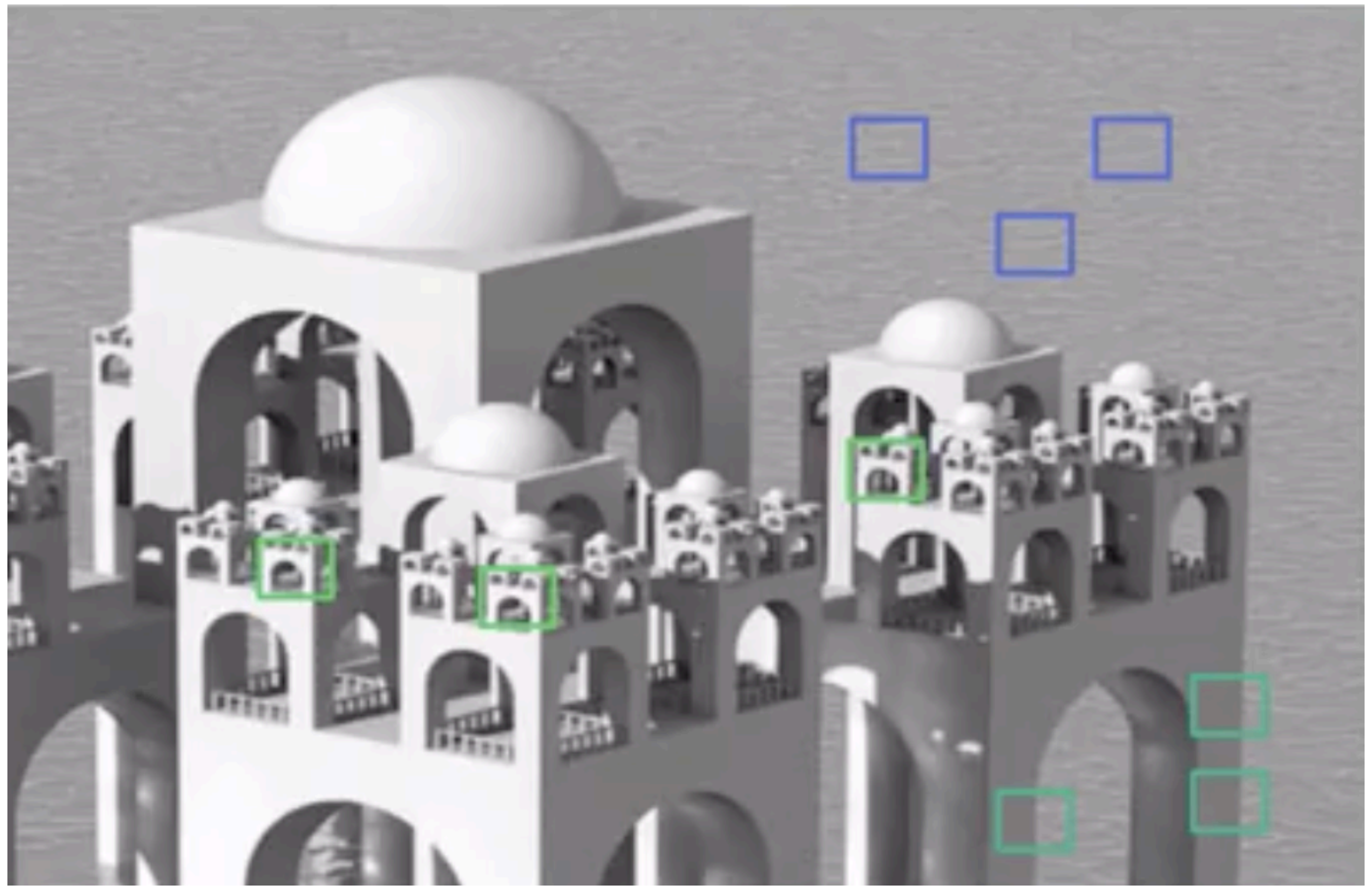
Assumptions

- Images have repetitive textures
- Images have self-similarity

Algorithms

- No averaging around the pixel
- For over all the image find the similar pixel and then average

$p_1 = p_0 + n_1$
$p_2 = p_0 + n_2$
....
$p_n = p_0 + n_n$



ij-nlm
CANDLE-J

BM3D

[Dabov, 2006]

- Block-Matching 3D
- Wavelet threshold
- Combine patches

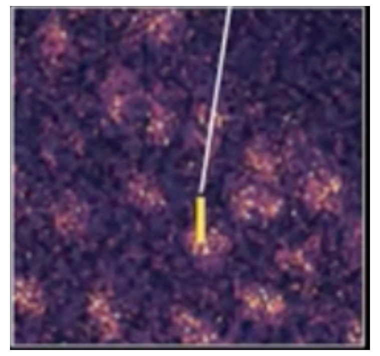
State-of-the-art in image denoising only in Matlab!

Supervised Learning

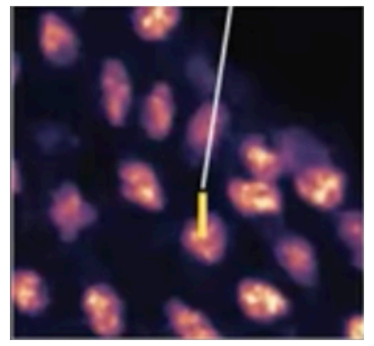
noisy image ⟵ **TRAINING** ⟶ ground-truth

noisy image **PREDICT** ⟶ clean image

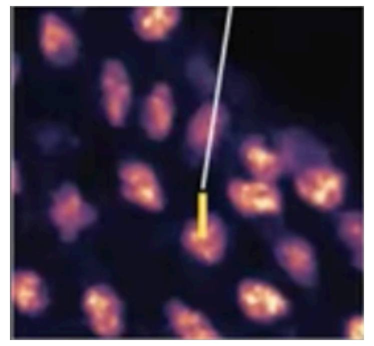
input



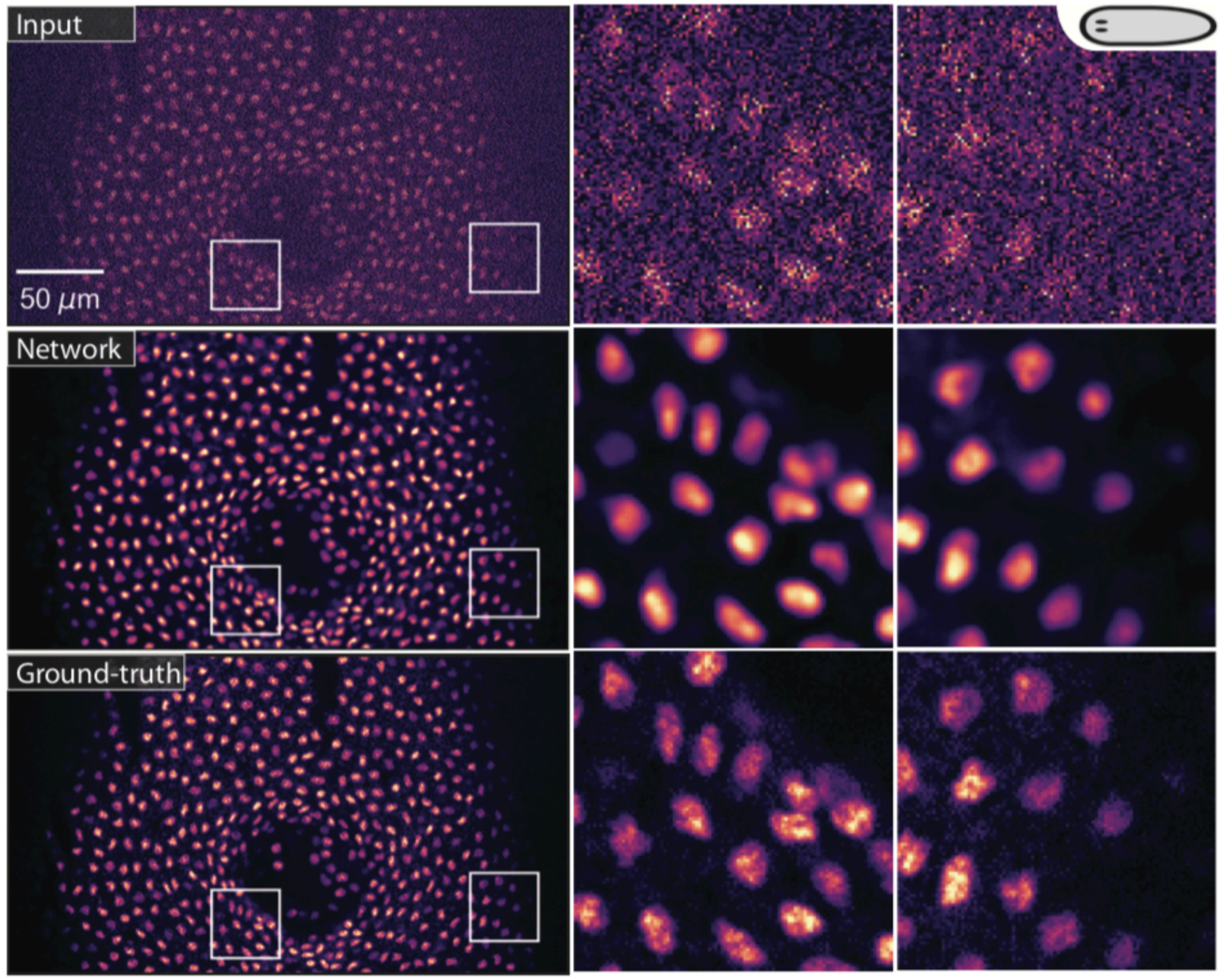
output



clean



Architecture: Resnet, U-net, ...



CARE

Weigert, Nature Methods, 2018

Video tutorial of Joran Deschamps, Denoising microscopy images with self supervised deep learning Nature Methods, Aneris Eurobioimaging, 2023

No ground-truth?

Simulation

- Add various noise quantity
- Add various noise type

Real data (microscope)

- Higher illumination
- Slow down the acquisition

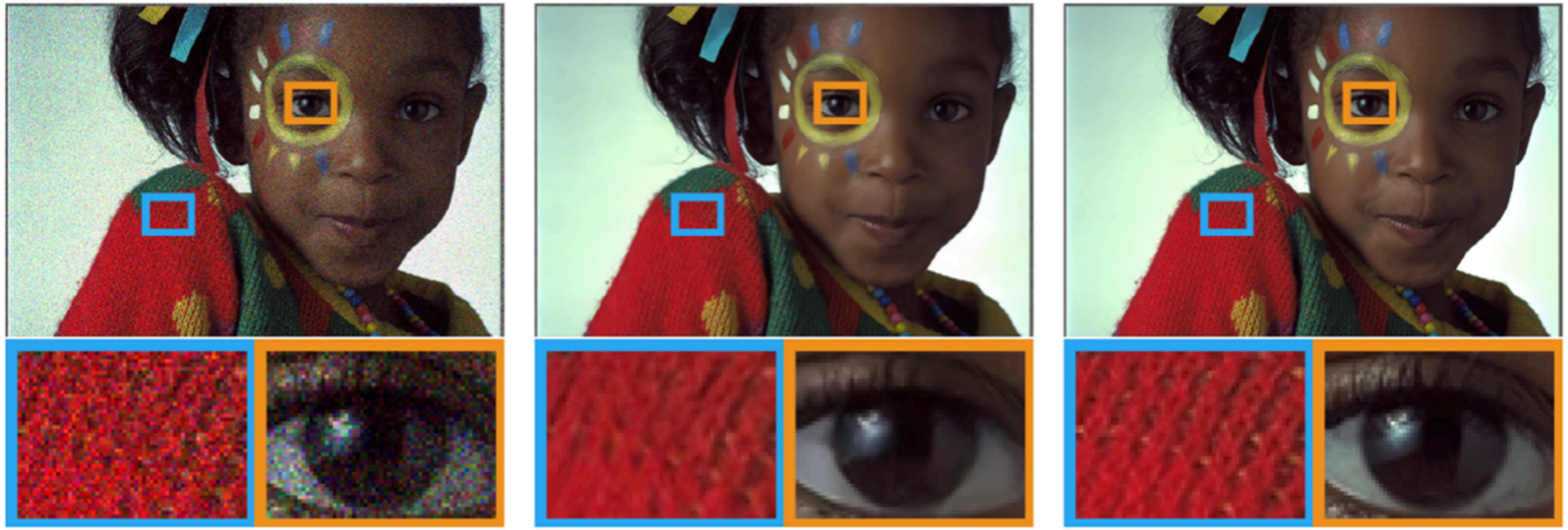
Self-Supervised Learning

noise2noise [Lehtinen, arXiv 2018] Learn bad images into good images by only looking at bad images

noisy image independent realisation ← **TRAINING** → noisy target independent realisation

noisy image → **PREDICT** → clean image

"if we were to acquire multiple images with the same signal, but different realizations of noise and average them, the result would approach the true signal"

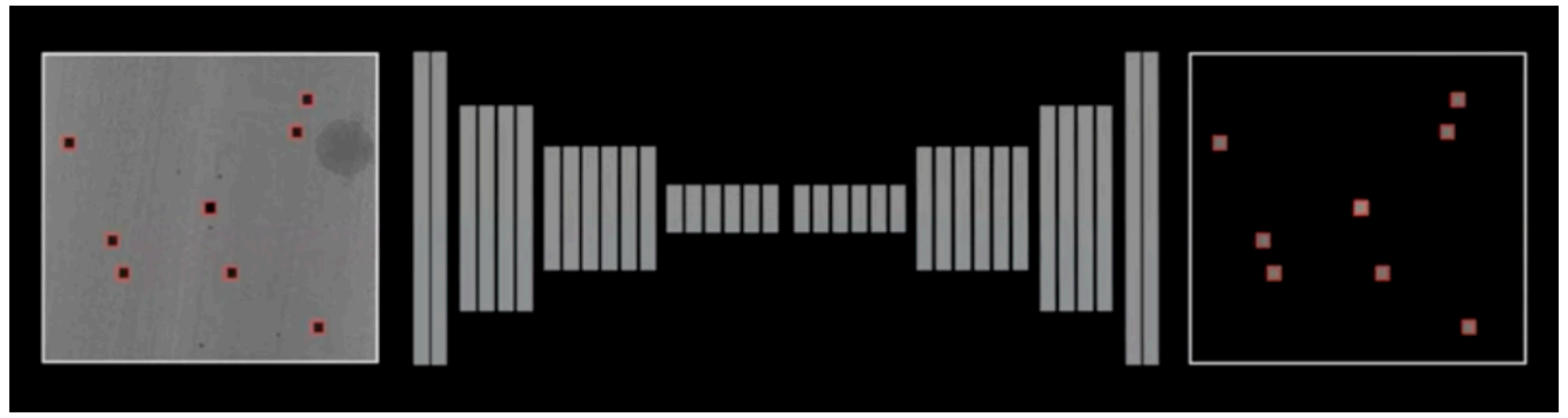


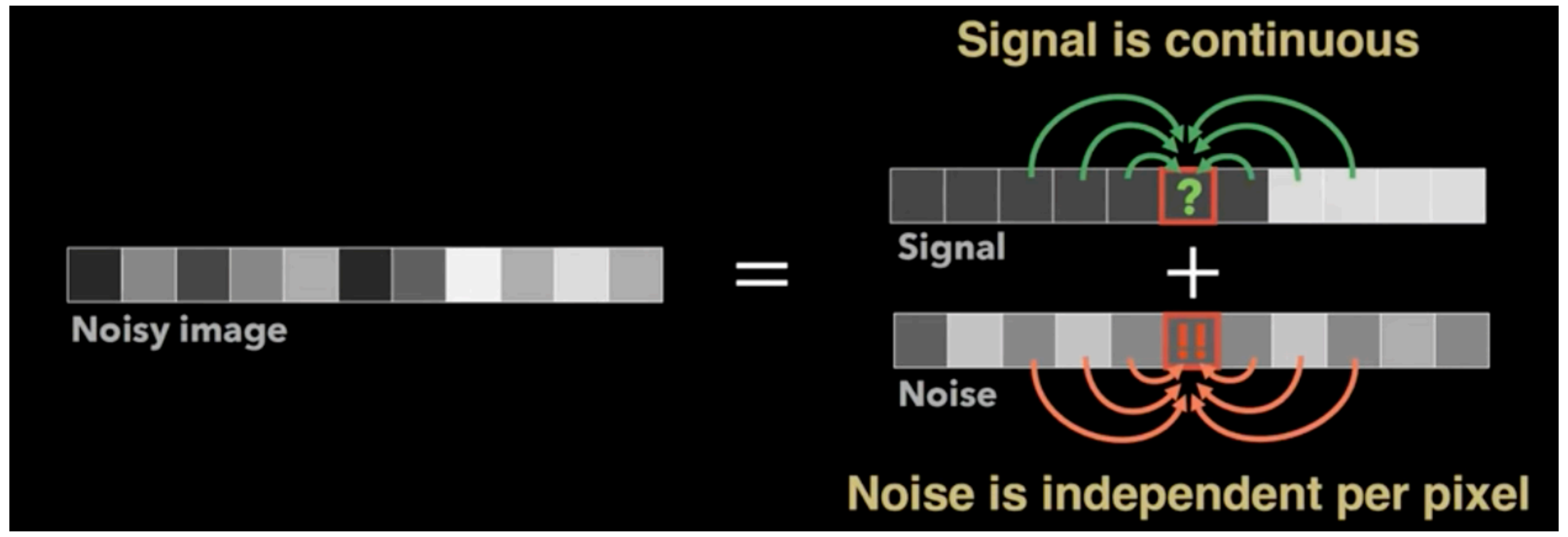
Input Our Ground truth

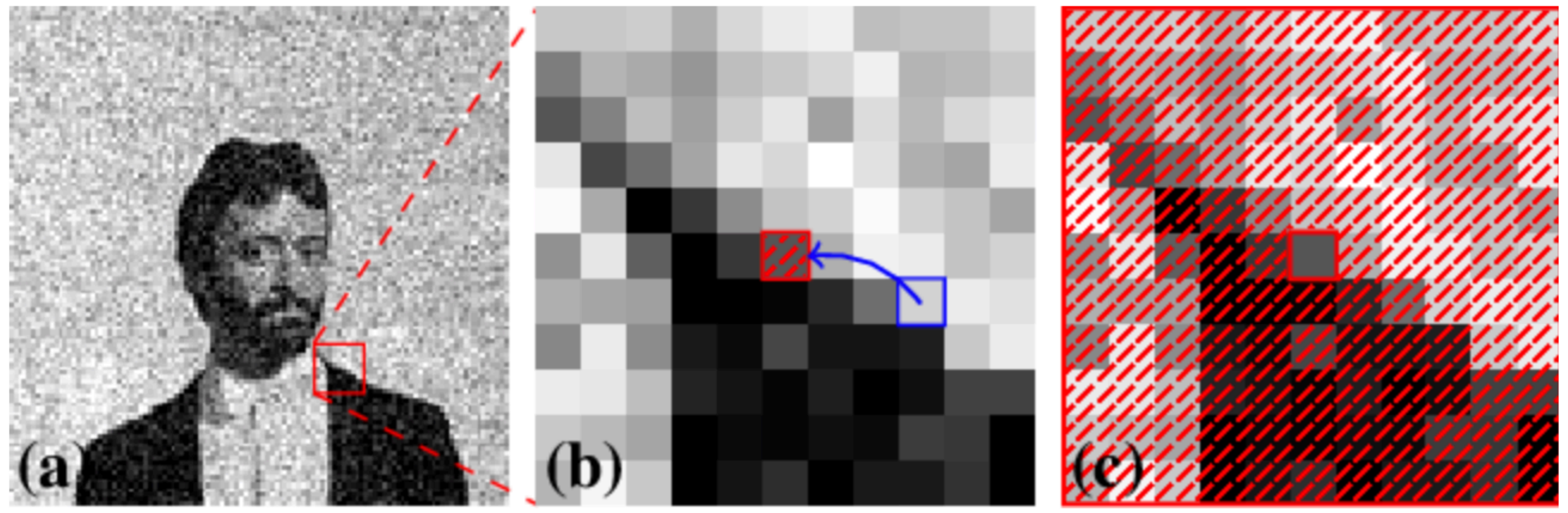
NVidia web site

Self-Supervised Learning

noise2void [Krull, IEEE 2019]

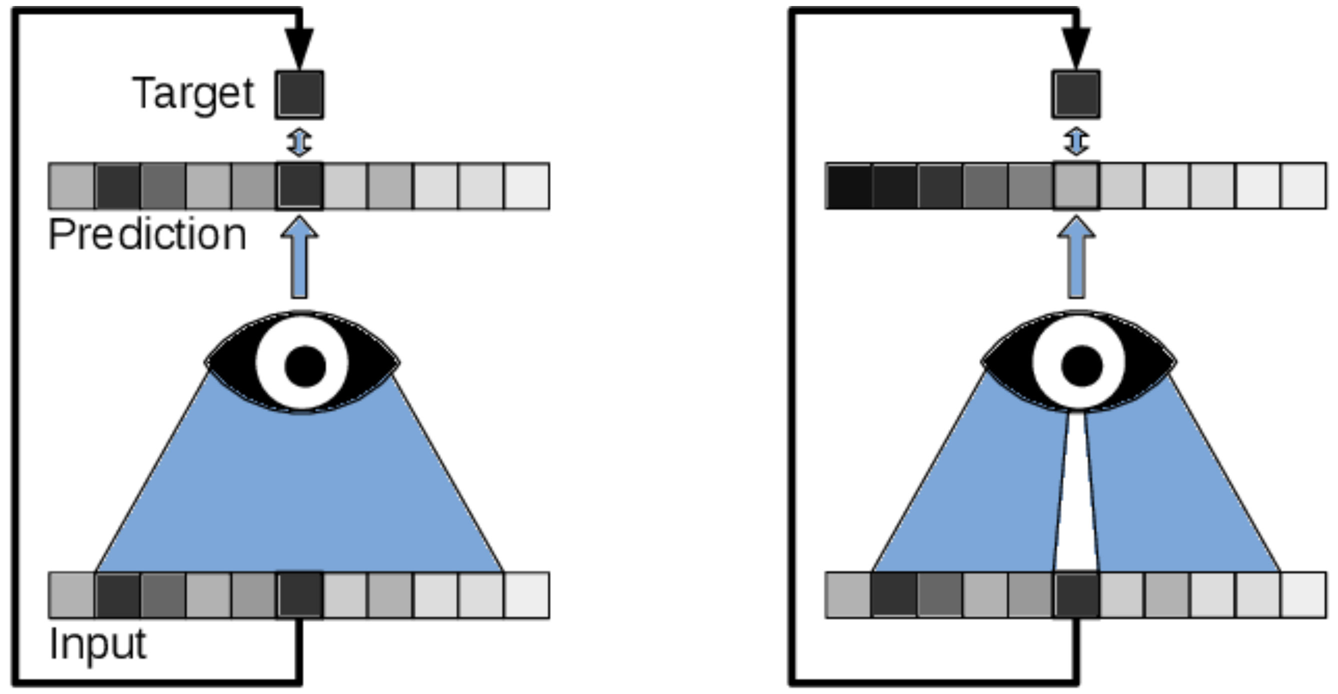


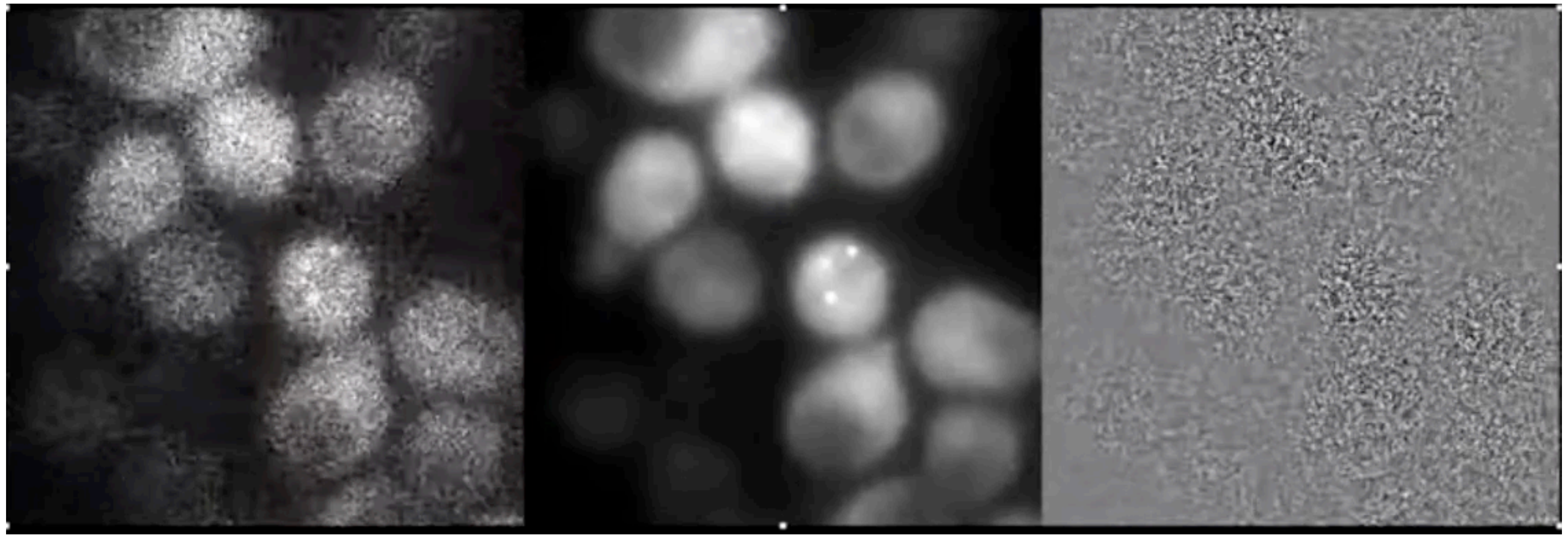


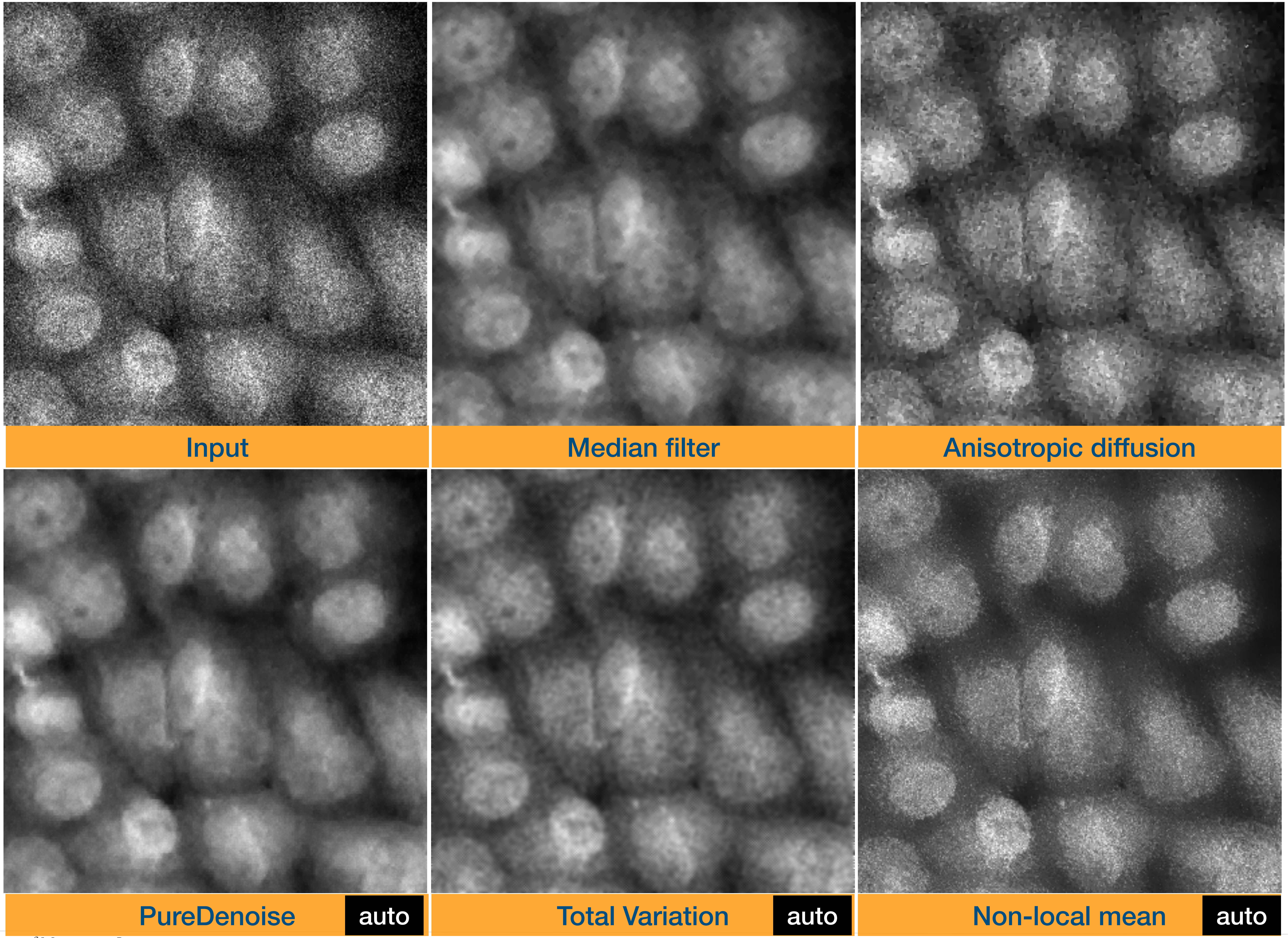


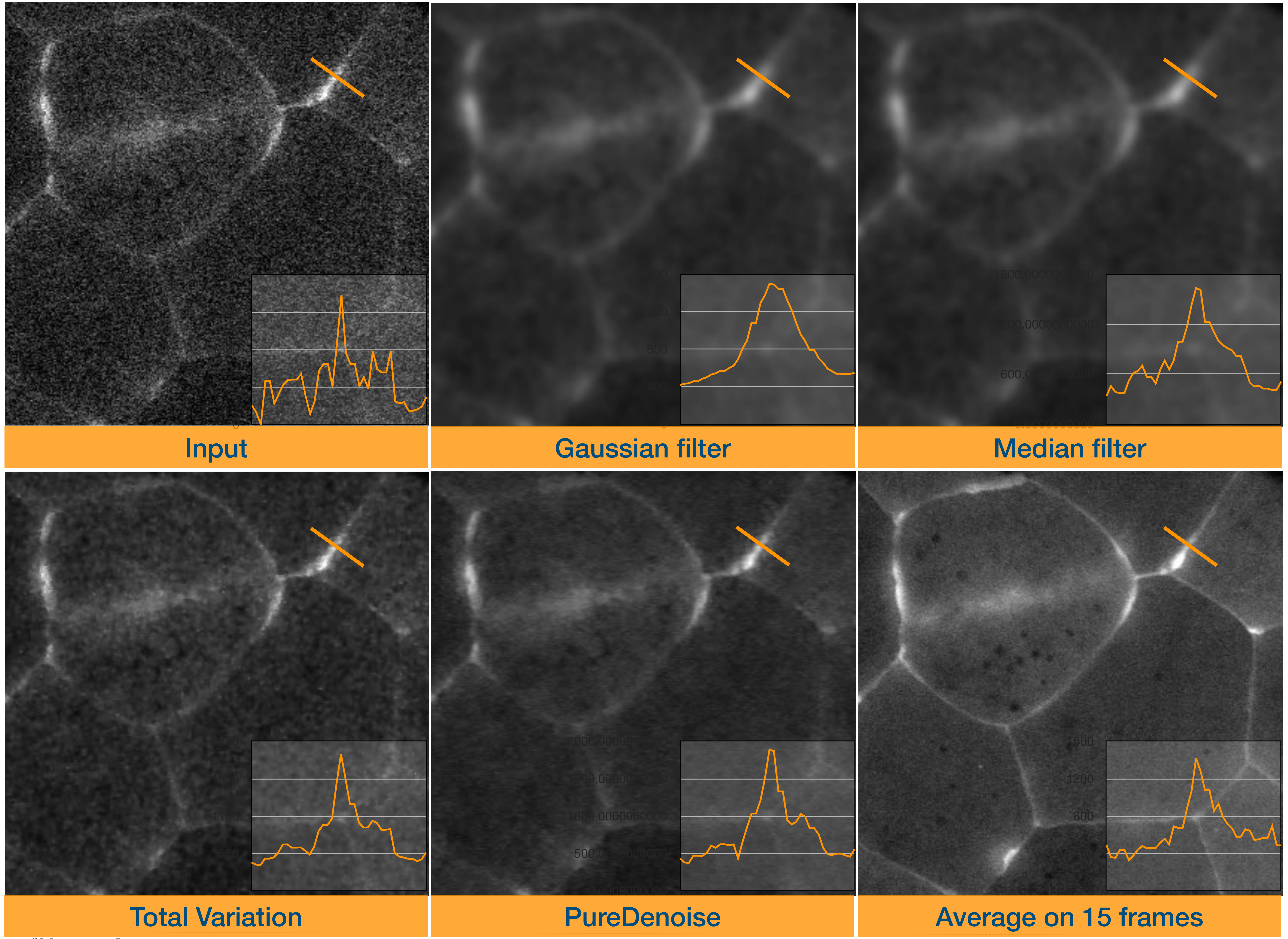
noise2void

- Fiji plugins
- Napari plugins
- Training on GPU
- Prediction on CPU






Input
Median filter
Anisotropic diffusion
PureDenoise
auto
Total Variation
auto
Non-local mean
auto


Input
Gaussian filter
Median filter
Total Variation
PureDenoise
Average on 15 frames

Take-Home Messages

Which method to choose?

- Characterization of the noise (frequency)
- Prior of the structure (**self similarity** is relevant in bioimage)
- Selection of parameters (between noise and structure, automatic)

✓ **Acquisition** is the key to avoid corrupted image

✓ Denoising is useful for **image simplification** (scattering, autofluo)

✓ **Joint** denoising and segmentation performs better (e.g. active contour)

✓ **Quantification** is generally affected by denoising

Acquisition

- Exposure time
- Denoising by averaging frames