PhD Summer School Seminar – 25/10/2023

Deep Learning for Microscopy Image Analysis

Human Technopole - Workshop

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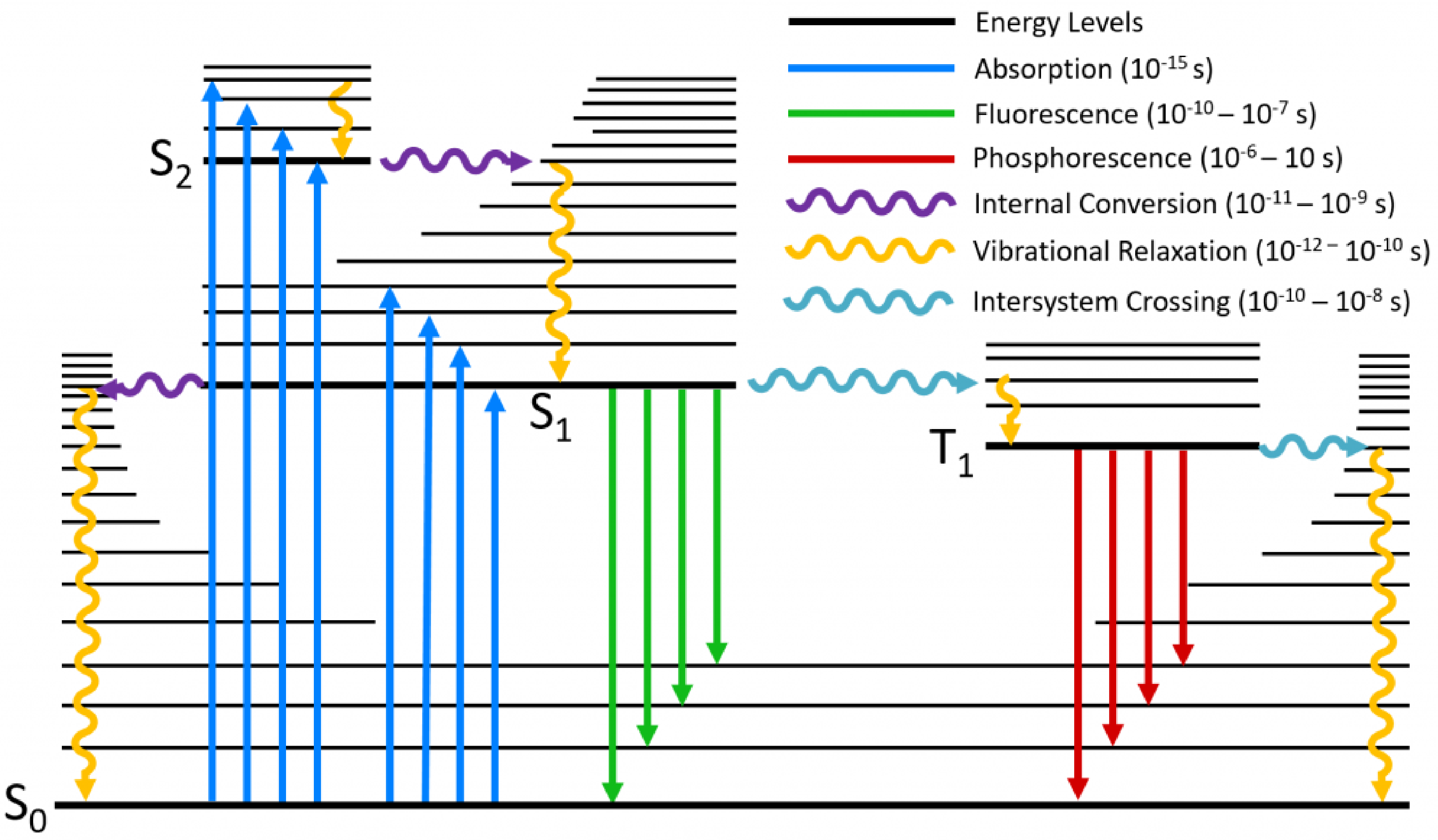
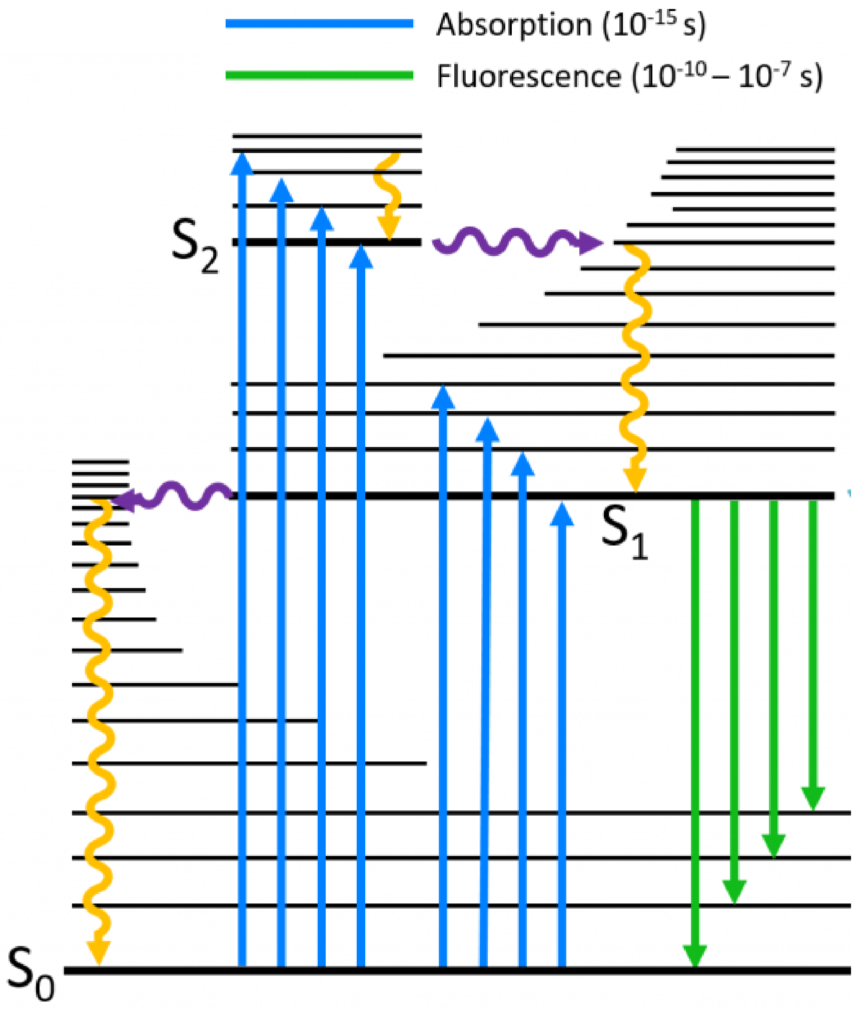
Fluorescence Overview



Fluorescence Overview

- Fluorescence:
  - Fast process (ns)
  - UV-VIS(-IR) Excitation
  - VIS Emission
- Fluorophores:
  - Moieties which can fluoresce
  - Direct Dyes
  - Labelled Antibodies
  - Nanoparticles
  - …



Fluorescence Microscopy

Pros:

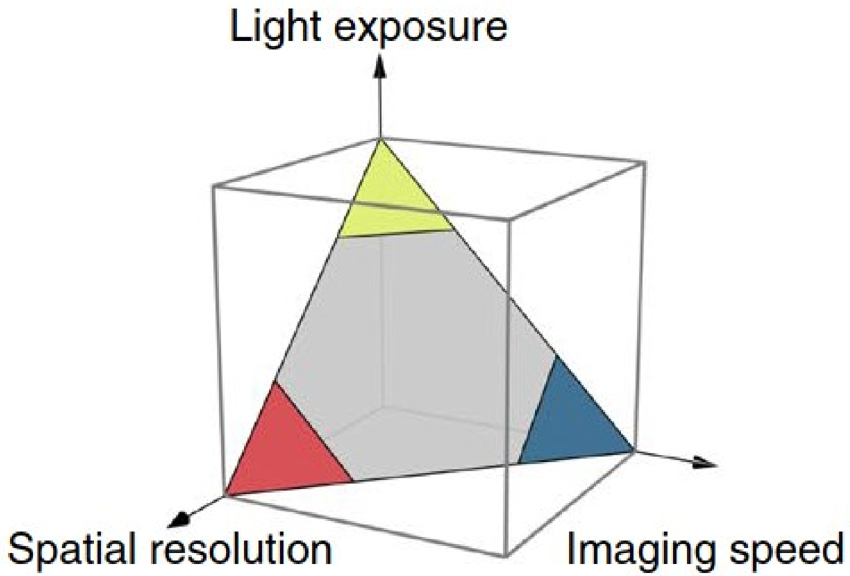
- Sufficient Time Resolution
- High Spatial Resolution
- 3D Reconstruction

Cons:

- Achievable fluorophore density
- Bleaching
- Photo-toxicity

Fluorescence Microscopy

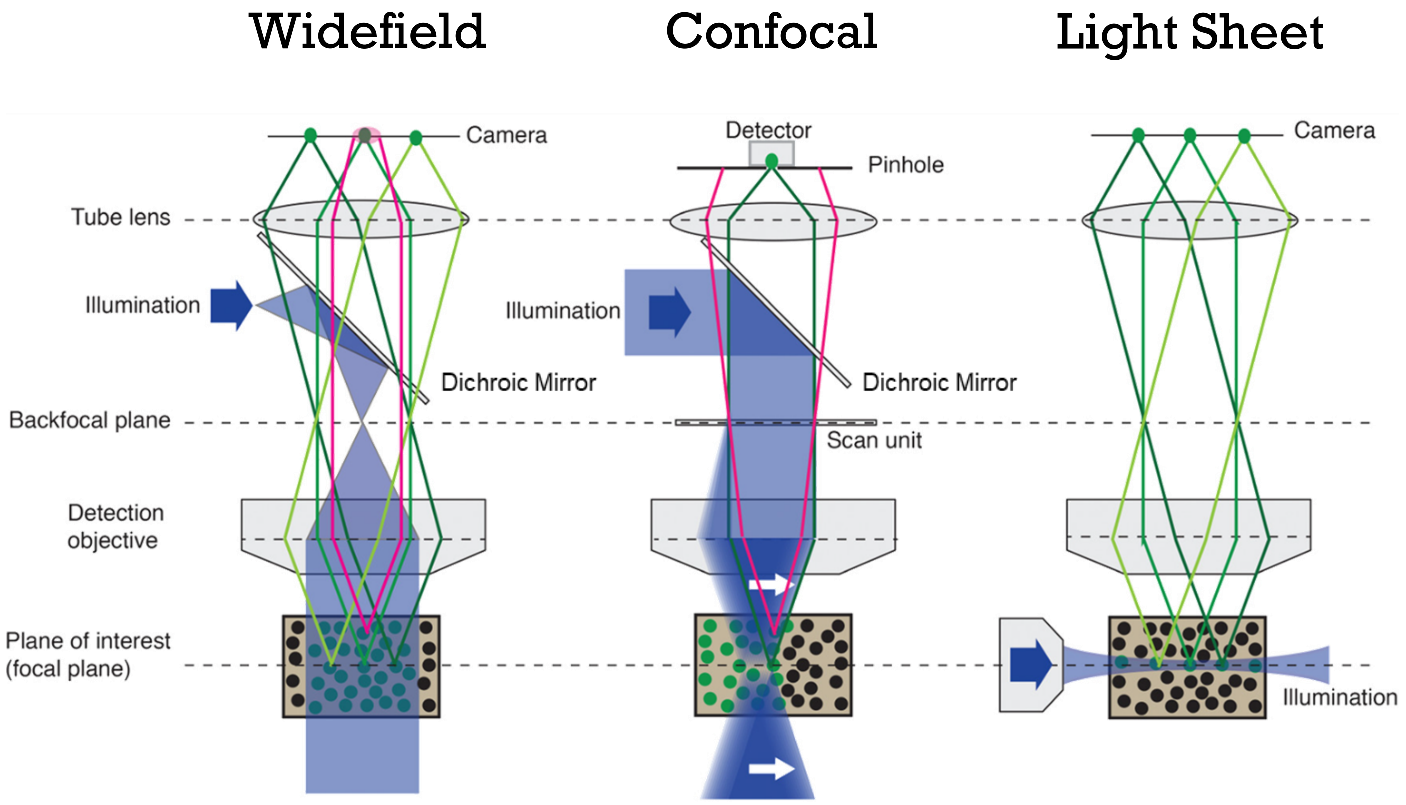
Dependance on:
- Photon Budget
- Sample Health



⇨ Final SNR of the Image

High SNR
= Good Quality
= Easier Processing
= Happy Scientist

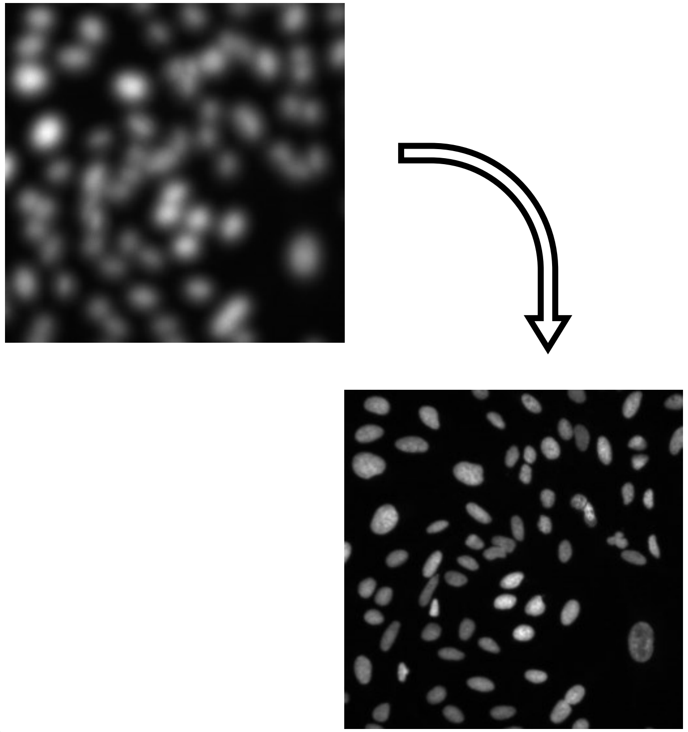
Microscopy Setup



Deep Learning in Microscopy

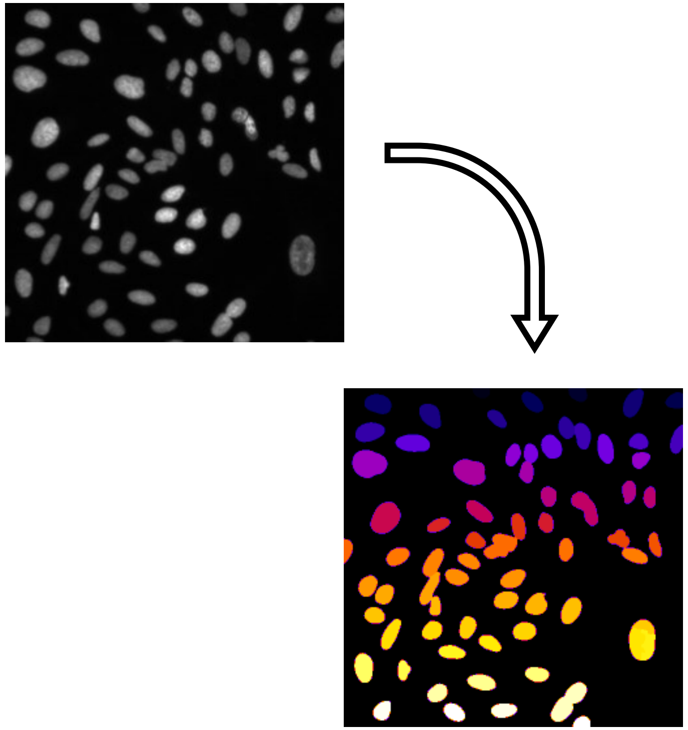
Restoration

image ⟶ image



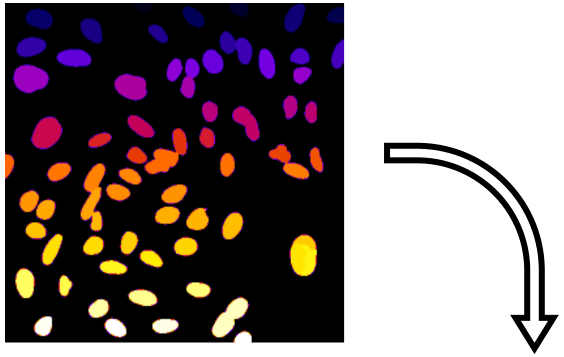
Segmentation

image ⟶ objects



Quantification

objects ⟶ features



ID	Area	Type	...
1	56.3	A	...
2	26.7	B	...
3	44.1	B	...
...	...	...	...

Ljosa, V., Sokolnicki, K. & Carpenter, A. Annotated high-throughput microscopy image sets for validation. *Nat Methods* **9**, 637 (2012). https://doi.org/10.1038/nmeth.2083

Image Restoration – Old Style

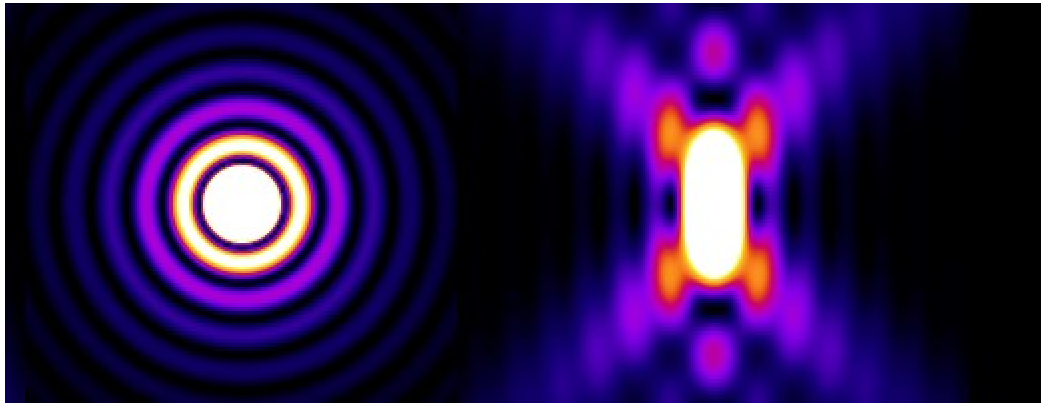
Theoretical Model of Image Formation = Deconvolution

- Computationally expensive
- Assumptions required for regularization
- Hard to adapt to custom setups
- Hard to control experimental conditions
- Noise is hard to model!

Image = Object * PSF + Noise

$$g = f * h = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} f(\vec{x}')h(\vec{x}-\vec{x}')\, d^3\vec{x}'$$

PSF = describes the optical response of the microscope during image formation

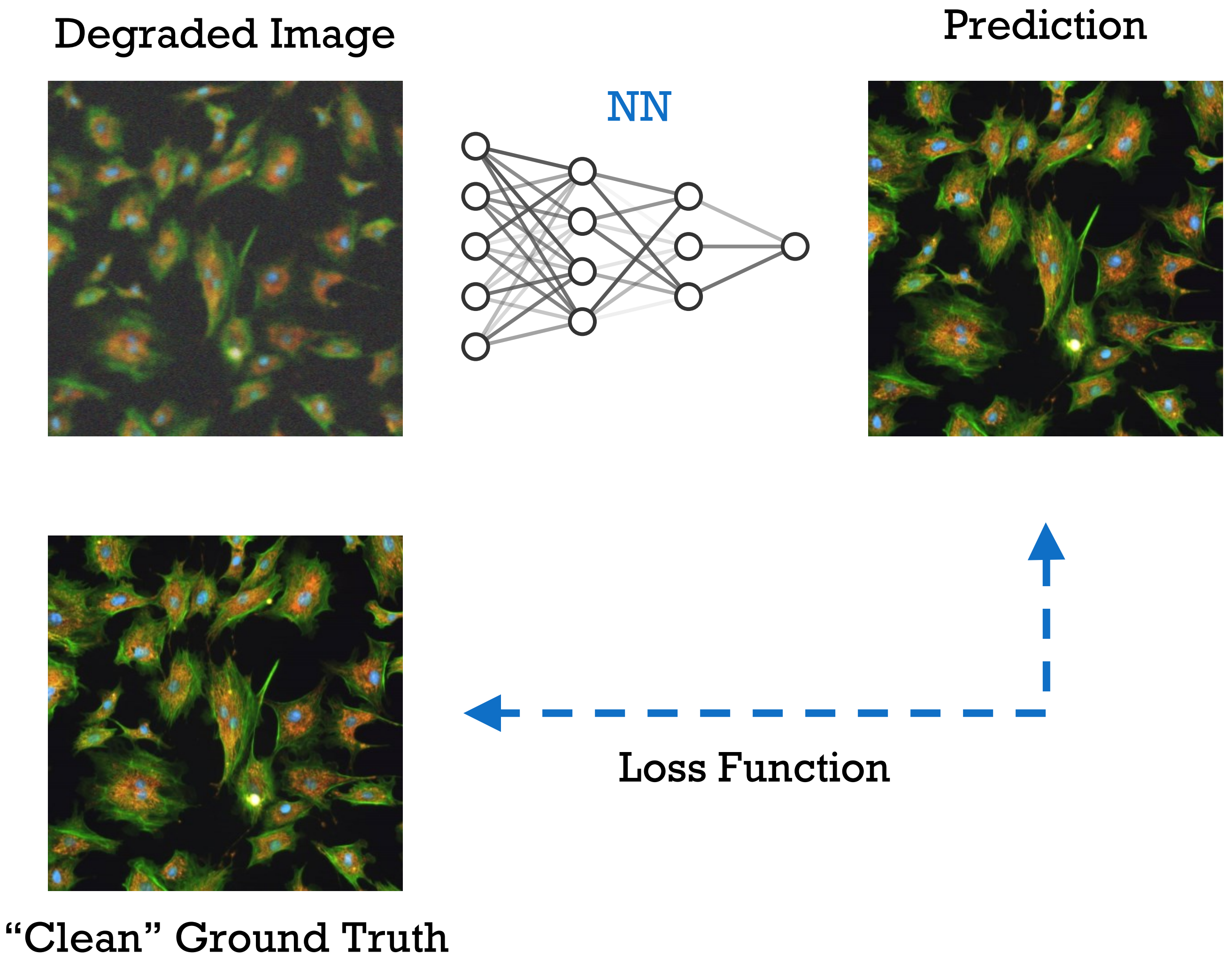


Examples of Inverse Filters	Examples of Iterative Algorithms
Wiener Deconvolution	Richardson-Lucy
Tikhonov Filtering	Jansson-Van Cittert
Linear Least Squares	Landweber
Naive Inverse Filtering	Nonlinear Tikhonov-Miller

Image Restoration – DL

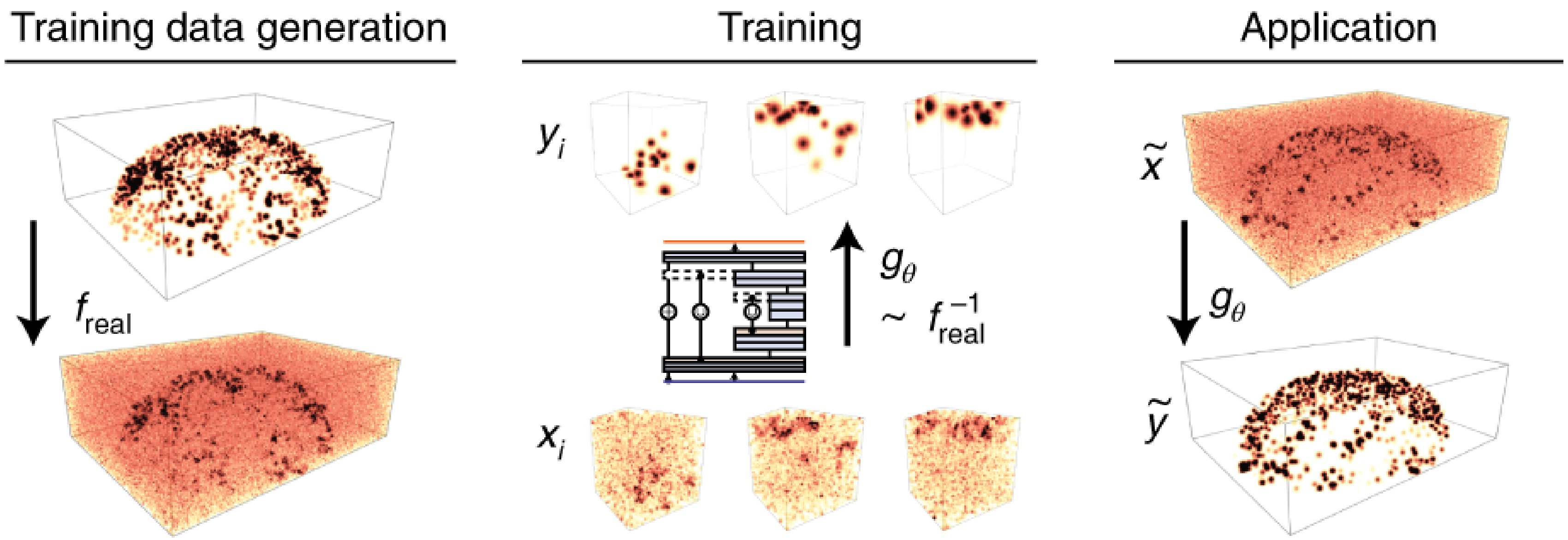
Data-Driven Approach:

- Computationally Expensive
- Adapts well to custom setups
- Adapts to different noise sources
- Involves Neural Networks



CARE: content-aware image restoration

Supervised Approach: Requires Matching Image Pairs



Weigert, M., Schmidt, U., Boothe, T. *et al.* Content-aware image restoration: pushing the limits of fluorescence microscopy. *Nat Methods* 15, 1090–1097 (2018).

CARE: content-aware image restoration

Dataset Generation:

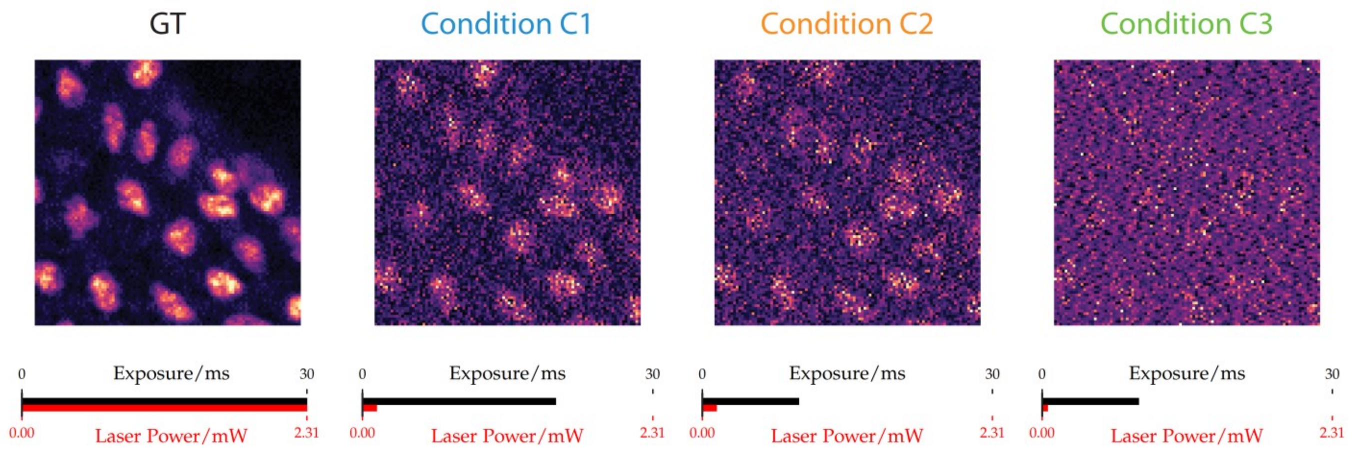
- Supervised Approach
- Multiple Microscope Acquisitions
- Registration

High SNR:
- Longer Exposure Time
- Higher Light Intensity
- Slower Acquisition

Low SNR:
- Faster Exposure Time
- Less Light Intensity
- Faster Acquisition

SNR

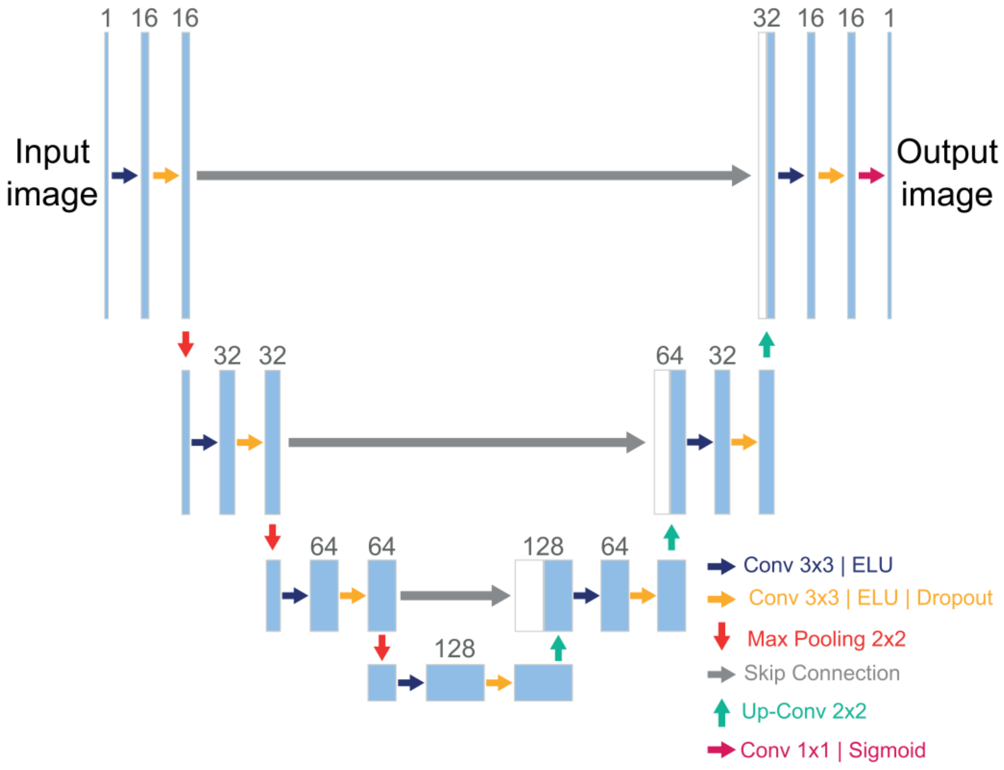
High SNR | Medium SNR | Low SNR | Very Low SNR



Weigert, M., Schmidt, U., Boothe, T. *et al.* Content-aware image restoration: pushing the limits of fluorescence microscopy. *Nat Methods* 15, 1090–1097 (2018).

CARE: content-aware image restoration

U-Net Architecture



Training:

- U-Net Architecture ~ 10^6 parameters
- 100 3D-stacks size $1024 \times 1024 \times 400$ ~ 80 GB
- 17000 patches $64 \times 64 \times 16$
- NVIDIA GeForce GTX 1080
- MSE Loss + Adam Optimizer
- 4-5 hours of training

$$NRMSE = \frac{\sum(s_i - o_i)^2}{\sum o_i^2}$$

$$\mathrm{SSIM}(\mathbf{x},\mathbf{y}) = [l(\mathbf{x},\mathbf{y})]^{\alpha} \cdot [c(\mathbf{x},\mathbf{y})]^{\beta} \cdot [s(\mathbf{x},\mathbf{y})]^{\gamma}$$

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Some CARE Examples

Disadvantages of CARE

- Ground Truth (GT) MUST be obtainable!
  - Very Fast Cellular Processing…
  - Photo-stability of Sample…
  - Cryo-EM…

- GT must be SUFFICIENTLY good quality!
- Training data must sample ALL visual features of interest!

So… what do you do if you just have noisy images?

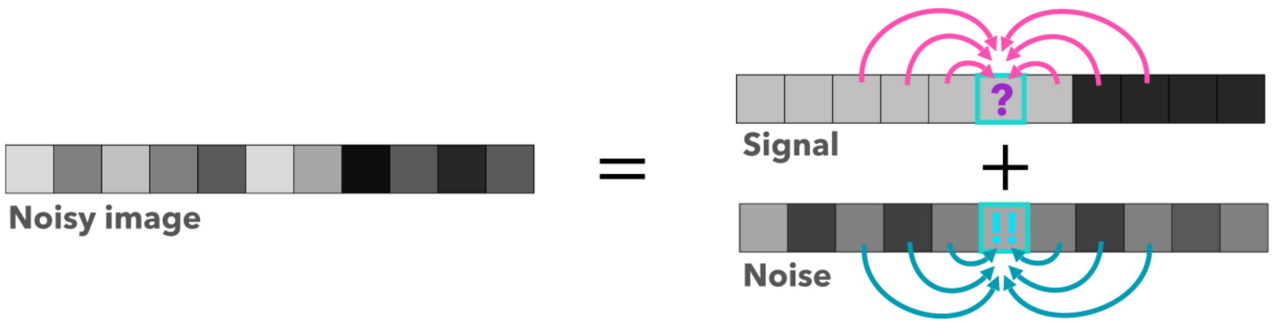
Noise2Void (N2V)

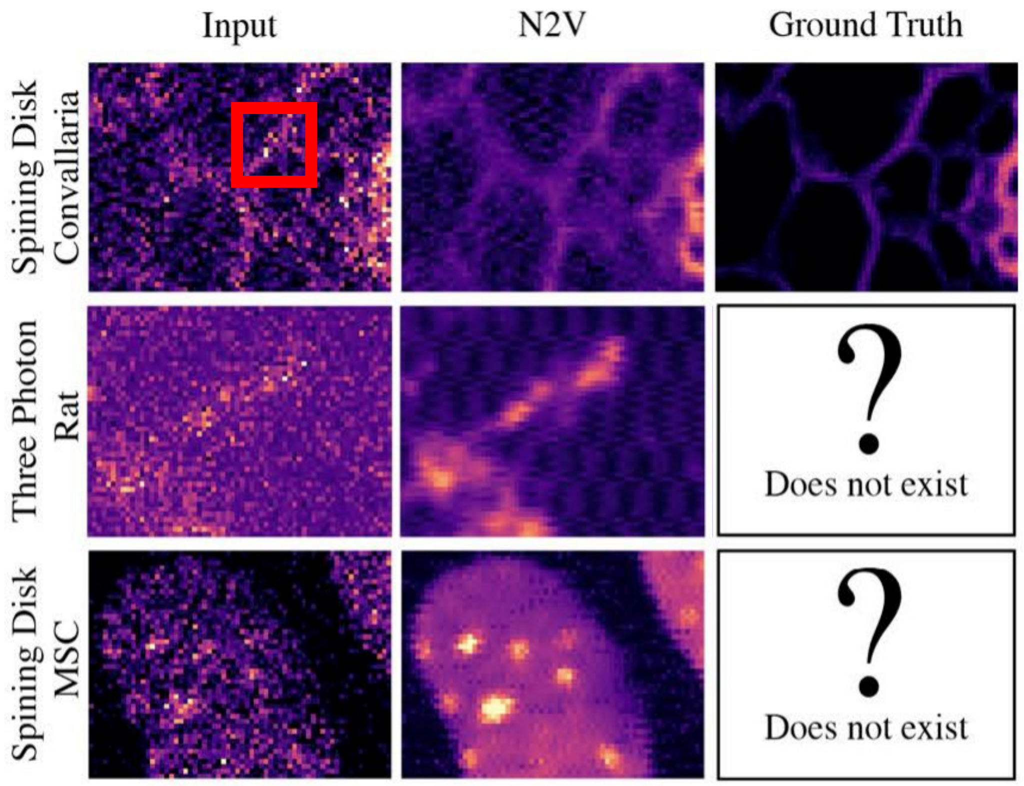
Self-Supervised Denoising through a blind-spot Convolutional Neural Network

=

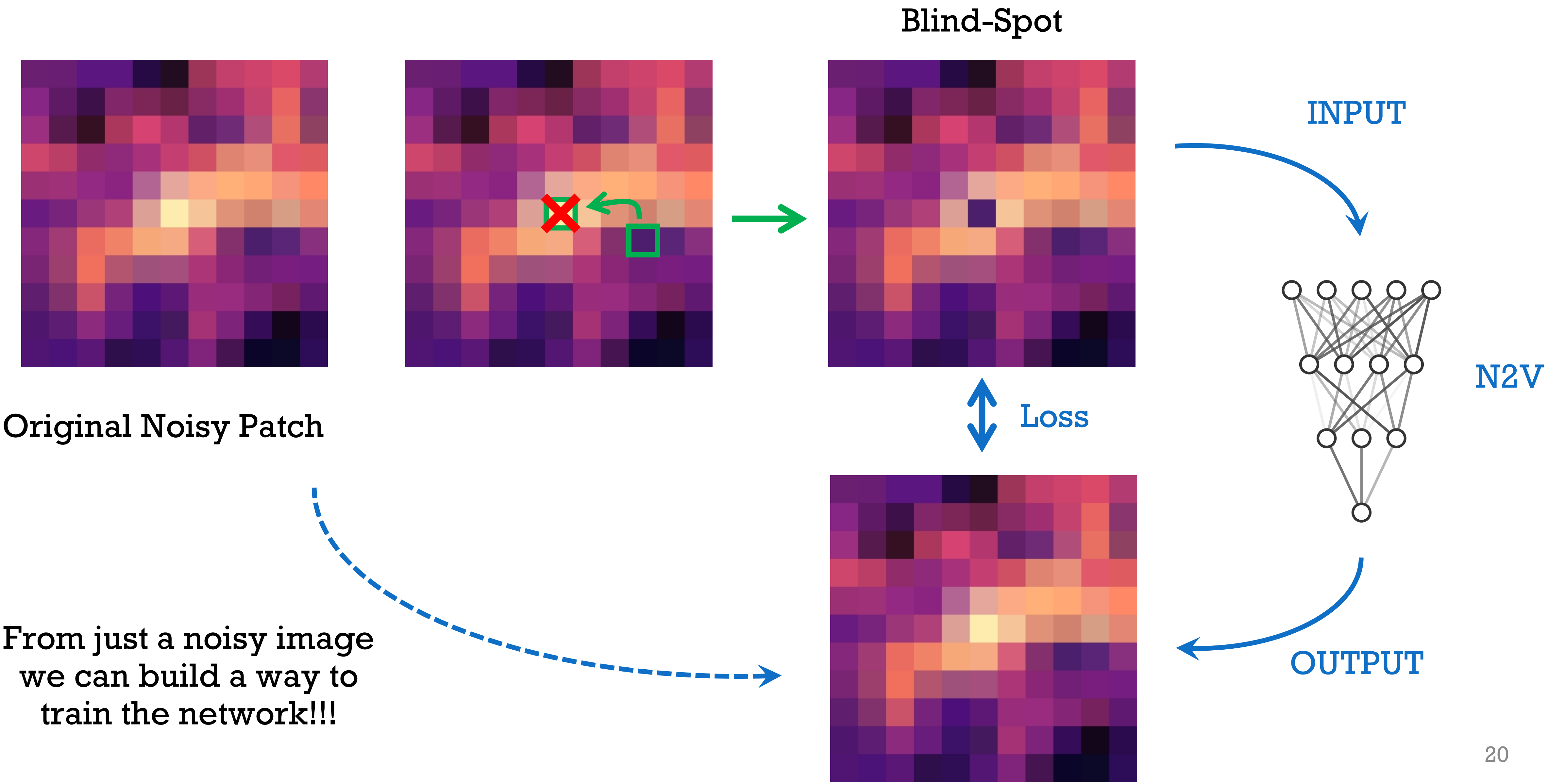
We can obtain denoised results without the need for paired images or ground truth!!!

Image = Signal + Noise



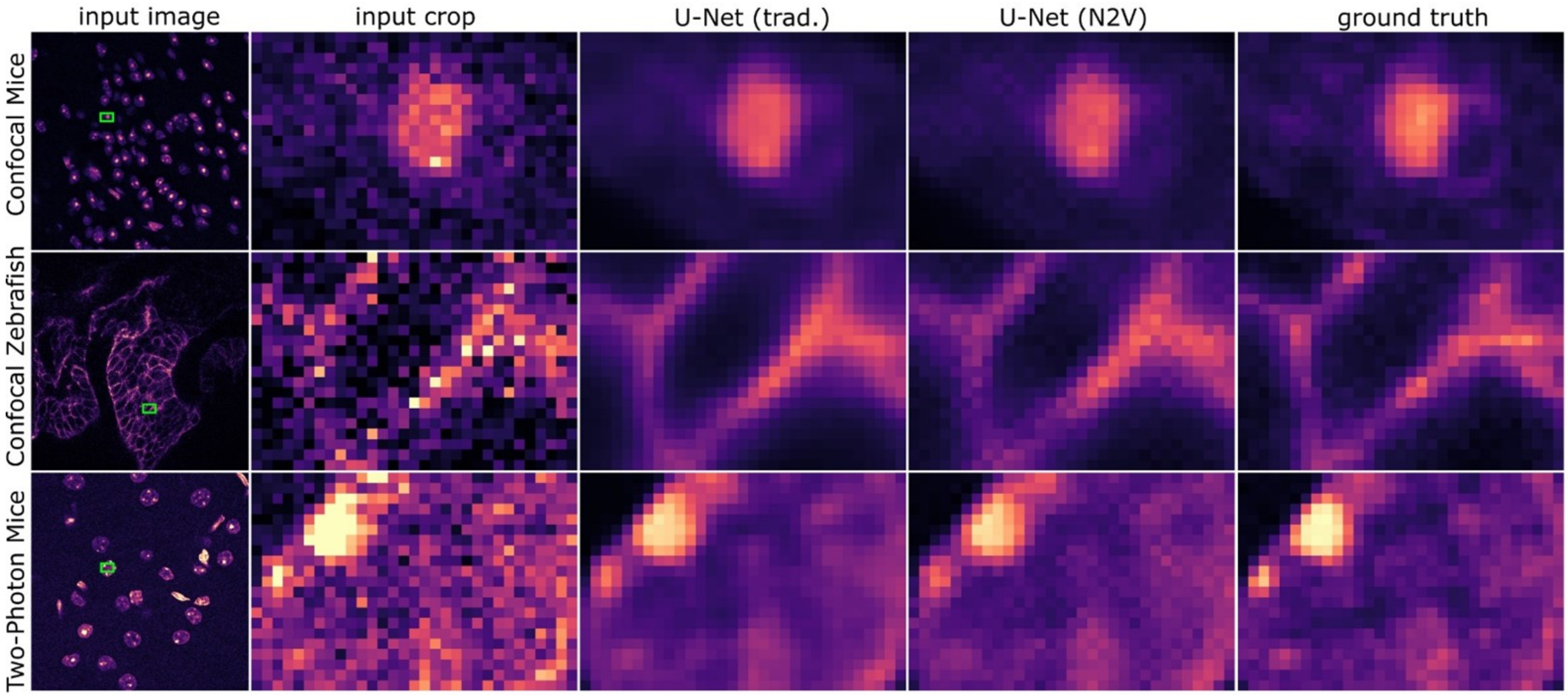


How does the blind-spot work?



From just a noisy image
we can build a way to
train the network!!!

Noise2Void (N2V)



Noise2Void

Learning Denoising from Single Noisy Images

Alexander Krull, Tim-Oliver Buchholz, and Florian Jug

Limitations of N2V

Useful for Per-Pixel-Noise:

- Low light images
- Shot noise
- Readout noise

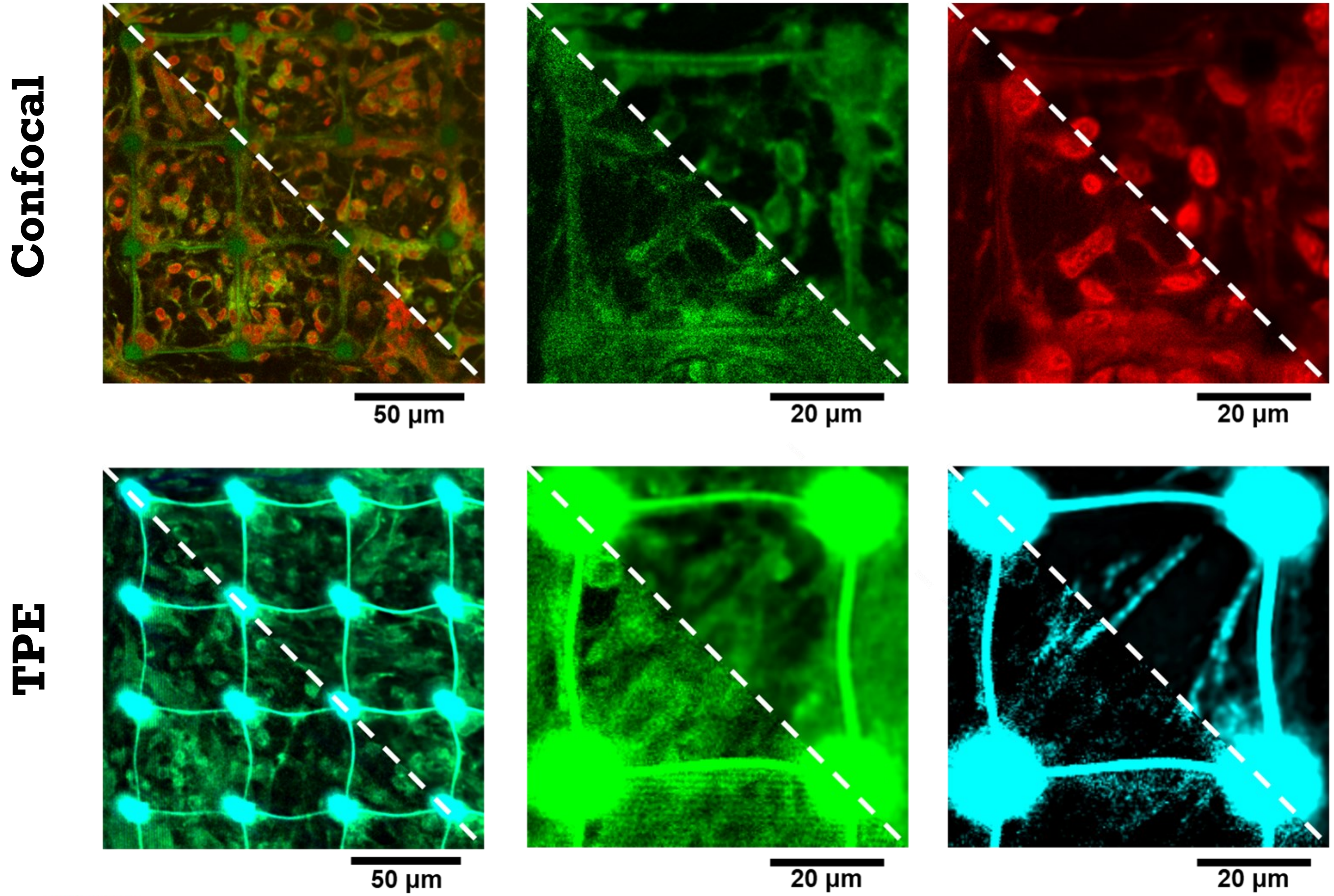
NOT Suitable for:

- Deconvolution
- Compression Artifacts
- Structured Noise

Noisy Image with Pattern | Original Image | N2V Output

N2V tends to highlight the Checker-box pattern!!

N2V on MY images



3D-N2V model

- NVIDIA GeForce RTX 3090
- Multi-channel processing
- Patch-size = 64x64x8 pixels
- 200 epochs ~ 1-2 hours

Confocal:

- Green Auto-Fluorescence
  (Exc: 488 nm, Em: 500-550 nn)
- Red Draq-5
  (Exc: 633 nm; Em: 645-720 nm)

TPE:

- Green Auto-Fluorescence
  (Exc: 800 nm, Em: 535/50 nm)
- SHG + autofluorescence
  (Exc: 800 nm; Em: 400/40 nm)

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