

Content-aware image restoration: pushing the limits of fluorescence microscopy

Author: Weigert, M., Schmidt, U., Boothe, T., Müller, A., Dibrov, A., Jain, A., Wilhelm, B., Schmidt, D., Broaddus, C., Culley, S., Rocha-Martins, M., Segovia-Miranda Fabian, Norden, C., Henriques R., Zerial, M., Solimena, M., Rink J., Tomancak, P., Royer, L., Jug, F. and Myers, E. W.

[Published 26 November 2018](#)

Slides compiled by Mengzhou Li

Deep Learning in fluorescent microscopy

➤ Main work

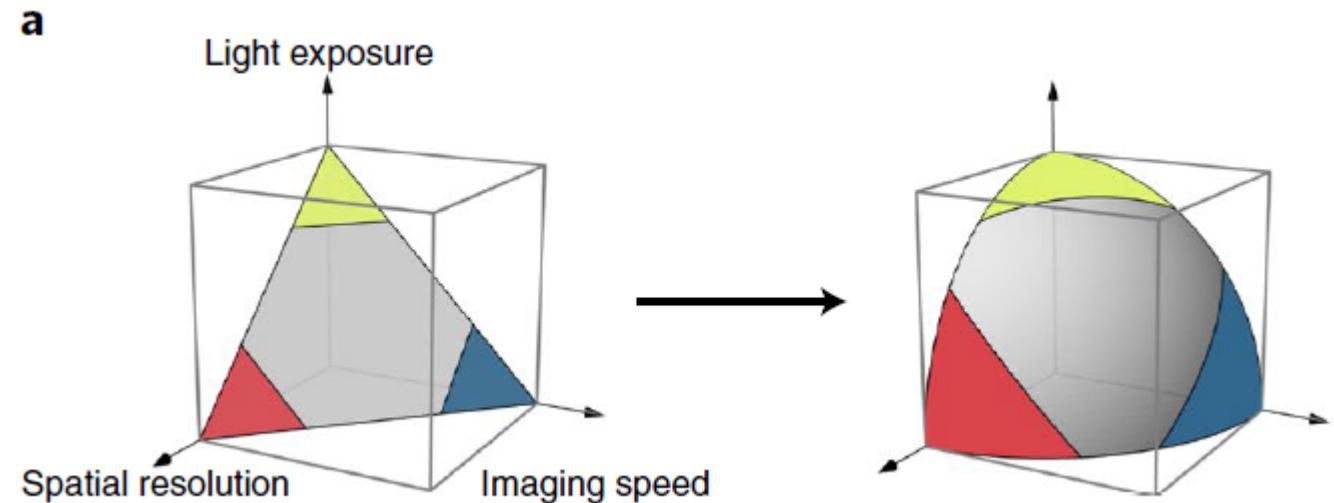
- Low SNR denoising (60-fold fewer photons)
- Isotropic resolution restoration (10-fold fewer axial slices)
- Deconvolution (20-times-higher frame rate)

➤ What we can learn

- Training data generation strategy
- Restoration reliability measures

Trade-offs in fluorescent microscopy

- **Spatial resolution**
- **Imaging speed**
- **Imaging depth**
- **Light exposure**



With the volume being limited by the maximal photon budget compatible with sample health.

Image restoration enlarges the design space.

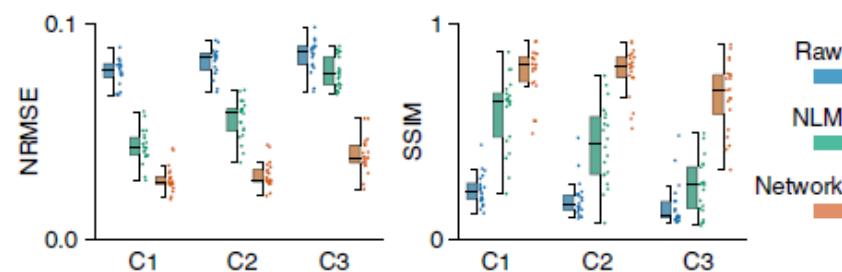
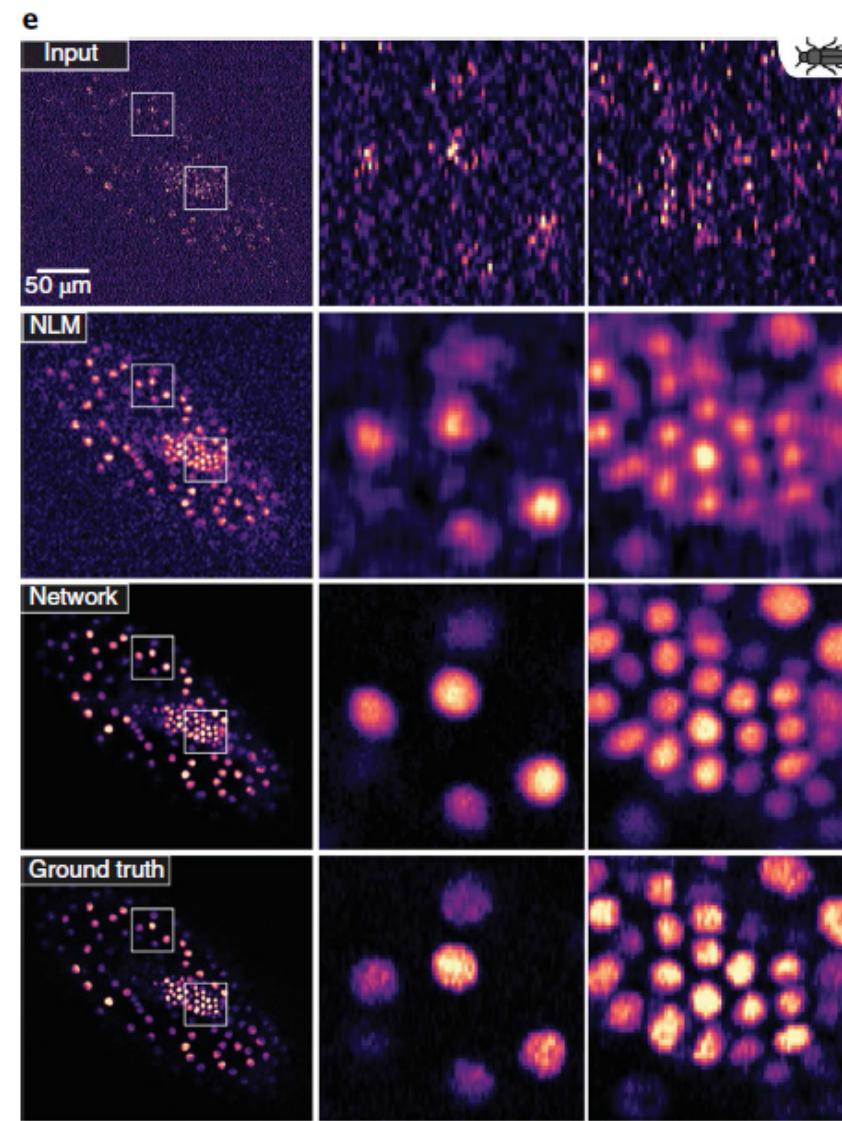
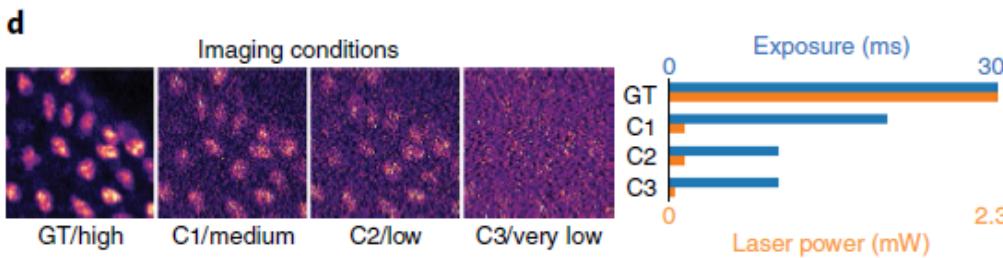
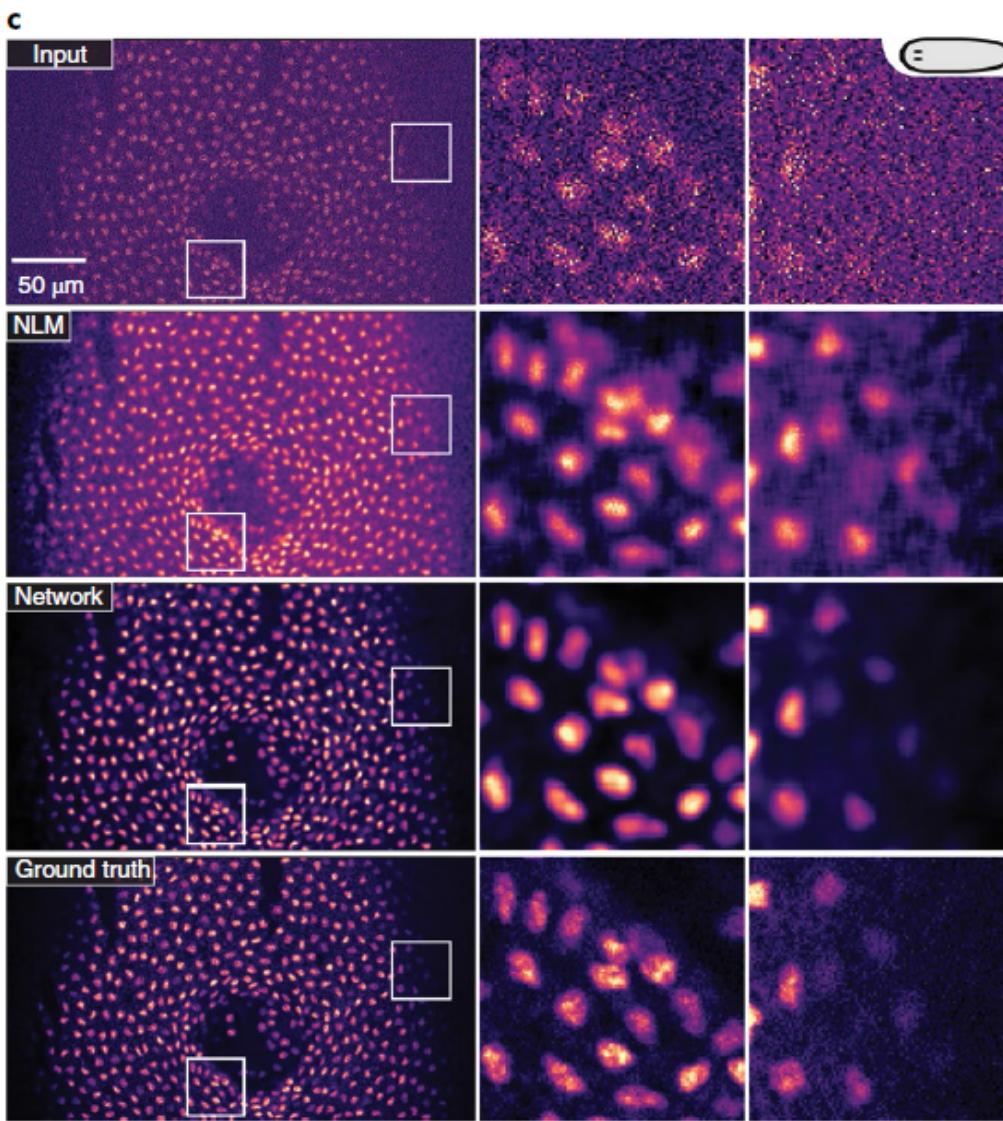
High Speed Low Light Case with Denoising

A typical example:

Flatworm *Schmidtea mediterranea* (a model organism for studying tissue regeneration)

Exceptionally sensitive to even moderate amounts of laser light.

[video](#)



Physically acquired training data:
Obtain **training data from fixed worm**; and apply the trained network on live worm.

Very similar to CT imaging case to try our best to reduce the dose.

Axially Under-sampling Case with Isotropic Resolution Restoration

Anisotropic resolution

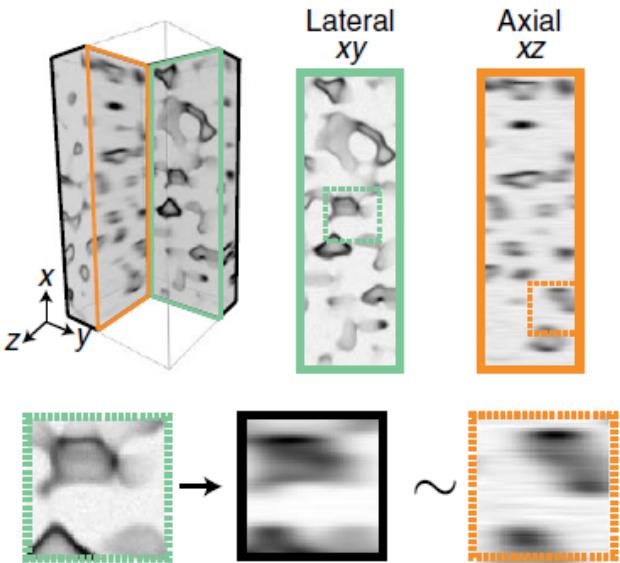
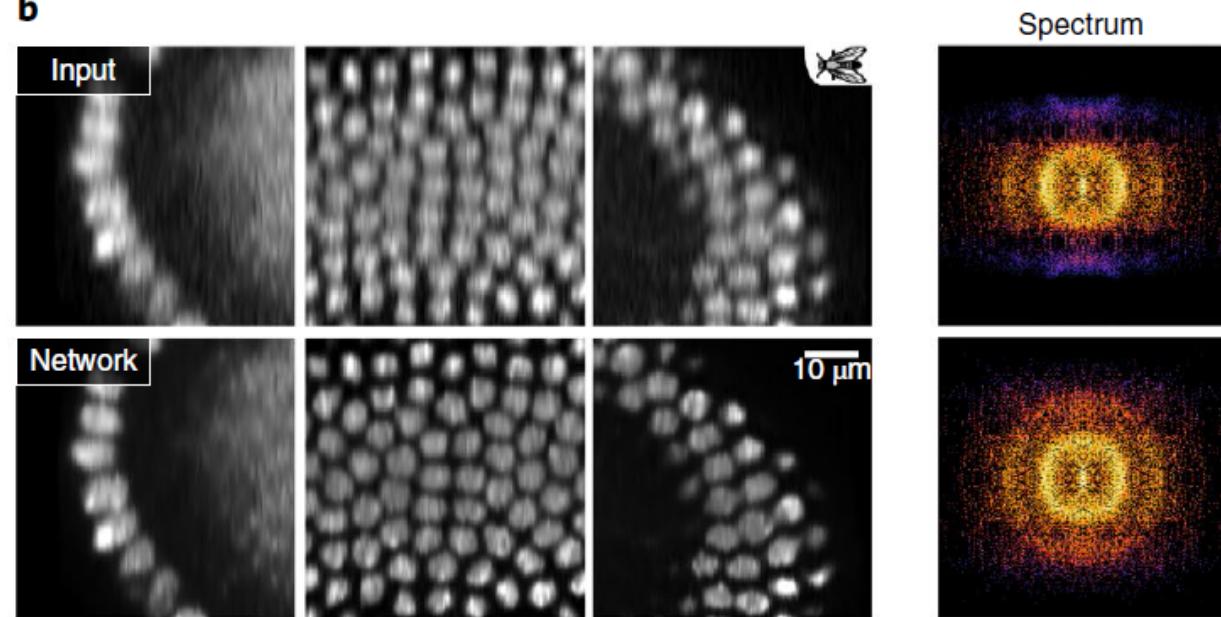
- Inherent axial elongation of the optical PSF
- Low axial sampling rate of volumetric acquisitions (fast imaging)

Semi-synthetic training data generation

- Treat high resolution lateral slice as a xz image (GT)
- Convolute GT with xz PSF to achieve axial blurring
- Down-sample the result to obtain the synthetic image (Input)
- Pair the GT (real data) and Input (synthetic) as the training data

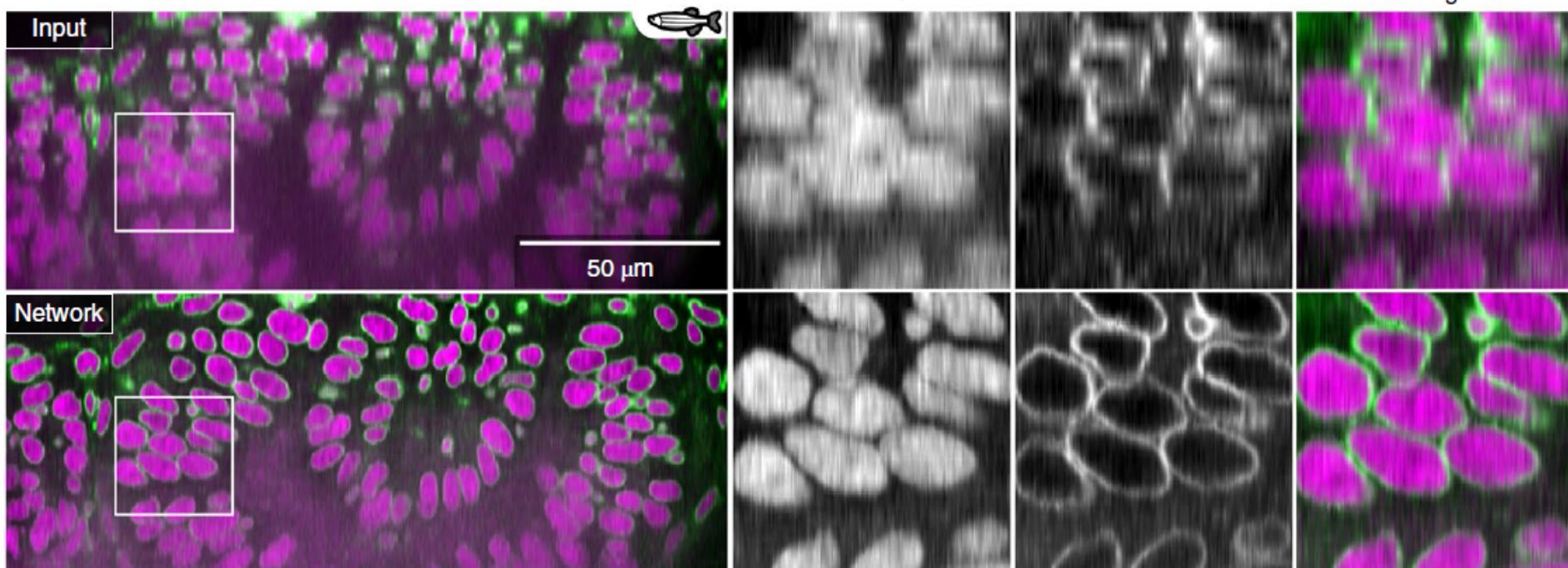
a

Training data generation

**b**

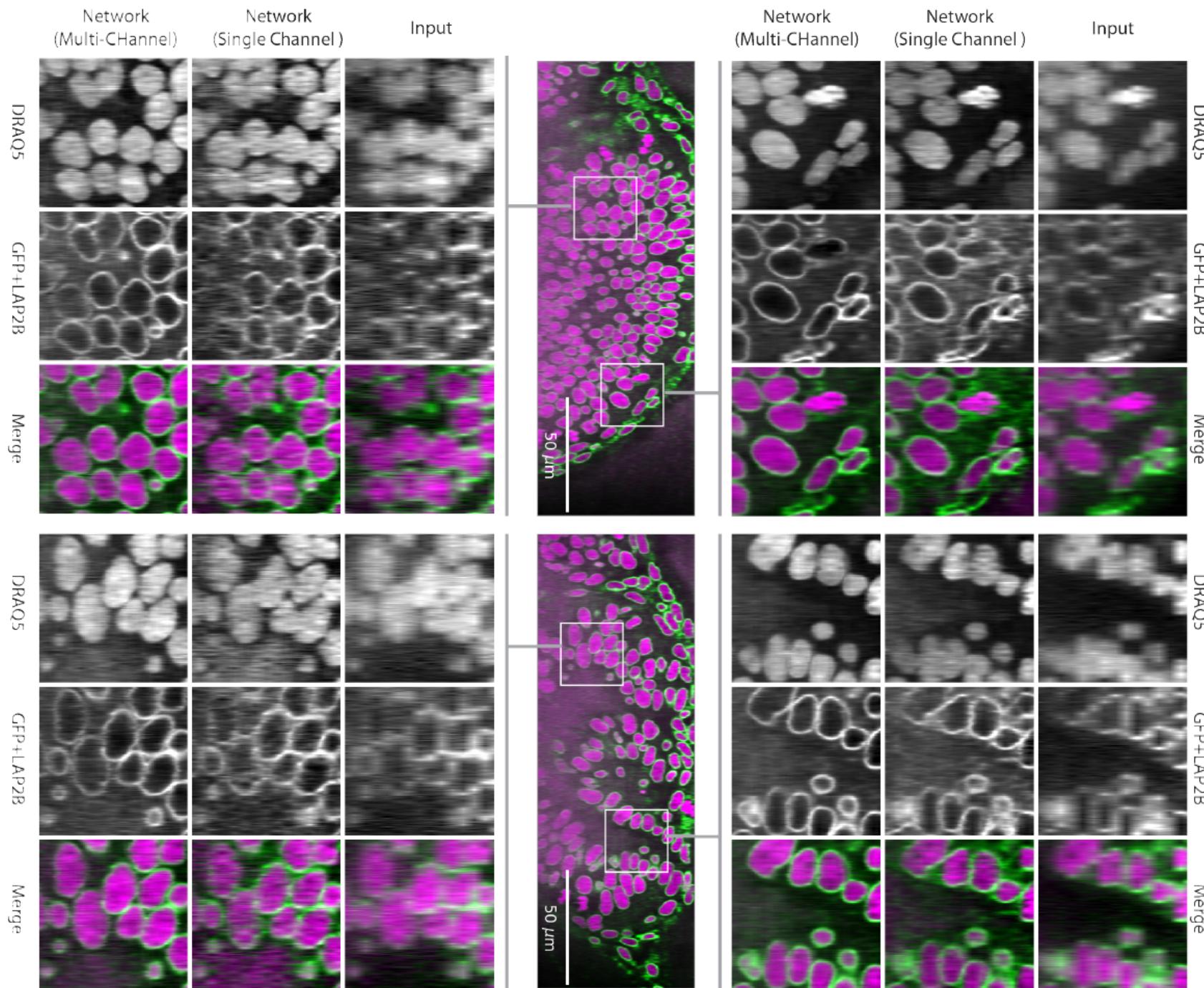
- Quasi-real ground truth
- Simulated degraded images
- Real testing data

It is almost impossible to get the real ground truth.

c

Very similar to MRI imaging case, subsampled along z direction to achieve fast imaging.

Still noisy at deep depth, due to the ineffectiveness of the model (PSF changes).



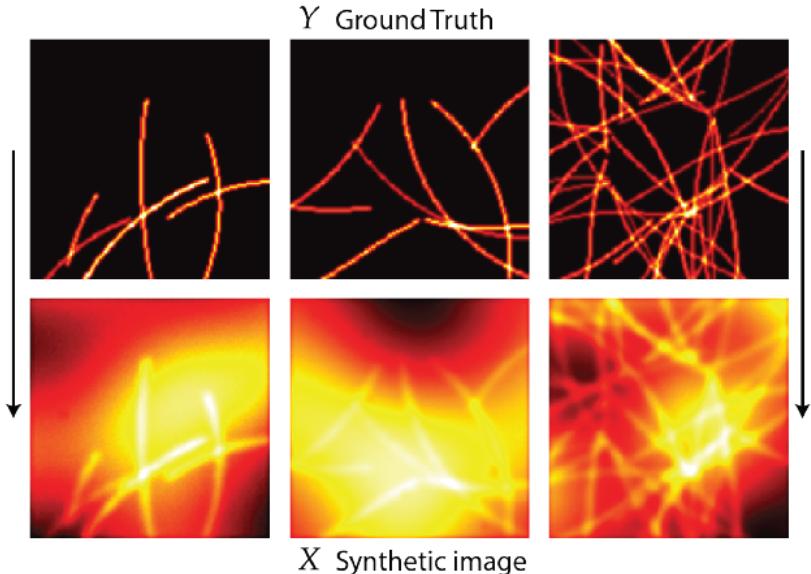
Interesting thing is that, networks learned to exploit correlations between channels, leading to a better overall restoration quality compared to results based on individual channels.

Deconvolution with Synthetic training data

Synthetic training data generation

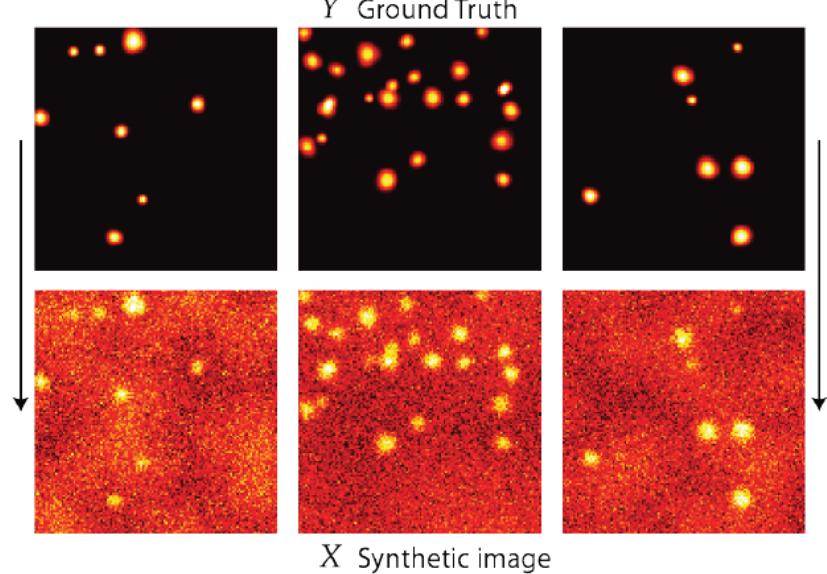
- Synthetic generative models of tubular and point-like structures
- Generated structures as ground truth (GT)
- Computationally modified them to resemble actual microscopy data
- Pair the GT and microscopy data as the training data

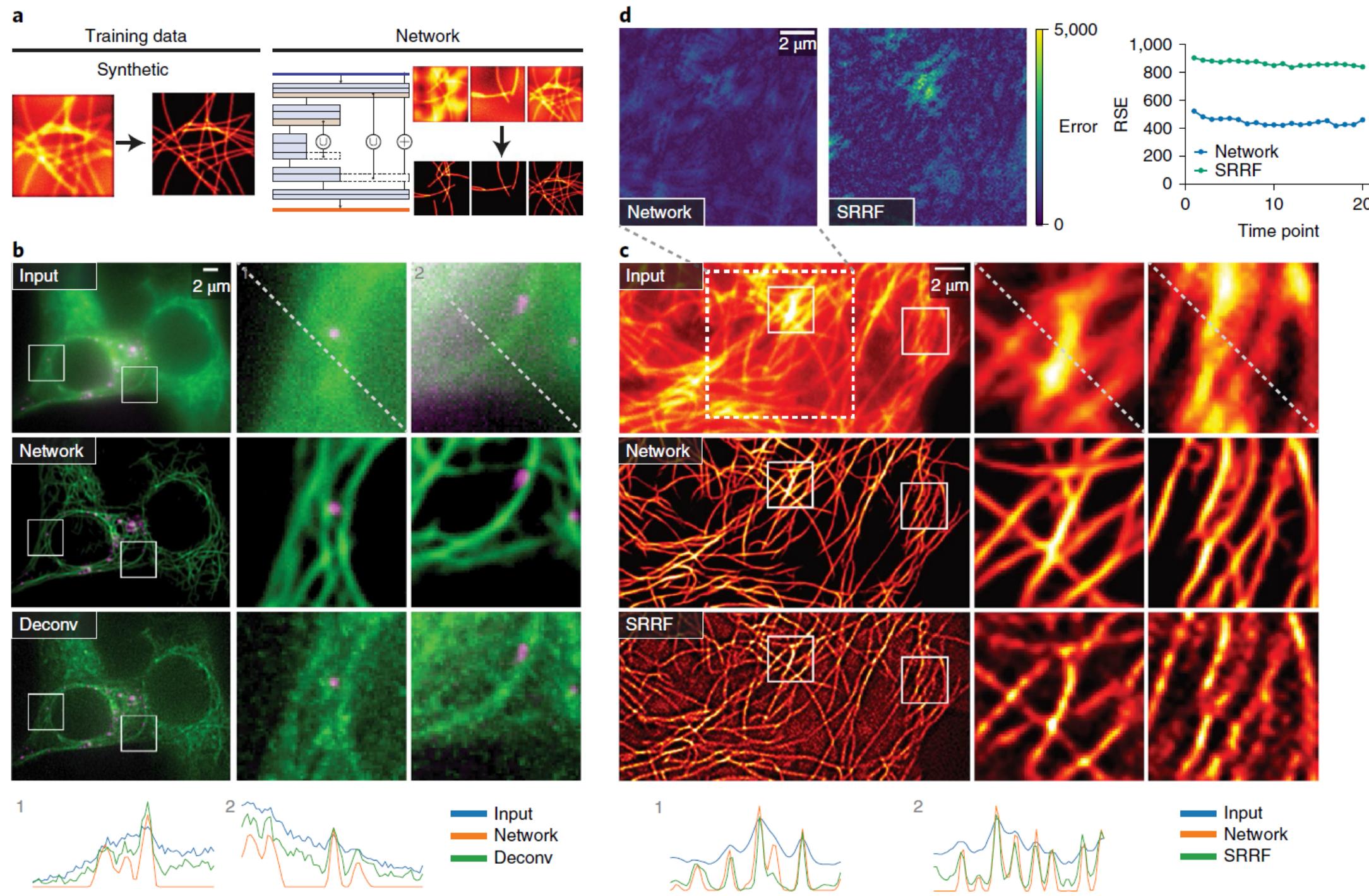
microtubules



GT
Low frequency
Perlin-noise
Microscope PSF
Poisson and Gaussian noise
Microscope image

Granules





Real testing data

Networks and Restoration Reliability

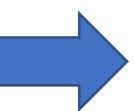
3D U-Net for 3D denoising for low-SNR stacks

2D U-Net for isotropic reconstruction and deconvolution

➤ A small adaption on **loss function**

- Output is a Laplace distribution (mean value and scale value)
- Scales indicate the per-pixel confidence intervals.

$$L_{\text{mse}}(\theta) = \frac{1}{T} \frac{1}{N} \sum_{t=1}^T \sum_{i=1}^N (y_i^t - g_\theta(x^t)_i)^2,$$



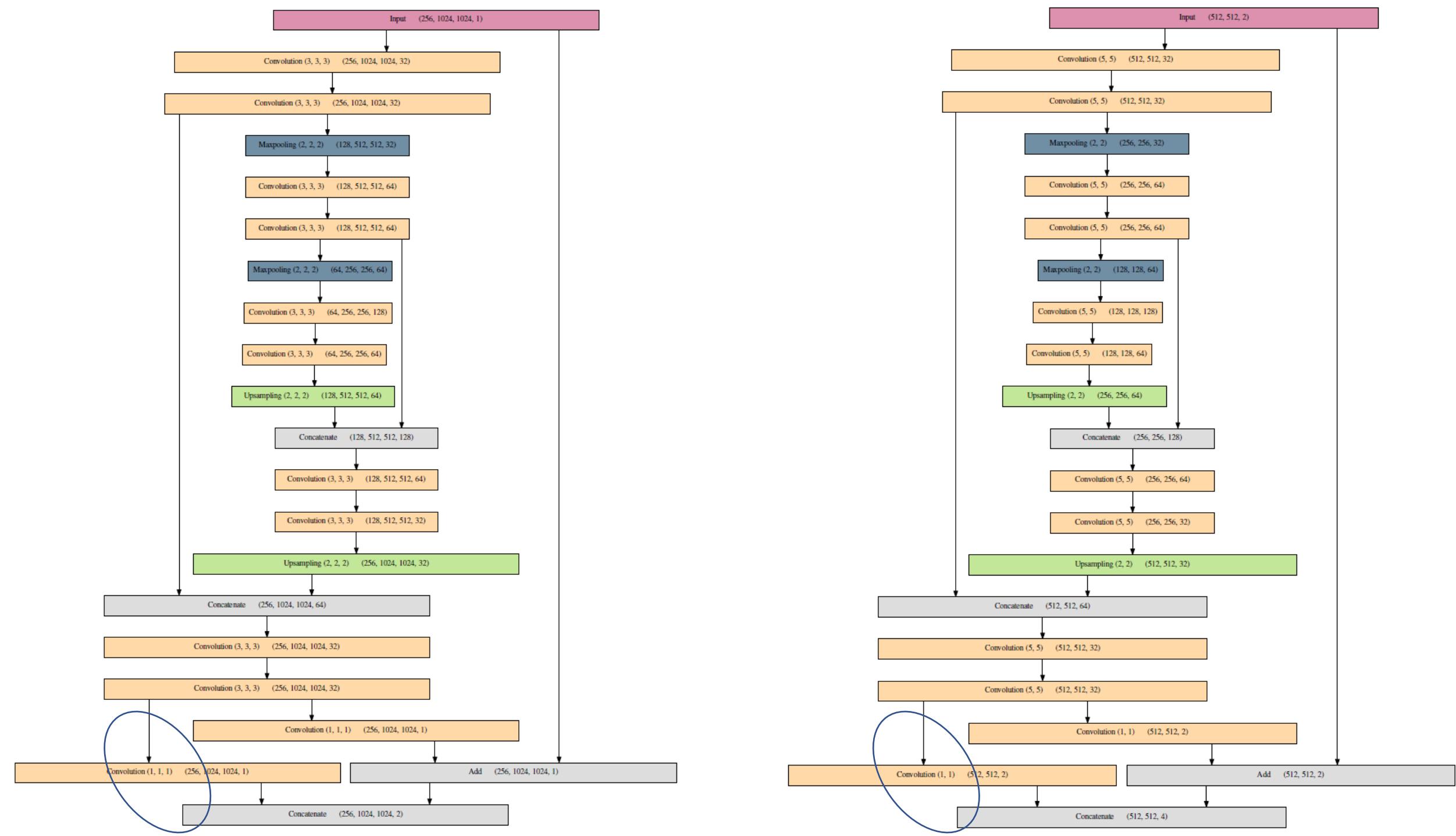
$$L_{\text{laplace}}(\theta) = \frac{1}{T} \frac{1}{N} \sum_{t=1}^T \sum_{i=1}^N \frac{|y_i^t - \mu_\theta(x^t)_i|}{\sigma_\theta(x^t)_i} + \log \sigma_\theta(x^t)_i,$$

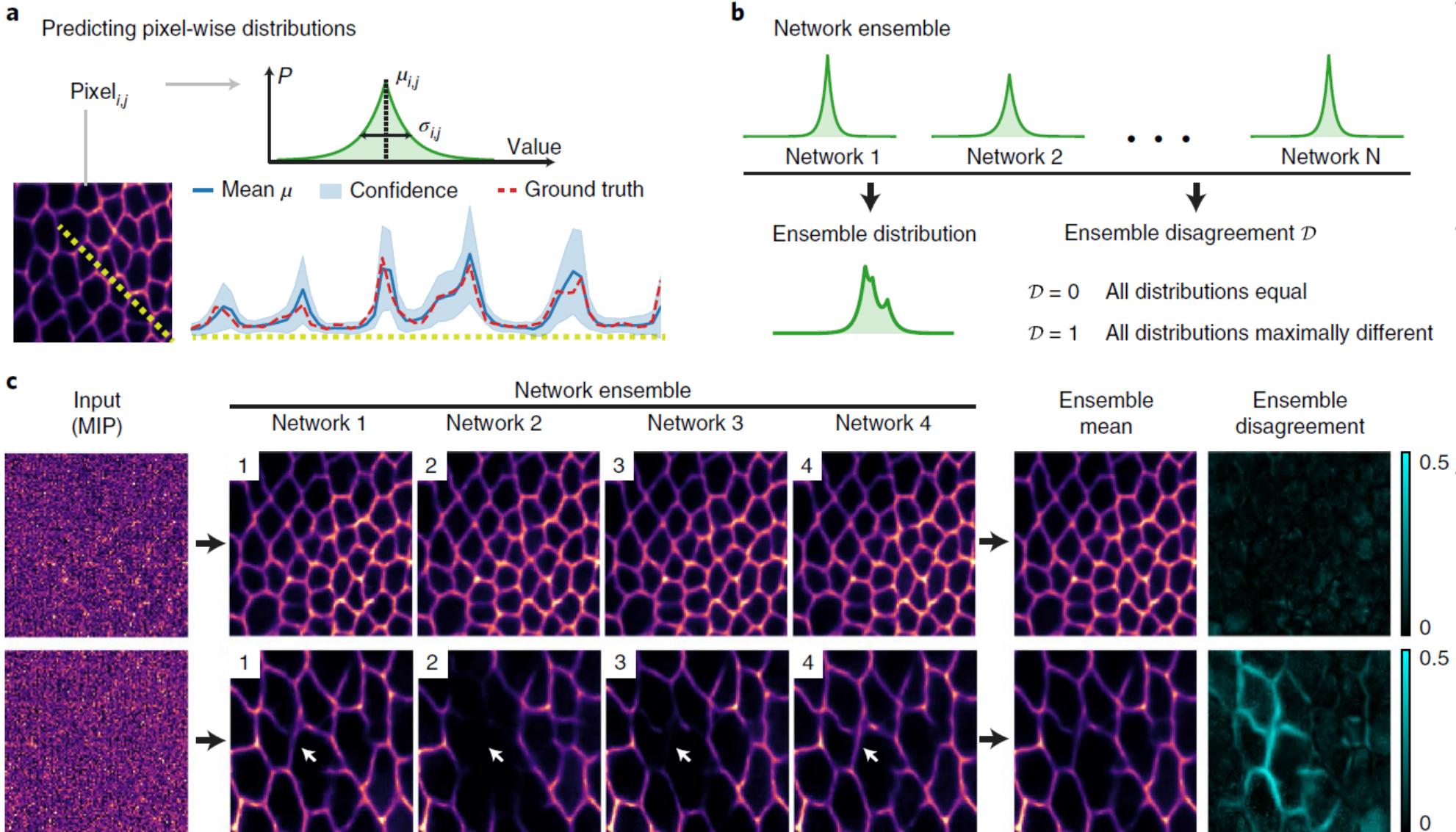
$$\mathcal{L}_{\text{gauss}}^{\text{homoscedastic}}(\theta) = \prod_{t=1}^T \prod_{i=1}^N p_{\text{gauss}}(y_i^t; g_\theta(x^t)_i, \sigma) \quad \text{with}$$

$$p_{\text{gauss}}(z; \mu, \sigma) = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left(-\frac{(z-\mu)^2}{2\sigma^2}\right)$$

$$\mathcal{L}_{\text{laplace}}^{\text{heteroscedastic}}(\theta) = \prod_{t=1}^T \prod_{i=1}^N p_{\text{laplace}}(y_i^t; \mu_\theta(x^t)_i, \sigma_\theta(x^t)_i).$$

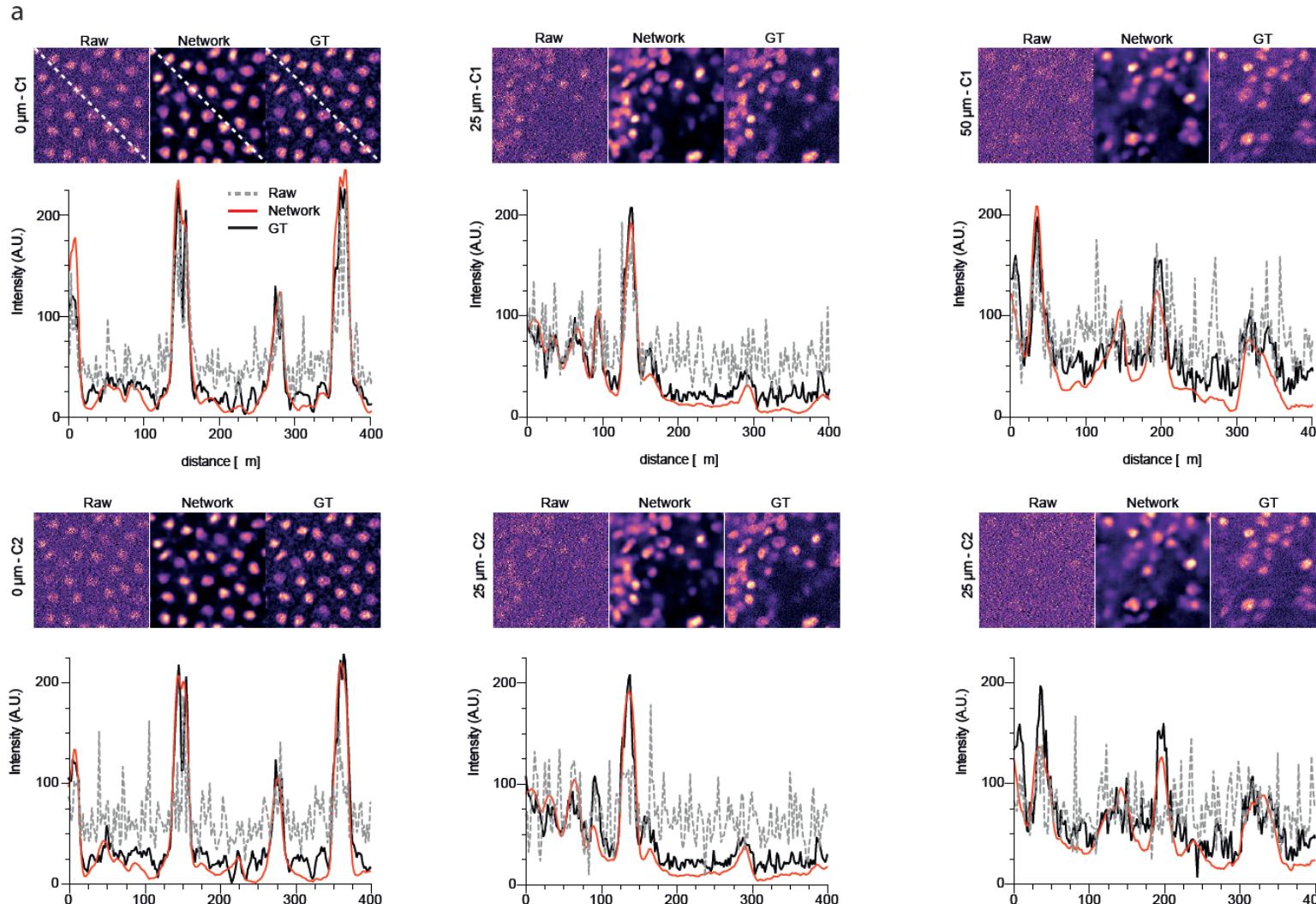
$$p_{\text{laplace}}(z; \mu, \sigma) = \frac{1}{2\sigma} \exp\left(-\frac{|z-\mu|}{\sigma}\right),$$



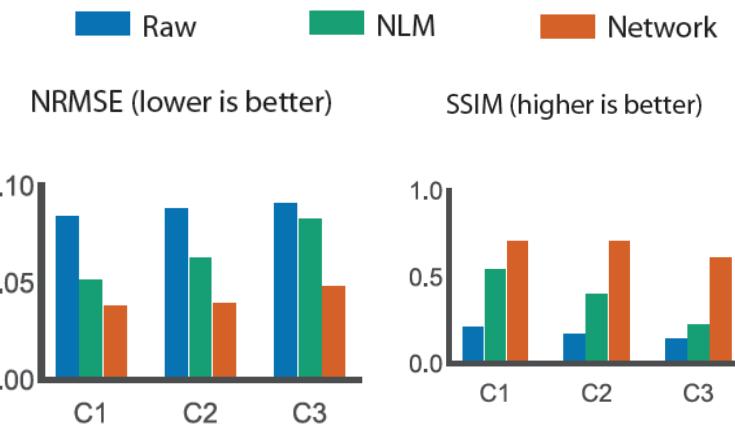


- Variances tend to increase with intensity
- Ensembles of about five CARE networks
- A measure D quantifies the probabilistic ensemble disagreement per pixel

Imperfectionness



- Must not be used for intensity-based quantifications such as, for example, fluorophore counting



Thanks for your attention!

Wish you all get papers published on Nature journals ☺