Machine Learning for Medical Imaging in Super-Resolution Microscopy:

Approaches to Cytological

Image Reconstruction and Enhancement

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Scientific Machine Learning
opencampus.sh
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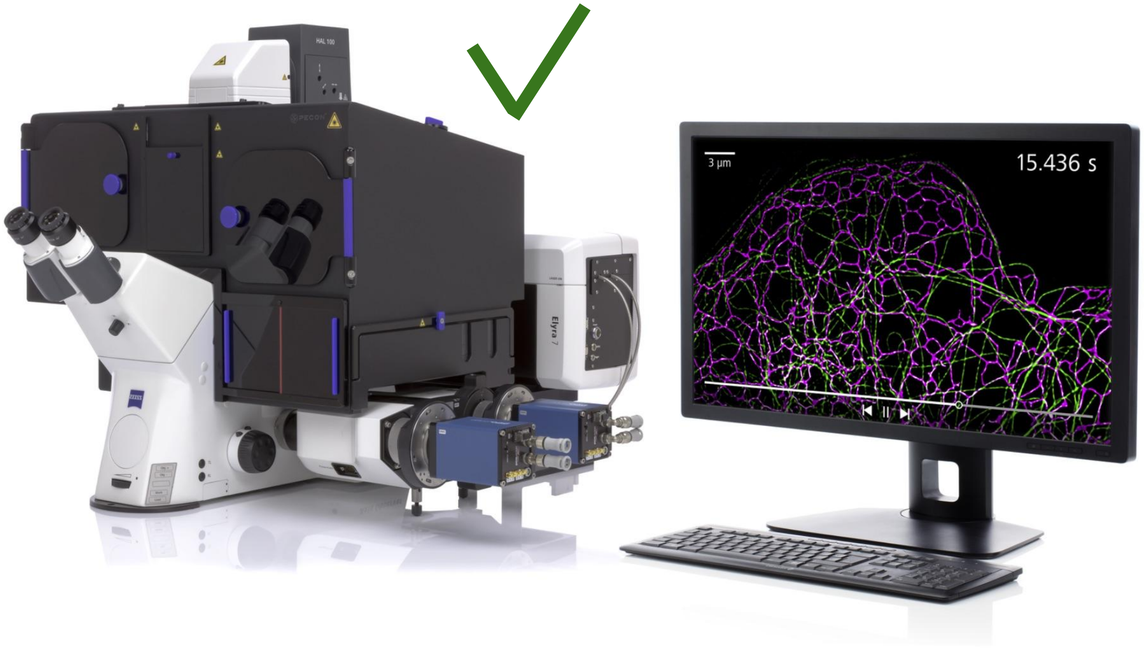
Content

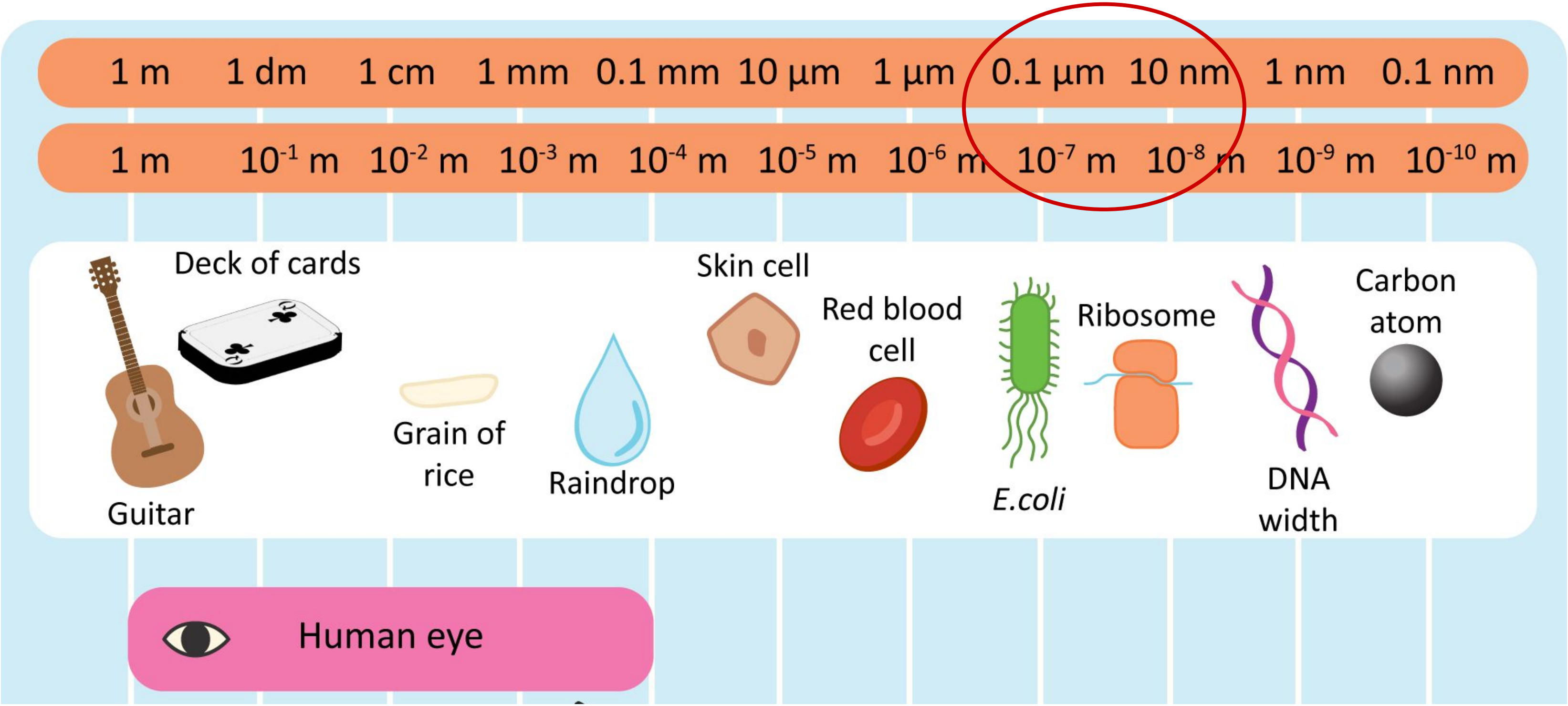
- Introduction to medical imaging
- Data exploration
- Approaches
- Results & Summary

Microscopy Imaging "problems"

- **Image reconstruction**
- **Noise**
- Blurry / low resolution images
- Low signal or high background
- Uneven illumination or artifacts

Super-resolution microscopy

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1 dm
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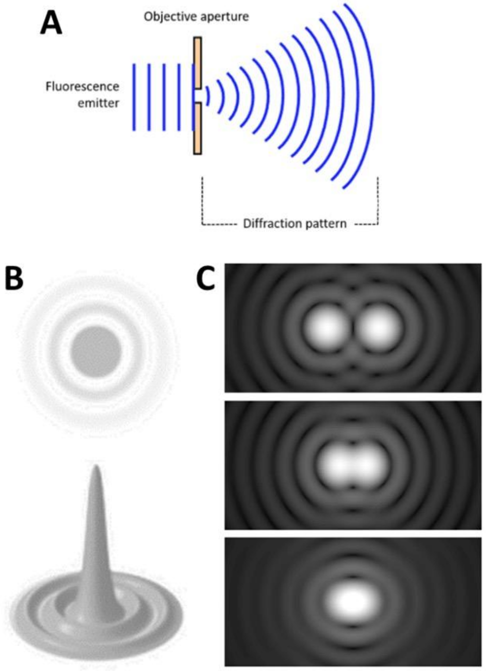
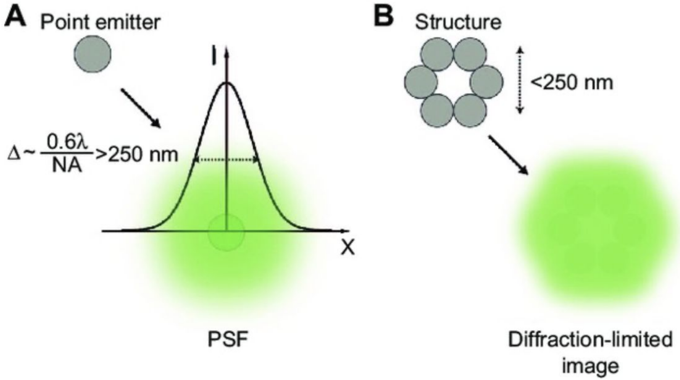
10^{-7} m
10^{-8} m
10^{-9} m
10^{-10} m
Guitar
Deck of cards
Grain of rice
Raindrop
Skin cell
Red blood cell
E.coli
Ribosome
DNA width
Carbon atom
Human eye

Super-resolution microscopy

Optics of microscopy

- PSF= Point spread Functions
- diffraction





https://www.teledynevisionsolutions.com/de-DE/learn/learning-center/scientific-imaging/what-is-super-resolution-microscopy/

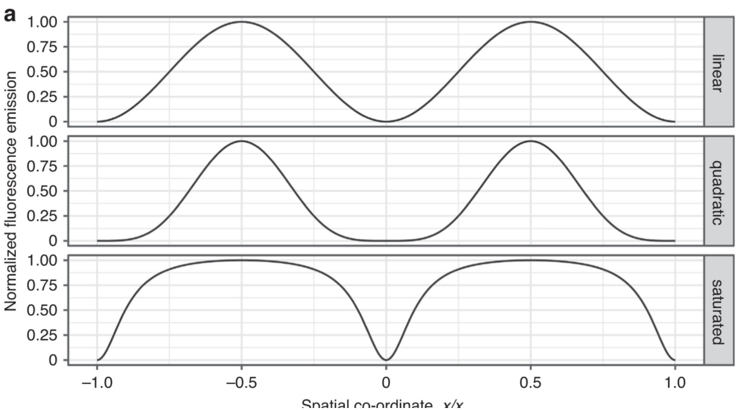
Super-resolution microscopy

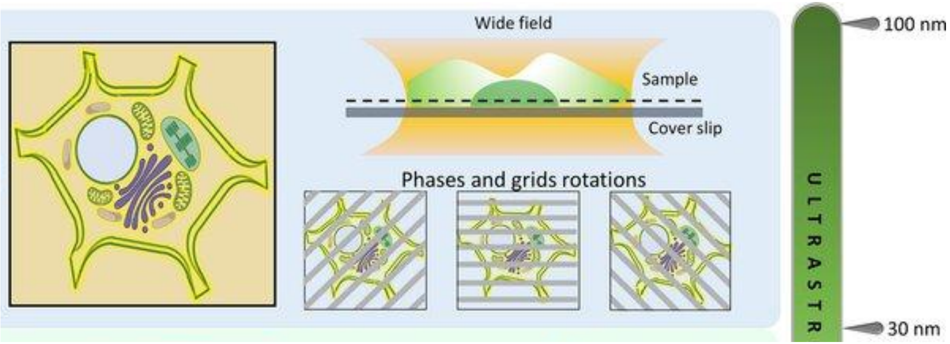
Method

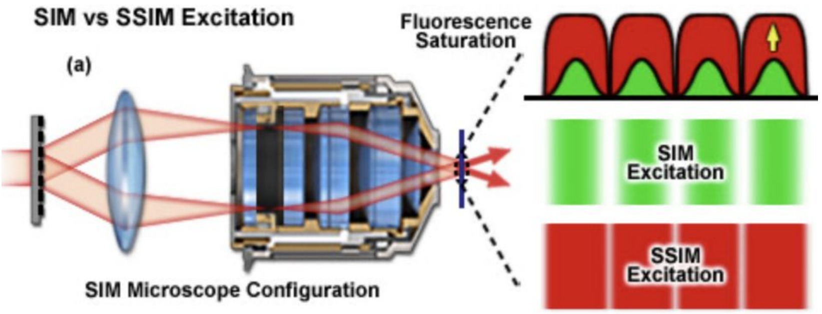
- SIM=structured illumination microscopy
  - linear
  - non-linear

Fig. 6: Comparison of nonlinear and linear SIM.

From: Superresolution structured illumination microscopy reconstruction algorithms: a review





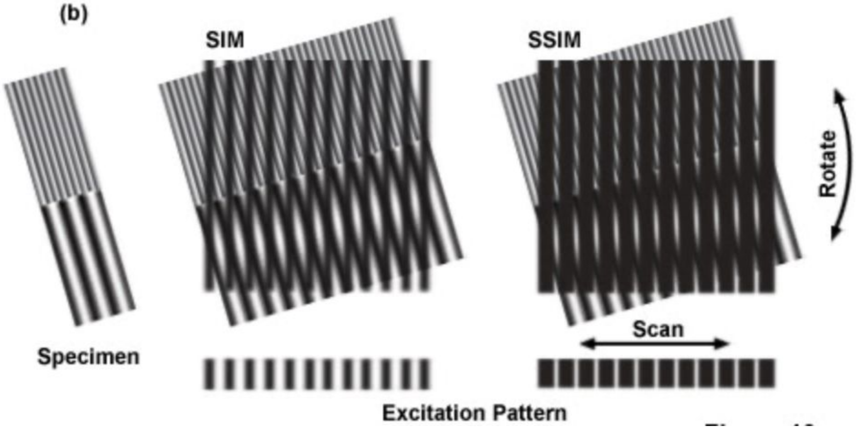


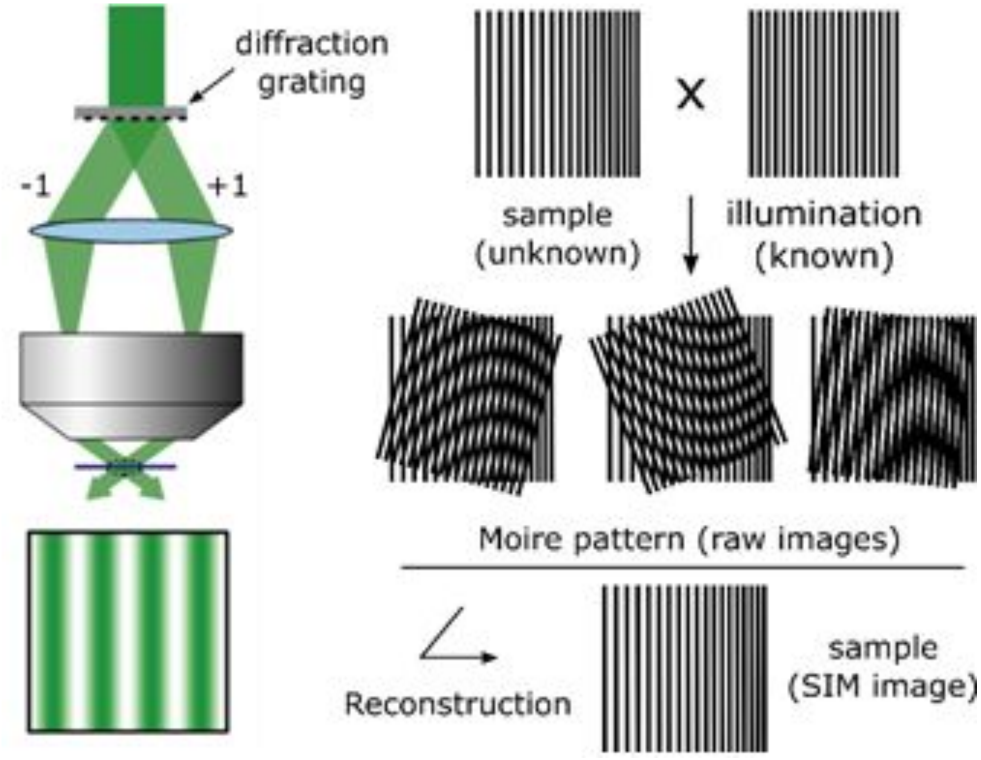
https://zeiss-campus.magnet.fsu.edu/print/superresolution/supersim-print.html

Moiré patterns

illumination grids

- Moiré patterns





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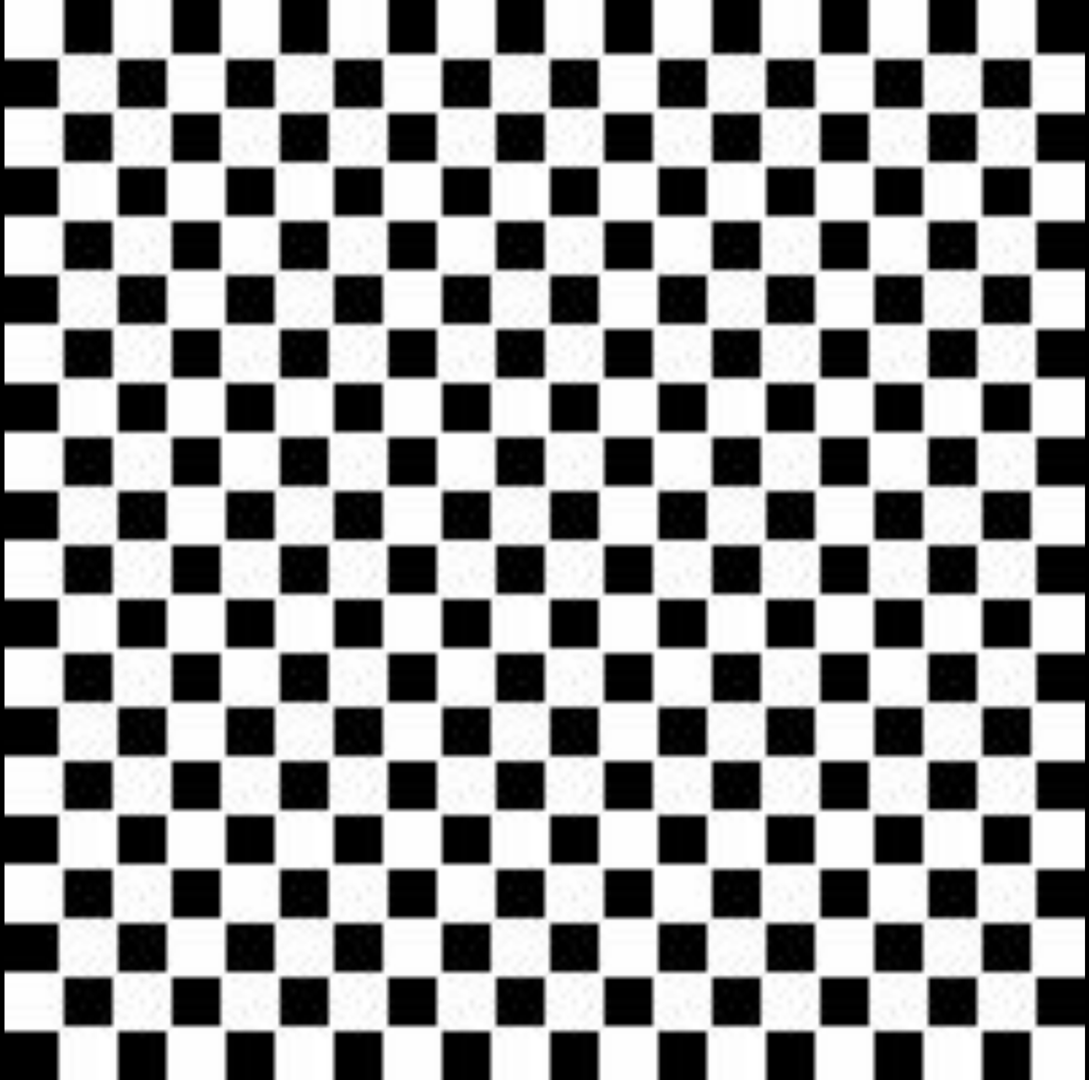


Computation

Image = Object + PSF

mimicking optics

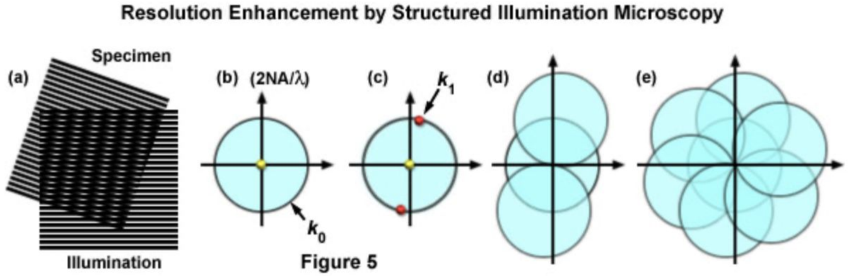
- Application limitations
- PSF

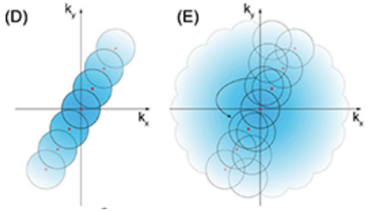
$$f_{SIM} = f_{det} + f_{ill}$$

spatial frequency = max optical f + max illumination f

System method: SIM

- illumination grids
    - Moiré patterns
- Mapping



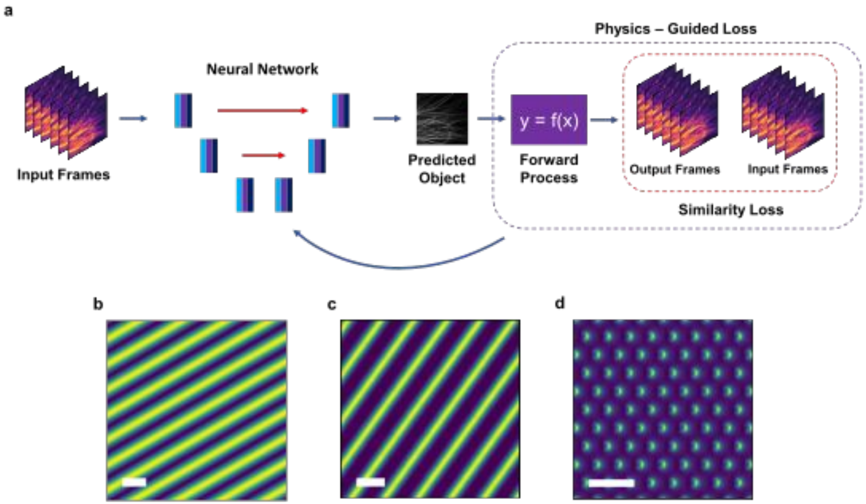


Original Publication

a) Linear SIM

b) Nonlinear SIM

c) plasmonic SIM



Research Article
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Untrained, physics-informed neural networks for structured illumination microscopy

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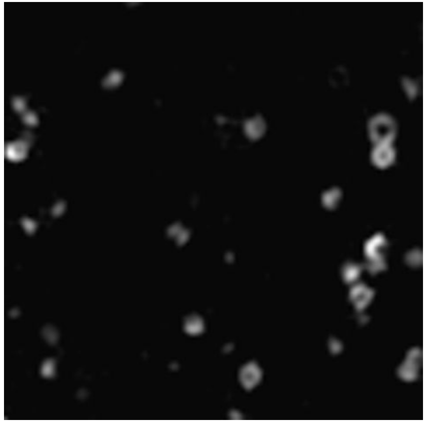
Abstract: Structured illumination microscopy (SIM) is a popular super-resolution imaging technique that can achieve resolution improvements of 2× and greater depending on the illumination patterns used. Traditionally, images are reconstructed using the linear SIM reconstruction algorithm. However, this algorithm has hand-tuned parameters which can often lead to artifacts, and it cannot be used with more complex illumination patterns. Recently, deep neural networks have been used for SIM reconstruction, yet they require training sets that are difficult to capture experimentally. We demonstrate that we can combine a deep neural network with the forward model of the structured illumination process to reconstruct sub-diffraction images without training data. The resulting physics-informed neural network (PINN) can be optimized on a single set of diffraction-limited sub-images and thus does not require any training set. We show, with simulated and experimental data, that this PINN can be applied to a wide variety of SIM illumination methods by simply changing the known illumination patterns used in the loss function and can achieve resolution improvements that match theoretical expectations.

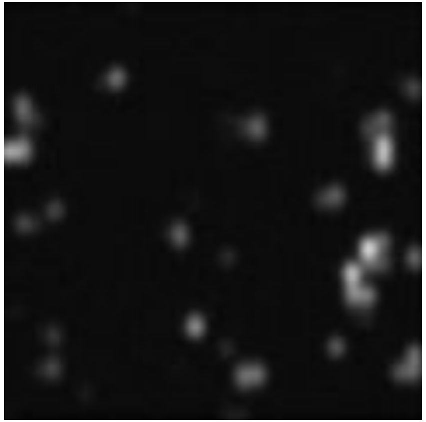
https://opg.optica.org/oe/fulltext.cfm?uri=oe-31-5-8714
https://github.com/Zach-T-Burns/Untrained-PINN-for-SIM

Dataset

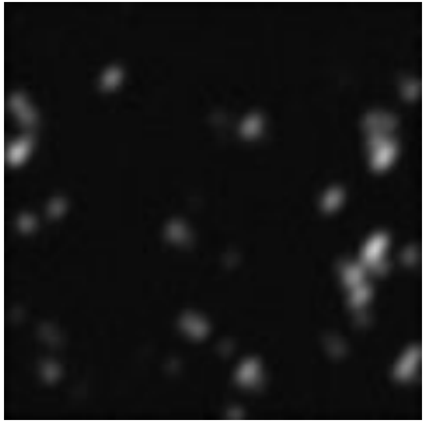
- microscopy images of Clathrin-coated pits (CCP), endoplasmatic reticula (ER), microtubules (MT) and F-actin
- 300 samples
    - small dataset ➜ everyday challenge in many scientific fields
- for each sample:
    - low resolution image
    - ground truth (high resolution)
    - results with different illumination patterns
- 25 illumination patterns
- PSF (Point Spread Function)



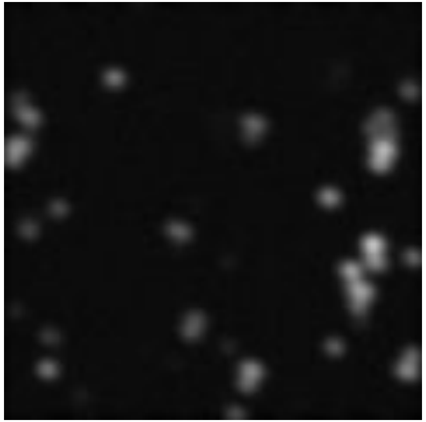
CCP, ground truth



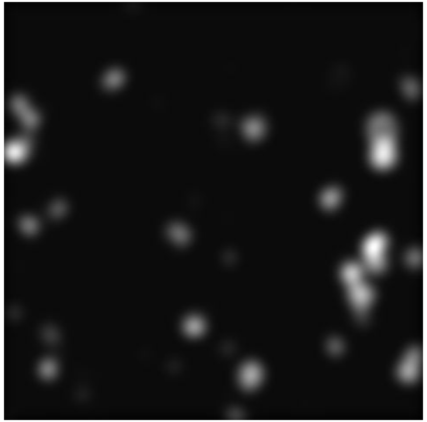
CCP, pattern 1



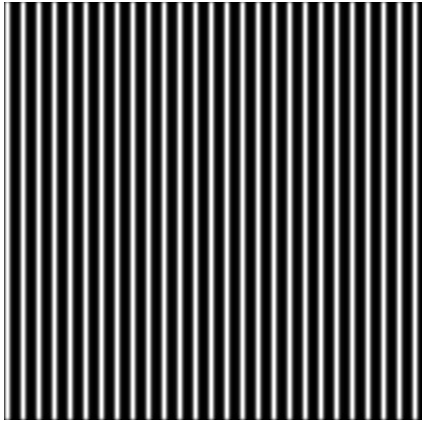
CCP, pattern 10



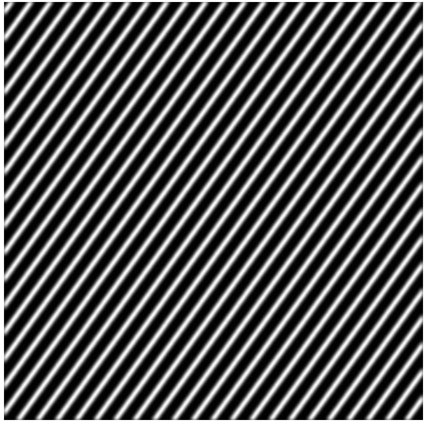
CCP, pattern 20



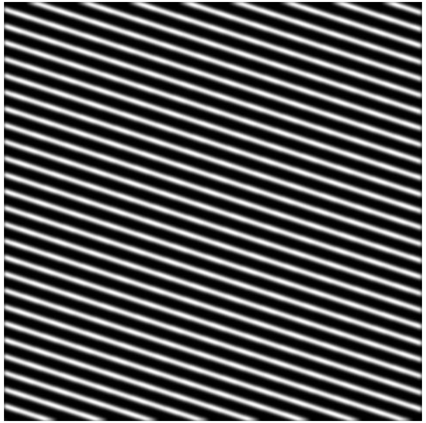
CCP, low resolution



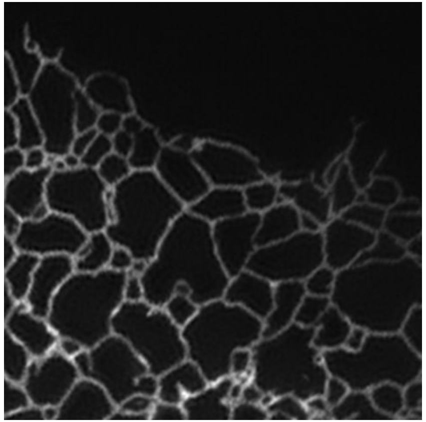
pattern 1



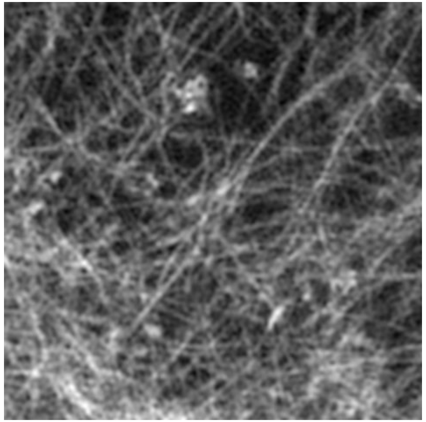
pattern 10



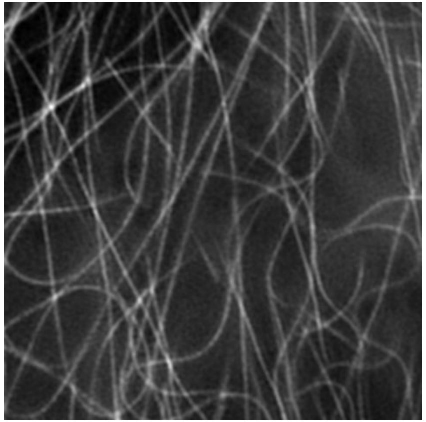
pattern 20



ER, ground truth

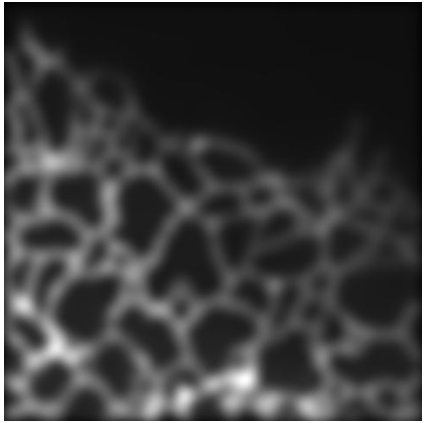


F-actin, ground truth

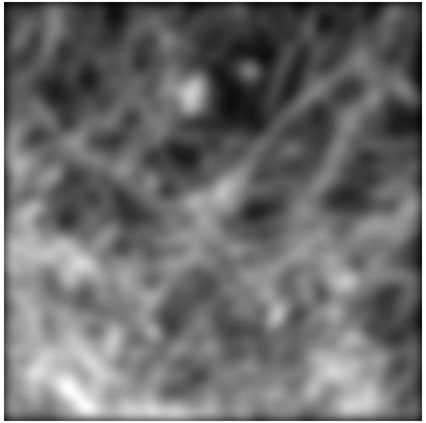


MT, ground truth

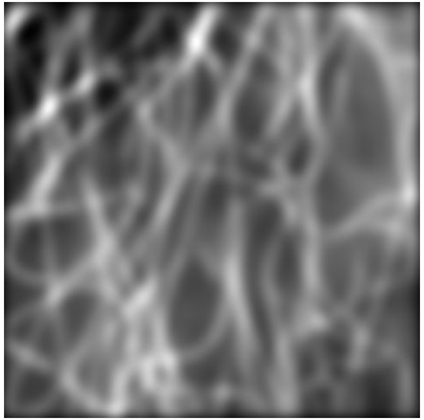
PSF



ER, low resolution



F-actin, low res.



MT, low resolution

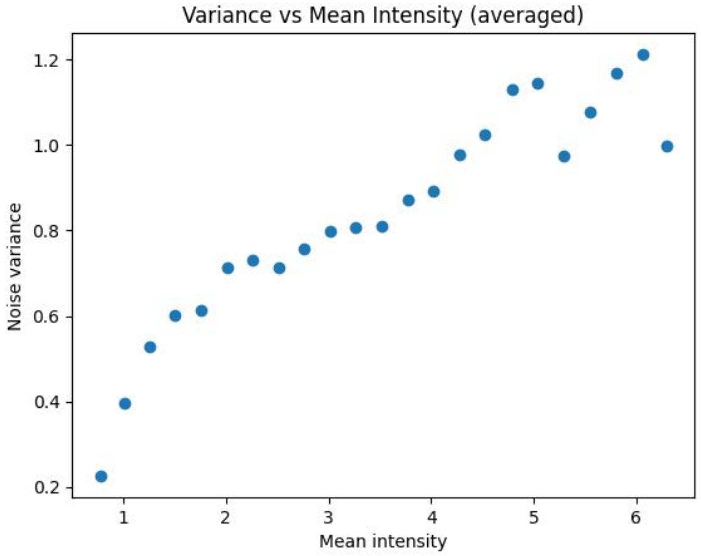
Main Noise Sources:

- Shot noise (**Poisson**): photon counting noise increases with intensity
- Camera readout noise (**Gaussian**): signal-independent electronic noise
- Background fluorescence: adds low-frequency intensity bias

What We Observed in the Data:

- Variance increases approximately linearly with mean intensity
- Residuals are zero-mean and approximately Gaussian after averaging
- → Indicates a Poisson–Gaussian noise model

Idea: Noise-Aware PINN Reconstruction



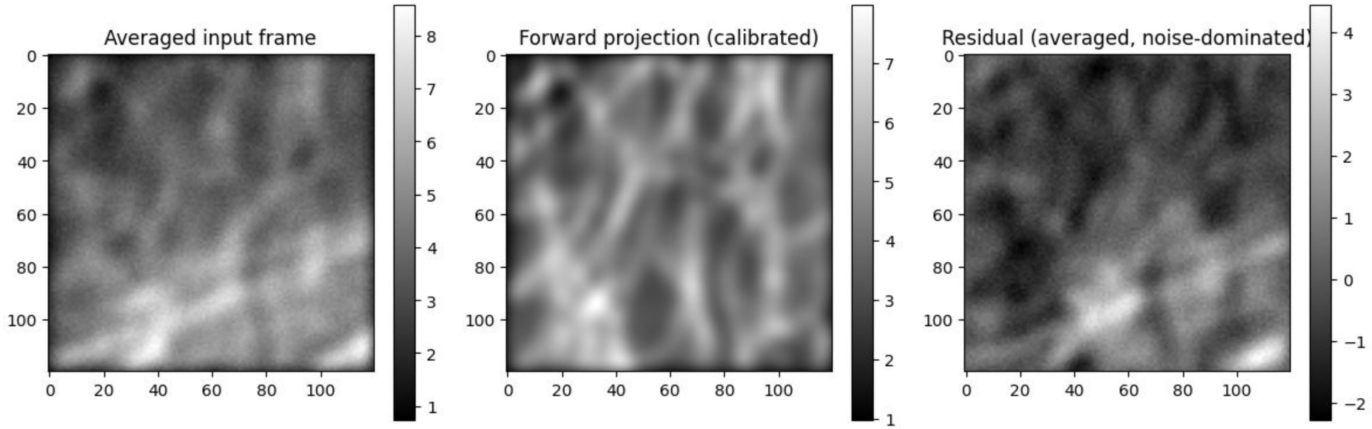
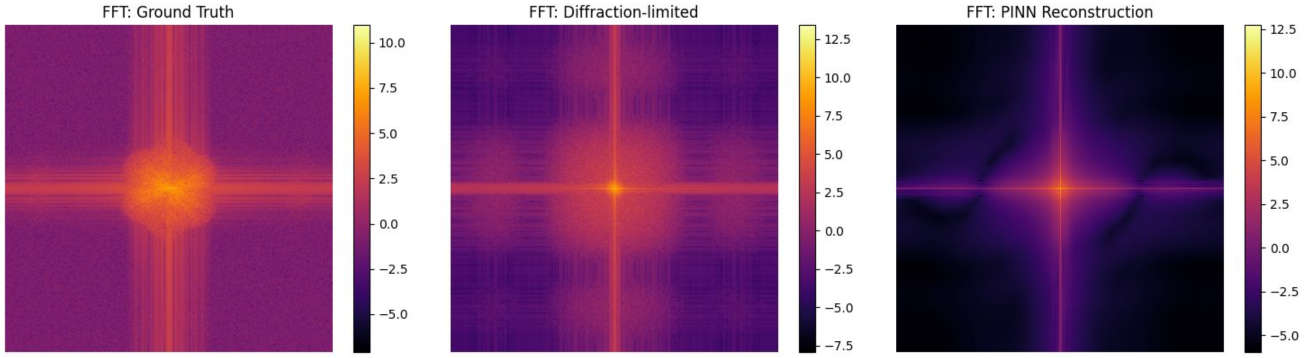


Image reconstruction:

- Reproduces the measured SIM frames
- Obeys the microscope forward model
- Uses a noise-aware loss function

Step	Purpose	Impact
Frame averaging	Reduce random photon noise	Improves SNR and stability
Intensity normalization	Keep values in a stable range	Enables stable optimization
Poisson loss	Match photon-counting noise statistics	Physically consistent fitting
Weak TV regularization	Suppress noise amplification	Stabilizes reconstruction while preserving structure

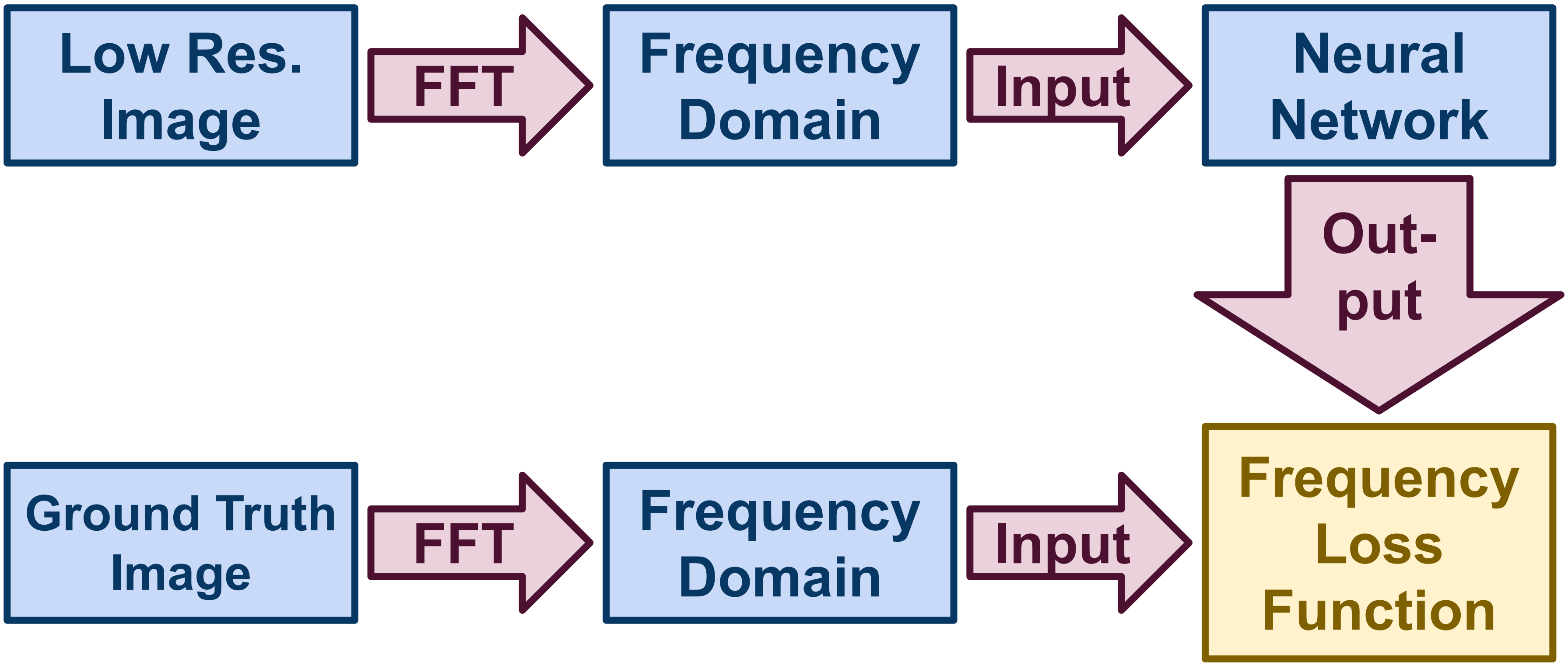


Frequency Domain Model

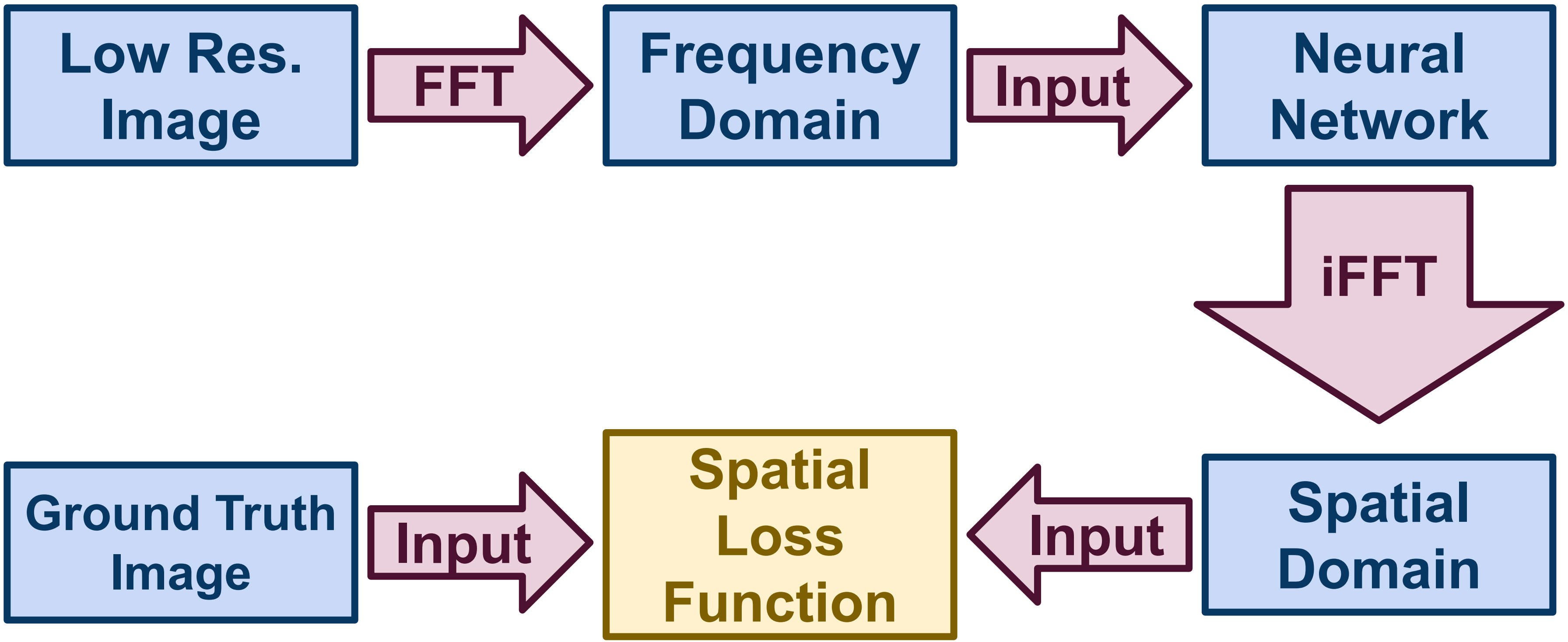
Idea:

- Input and loss function for training in frequency domain
- Training loss function:
  - Experiments with different functions
  - best result: combination of amplitude loss, radial loss and spatial loss
- Evaluation in spatial domain
  - for comparability with PINN model
  - Evaluation loss function adapted to PINN model (from Zach. T. Burns paper/repo)
- Same NN architecture as the PINN model (for comparability)

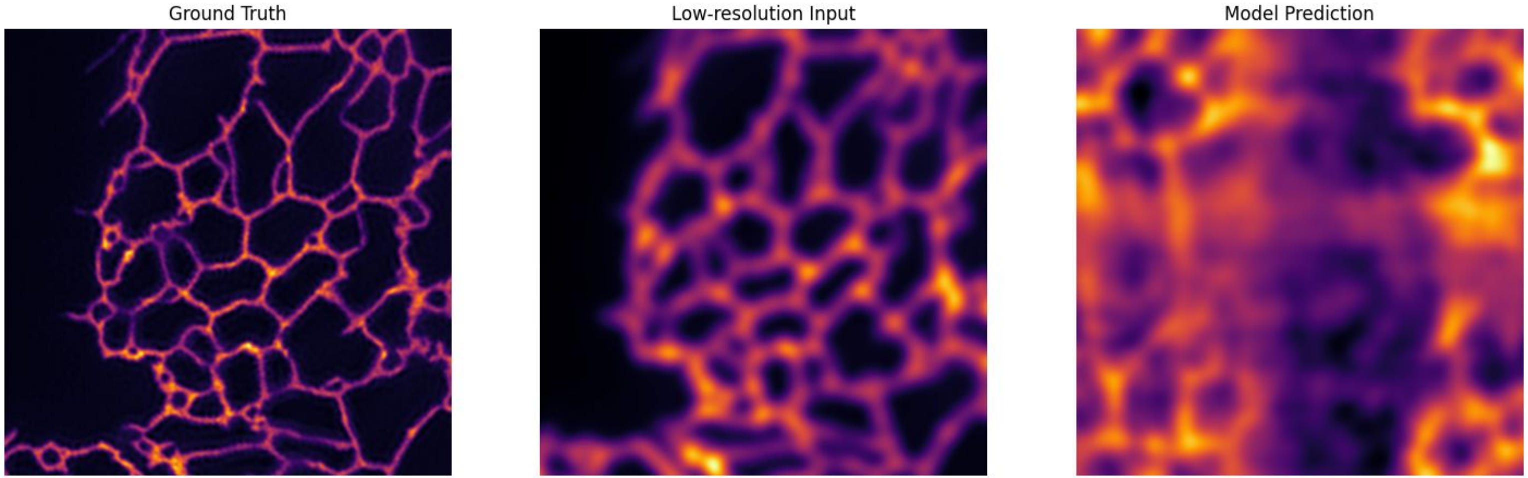
Frequency Domain Model: Training



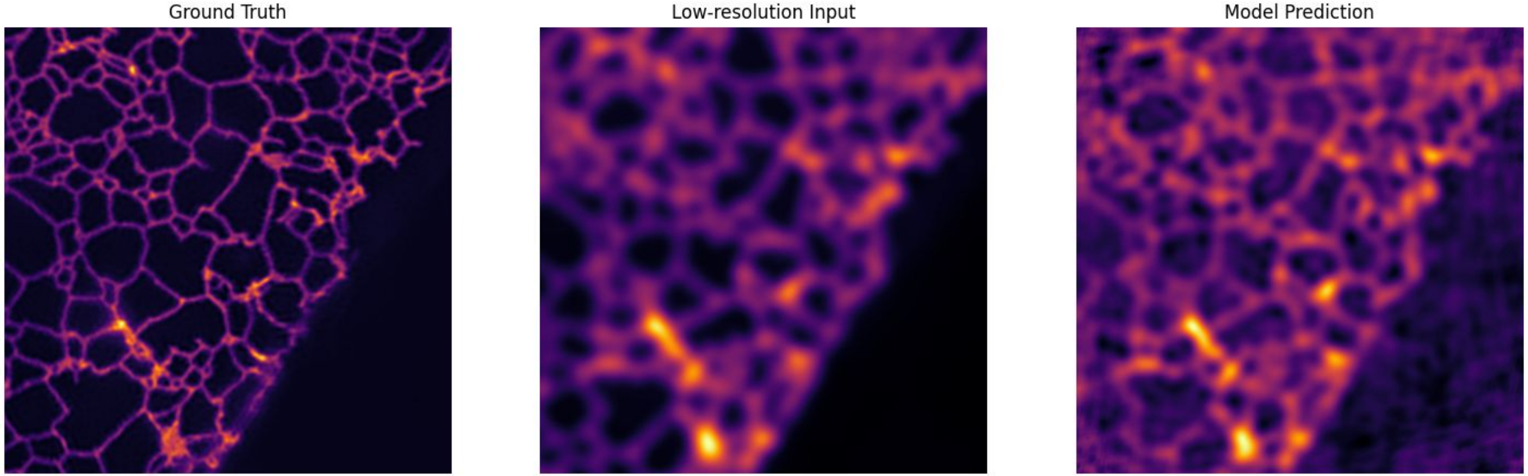
Frequency Domain Model: Evaluation

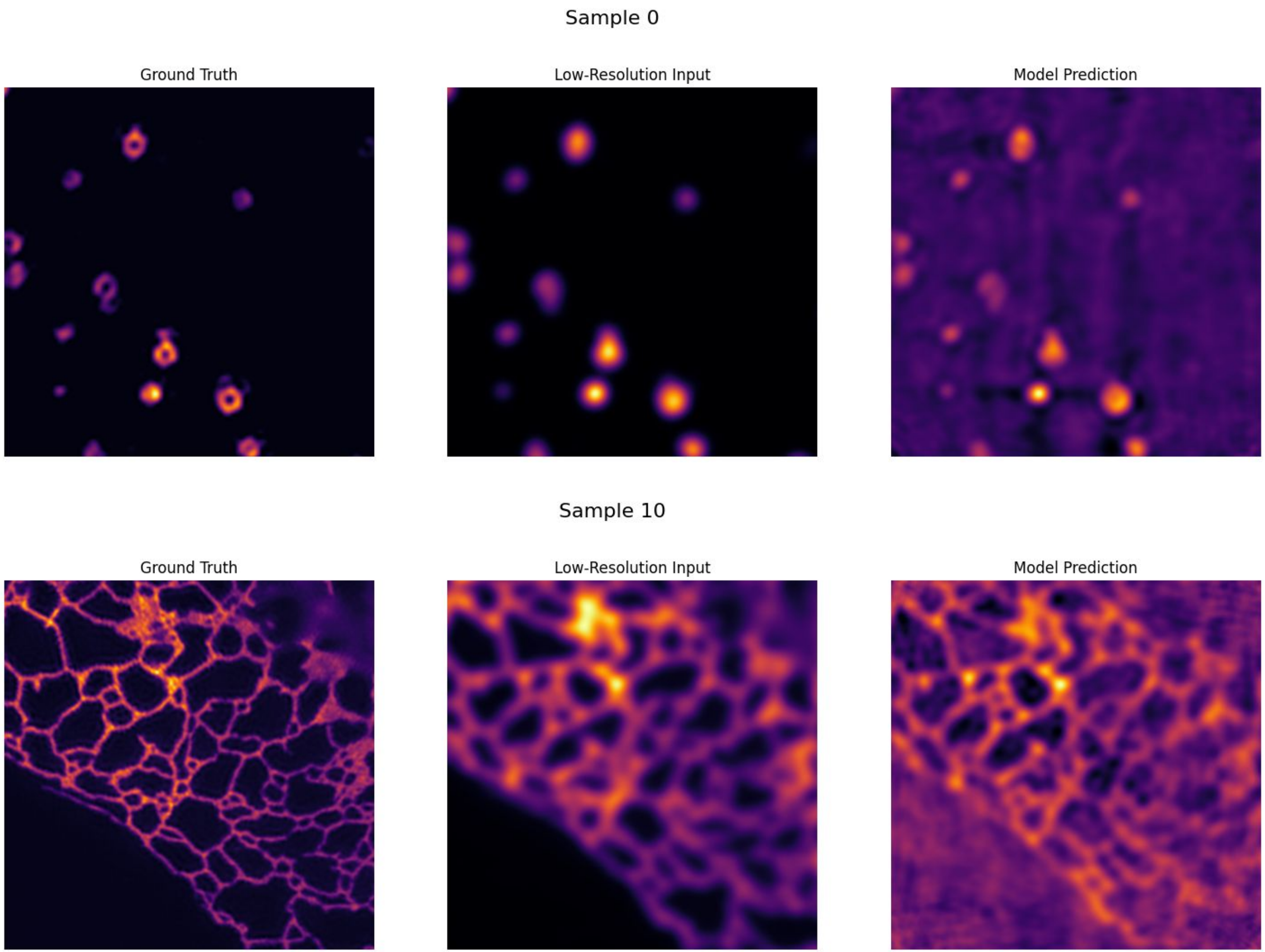


Training sample, at training loss 0.080636 (pure frequency loss), test loss 0.999989.

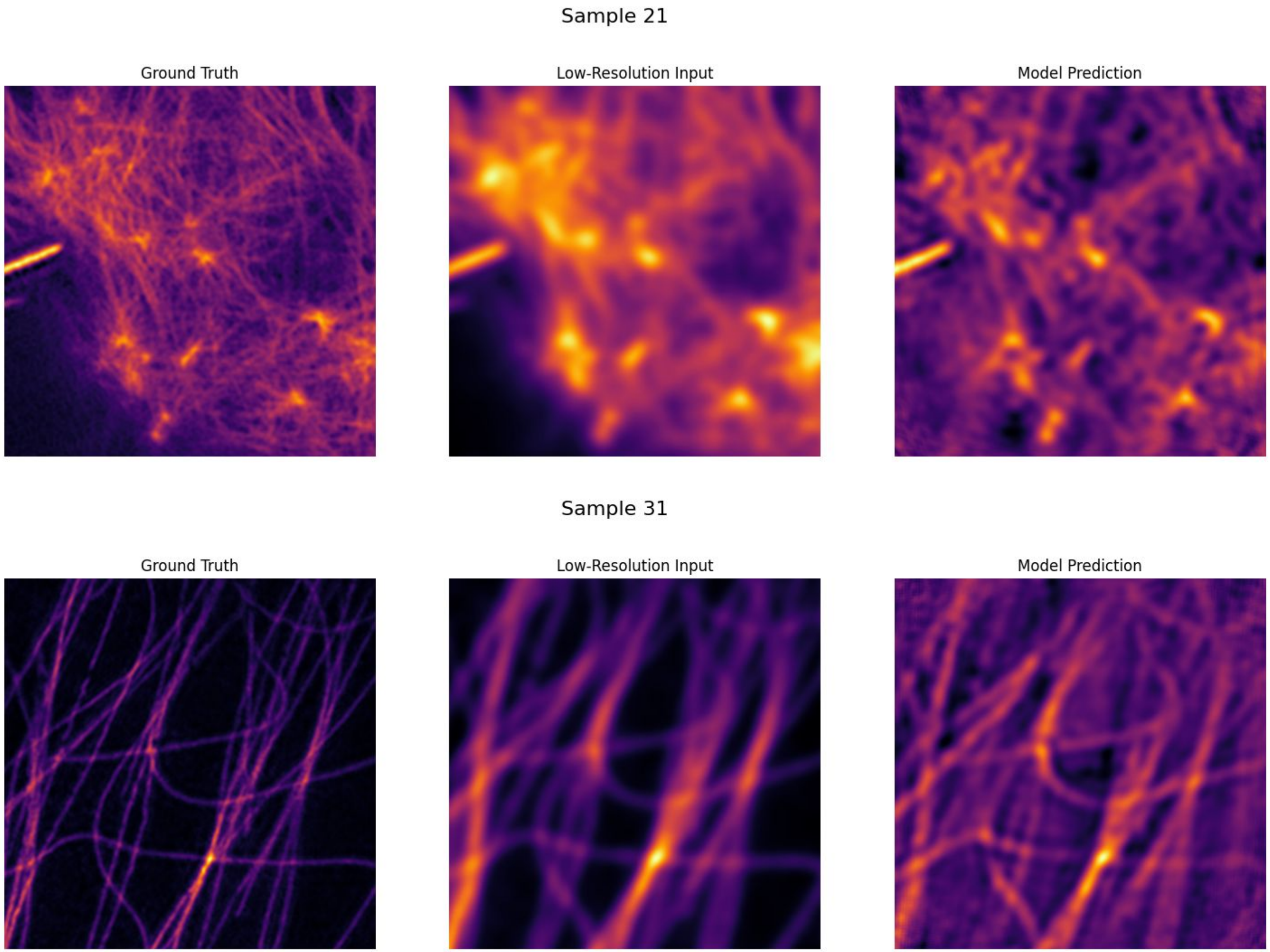


Training sample, at training loss 0.267202 (phase, radial & spatial loss), test loss 0.368902.





Test samples, at training loss 0.267202 (amplitude, radial & spatial loss), test loss (spatial) 0.368902.



Test samples, at training loss 0.267202 (amplitude, radial & spatial loss), test loss (spatial) 0.368902.

Frequency Domain Model: Results

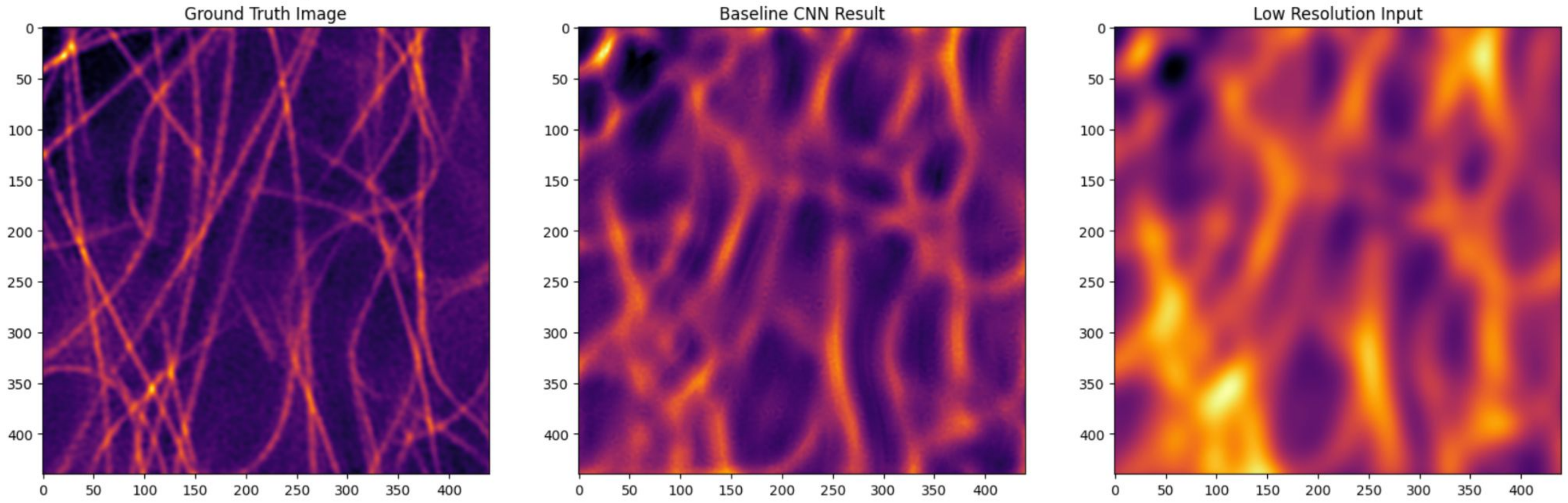
- Different Training Loss Functions

  ➜ Very different results

- (Very) high test loss (even with low training loss)
- Even predicted images of training data looked bad
- Reasons:
  - Not enough training data
  - Overfitting
  - High sensitive to errors in frequency domain (small errors in phase lead to massive errors in spatial domain)

Baseline CNN

- U-Net
  - Image segmentation



Method	**Training Loss**	**Comments**
CNN	MSE= 0.0054	1000 Epochs ~ 1h GPU
Supervised	MSE = 0.00012 (extremely low -> overfitting)	1500 Epochs ~ 1.5h GPU > Method relies on large dataset
Supervised +Batch Augmentation	MSE = 0.00035 (extremely low -> overfitting)	1000 Epochs ~ 1h GPU
Frequency Domain	Frequency Loss = 0.080636 (Test Loss SSIM = 0.999989)	30 Epochs
Frequency Domain	Combined Frequency & Spatial Loss = 0.235363 (Test Loss SSIM = 0.368902)	111 Epochs
Original Method from Publication: Self supervised PINNs	NL SIM= 0,1092/0,0738/0,1086	100 Epochs ~ 1h GPU

Summary

- Some algorithms cause deterioration of image quality
- Small errors in frequency domain can cause huge errors in spatial domain
- Small training dataset ➜ prone to overfitting
- Advantage for unsupervised approaches: large image databases without ground truth (≙ target) can be used
- Complex topic (microscopy + optical systems + imaging algorithms)

Sources

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- https://zeiss-campus.magnet.fsu.edu/articles/superresolution/index.html

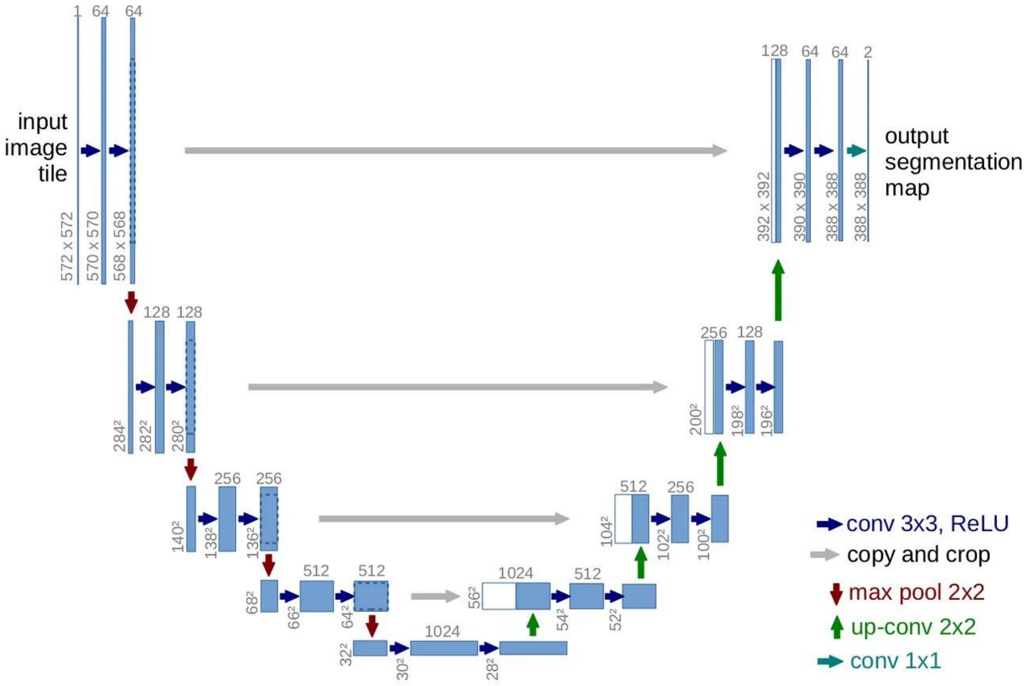
Thank you!


http://www.CHRISTOON.COM
YOU MIGHT CALL THEM SCIENTISTS, BUT FOR ME THEY ARE "PAPARAZZI"!
KOMARNITSKI

Additional Insights

U-Net Architecture

-special type of CNN



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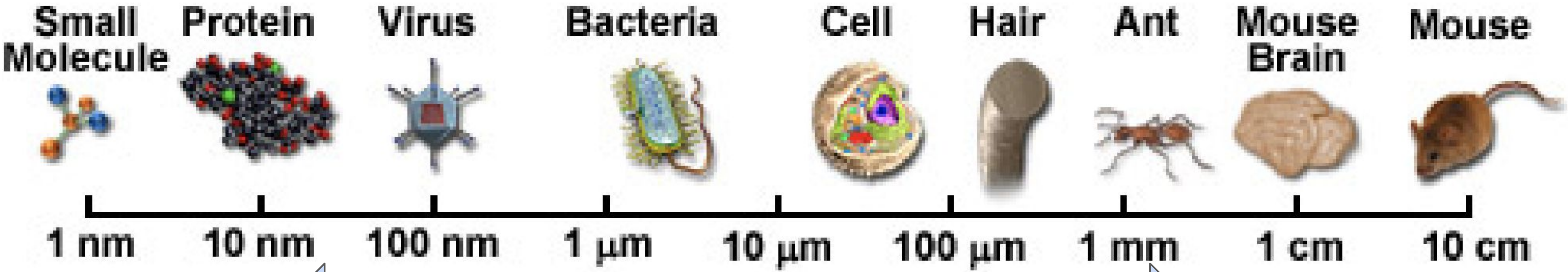
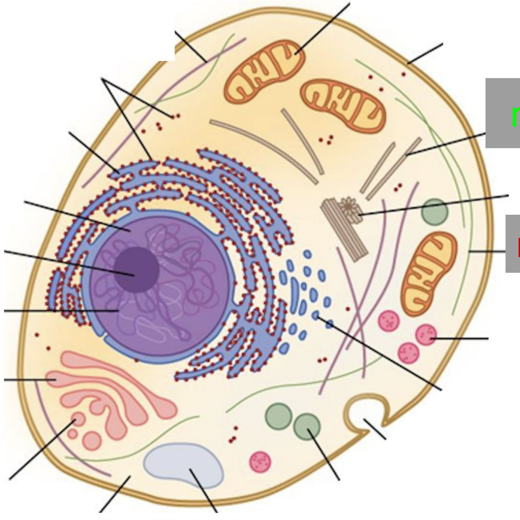
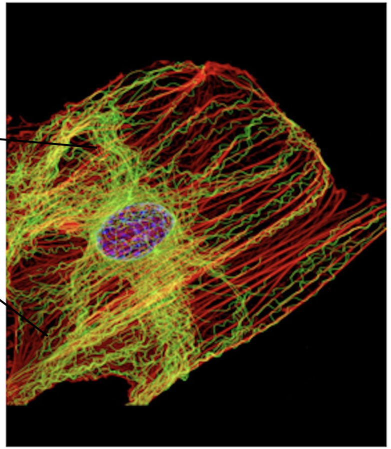


Super-resolution microscopy methods



microtubules

microfilaments



Fluorescent microscopy; microtubules (green) and microfilaments (red)