Scalable and *reliable* deep learning for computational microscopy

Lei Tian

Computational Imaging Systems Lab

Department of Electrical and Computer Engineering

Boston University

leitian@bu.edu

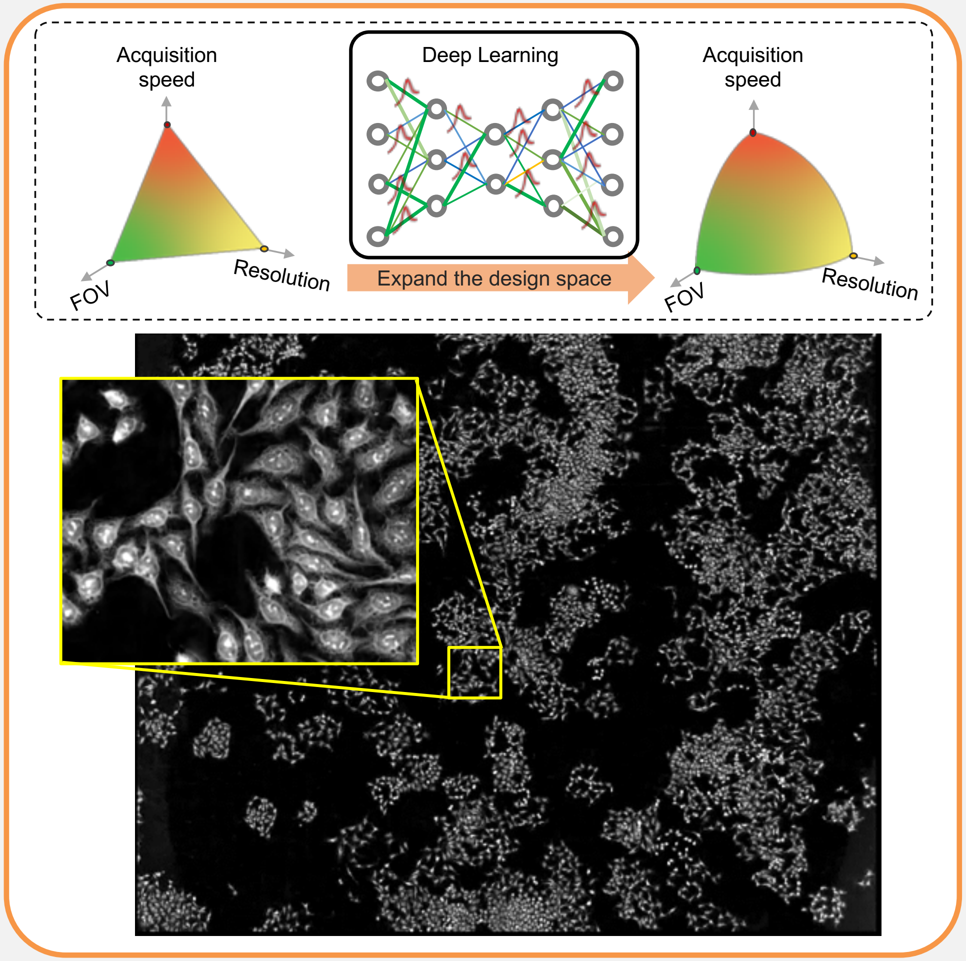
http://sites.bu.edu/tianlab/

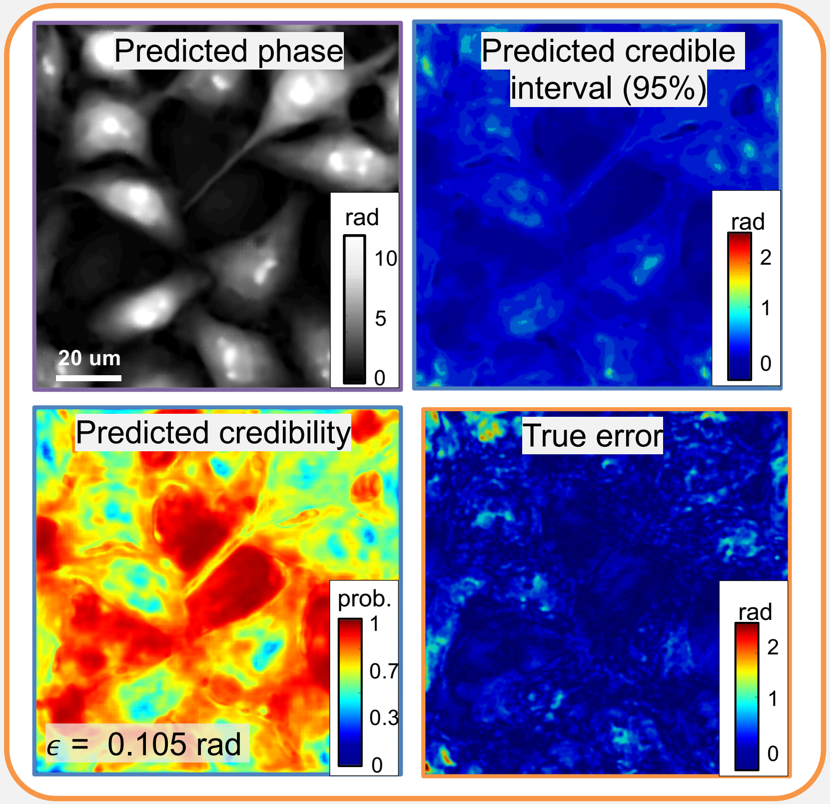
Boston University College of Engineering
Department of Electrical & Computer Engineering

Neurophotonics Center
Building and sustaining a community of developers, early adopters, and users.
http://bu.edu/neurophotonics

Photonics Center

Scalable and *reliable* deep learning for computational microscopy





- » ***Physics-guided*** measurement design for efficient large-SBP imaging
- » ***Uncertainty quantification*** towards reliable deep learning

[1] Xue, Cheng, Li, Tian , "Illumination coding meets uncertainty learning: toward reliable AI-augmented phase imaging", *arXiv* (2019).

Computational Phase Imaging

Hardware & Acquisition design

input field (amplitude & phase) → imaging system → detector (measures only intensity)

Computational strategy can also influence the hardware design & data capture strategy

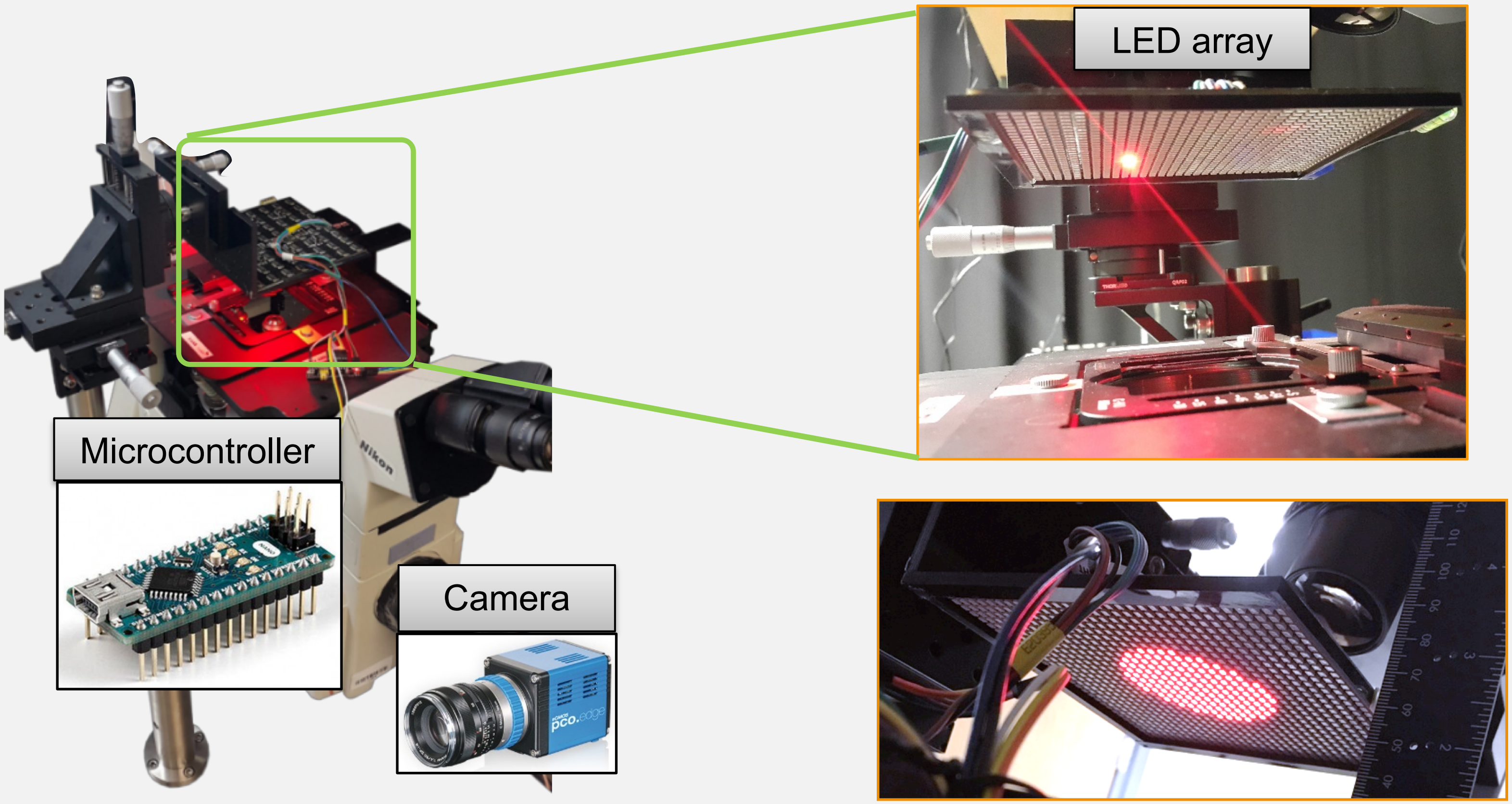
Computation

find x

such that Intensity = $|\mathbf{A}x|^2$

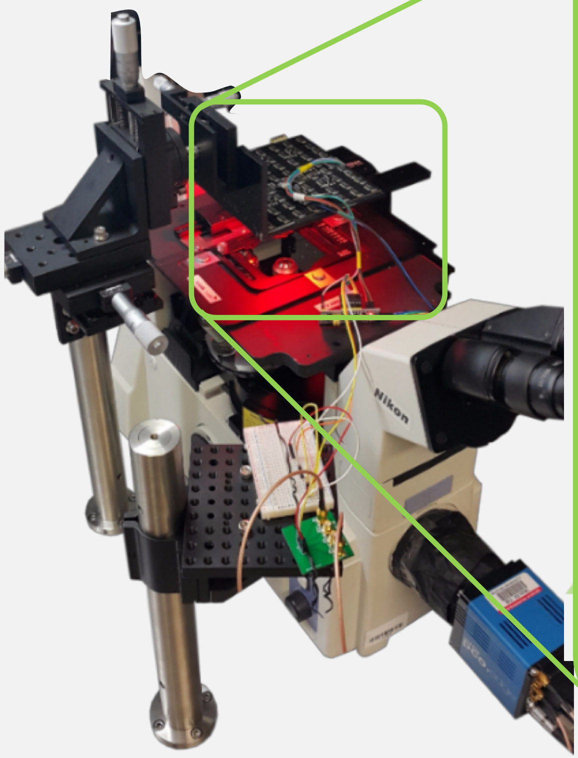
- *Model* based inversion
- *Learning* based inversion

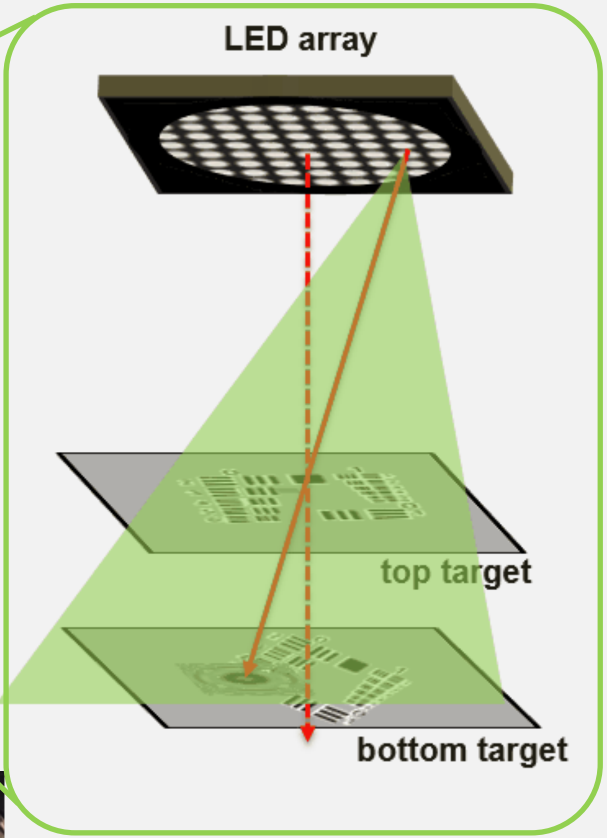
Computational microscopy using an LED array



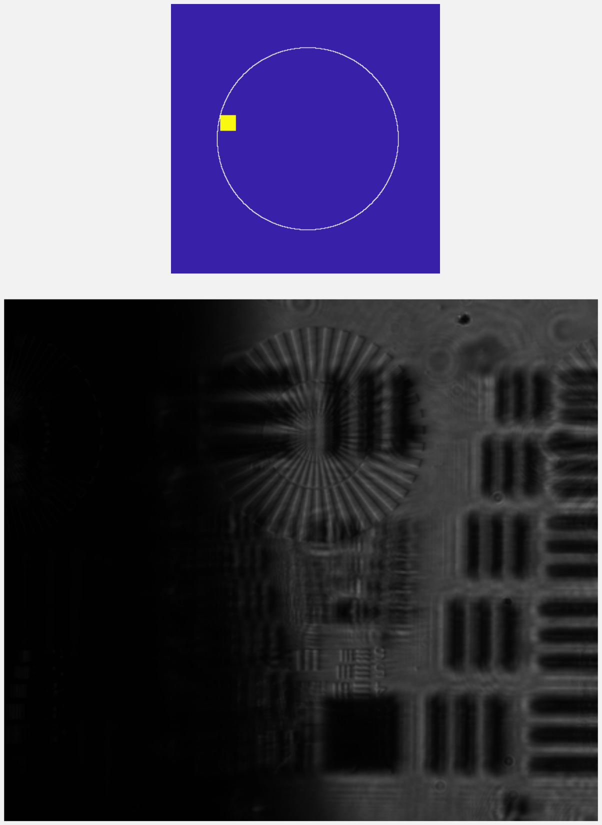
Programmable illumination pattern

Computational microscopy using an LED array



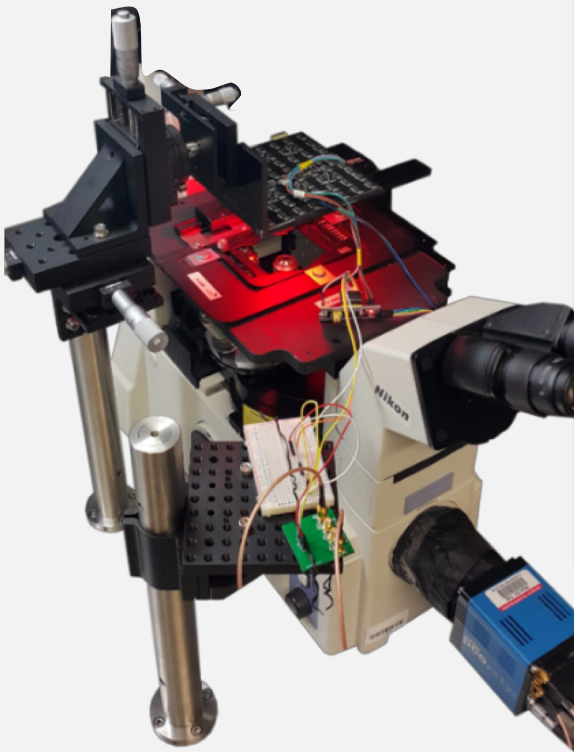


scan illumination
in (θ_x,θ_y)

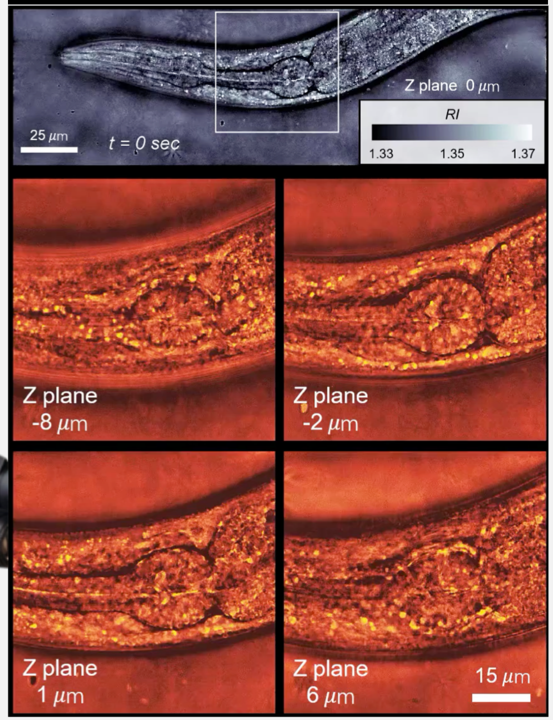


- Each LED encodes distinct **angular** information
- **Intensity-only** measurement (no interferometry)
- Any **phase, diffraction** & **scattering** information is recovered by optimization algorithms

Multimodal computational microscopy

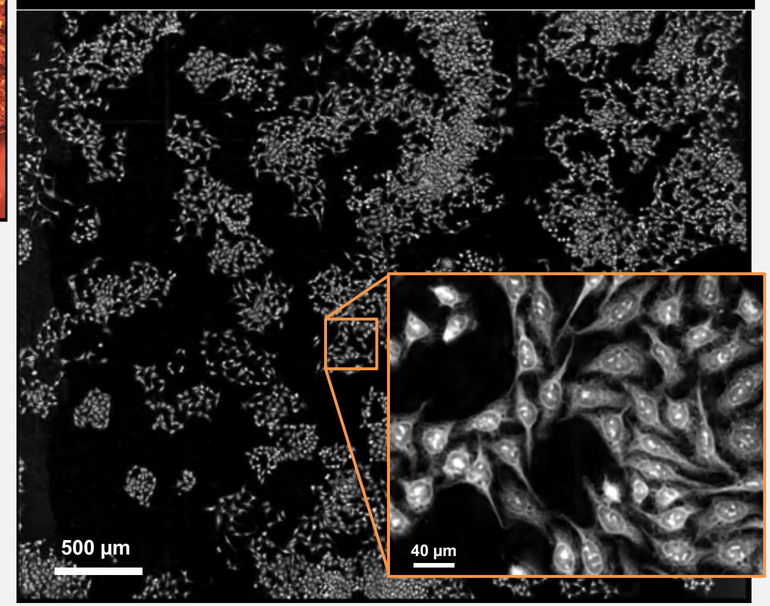


3D imaging



Ling, Tahir, Lin, Lee, Tian, Biomed. Opt. Express 9, 2130-2141 (2018).

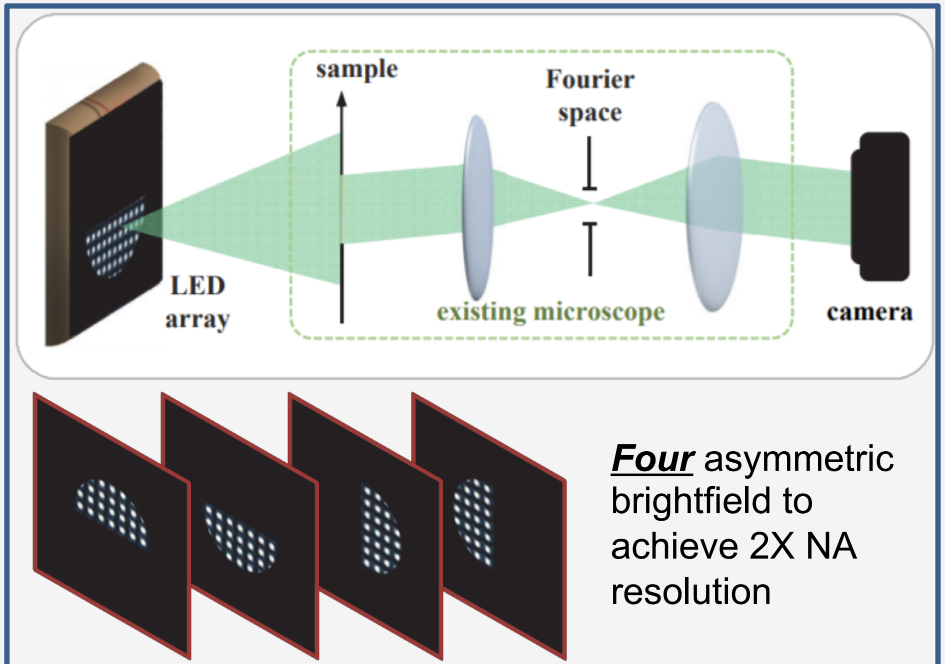
Phase & High resolution



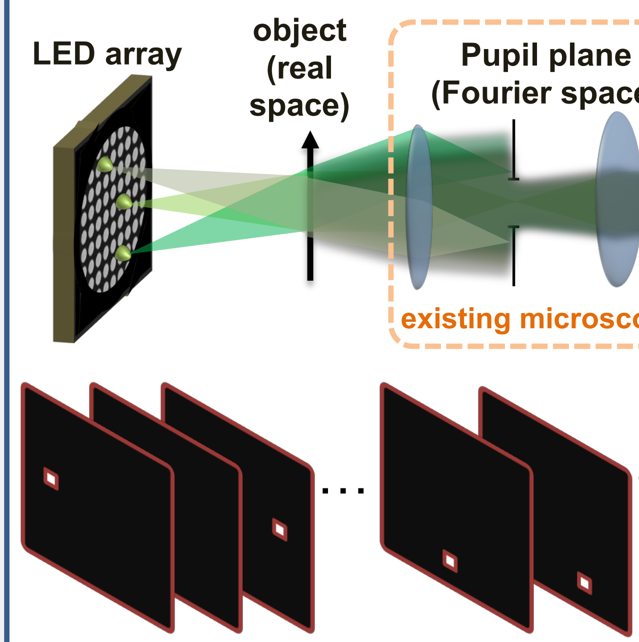
Xue, Cheng, Li, Tian , “Illumination coding meets uncertainty learning: toward reliable AI-augmented phase imaging”, *arXiv* (2019)

Physical model based phase microscopy

- Asymmetric illumination encodes both phase and high resolution information
  - Differential Phase Contrast Microscopy
  - Fourier Ptychographic Microscopy

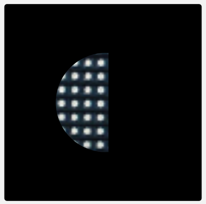


Four asymmetric brightfield to achieve 2X NA resolution

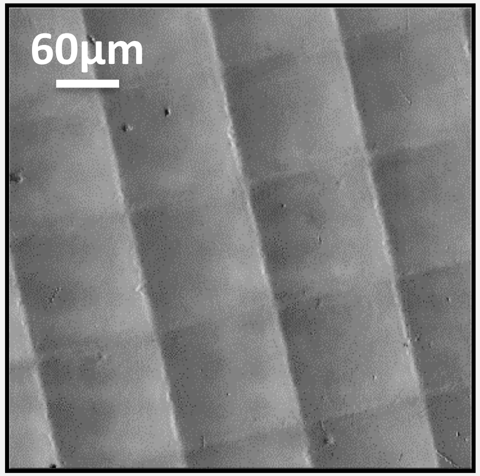


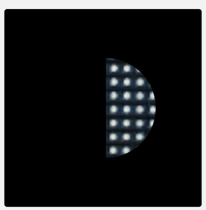
Many brightfield and darkfield to achieve >2X NA resolution

Differential phase contrast (DPC) by asymmetric illumination

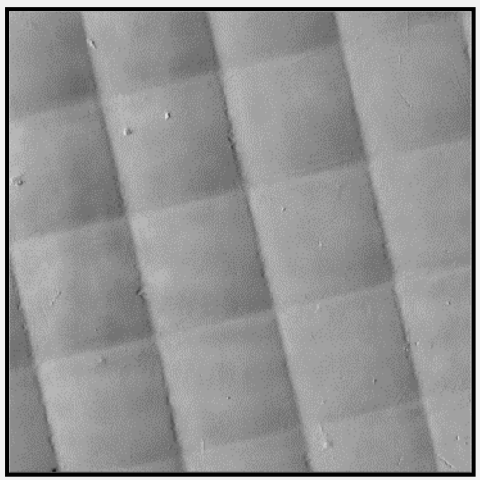


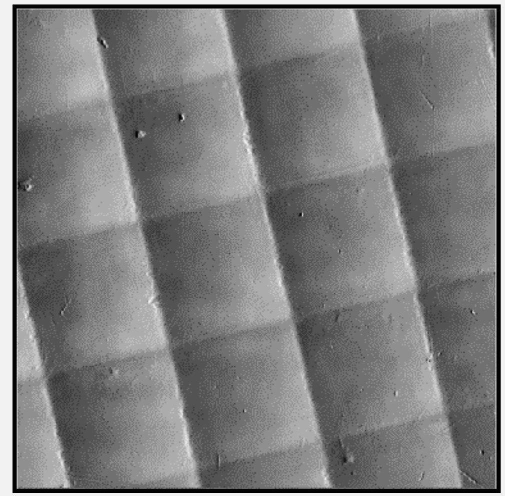
Left





Right



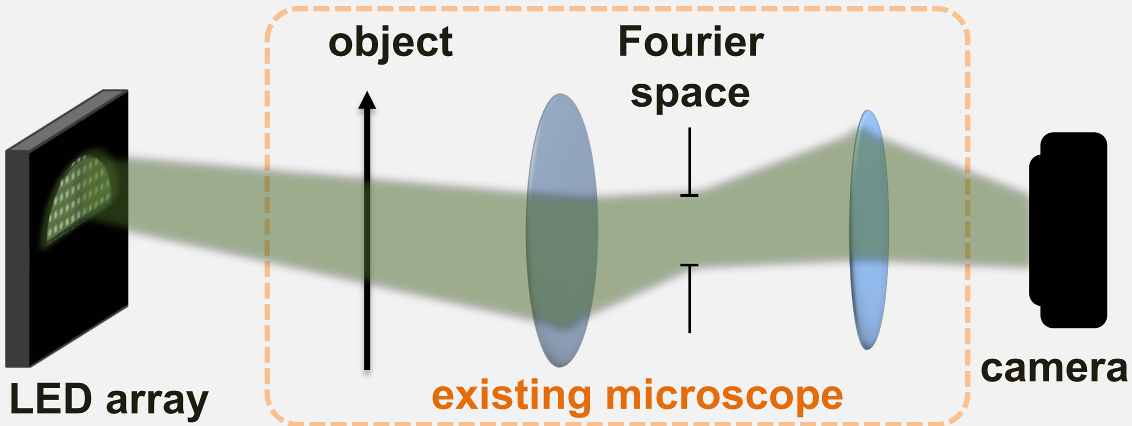


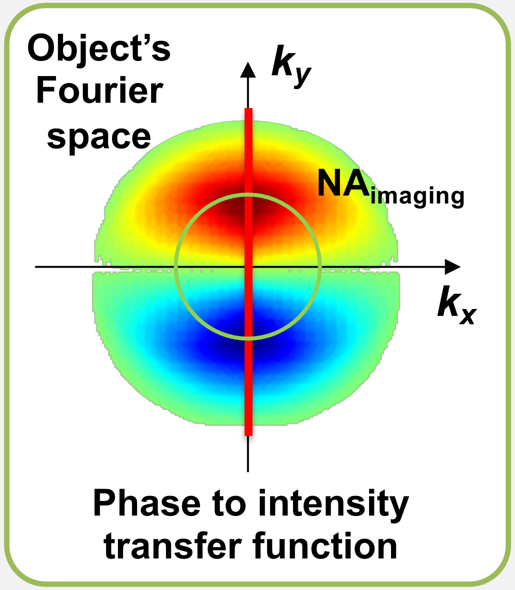
$$\text{DPC} = \frac{\text{Left} - \text{Right}}{\text{Left} + \text{Right}}$$

related to the gradient of phase[1,2]

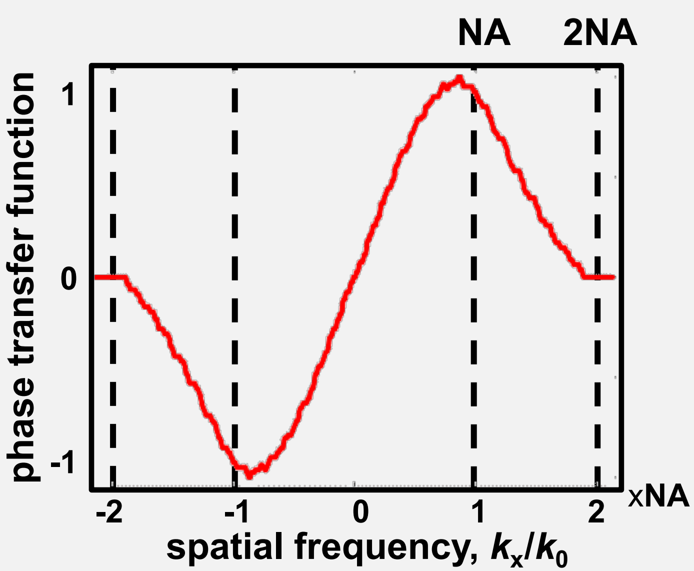
Kachar, Science 227, 27 (1985).
[1] Mehta, Sheppard, Opt. Lett. 34, 1924 (2009).
[2] Ford, Chu, Mertz, Nat. Methods 9, 1195 (2012).

Phase transfer function for DPC



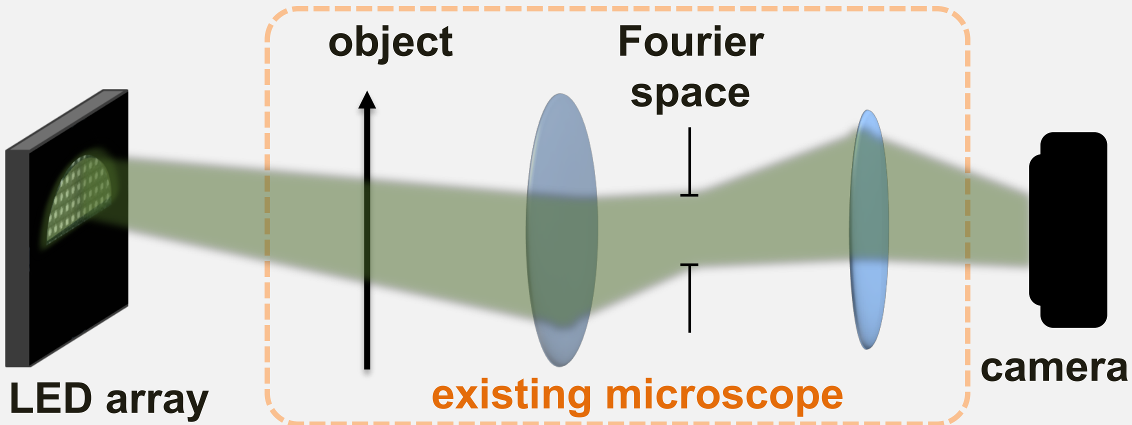


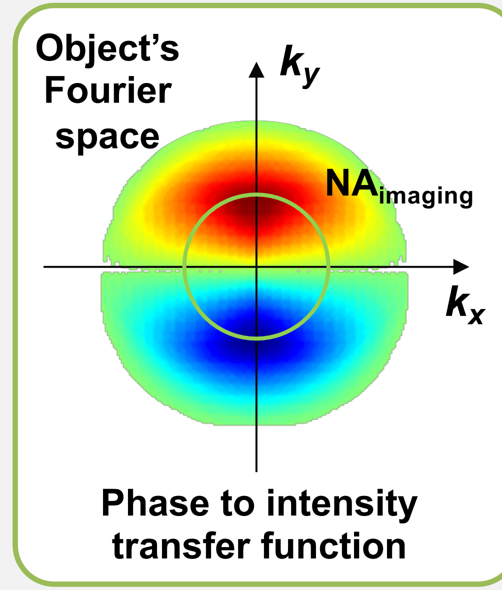
- But…transfer function means linear?
  → weak object approximation
- 2x better resolution than coherent case (e.g. interferometry)

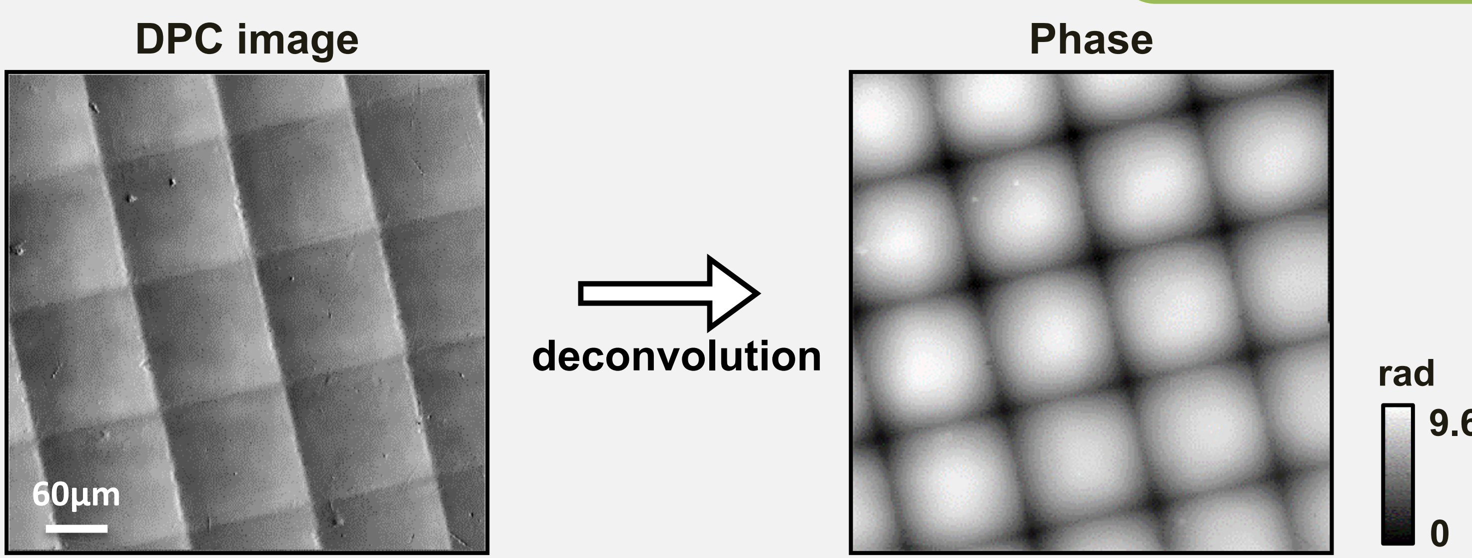


Tian, Waller, *Opt. Express* 23(9), 11394-11403 (2015).

Phase reconstruction from DPC measurements





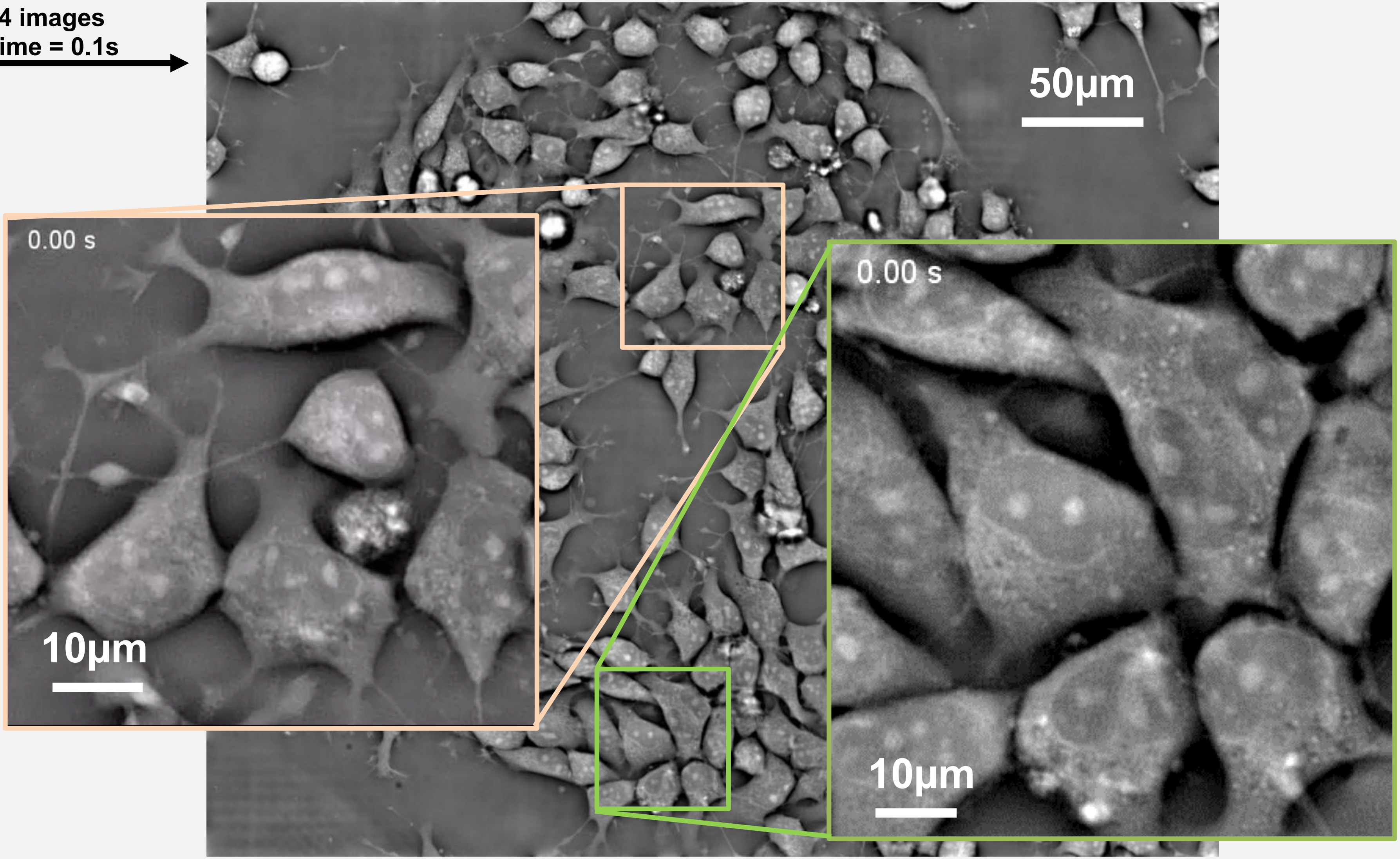


Tian, Waller, *Opt. Express* 23(9), 11394-11403 (2015).

Real-time DPC in vitro

10 Hz
resolution ~0.4 µm

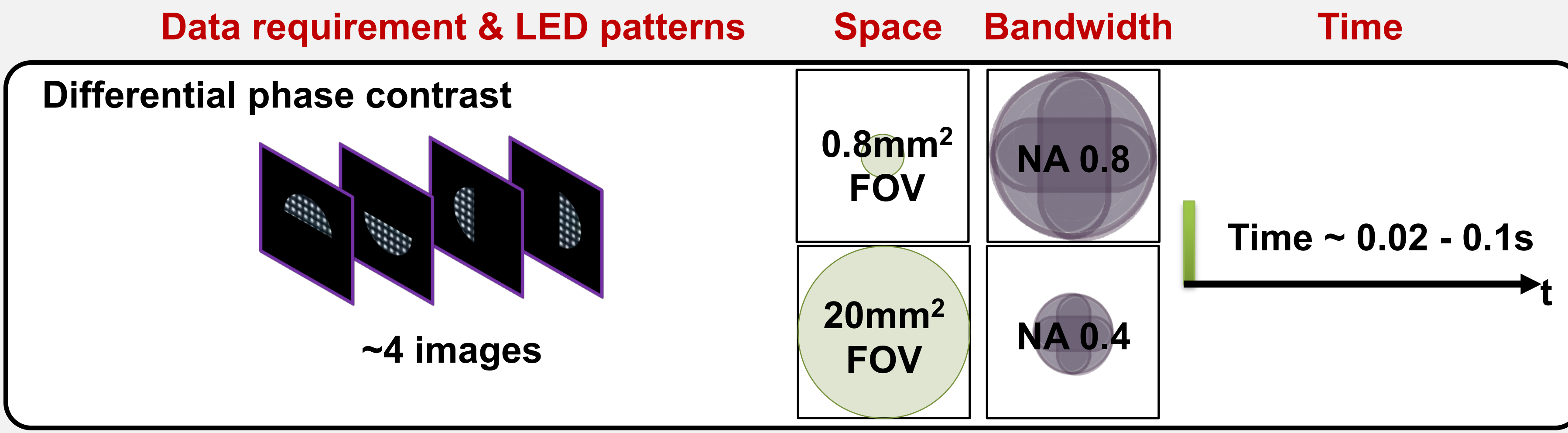
4 images
Time = 0.1s



Neural Progenitor Cell

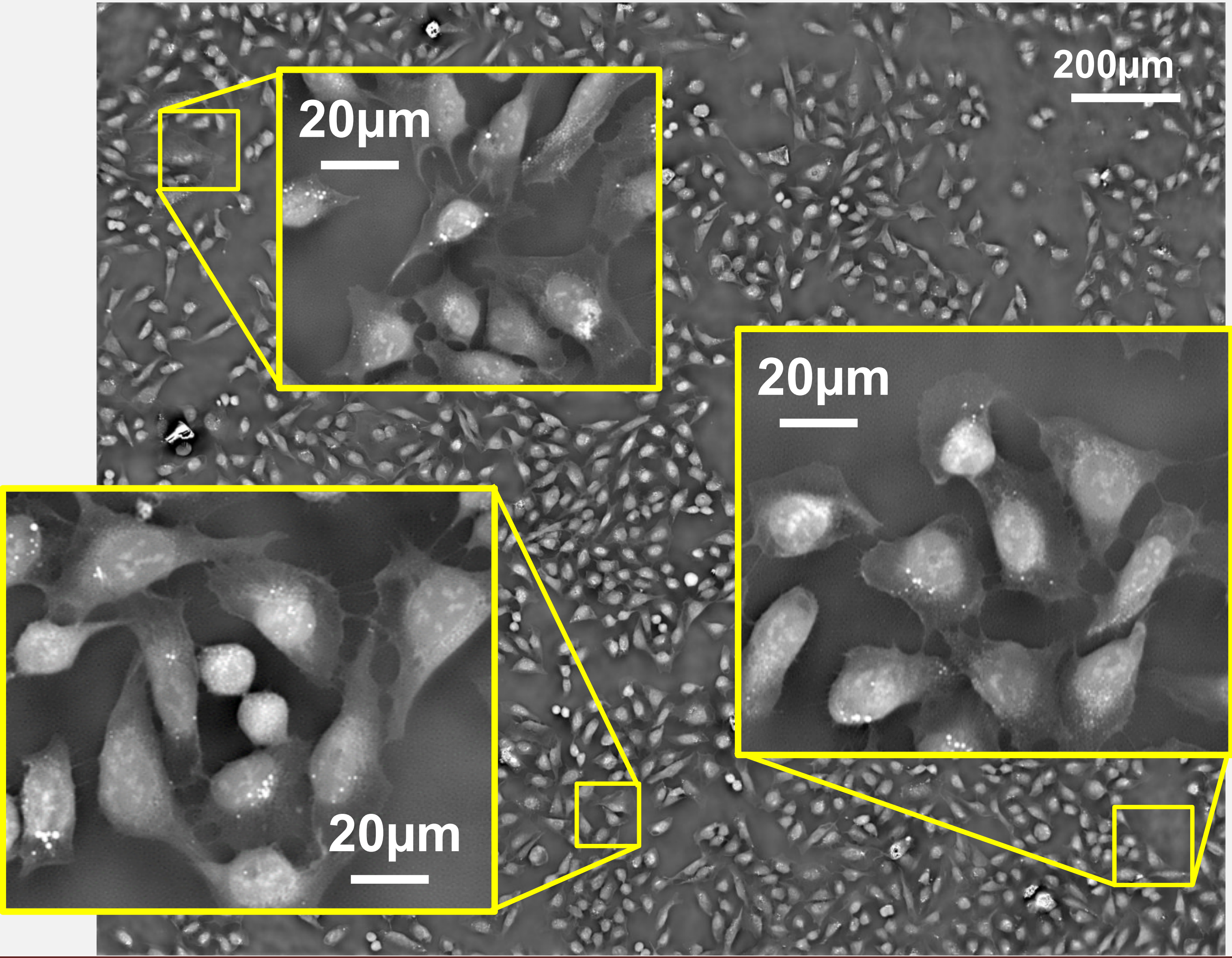
L. Tian and L. Waller, *Opt. Express* 23(9), 11394-11403 (2015).

Spatio-temporal bandwidth engineering by computational microscopy



+ Fast
- Must trade space for spatial bandwidth!

Wide field-of-view *and* high resolution for high-throughput, multi-scale imaging



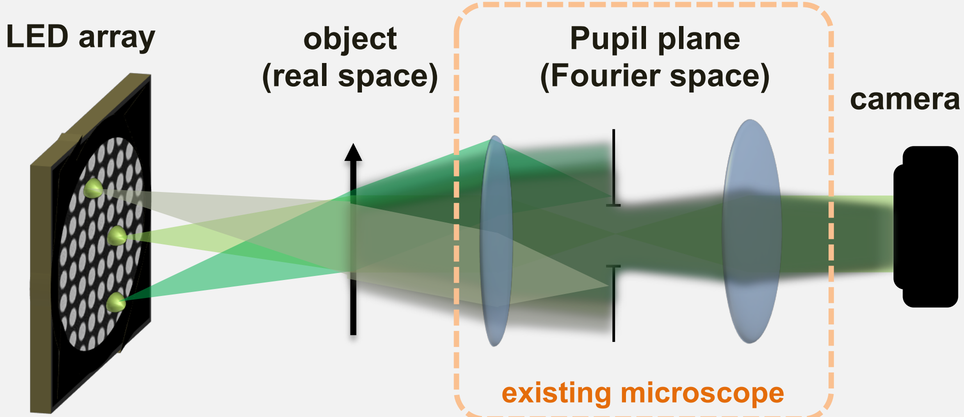
~10,000 cells

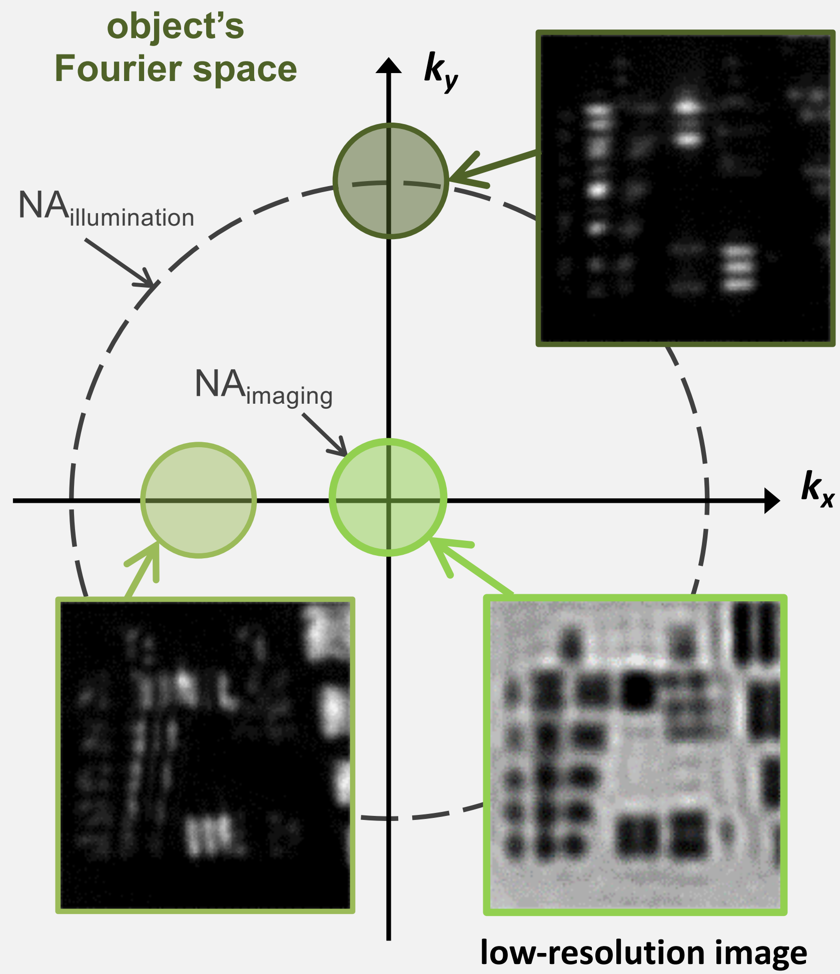
2.1mmx1.7mm
resolution ~400nm

13k x 11k pixels
reconstructed

Unstained
Human Bone
Osteosarcoma
Epithelial U2OS
sample

Fourier ptychography: synthetic aperture + phase retrieval





- Capture data with low magnification objective lens:
  - wide field-of-view
  - but… small bandwidth
- Improve resolution by synthetic aperture
  - $NA_{final} = NA_{illumination} + NA_{imaging}$

Zheng, Horstmeyer, Yang, *Nat. Photon.* (2013)
Tian, Li, Ramchandran, Waller, *Biomed. Opt. Express* (2014).

Phase retrieval by nonlinear optimization

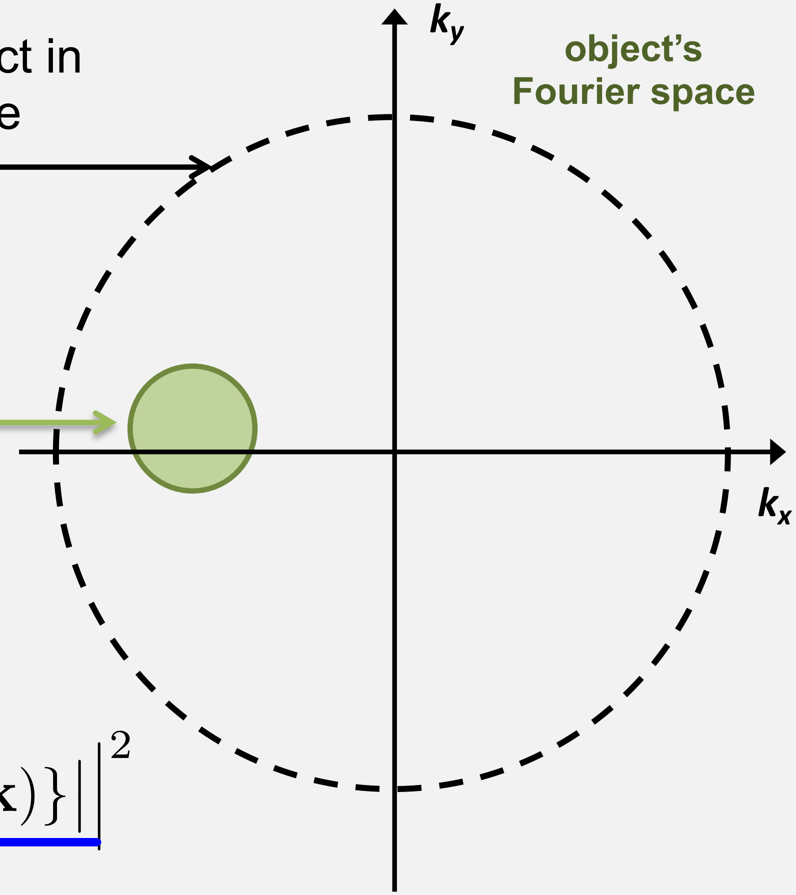
Forward Model

Estimated Object in Fourier space

$$\hat{I}_\ell(\mathbf{r}) = |\,\mathcal{F}^{-1}\{P(\mathbf{k}+\mathbf{k}_\ell)O(\mathbf{k})\}|^2$$

Estimated Intensity

Pupil function



Inverse problem

$$\min_{O(\mathbf{k})} \sum_\ell \sum_{\mathrm{r}} \Big| \underbrace{\sqrt{I_\ell(\mathbf{r})}}_{\text{Measured Amplitude}} - \underbrace{\big|\mathcal{F}^{-1}\{P(\mathbf{k}+\mathbf{k}_\ell)O(\mathbf{k})\}\big|}_{\text{Estimated Amplitude}} \Big|^2$$

Phase diversity:
Fienup, *Appl. Opt.* (1982).
Paxman, Schulz, Fienup, *JOSA A* (1992).
Guizar-Sicairos, Fienup, *Opt. Express* (2008).

Ptychography:
J. Rodenburg, H. Faulkner, *Appl. Phys. Lett.* (2004).
P. Thibault, et al, *Ultramicroscopy* (2009).

Fourier Ptychography:
Zheng, Horstmeyer, Yang, *Nat. Photon.* (2013).
Ou, Yang, *Opt. Express* (2013).
Tian, Li, Ramchandran, Waller, *Biomed. Opt. Express* (2014).

Phase retrieval by nonlinear optimization

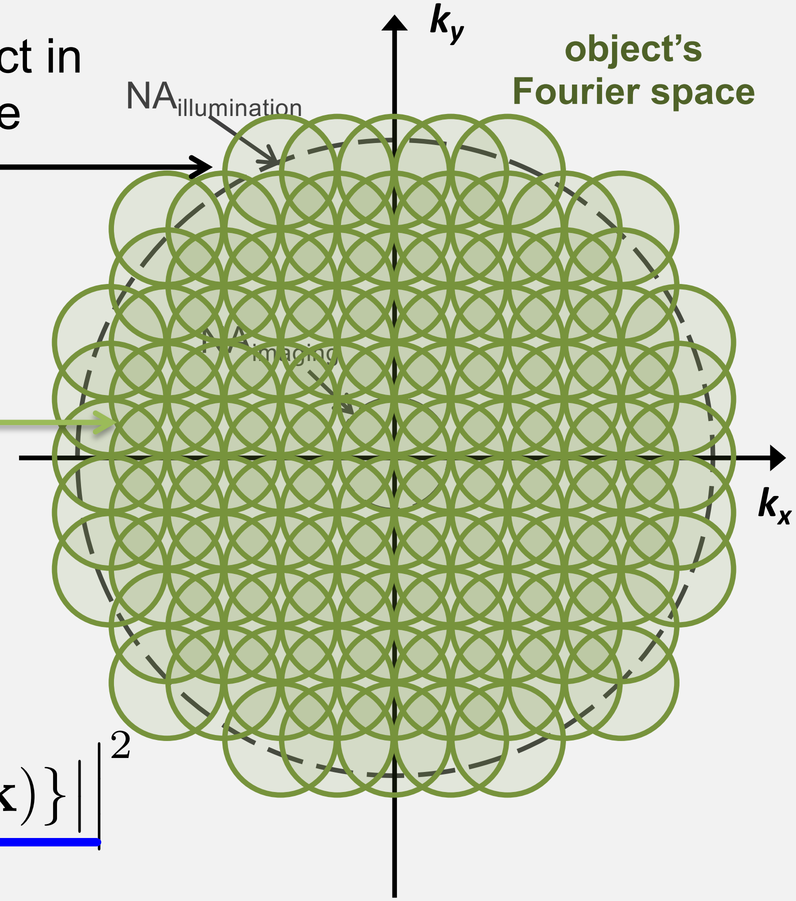
Forward Model

Estimated Object in Fourier space

$$\hat{I}_\ell(\mathbf{r}) = |\,\mathcal{F}^{-1}\{P(\mathbf{k}+\mathbf{k}_\ell)O(\mathbf{k})\}|^2$$

Estimated Intensity

Pupil function



Inverse problem

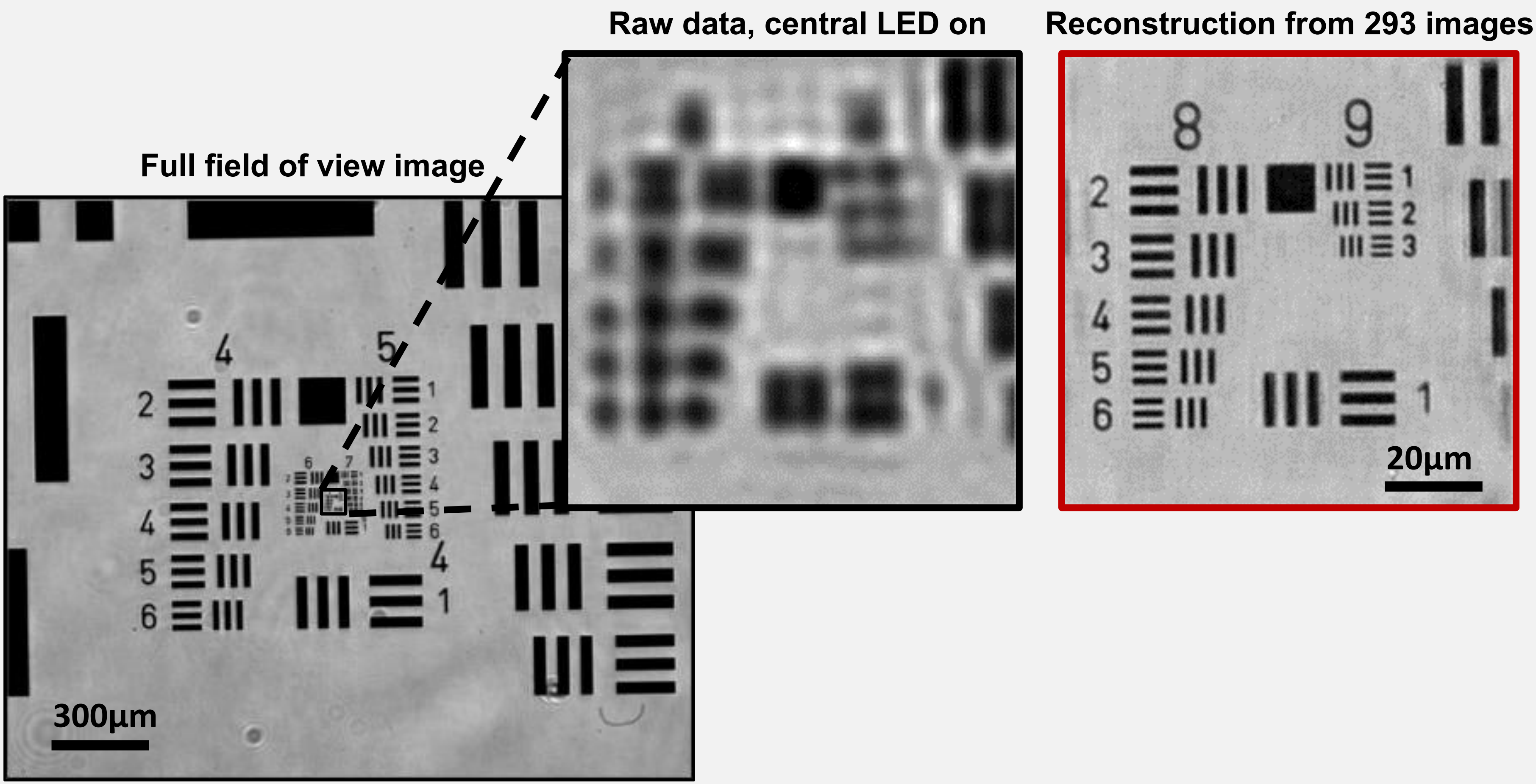
$$\min_{O(\mathbf{k})} \sum_\ell \sum_{\mathrm{r}} \left| \underbrace{\sqrt{I_\ell(\mathbf{r})}}_{\text{Measured Amplitude}} - \underbrace{\left|\mathcal{F}^{-1}\{P(\mathbf{k}+\mathbf{k}_\ell)O(\mathbf{k})\}\right|}_{\text{Estimated Amplitude}} \right|^2$$

Phase diversity:
Fienup, *Appl. Opt.* (1982).
Paxman, Schulz, Fienup, *JOSA A* (1992).
Guizar-Sicairos, Fienup, *Opt. Express* (2008).

Ptychography:
J. Rodenburg, H. Faulkner, *Appl. Phys. Lett.* (2004).
P. Thibault, et al, *Ultramicroscopy* (2009).

Fourier Ptychography:
Zheng, Horstmeyer, Yang, *Nat. Photon.* (2013).
Ou, Yang, *Opt. Express* (2013).
Tian, Li, Ramchandran, Waller, *Biomed. Opt. Express* (2014).

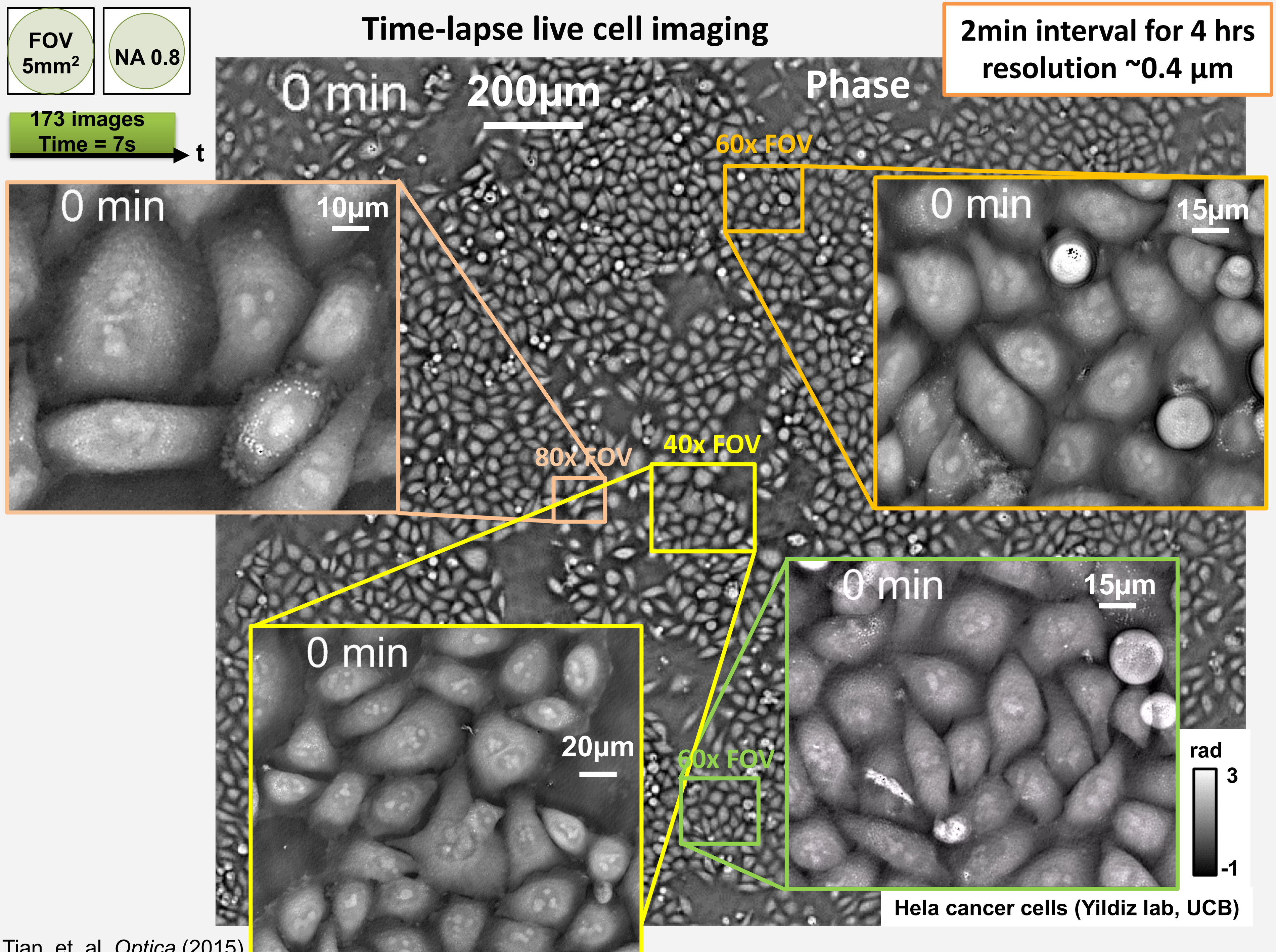
Fourier Ptychography[1] achieves resolution beyond the objective's diffraction limit



4x 0.1NA → $NA_{reconstructed}$ = 0.6

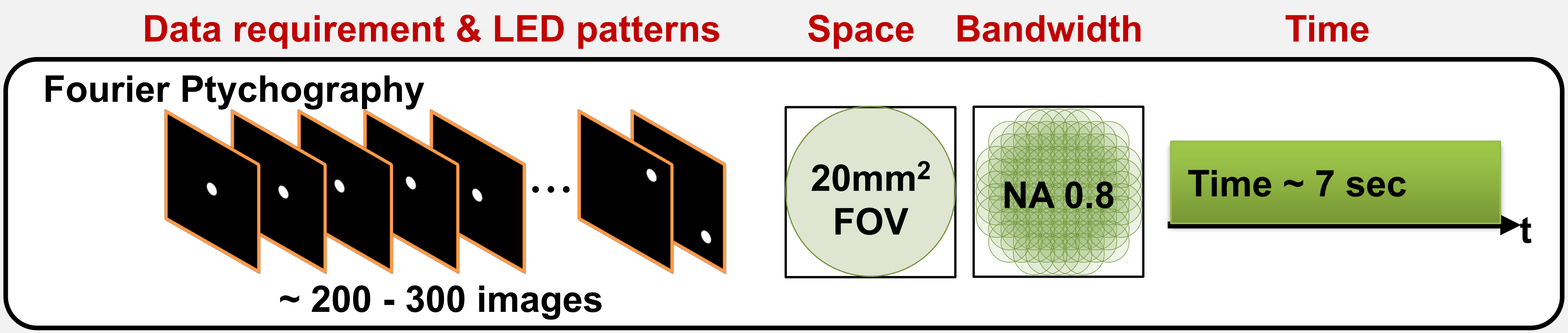
[1] Zheng, Horstmeyer, Yang, *Nat. Photon.* (2013)
[2] Tian, Li, Ramchandran, Waller*, Biomed. Opt. Express* (2014)

Time-lapse live cell imaging



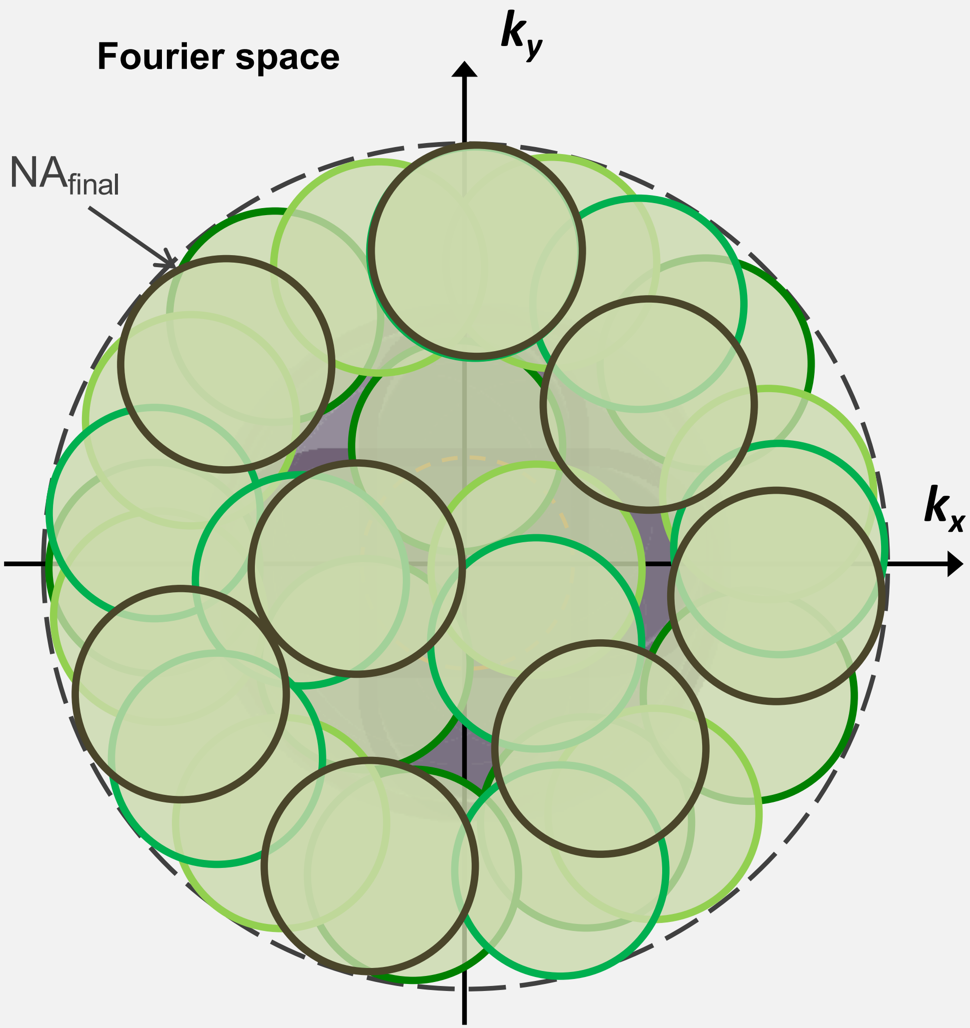
Tian, et. al, *Optica* (2015)

Spatio-temporal bandwidth engineering by computational microscopy



+ Large Space-Bandwidth Product
- Must trade time and large-data requirement!

Hybrid Multiplexing: DPC + random darkfield



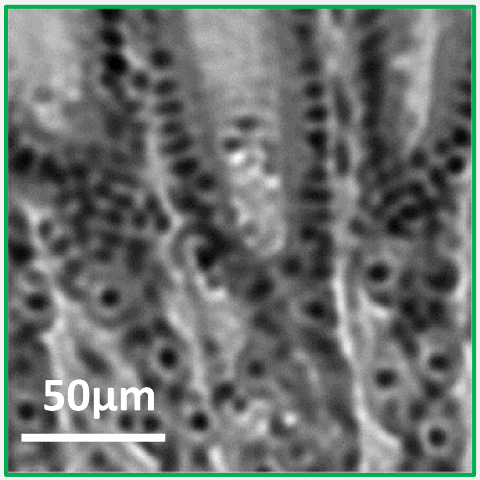
Coding strategy:

1) DPC covers 2NA with only 4 images for all brightfield LEDs
2) Random coding for 8-multiplexed darkfield LEDs

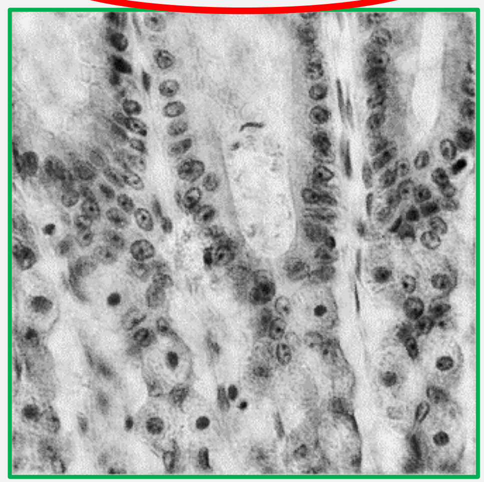
Tian, Li, Ramchandran, Waller, *Biomed. Opt. Express* (2014)
Tian, Liu, Yeh, Chen, Zhong, Waller, "Computational illumination for high-speed *in vitro* Fourier ptychographic microscopy", *Optica* (2015)

Multiplexing reduces acquisition time *and* data size

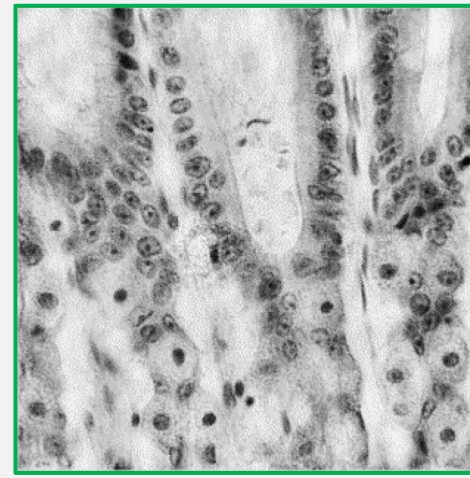
low resolution zoom-in



Sequential method
293 images



Multiplexing
40 images



4x 0.1NA → $NA_{reconstructed} = 0.6$

Only require 13% of data!

Tian, Li, Ramchandran, Waller, *Biomed. Opt. Express* (2014)

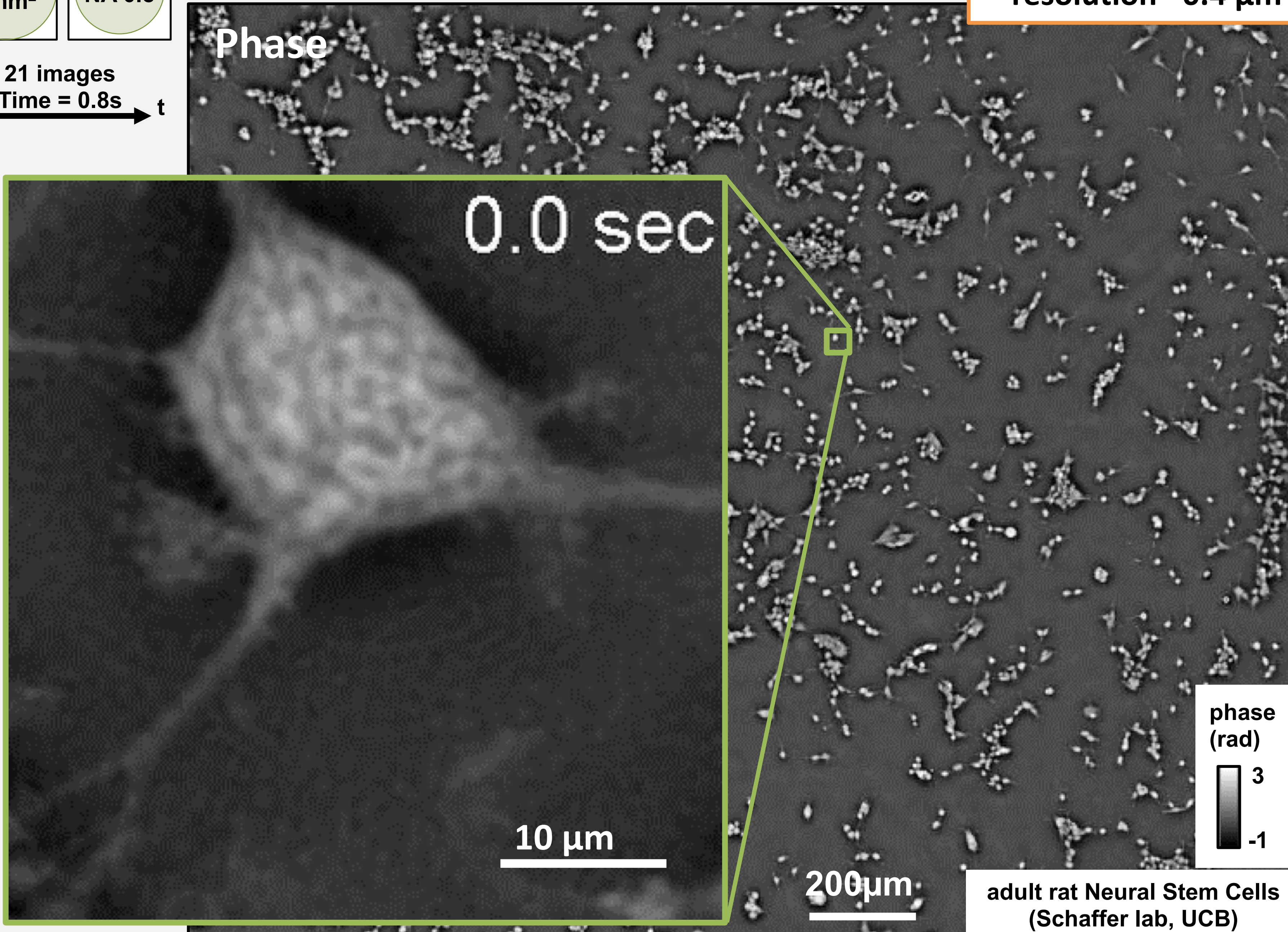
Multi-scale live cell imaging

FOV 5mm^2

NA 0.8

0.8s acquisition time resolution ~0.4 µm

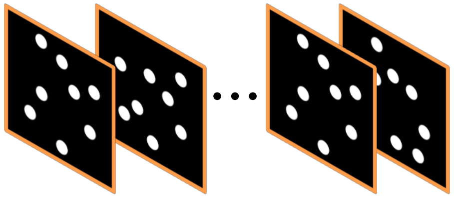
21 images
Time = 0.8s
t



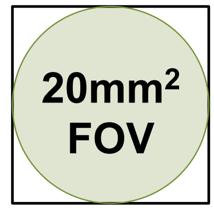
Spatio-temporal bandwidth engineering by computational microscopy

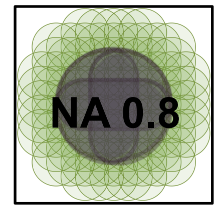
Data requirement & LED patterns **Space** **Bandwidth** **Time**

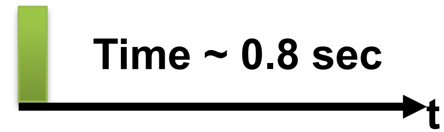
Multiplexed FPM



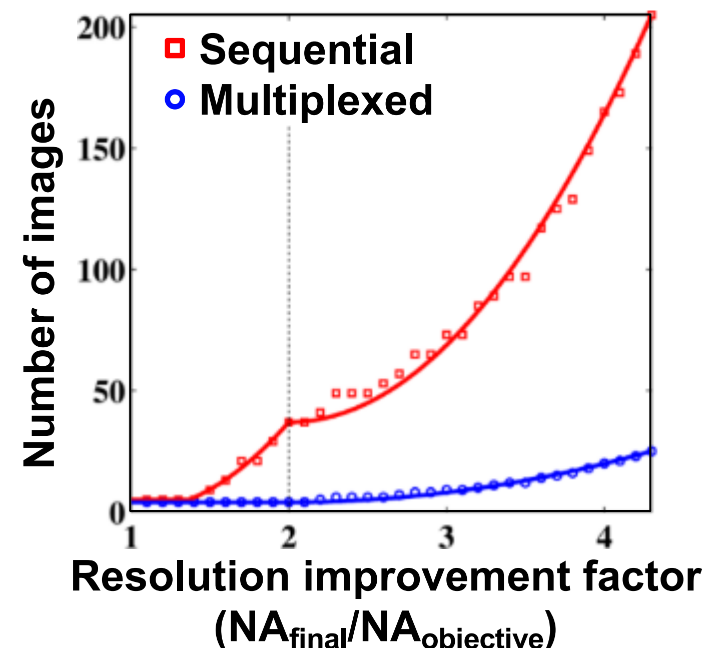
~ 20 - 30 images





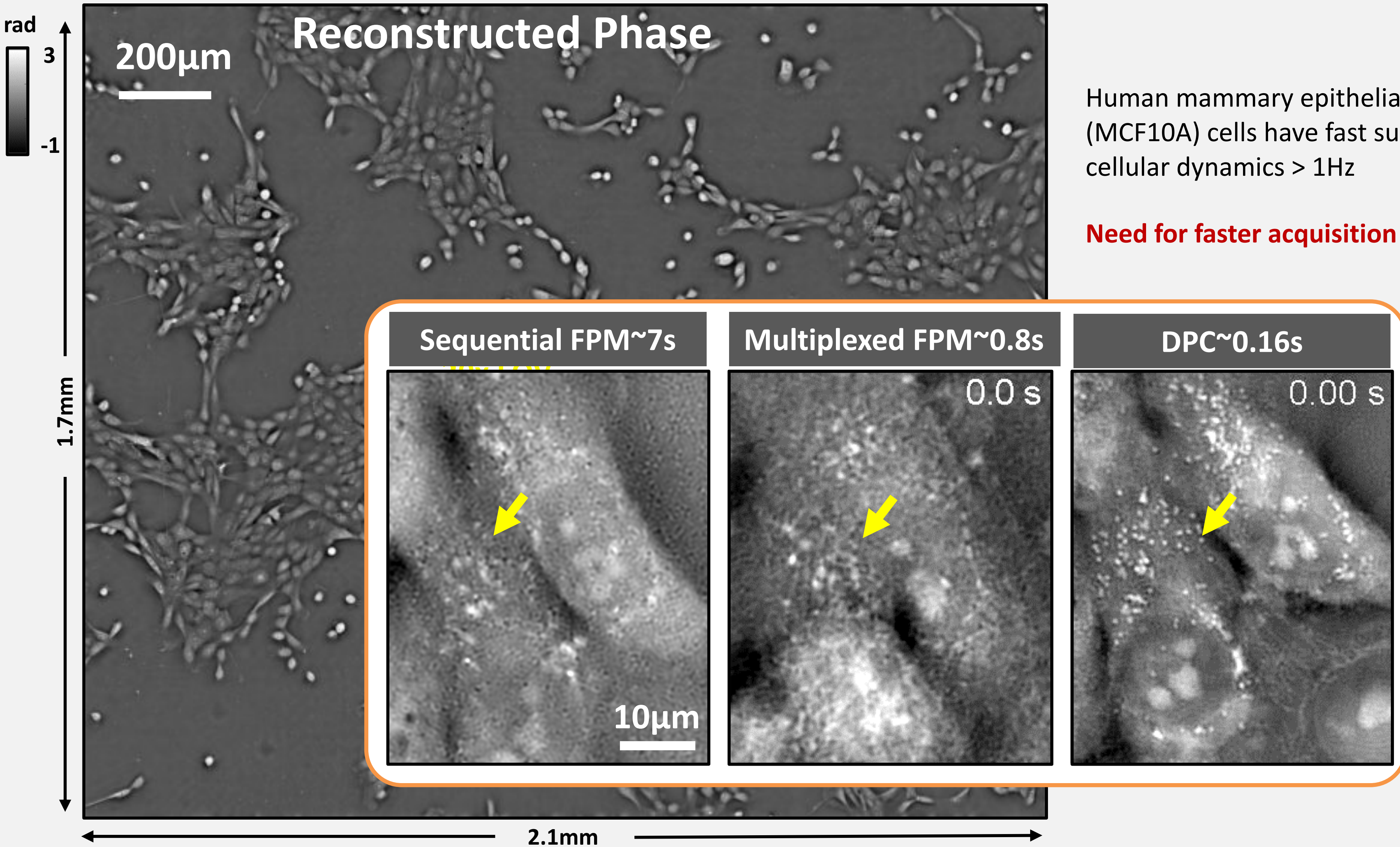


+ Large Space-Bandwidth Product
+ Faster acquisition
- poor scalability for large space-bandwidth product (SBP) imaging



[1] Tian, Liu, Yeh, Chen, Zhong, Waller, "Computational illumination for high-speed *in vitro* Fourier ptychographic microscopy", *Optica* (2015)

Fast dynamics create motion blurs



Human mammary epithelial (MCF10A) cells have fast sub-cellular dynamics > 1Hz

Need for faster acquisition!

Computational Phase Imaging

Hardware & Acquisition design

input field (amplitude & phase) → imaging system → detector (measures only intensity)

Computational strategy can also influence the hardware design & data capture strategy

Computation

find x

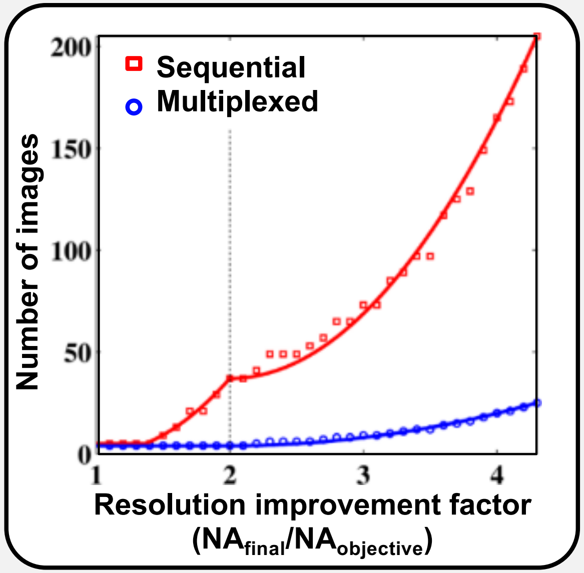
such that Intensity = $|\mathbf{A}x|^2$

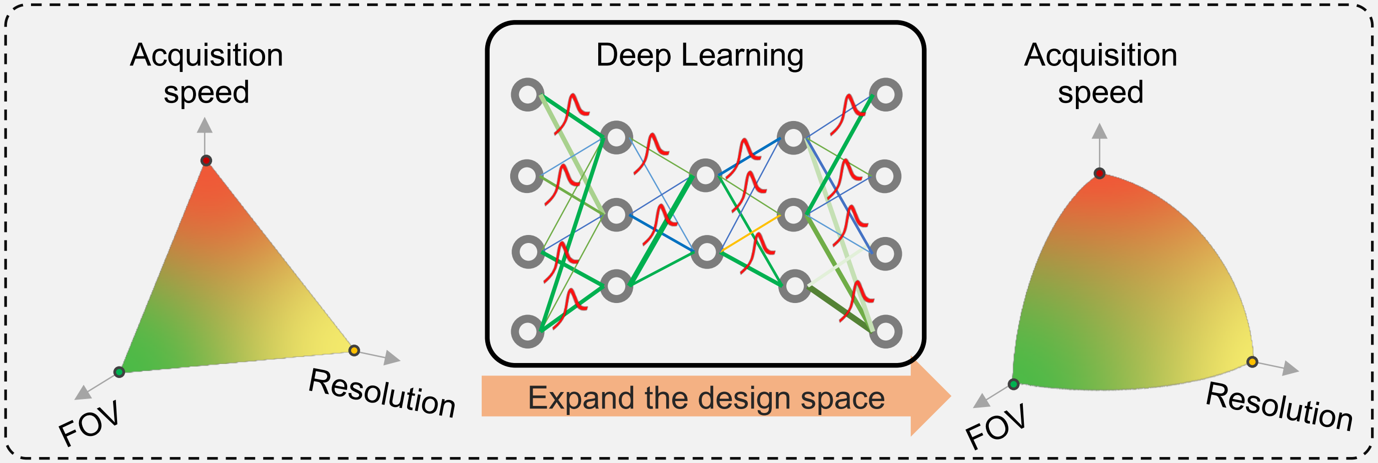
- *Model* based inversion
- ***Learning* based inversion**

How to improve scalability for large-SBP imaging?

» The number of measurements increases ***quadratically*** with final resolution[1]

» How to improve ***scalability*** for Spatio-temporal bandwidth engineering?

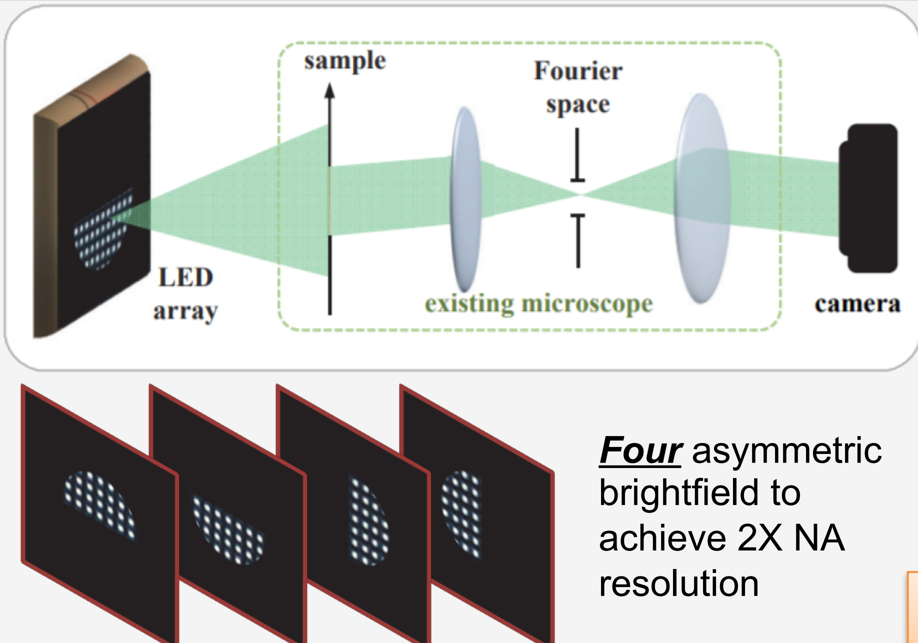




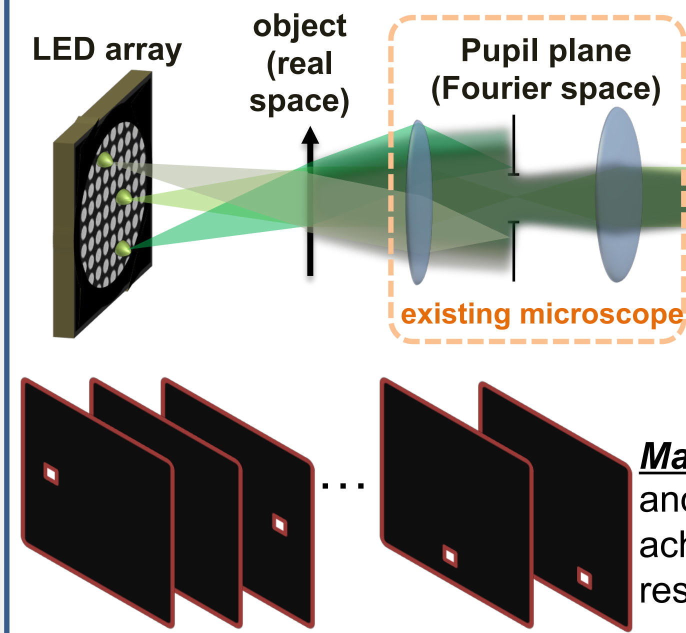
[1] Tian, Liu, Yeh, Chen, Zhong, Waller, "Computational illumination for high-speed *in vitro* Fourier ptychographic microscopy", *Optica* (2015)

Physics-guided deep learning for efficient large-SBP phase imaging

- Asymmetric illumination encodes both phase and high resolution information
  - Differential Phase Contrast Microscopy
  - Fourier Ptychographic Microscopy



Four asymmetric brightfield to achieve 2X NA resolution

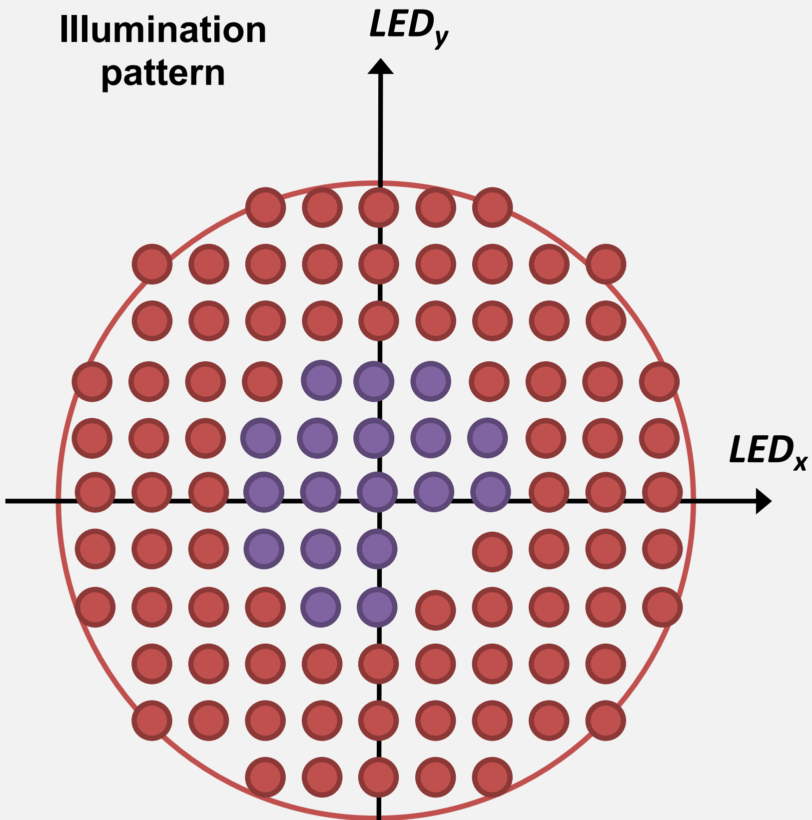


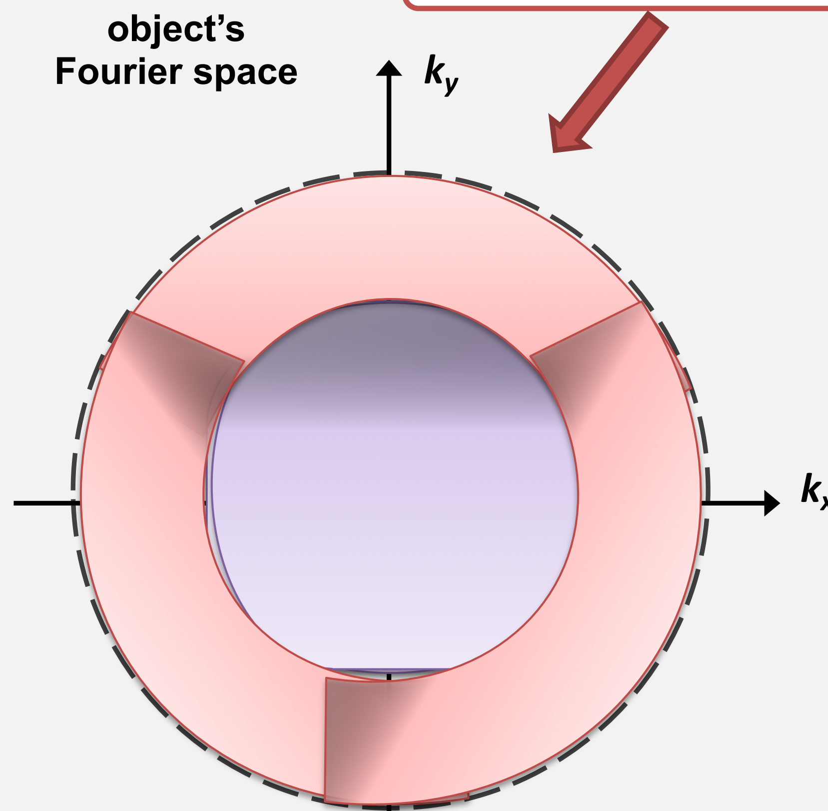
Many brightfield and darkfield to achieve >2X NA resolution

Design more efficient measurement by combining **physics** and **deep learning**

Physics-guided measurements for deep learning using multiplexed illumination

Same Fourier coverage, Highly multiplexed



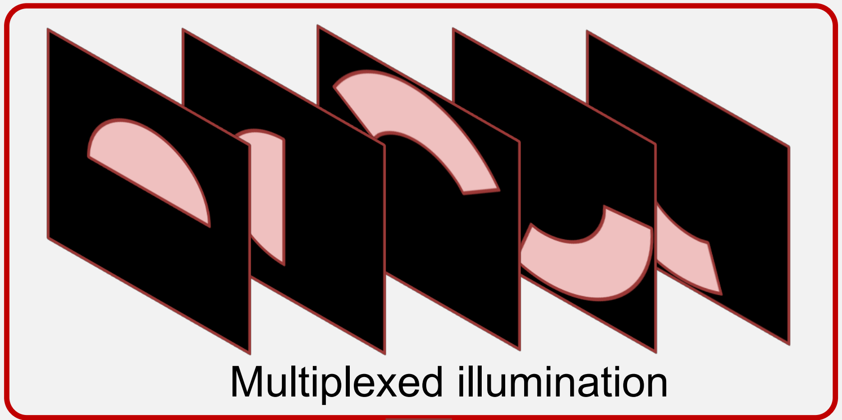


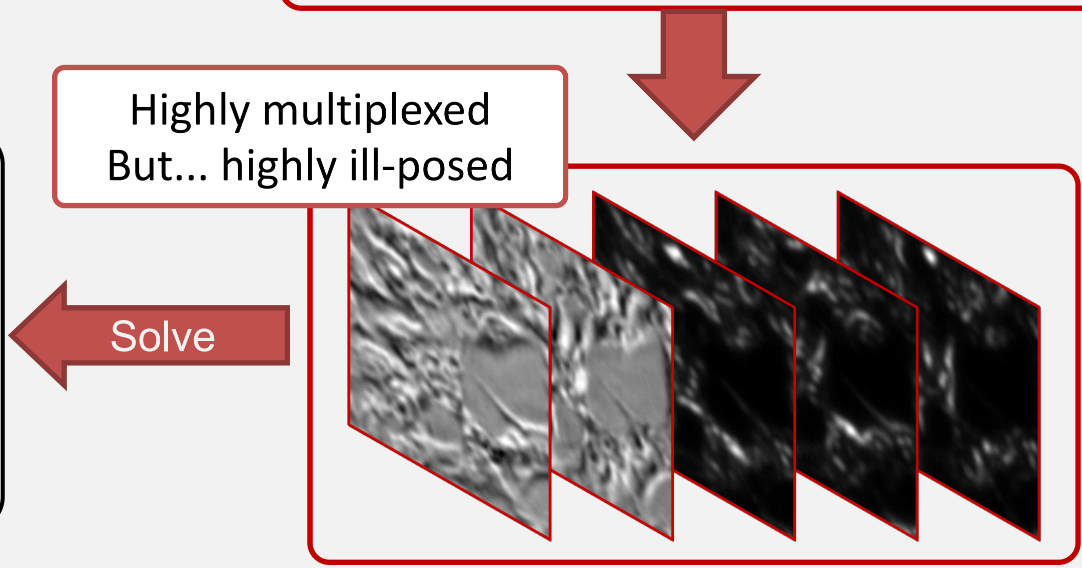
- Asymmetric illumination from **2 brightfield** & **3 darkfield**
- multiplex phase and high resolution efficiently

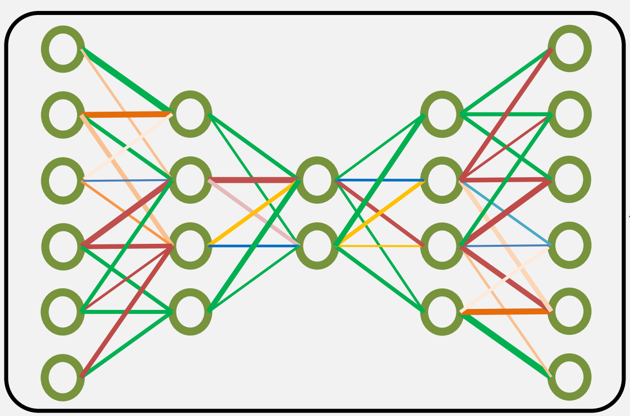
Xue, Cheng, Li, Tian, "Illumination coding meets uncertainty learning: toward reliable AI-augmented phase imaging", *arXiv* (2019)

Physics-guided measurements for deep learning using multiplexed illumination

» 5 measurements ***regardless of final resolution*** $\boldsymbol{NA_{final}}$





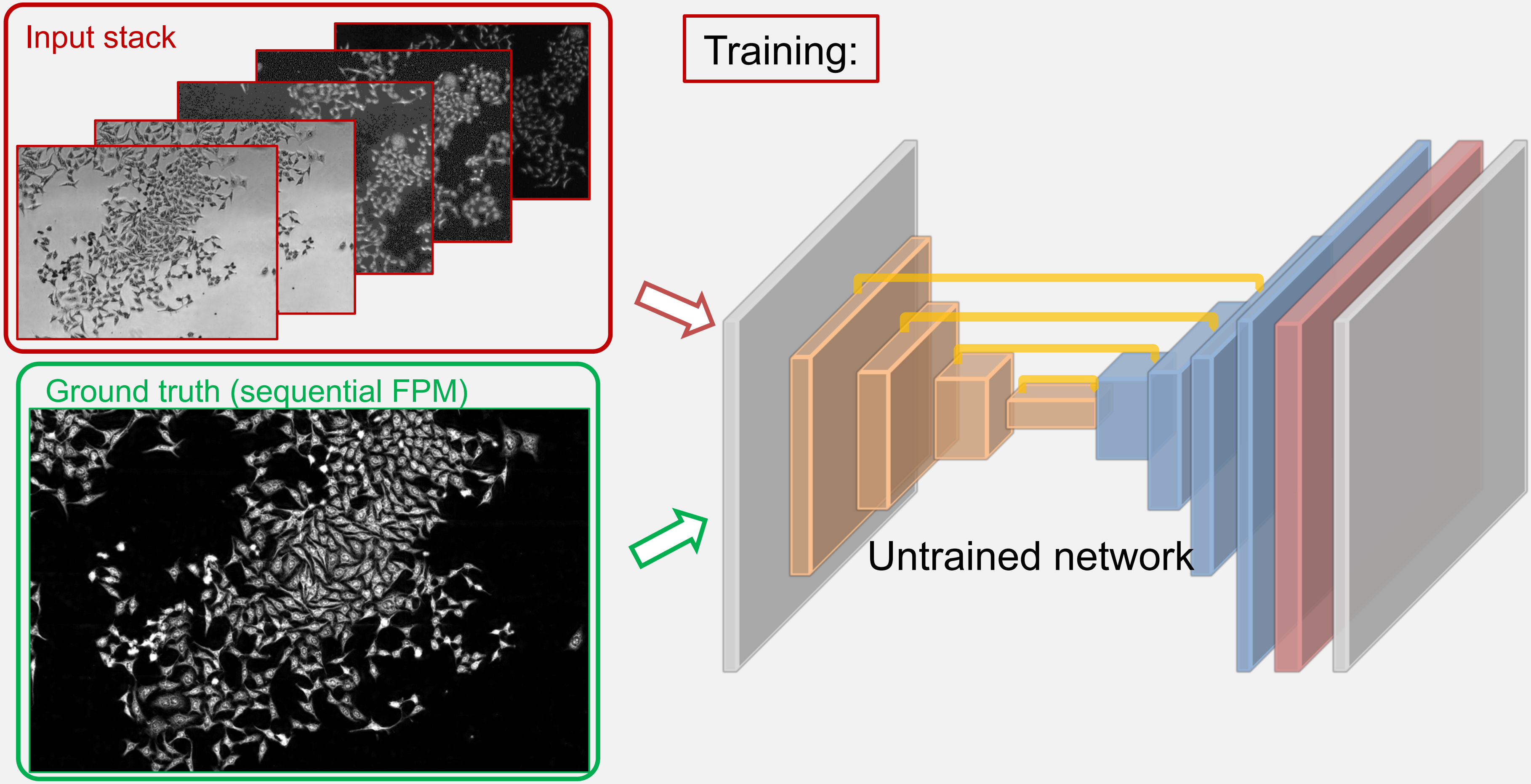


Deep Neural Network (DNN)

[1] Bostan, Soltanolkotabi, Ren, Waller, “Accelerated Wirtinger flow for multiplexed Fourier ptychographic microscopy”, *arXiv* (2018)
[2] Chen, Fannjiang, “Coded aperture ptychography: uniqueness and reconstruction”, *Inverse Problems* (2018)

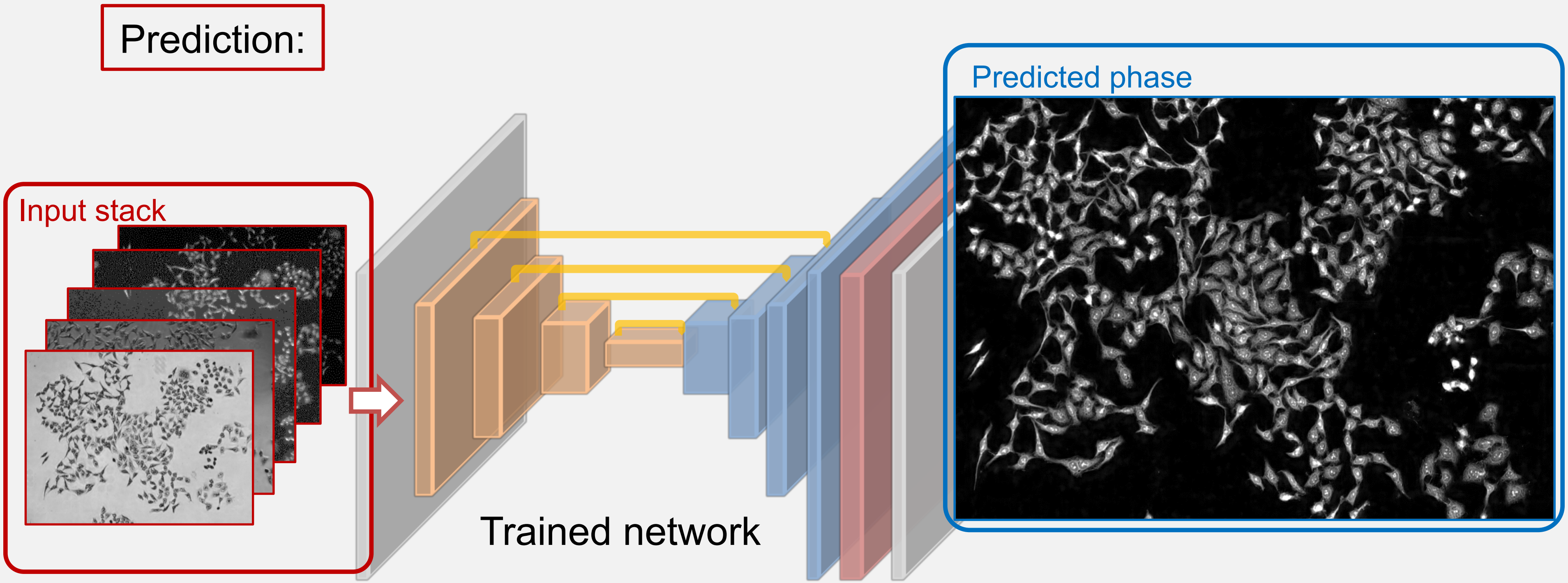
Deep neural network can solve highly ill-posed inverse problems

» Network architecture: a customized "U-Net"[1-2]



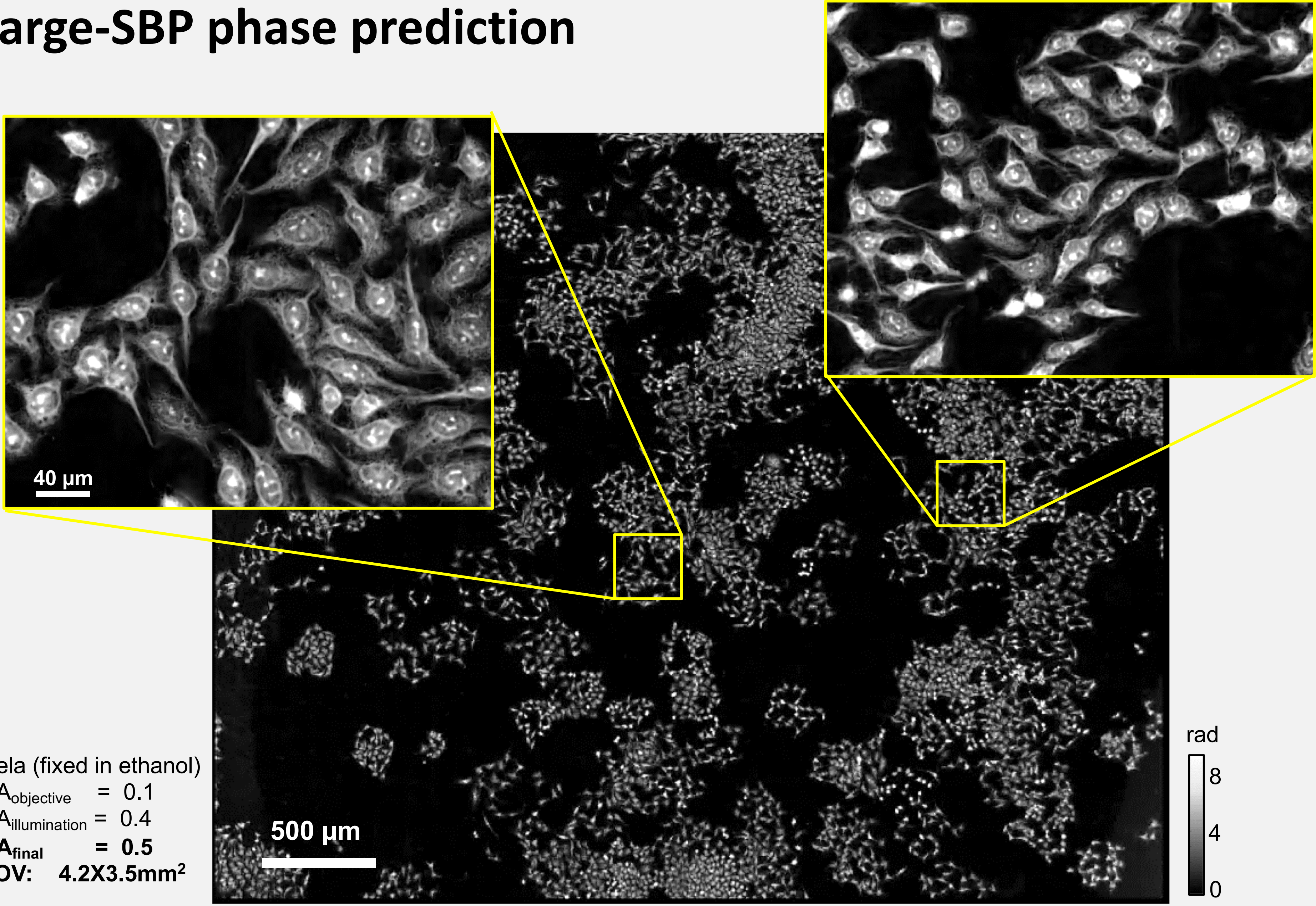
[1] Ronneberger, Fischer, Brox, "U-Net: Convolutional Networks for Biomedical Image segmentation", *MICCAI* (2015)
[2] Falk, et. al., "U-Net: deep learning for cell counting, detection and morphometry", *Nat. Methods* (2018)
[3] Xue, Cheng, Li, Tian , "Illumination coding meets uncertainty learning: toward reliable AI-augmented phase imaging", *arXiv* (2019)

Deep neural network can solve highly ill-posed inverse problems



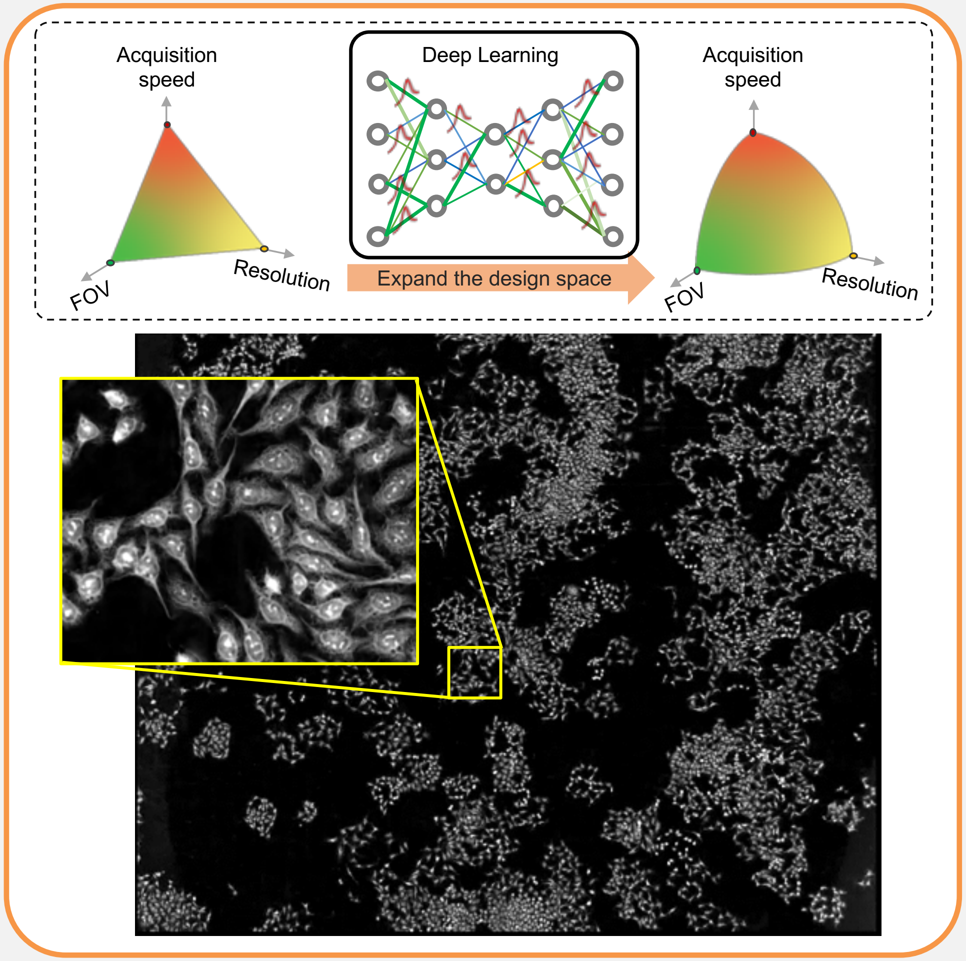
[1] Xue, Cheng, Li, Tian , “Illumination coding meets uncertainty learning: toward reliable AI-augmented phase imaging”, *arXiv* (2019).
[2] Sinha, Ayan, et al. "Lensless computational imaging through deep learning." Optica 4.9 (2017): 1117-1125.
[3] Goy, Alexandre, et al. "Low photon count phase retrieval using deep learning." Physical review letters 121.24 (2018): 243902.
[4] Li, Shuai, et al. "Imaging through glass diffusers using densely connected convolutional networks." optica 5.7 (2018): 803-813.
[5] Nguyen, Thanh, et al. "Deep learning approach for Fourier ptychography microscopy." Optics express 26.20 (2018): 26470-26484.
[6] Rivenson, et al. "Phase recovery and holographic image reconstruction using deep learning in neural networks." Light: Science & Applications (2018).

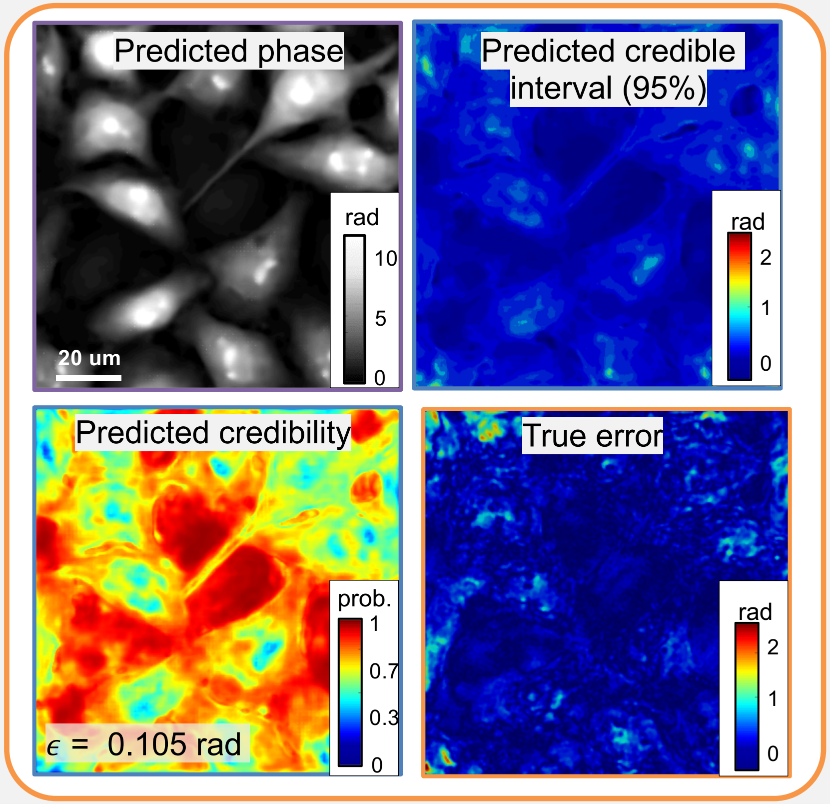
Large-SBP phase prediction



Hela (fixed in ethanol)
$NA_{objective}$ = 0.1
$NA_{illumination}$ = 0.4
NA_{final} = 0.5
FOV: 4.2X3.5mm^2

Scalable and *reliable* deep learning for computational microscopy





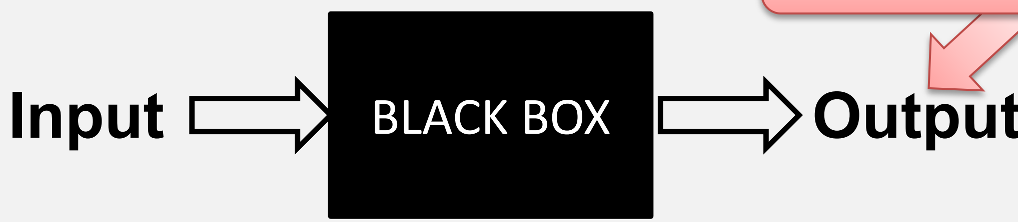
- » ***Physics-guided*** measurement design for efficient large-SBP imaging
- » ***Uncertainty quantification*** towards reliable deep learning

[1] Xue, Cheng, Li, Tian , "Illumination coding meets uncertainty learning: toward reliable AI-augmented phase imaging", *arXiv* (2019).

Need for uncertainty quantification for deep Learning applied to biomedical imaging

- Existing examples of DNN solving nonlinear, complex problems
  - Super resolution[1], phase imaging[2], holography[3], imaging through scattering[4], virtual staining/labeling[5], …
- Though effective, remains a black box

How much should we ***trust*** it?

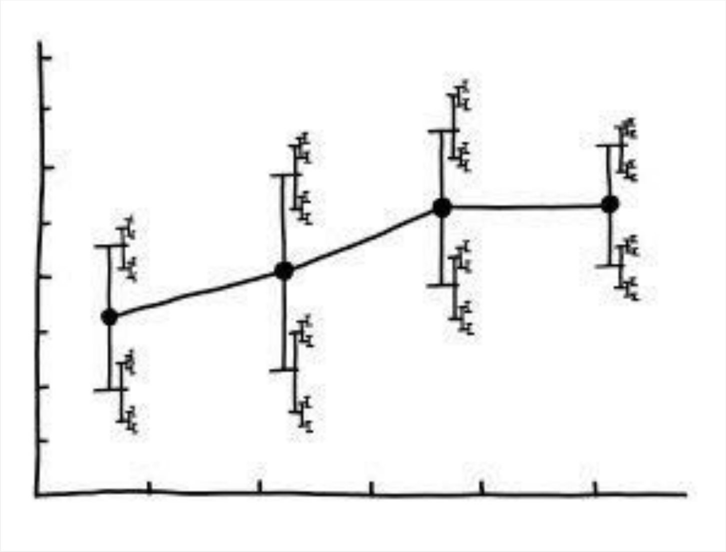


- Importance of ***uncertainty quantification***[7]

[1] Wang, et. al, "Deep learning enables cross-modality super-resolution in fluorescence microscopy", *Nat. Methods* (2019).
[2] Goy, Arthur, Li, Barbastathis, "Low photon count phase retrieval using deep learning", *Phys. Rev. Lett.* (2018)
[3] Rivenson, et al. "Phase recovery and holographic image reconstruction using deep learning in neural networks." Light: Science & Applications (2018).
[4] Li, Xue, Tian, "Deep speckle correlation: a deep learning approach toward scalable imaging through scattering media", *Optica* (2018)
[5] Christiansen, et al. "In silico labeling: Predicting fluorescent labels in unlabeled images." Cell 173.3 (2018): 792-803.
[6] Weigert, et. al.., "Content-aware image restoration: pushing the limits of fluorescence microscopy", *Nat. Methods* (2018)
[7] Begoli, Bhattacharya, Kusnezov, "The need for uncertainty quantification in machine-assisted medical decision making", *Nat. Mach. Intell.* (2019)

Why uncertainty quantification?

How to assess errors in DNN predictions?



DNN hallucinations

Uncertainties in DNN

» Two types of uncertainties in deep learning:

- ***Model*** uncertainty:
- Randomness in training process:
  - Stochastic gradient descent training algorithm
  - Network initialization

  → Trained model varies in different rounds!

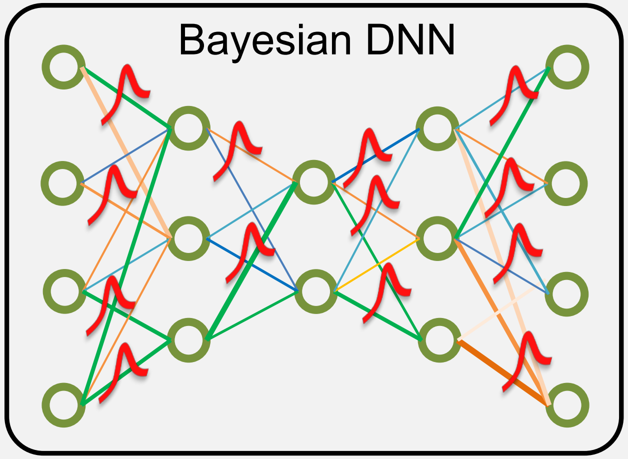
- ***Data*** uncertainty:
- Experimental noise:
  - Sensor noise
  - Misalignment
  - Spatial varying aberration
  - etc.

  → Can lead to prediction artifacts!

How do we quantify uncertainties?
→ Bayesian DNN

[1] Kiureghian, Ditlevsen, "Aleatory or epistemic? Does it matter?", *Struct. Saf.* (2009)
[2] Kendall, Gal, "What uncertainties do we need in Bayesian deep learning for computer vision", *NIPS* (2017)

Overview of Bayesian DNN



» Both DNN **weights** and **predictions** are random variables

» Apply Bayes' rule to DNN prediction…

Data uncertainty term

$$p(\boldsymbol{y}|\boldsymbol{x}^*,\boldsymbol{X},\boldsymbol{Y}) = \int \boxed{p(\boldsymbol{y}|\boldsymbol{x}^*,\boldsymbol{\omega})}\,\boxed{p(\boldsymbol{\omega}|\boldsymbol{X},\boldsymbol{Y})}\, d\boldsymbol{\omega}$$

Model uncertainty term

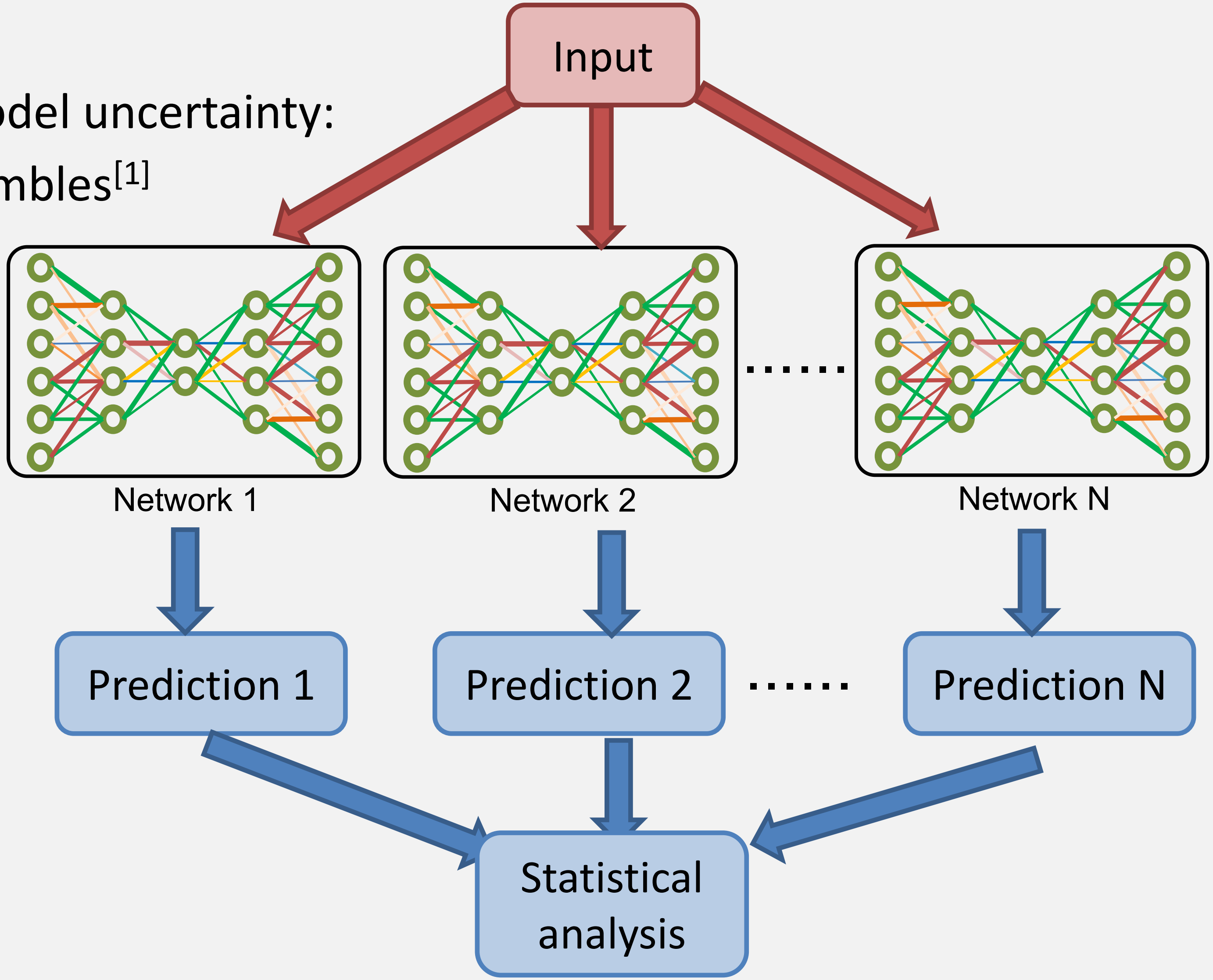
Probability of the **prediction** given
the **training** and **testing** data

[1] Kiureghian, Ditlevsen, "Aleatory or epistemic? Does it matter?", *Struct. Saf.* (2009)
[2] Kendall, Gal, "What uncertainties do we need in Bayesian deep learning for computer vision", *NIPS* (2017)

Model uncertainty quantifies stochasticity in DNN

- To quantify model uncertainty:
  - Deep Ensembles[1]

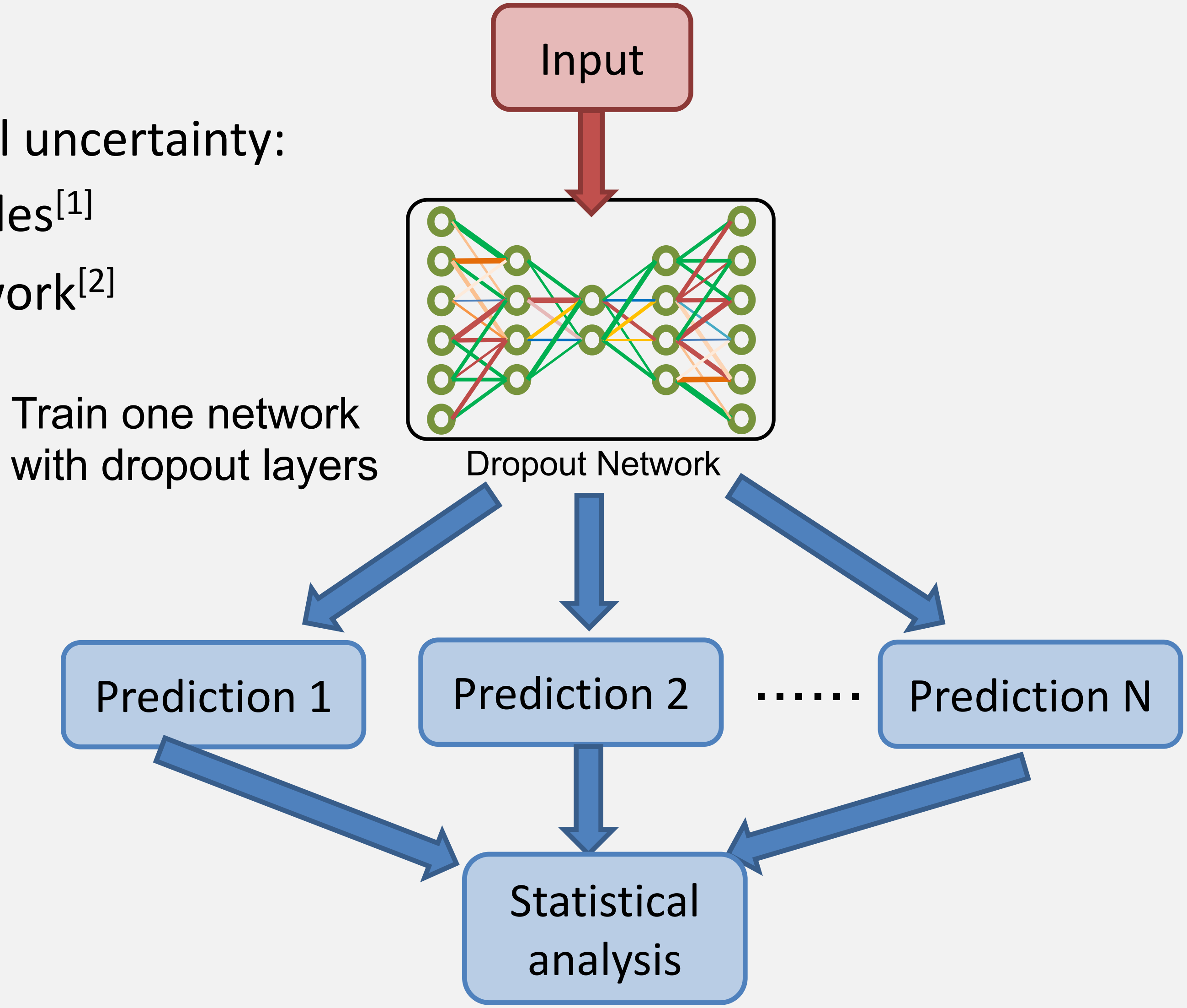
Train multiple networks with same data and structure



[1] Lakshminarayan, Pritzel, Blundell, "Simple and scalable Predictive Uncertainty Estimation using Deep Ensembles", *NIPS*, (2017)
[2] Gal, Ghahramani, "Dropout as a Bayesian Approximation: Representing Model Uncertainty in Deep Learning", *ICML* (2016)

Model uncertainty quantifies stochasticity in DNN

- To quantify model uncertainty:
  - Deep Ensembles[1]
  - Dropout Network[2]



[1] Lakshminarayan, Pritzel, Blundell, "Simple and scalable Predictive Uncertainty Estimation using Deep Ensembles", *NIPS*, (2017)
[2] Gal, Ghahramani, "Dropout as a Bayesian Approximation: Representing Model Uncertainty in Deep Learning", *ICML* (2016)

Data uncertainty quantifies randomness in data

- To quantify data uncertainty:
  - Commonly used loss function: mean squared error (MSE), mean absolute error (MAE), etc.

Assumes uniform noise across all measurements

  - **Our customized loss function**:

Pixel-wise variance allows quantification of ***non*-uniform** noise

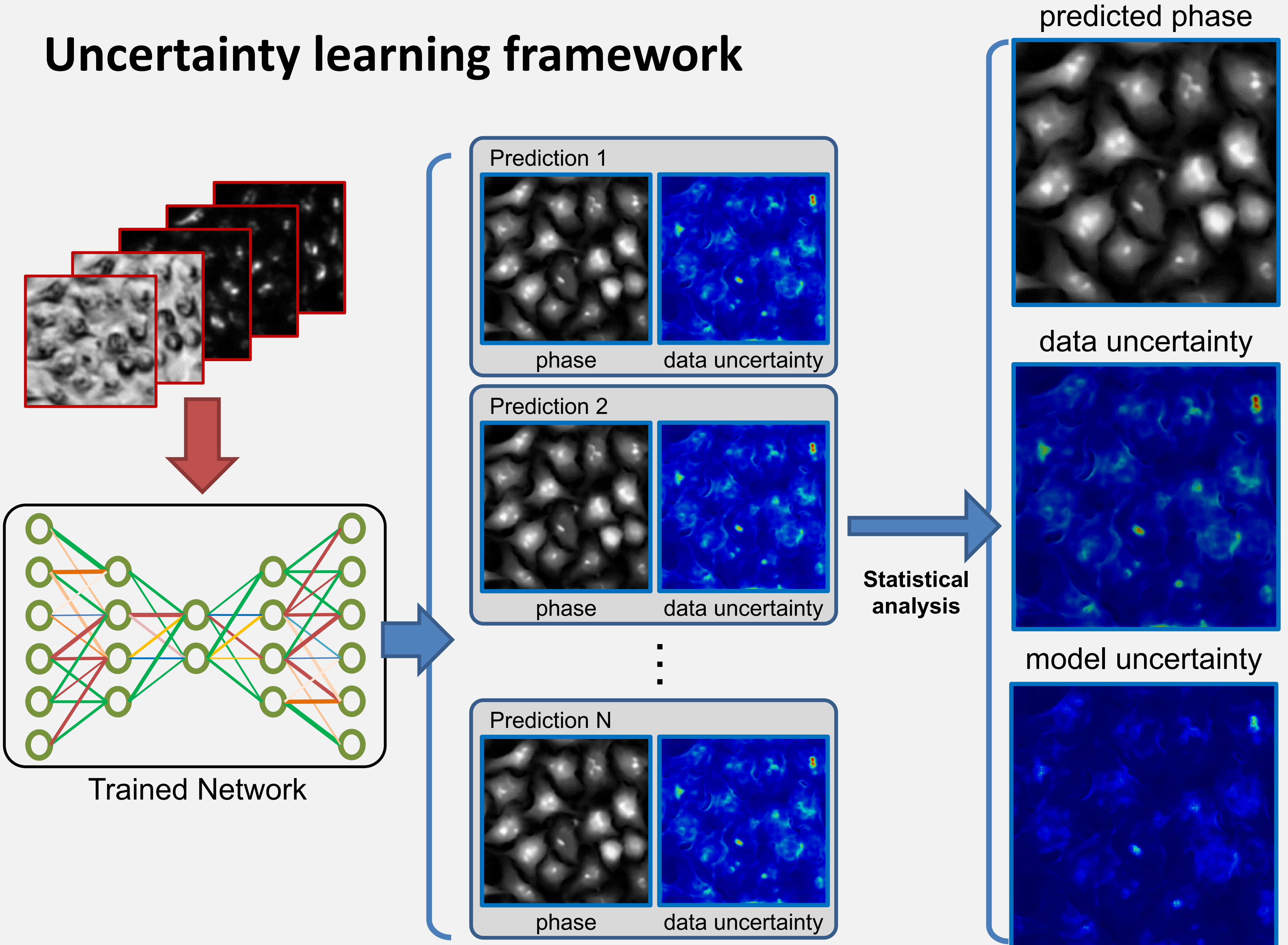
$$Loss = \sum \left[\frac{|y - \mu^{pred}|}{\sigma^{pred}} + \log(2\sigma^{pred}) \right]$$

- Only samples (x, y) needed for training
- Network "learns" pixel-wise mean & variance from data

μ^{pred}: pixel-wise mean
σ^{pred}: pixel-wise variance

Xue, Cheng, Li, Tian , "Illumination coding meets uncertainty learning: toward reliable AI-augmented phase imaging", *arXiv* (2019)

Uncertainty learning framework



Statistical analysis for uncertainty quantification

Total uncertainty:

$$\hat{\sigma}_i^2 \equiv var(y_i|\boldsymbol{x}^*,\boldsymbol{X},\boldsymbol{Y}) = \mathrm{E}[Var(y_i|\boldsymbol{\omega},\boldsymbol{x}^*)] + Var(\mathrm{E}[y_i|\boldsymbol{\omega},\boldsymbol{x}^*])$$

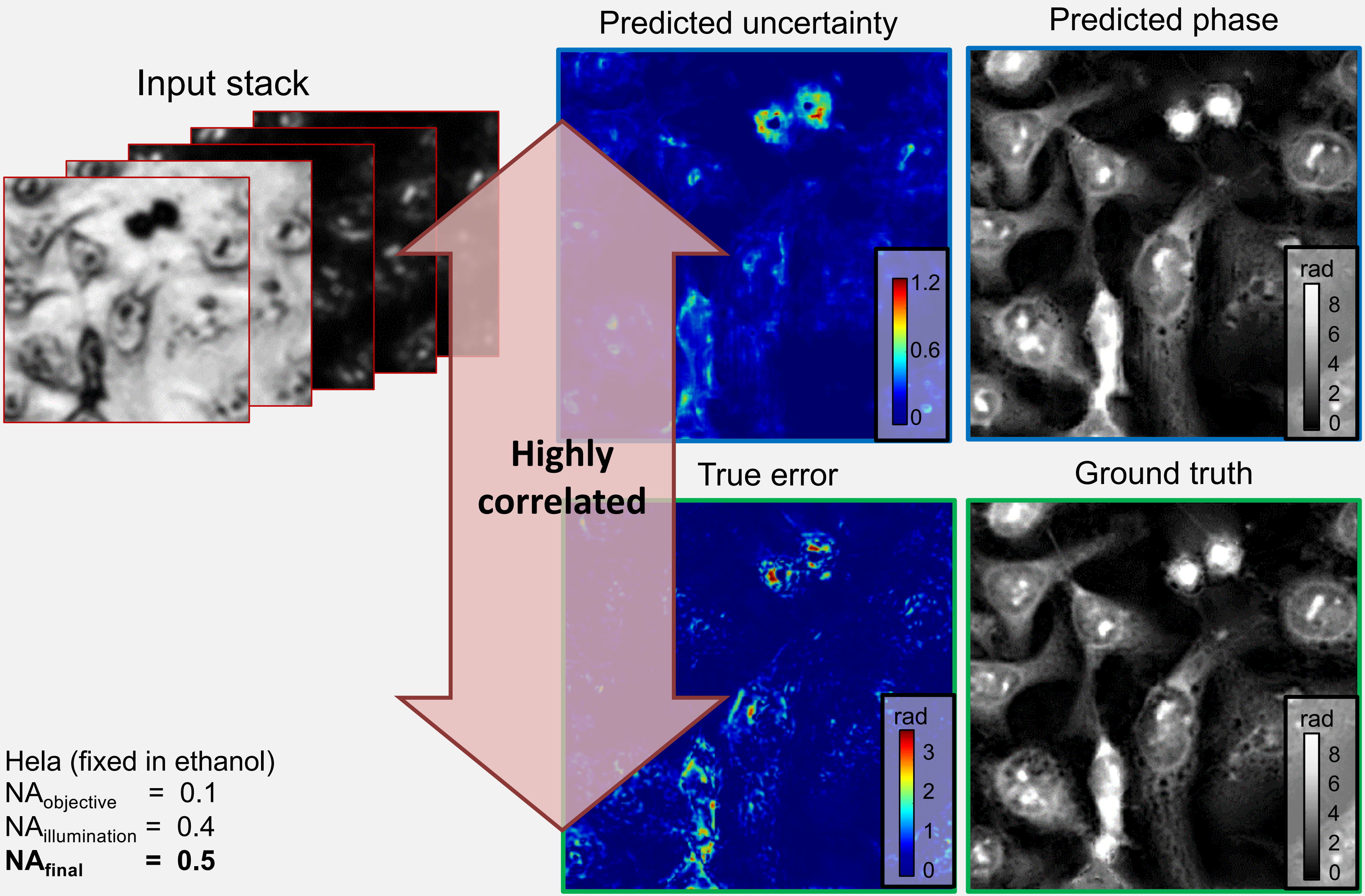
$$\approx \boxed{\frac{1}{T}\sum_{t=1}^{T} 2\left(\sigma_i^t\right)^2} + \boxed{\frac{1}{T}\sum_{t=1}^{T}\left(\mu_i^t - \hat{\mu}_i\right)^2} = (\sigma_i^D)^2 + (\sigma_i^M)^2$$

data uncertainty:
mean of the predicted variance

model uncertainty:
variance of the predicted mean (phase)

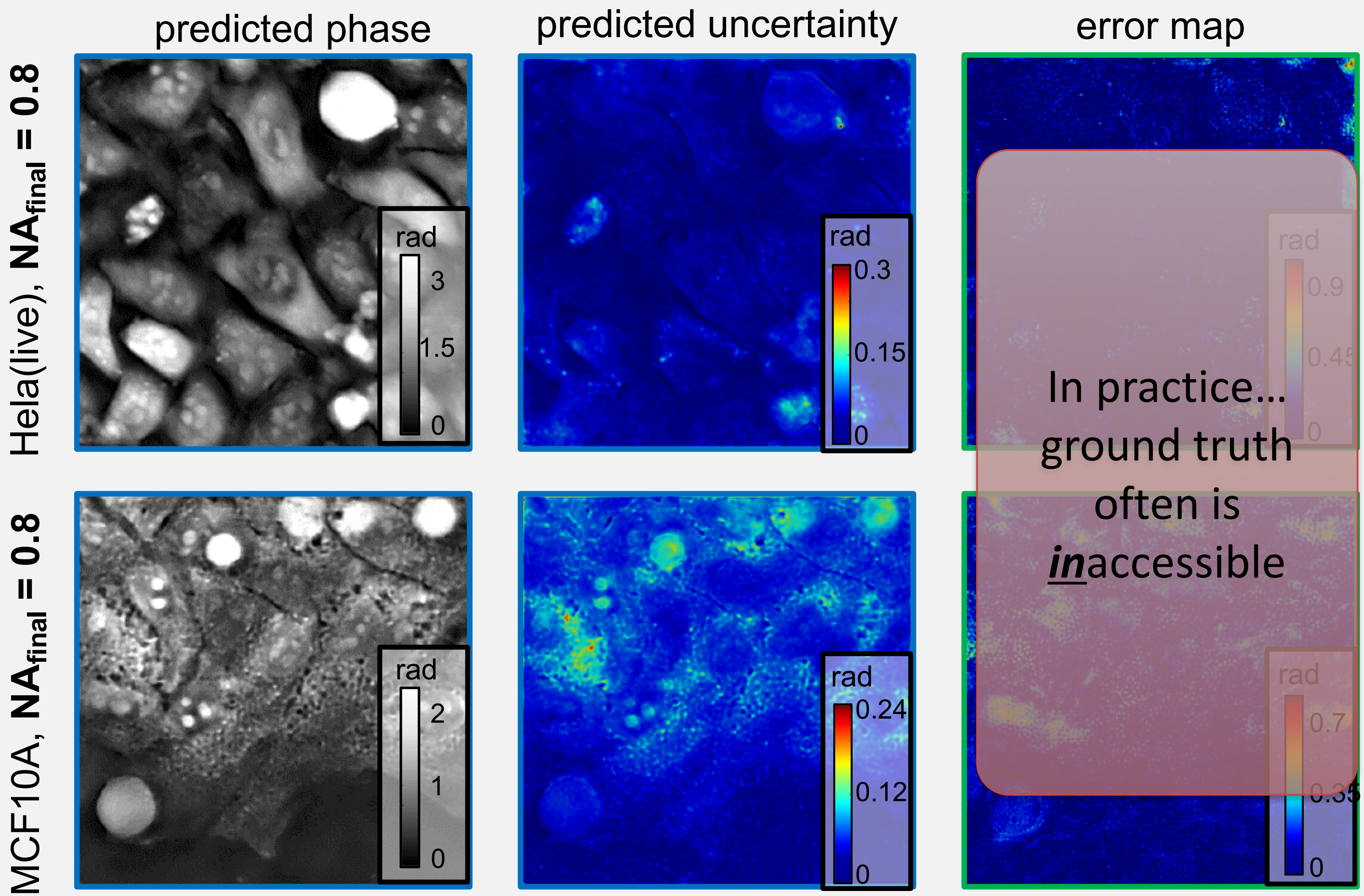
Xue, Cheng, Li, Tian , “Illumination coding meets uncertainty learning: toward reliable AI-augmented phase imaging”, *arXiv* (2019)

Predicted uncertainty correlates with true error



Hela (fixed in ethanol)
$NA_{objective} = 0.1$
$NA_{illumination} = 0.4$
$\mathbf{NA_{final} = 0.5}$

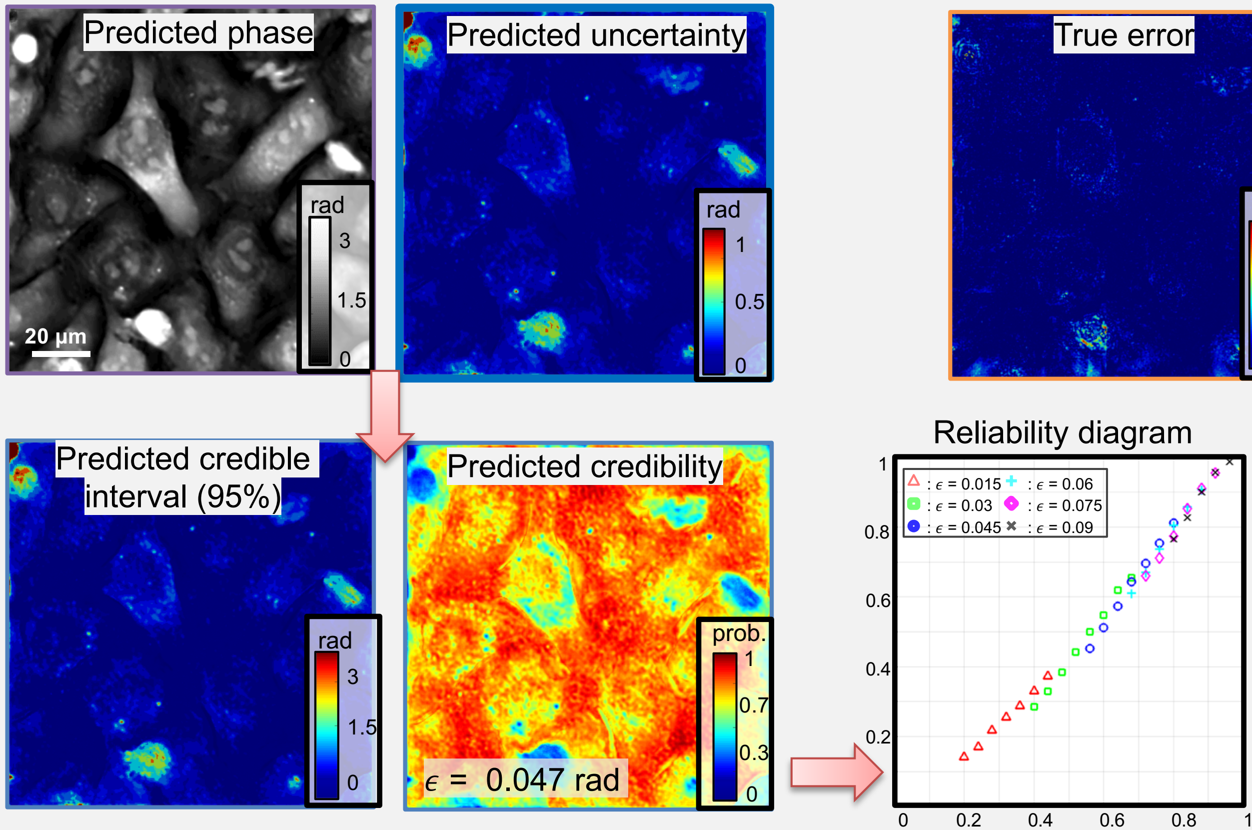
Scalability in cell types and resolution



Predicted uncertainty as surrogate to the true error

[1] Xue, Cheng, Li, Tian , "Illumination coding meets uncertainty learning: toward reliable AI-augmented phase imaging", *arXiv* (2019)

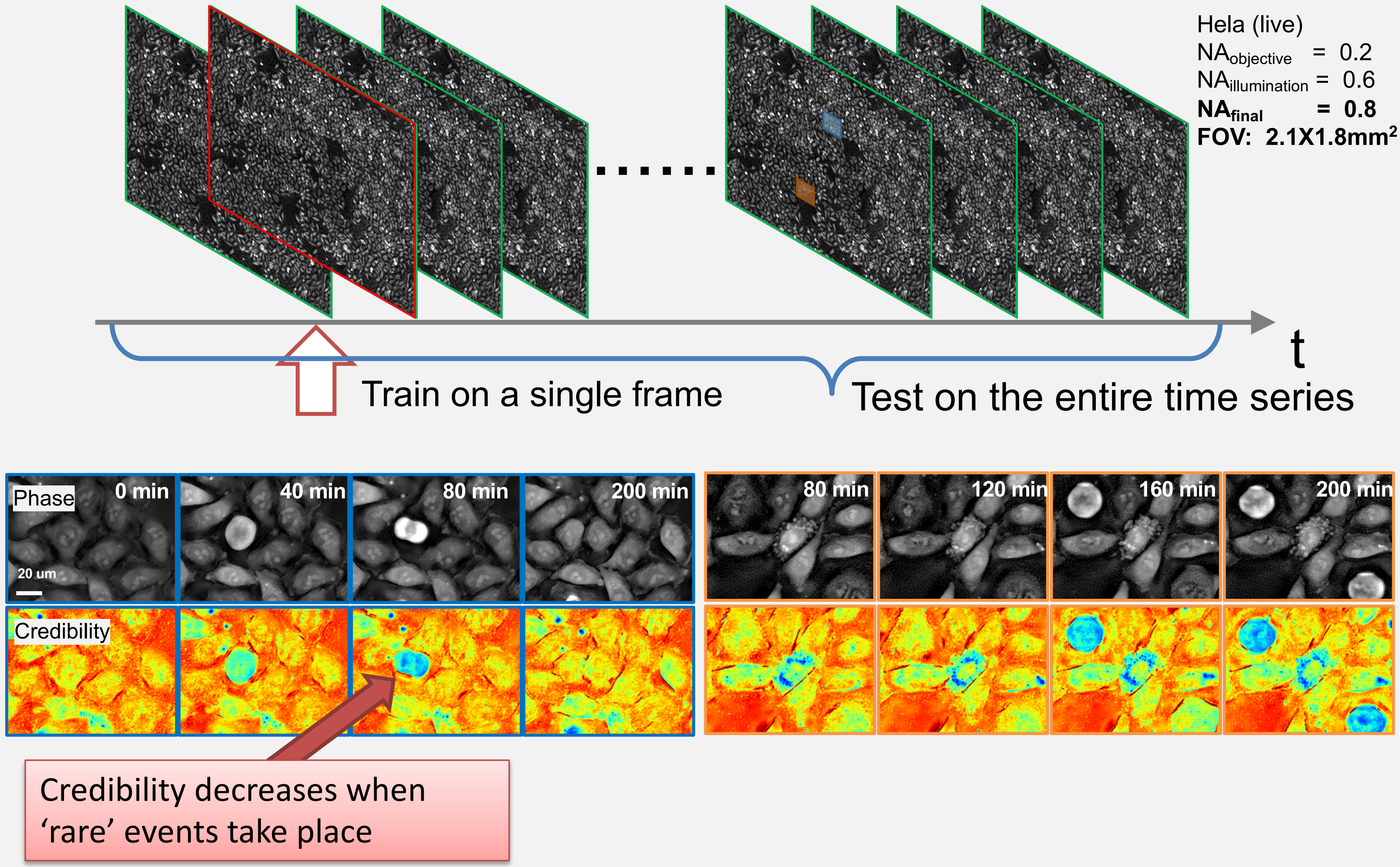
Reliability assessment by Bayesian statistical inference

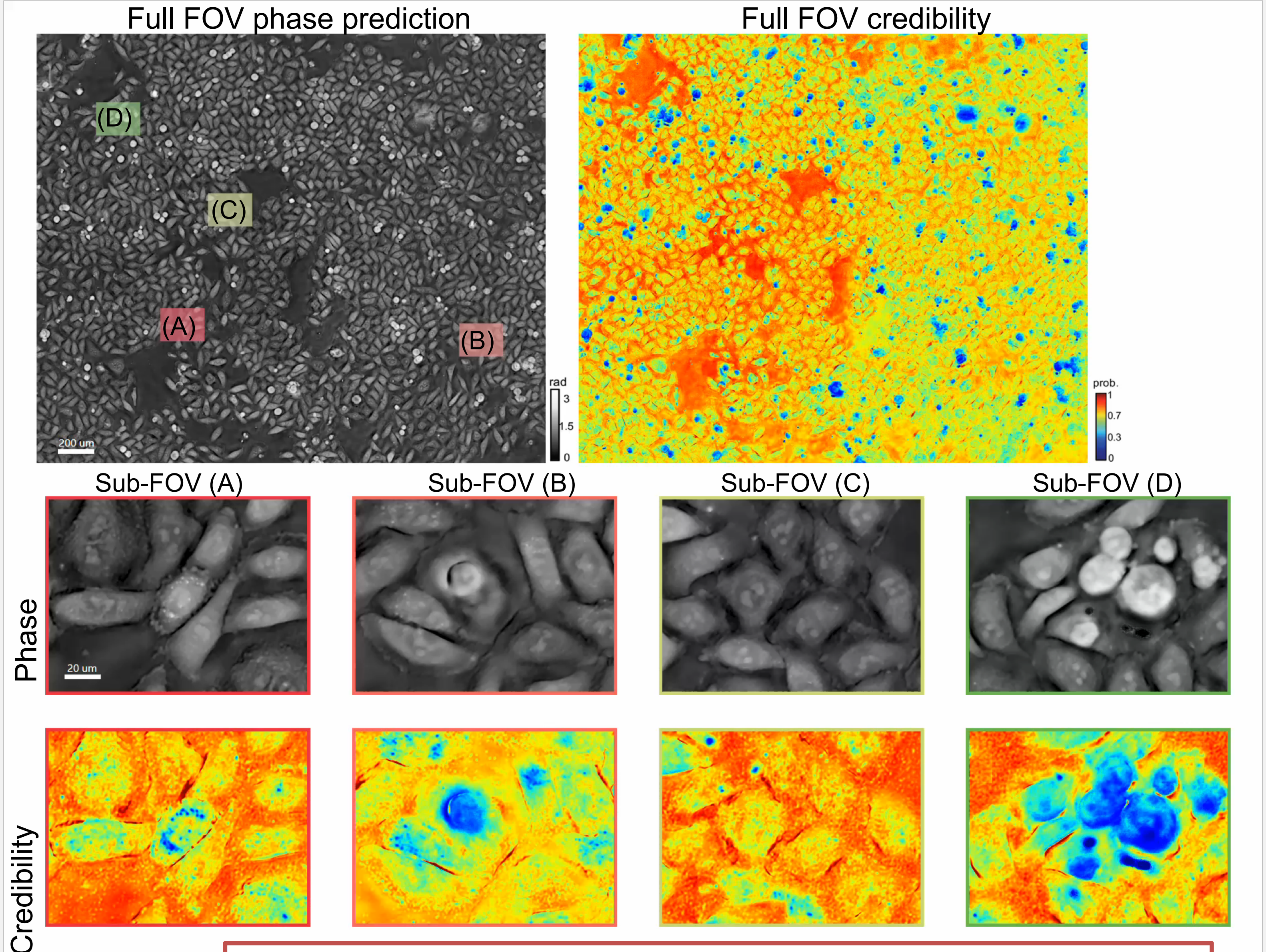


- Quantification of ***reliability*** of the predicted phase
  - **Credible interval** quantitatively estimates the *error bound* in the prediction
  - **Credibility** provides a *probabilistic* measure of the reliability of prediction in %
  - **Reliability diagram** measures *predicted credibility* vs *true accuracy*.

[1] Xue, Cheng, Li, Tian , “Illumination coding meets uncertainty learning: toward reliable AI-augmented phase imaging”, *arXiv* (2019)
[2] Niculescu-Mizil, Caruana, “Predicting good probabilities with supervised learning”, *ICML*, (2005)

Time series prediction and identification of rare events





Hela (live)

$NA_{objective} = 0.2$

$NA_{illumination} = 0.6$

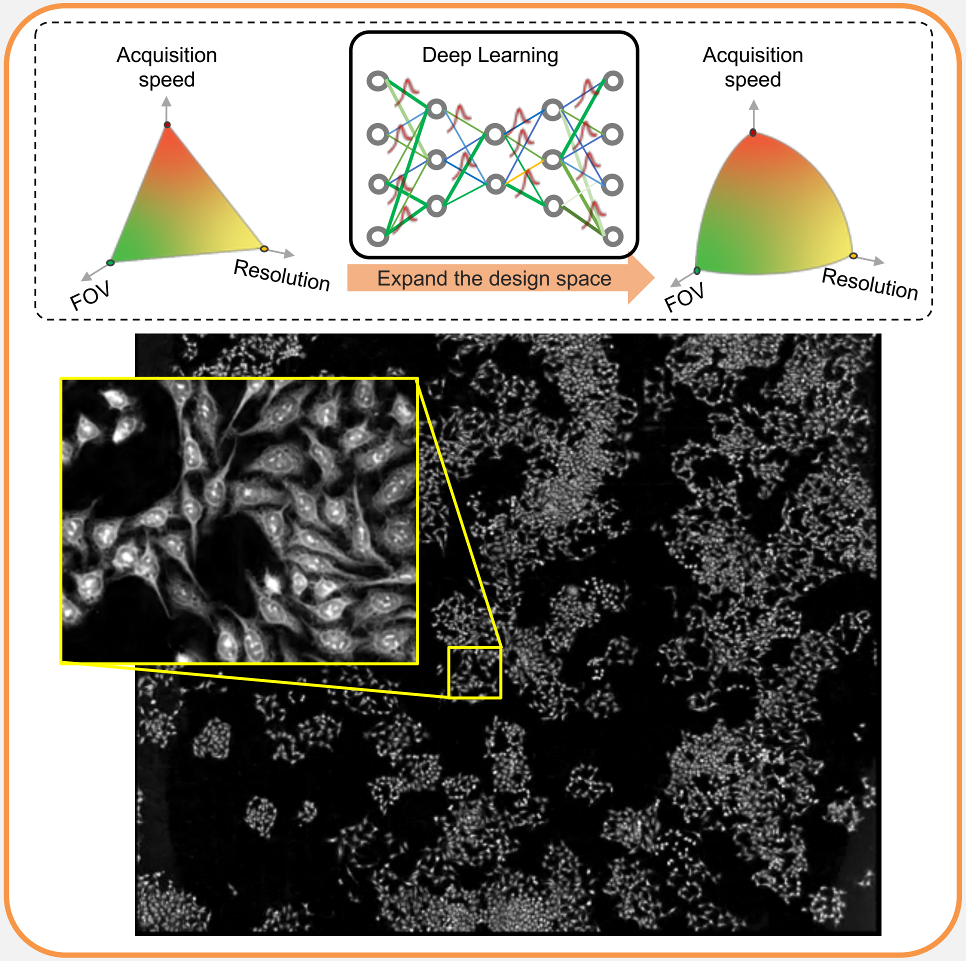
$\mathbf{NA_{final} = 0.8}$

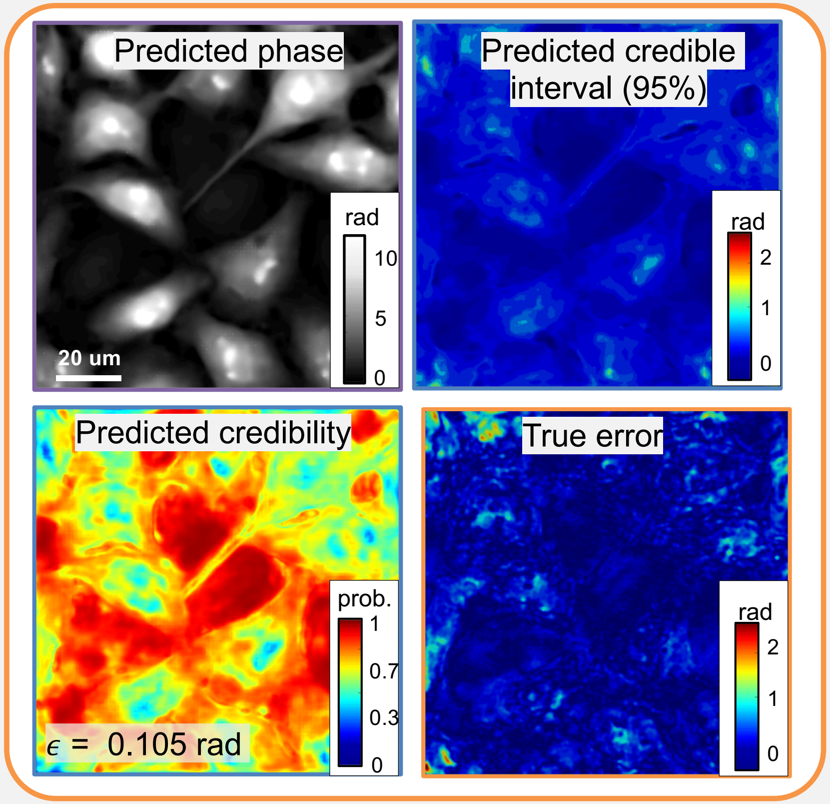
FOV: 2.1X1.8mm^2

Credibility maps identifies less confident prediction feature

- Hallucination artifacts marked with low credibility
- Improve training data → more robust prediction
- Rare biological events → (maybe) can facilitates new discovery

Scalable **and** ***reliable*** **deep learning for computational microscopy**





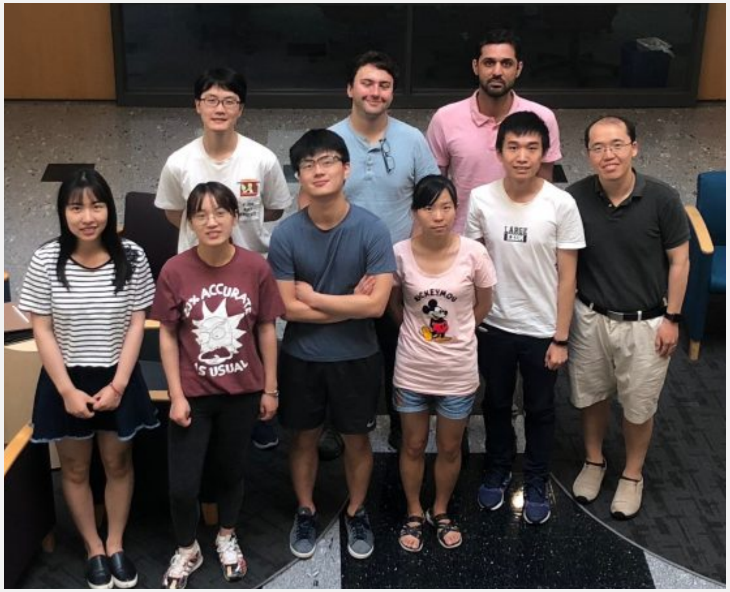
» ***Physics-guided*** measurement design for efficient large-SBP imaging

» ***Uncertainty quantification*** towards reliable deep learning

[1] Xue, Cheng, Li, Tian , “Illumination coding meets uncertainty learning: toward reliable AI-augmented phase imaging”, *arXiv* (2019).

Acknowledgements

- » Members @ BU Computational Imaging Systems Lab
  - » Yujia Xue, Shiyi Cheng, Yunzhe Li, Alex Matlock, Waleed Tahir, Jiaji Li
- » Postdoc group @ UC Berkeley Waller Lab
- » Collaborators:
  - » Jerome Mertz, David Boas, Roberto Paiella, Ian Davison (BU), Yi Ji (BUMC), Ulugbek Kamilov (WUSTL), Hakho Lee (MGH)



http://sites.bu.edu/tianlab/

- » Funding :

NIH National Institutes of Health

NSF

BU Boston University College of Engineering
Department of Electrical & Computer Engineering