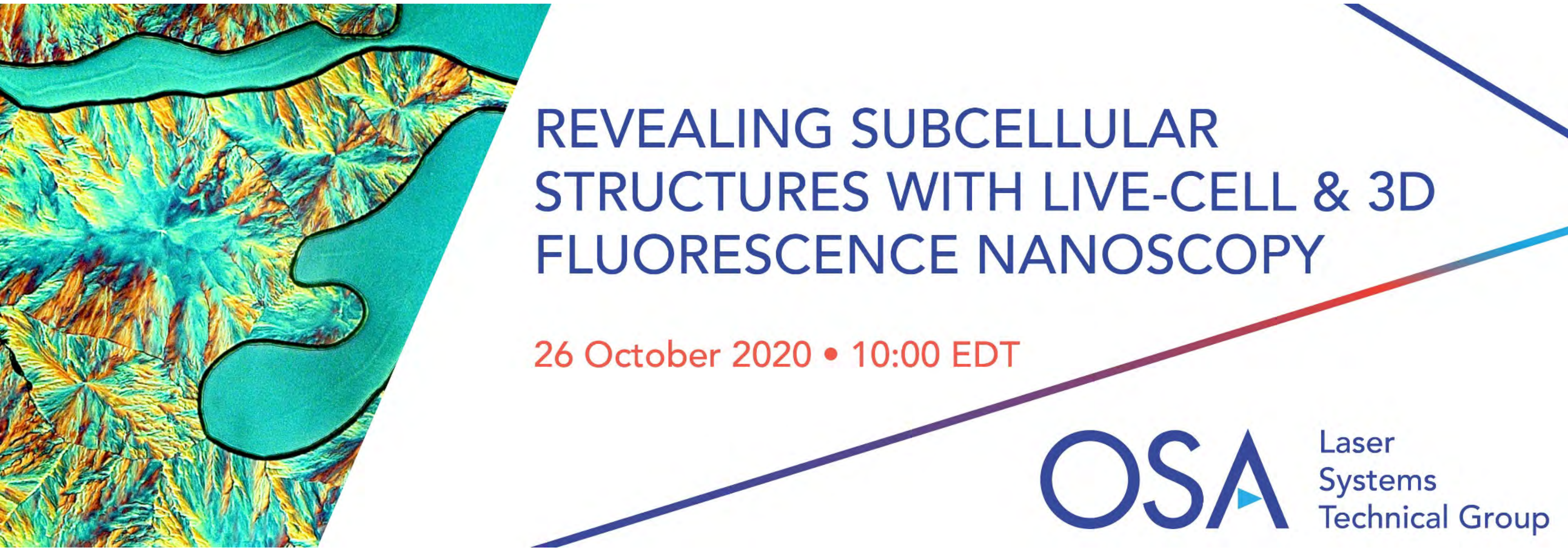
WE ARE ON

OSA Laser Systems Technical Group

Revealing Subcellular Structures with Live-cell and 3D Fluorescence Nanoscopy

Fang Huang, Purdue University

The OSA Laser Systems Technical Group Welcomes You!



OSA Laser Systems Technical Group

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OSA Laser Systems Technical Group

Technical Group at a Glance

- Focus
  - This group encompasses novel laser system development for a broad range of scientific, industrial, medical, remote sensing and other directed-energy applications.
- Mission
  - To benefit ***YOU***
  - Webinars, e-Presence, publications, technical events, business events, outreach
  - Interested in presenting your research? Have ideas for TG events? Contact us at osa.lasersystechgroup@gmail.com.
- Find us here
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OSA Laser Systems Technical Group

Today’s Webinar

Ultra high resolution imaging in whole cell and tissue specimens and multiplexed single molecule imaging using deep learning

Dr. Fang Huang

Weldon School of Biomedical Engineering, Purdue University, USA

Speaker’s Short Bio:

Dr. Fang Huang earned his bachelor degree in Physics at the University of Science and Technology of China in 2004 and his doctoral degree in Physics from the University of New Mexico in 2011. Before joining Purdue, Fang Huang was a Brown-Coxe Postdoctoral Fellow in Cell Biology at Yale School of Medicine. Huang received Excellence in Research Awards from Purdue, Maximize Investigator Research Award (MIRA) from NIH, 2016 Young Faculty Award from DARPA.

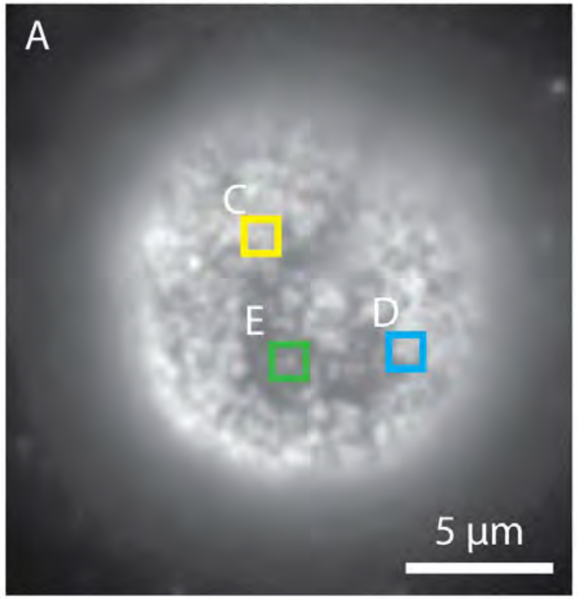
Ultra-high resolution imaging in whole-cell and tissue specimens and multiplexed single molecule imaging using deep learning

Fang Huang, Ph.D.

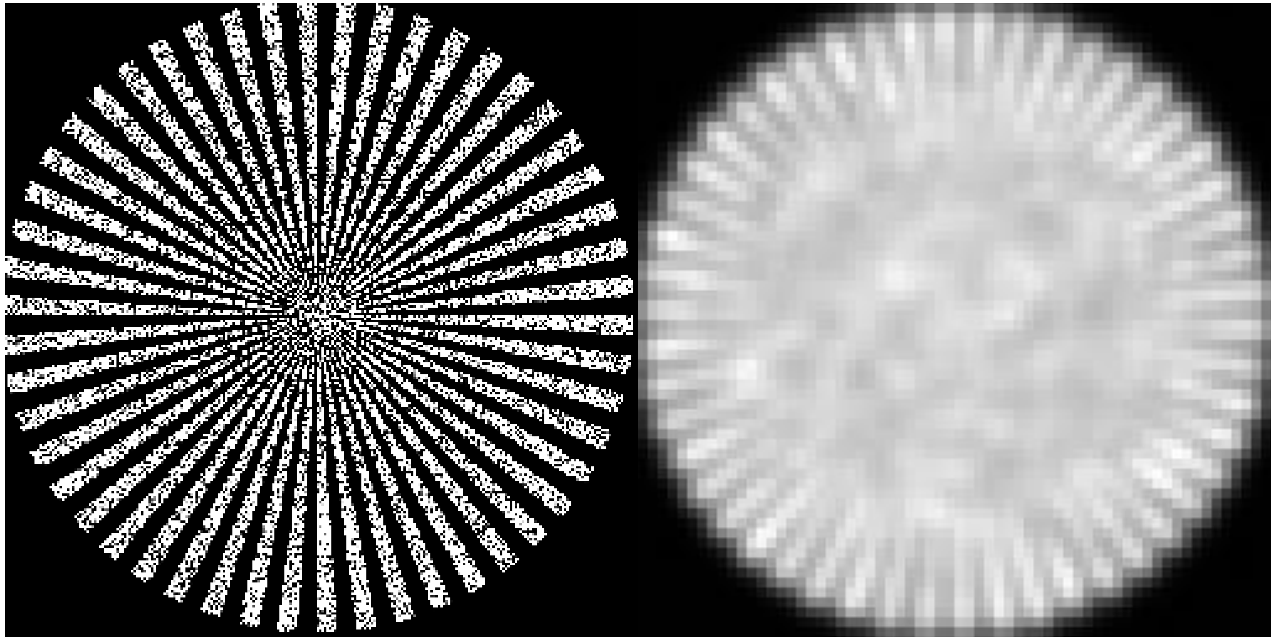
Assistant Professor
Weldon School of Biomedical Engineering

PURDUE UNIVERSITY
Weldon School of Biomedical Engineering
COLLEGE OF ENGINEERING

Diffraction limited



Concept of single molecule super-resolution imaging



PALM (E. Betzig, *Science*, 2006)
STORM (M. Rust, *Nature Methods*, 2006)
FPALM (S. Hess et al, *Biophys Journal*, 2006)
Nobel Prize in Chemistry, 2014

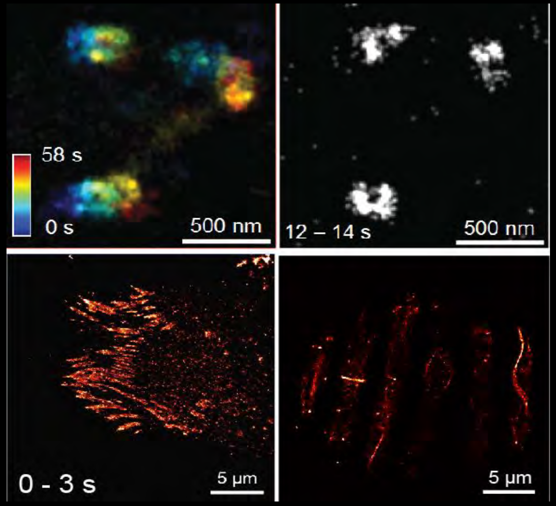
- Slow
- aberration and scattering
- Insufficient 3D resolution

Live cell dynamics
Super-res in tissues
Ultra-structural mapping in cell and tissues

Major directions in the lab

Dynamic Imaging in live Cells

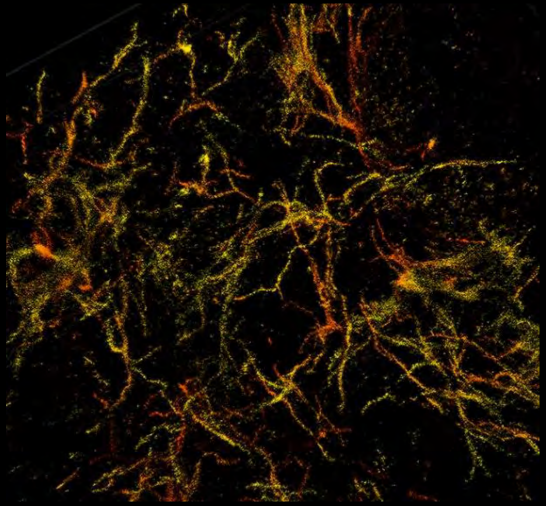
Super-resolution imaging on CCP, focal adhesion and actin (**live cell**)



(2011) Biomed. Opt. Express, 2(5):1377-93
(2013) Nat. Methods, 10(7): 653-8
(2016) PNAS, 113(40):E5876-E5885
(2017) Nat. Methods, 14(7): 760-1

Tissue to small animal

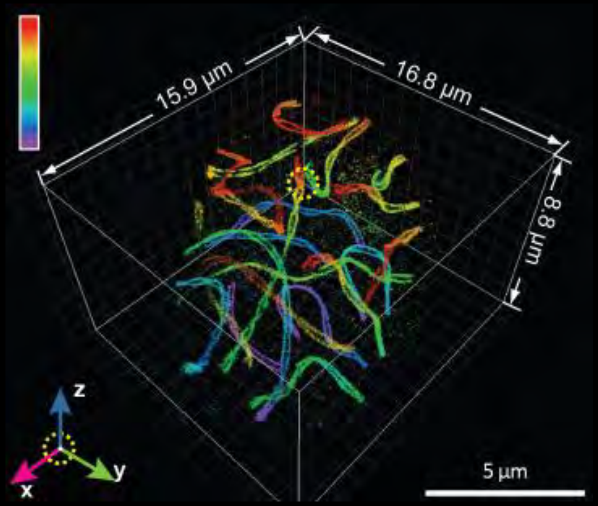
Super-resolution reconstruction of fibrillar Aβ in **mouse frontal cortex**



(2015) Optica 2(2):177-185
(2018) Nat. Methods, 15(7), 583-586
(2020) Nat. Methods, 17, 531–540

1-5 nm resolution

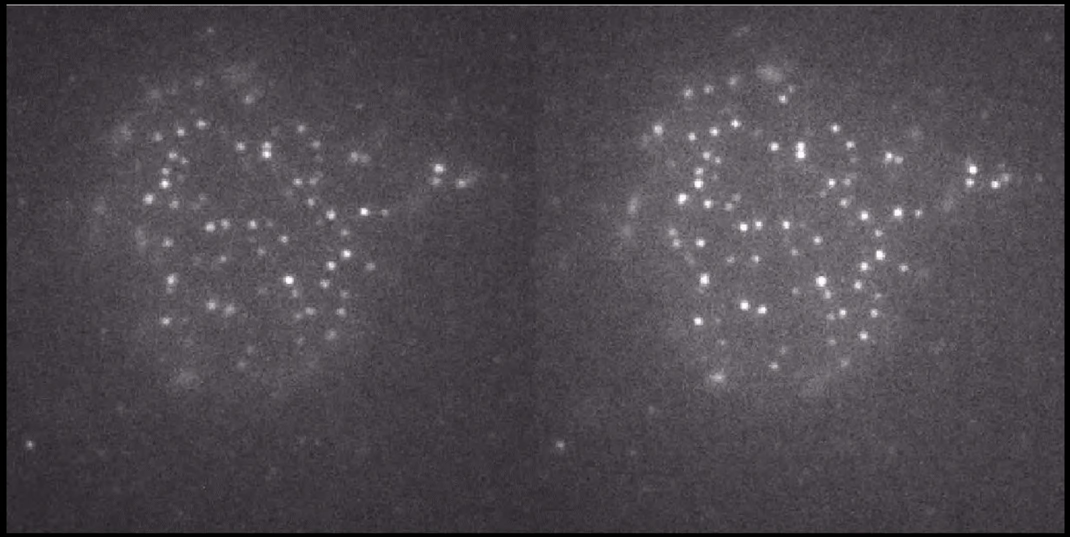
Interferometric SMSN reconstruction of synaptonemal complex



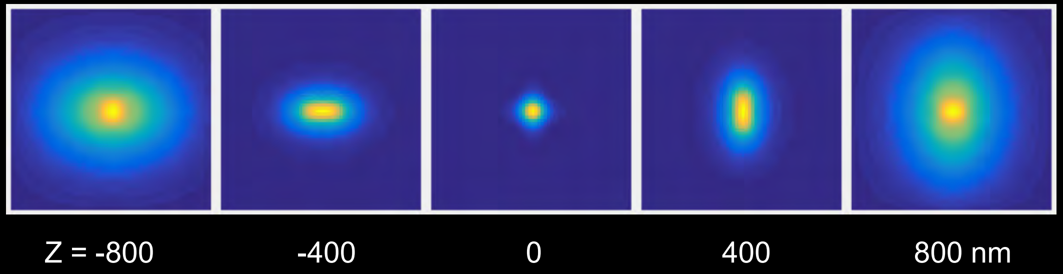
(2016) Cell, 166(4):1028-40
(2016) Developmental Cell, 38(5):478-92
(2018) Nat. Methods, 15(11), 913–916
(2020) Communications Biology, 3:220

Astigmatism

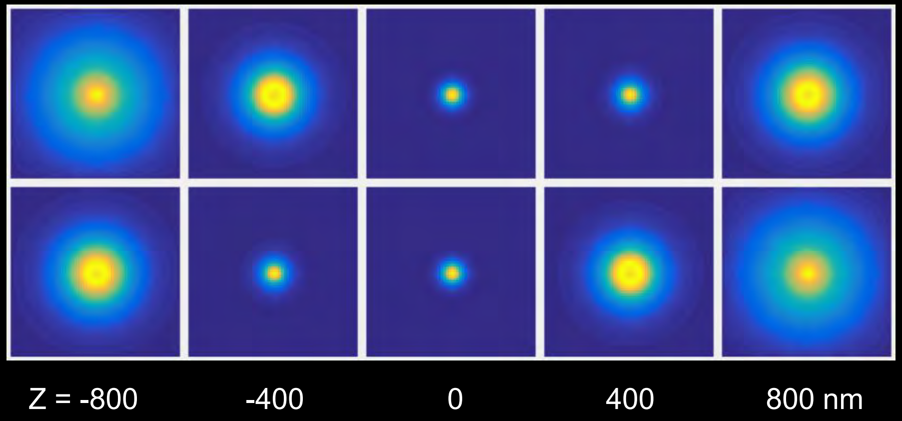
Biplane



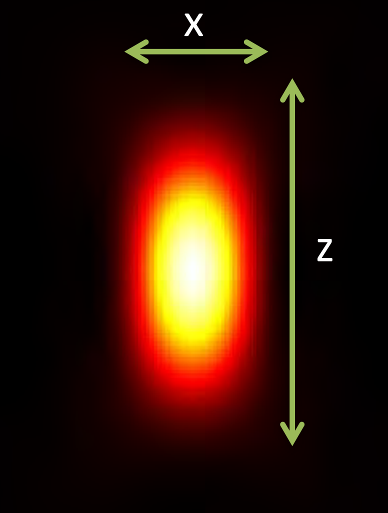
Astigmatism



Biplane

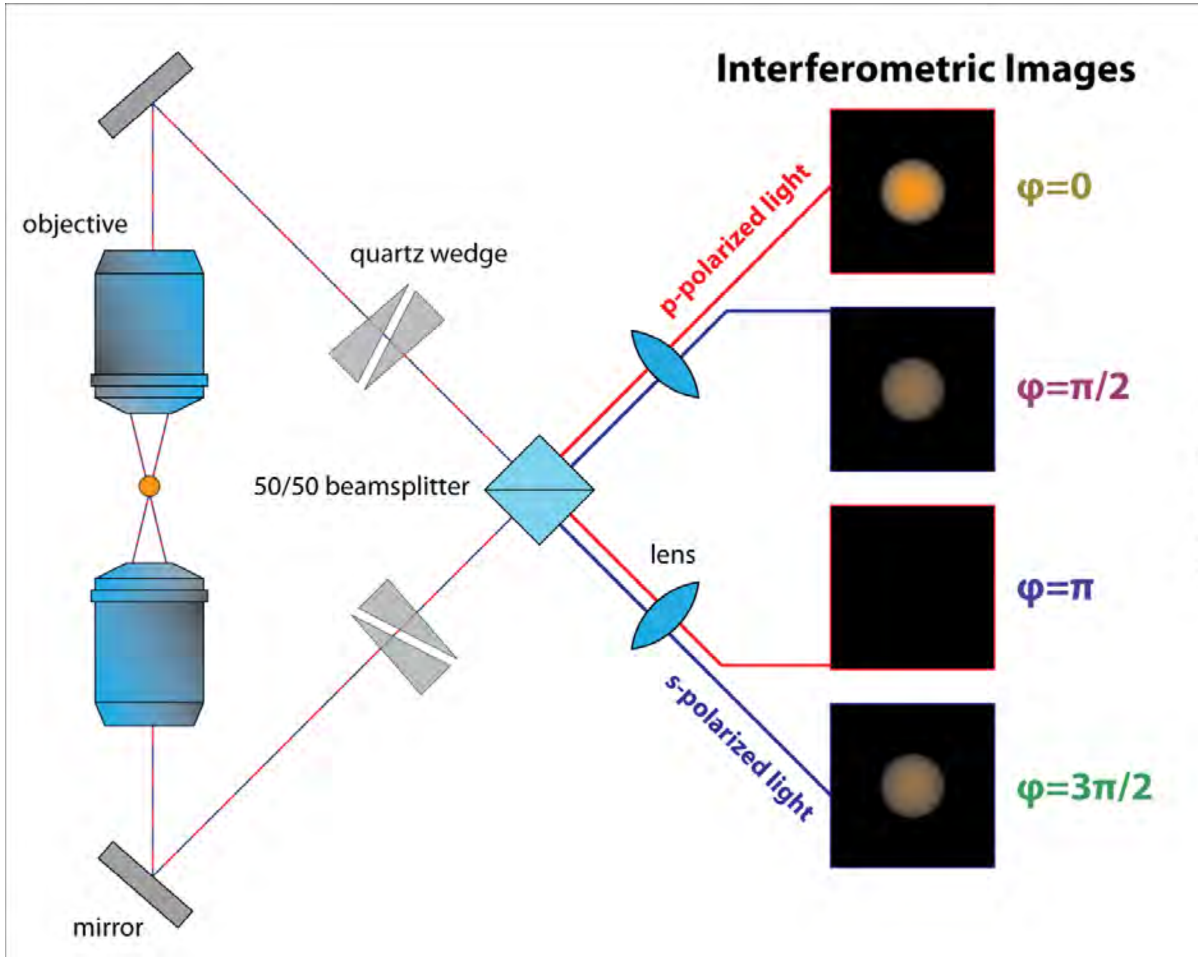


Conventional 3D super-resolution microscopy



Significantly worse resolution in axial direction
Deteriorating resolution in thick samples

4Pi/Interferometric detection of single molecules

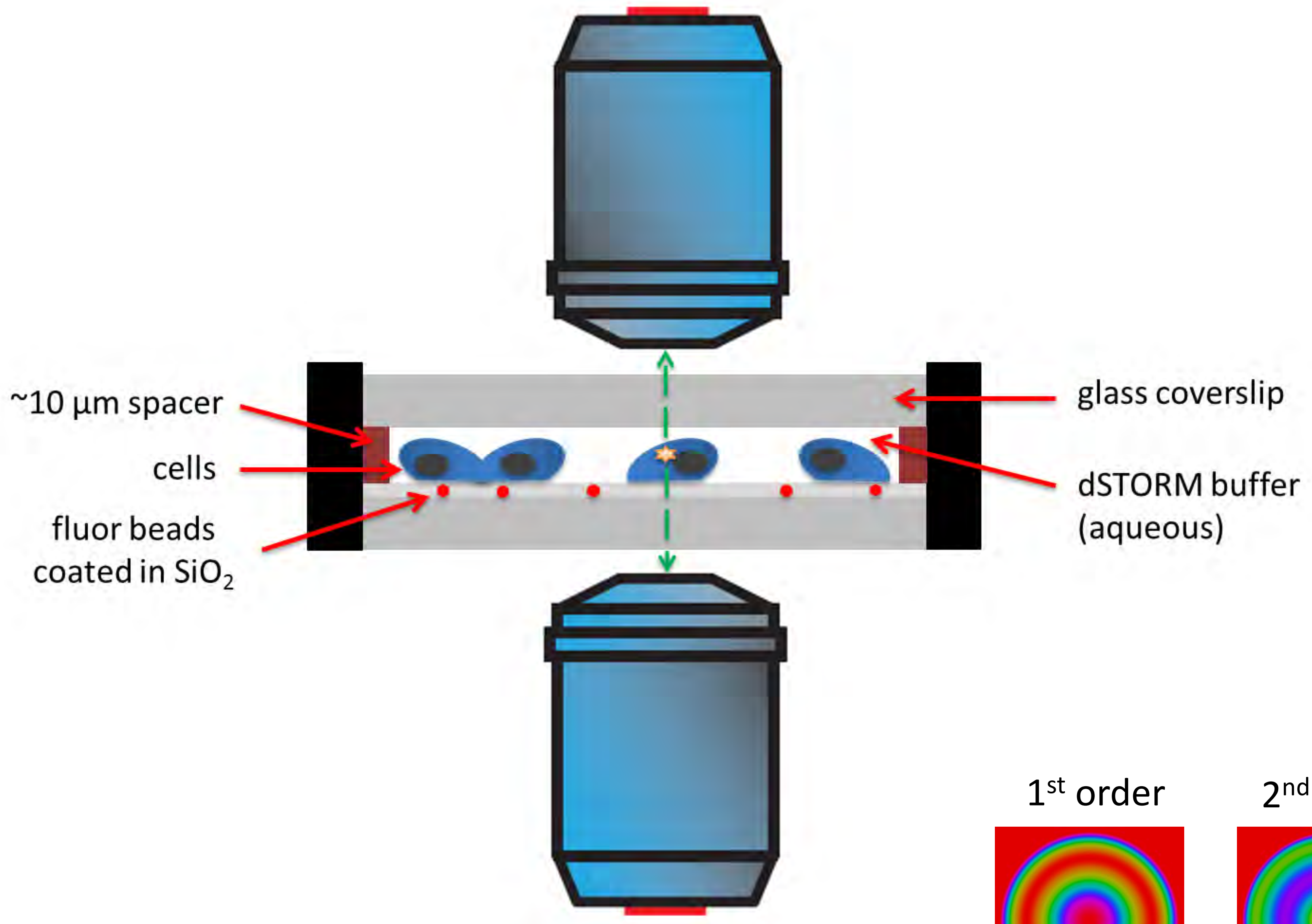


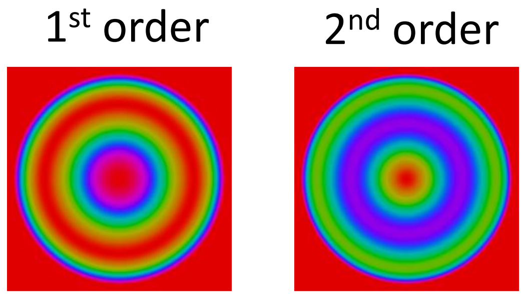
Huang and Sirinakis *et al.*, *Cell* **2016,** 166:4, 1028-40, 2016

Aquino, D. *et al. Nat. Methods* **2011**, *8*, 353-359

Shtengel *et al.* PNAS **2008**, *106*, 9 3125–3130

W-4PiSMSN : PSF Shaping and Aberration Correction

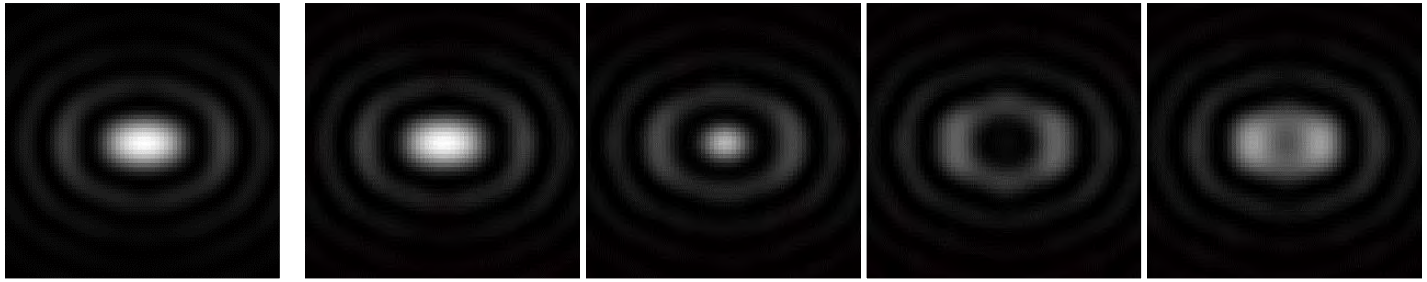




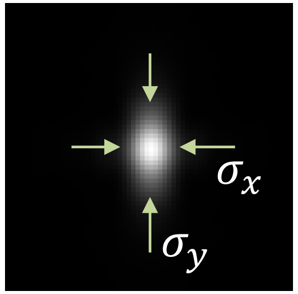
W-4PiSMSN : Novel Algorithms

Cell **166:4**, 1028-40, 2016

Point Spread Function of the W-4PiSMS



sum



Extra information!

1. General shape
2. Interference

ranking

$$M = \frac{\sigma_x^3}{\sigma_y} - \frac{\sigma_y^3}{\sigma_x} \xrightarrow{\text{unwrap}} \varphi$$

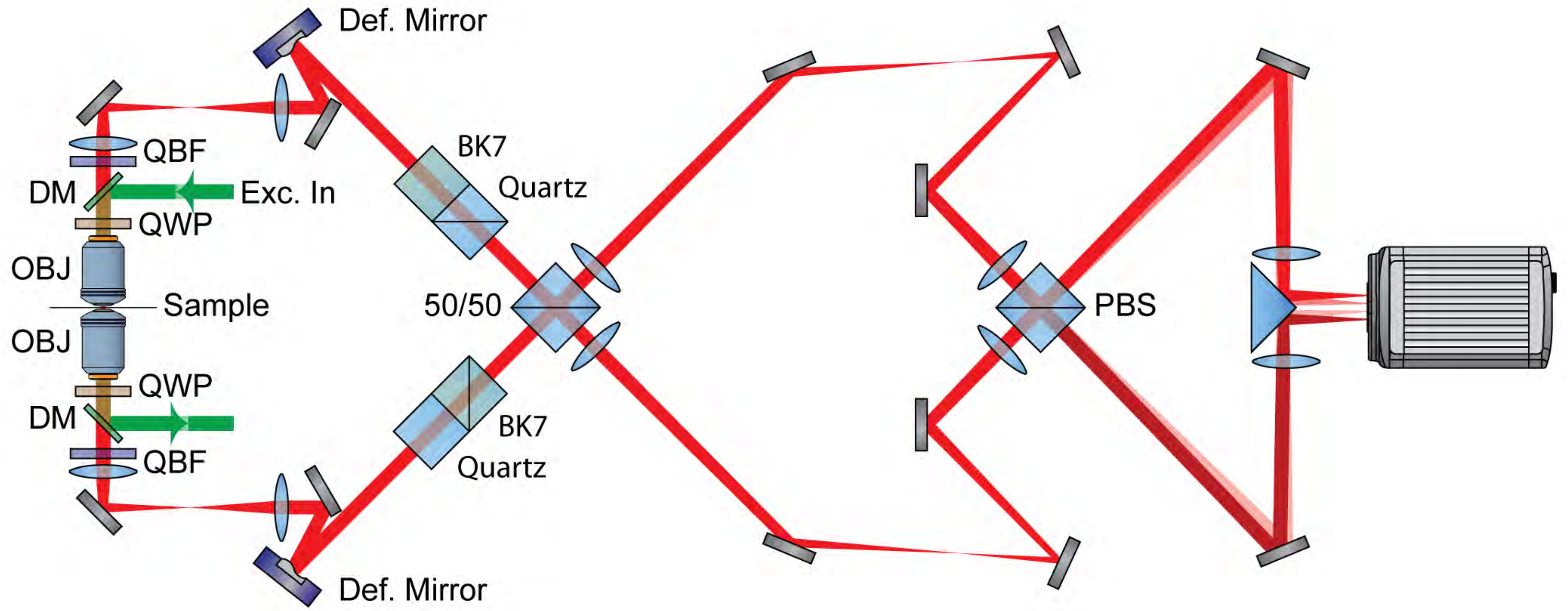
pin-point

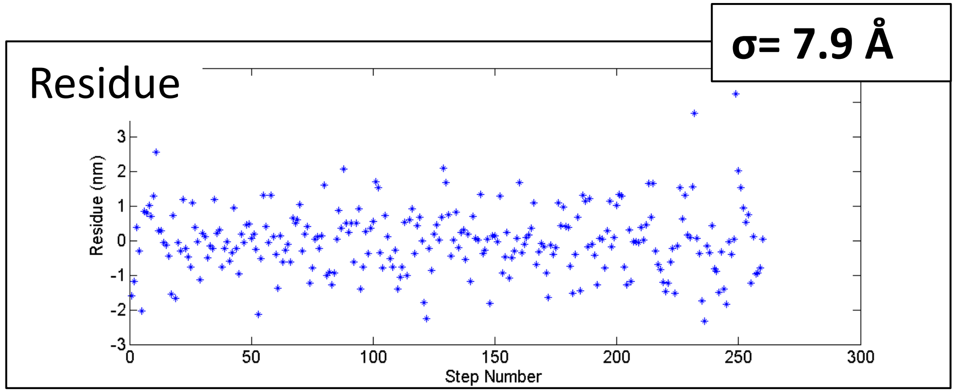
Interference phase

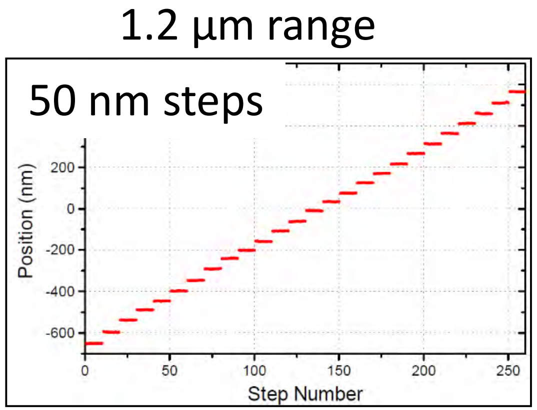
- Adaptive ridge-finding algorithm to account for aberration distortion on PSF
- Interferometric PSF with pupil function to translate measured phase into axial position
- Redundancy based drift correction with 3D point cloud data
- Correlation based optical sections merging aided by redundancy

W-4PiSMSN : Simplified Schematics

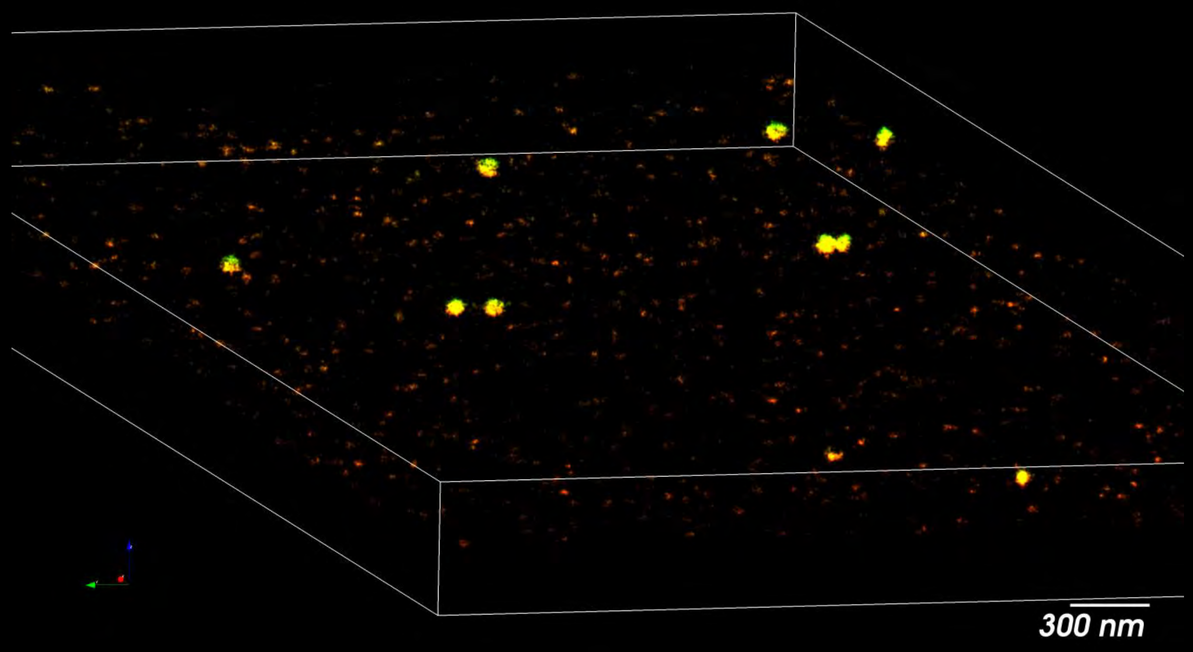
Cell **166:4**, 1028-40, 2016



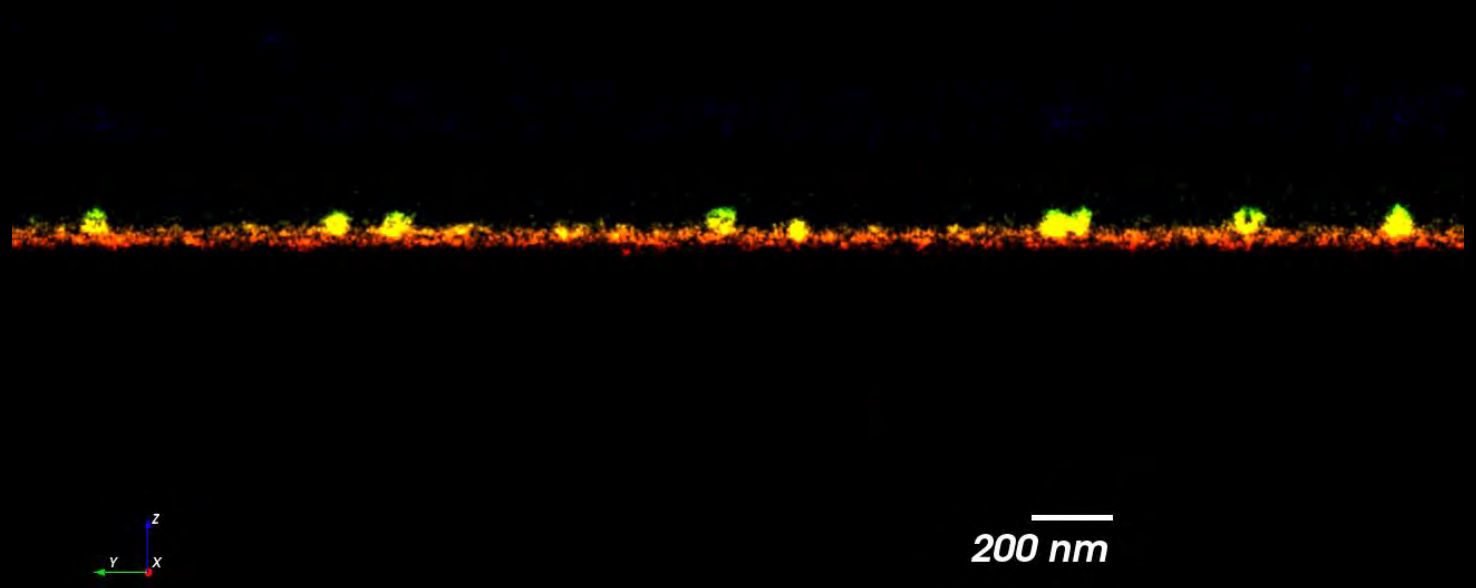




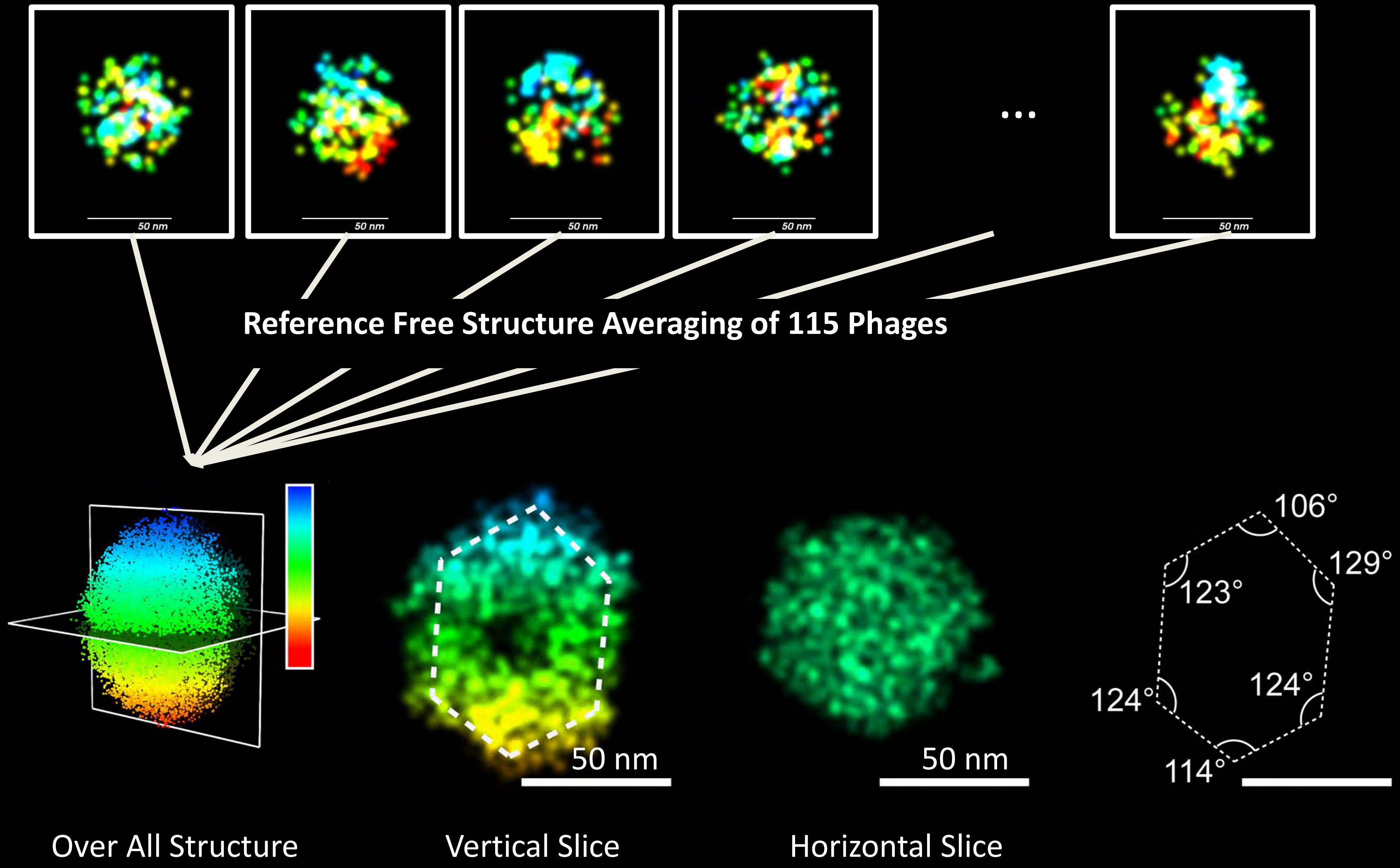
W-4PiSMSN : Bacteriophage T7



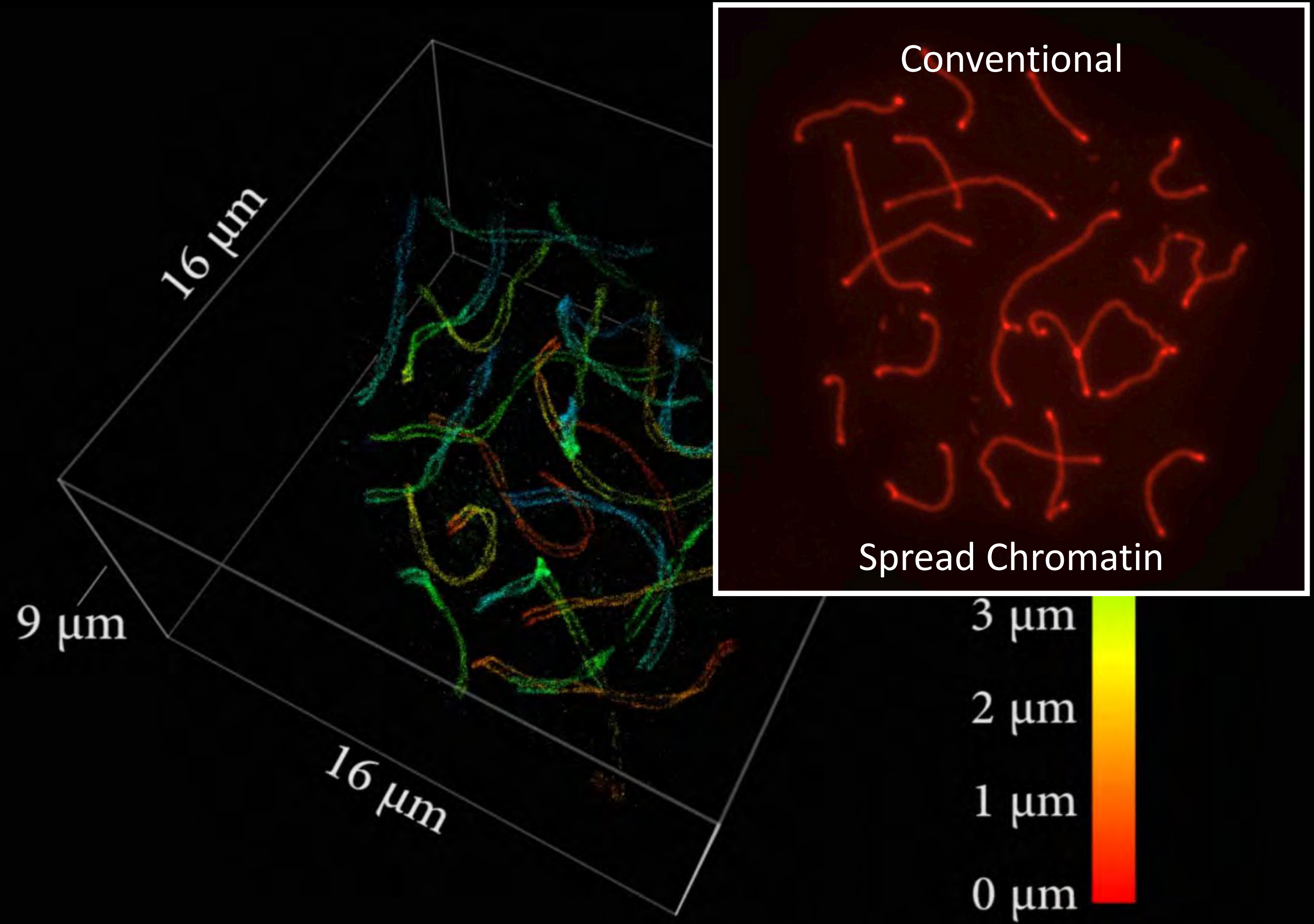
W-4PiSMSN : Bacteriophage T7



W-4PiSMSN : Rotating and Aligning 100+ Bacteriophages



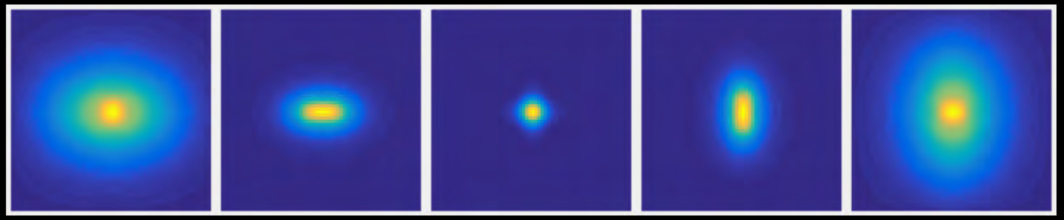
W-4PiSMSN: Synaptonemal complex in Spermatocytes



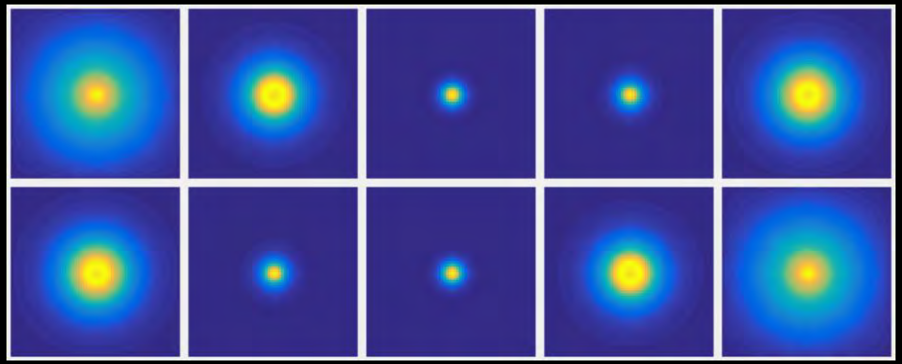
Whole Spermatocyte
9 µm thick cell
21 optical sections

Cell **166:4**, 1028-40, 2016

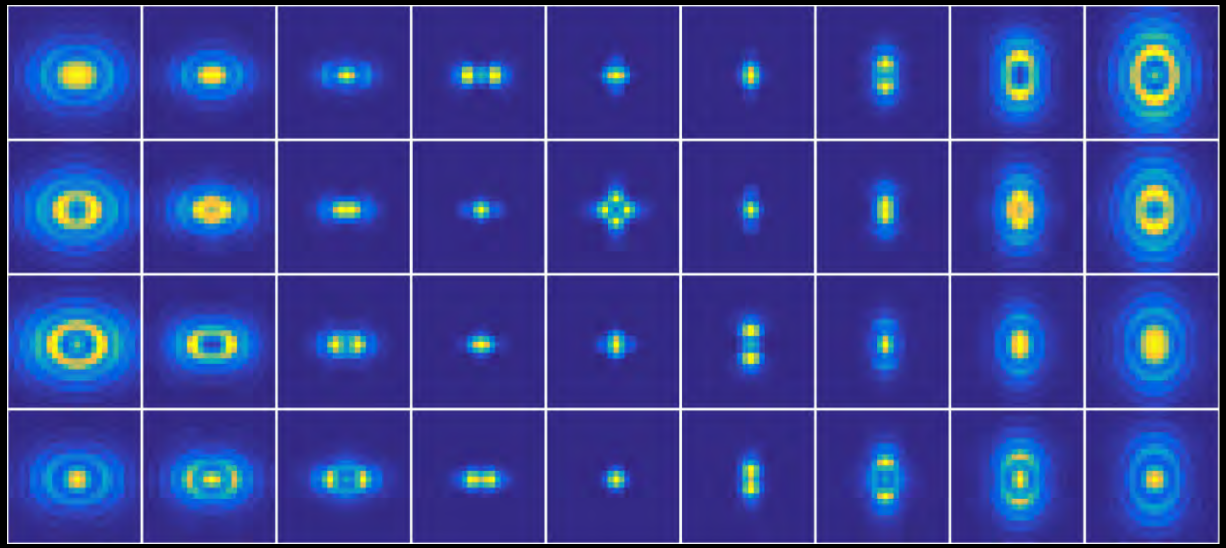
Astigmatism



Biplane



4Pi based single molecule imaging



Single molecule emissions
carry multiplexed information

Where is each molecule located?
What is its orientation?
are their wavefronts distorted?

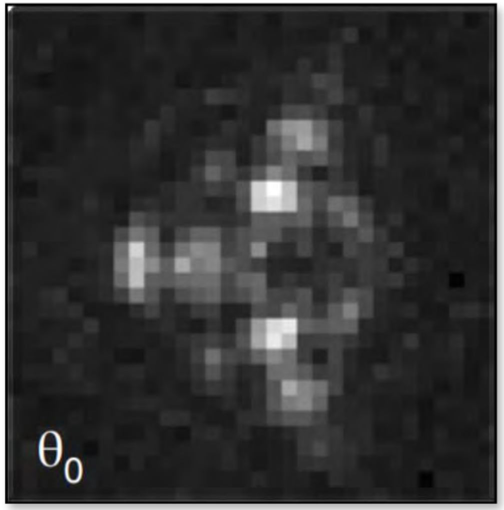
Molecular location

Dipole orientation

Wavefront

Estimate from individual emission patterns?

Question: How much coma do we have?



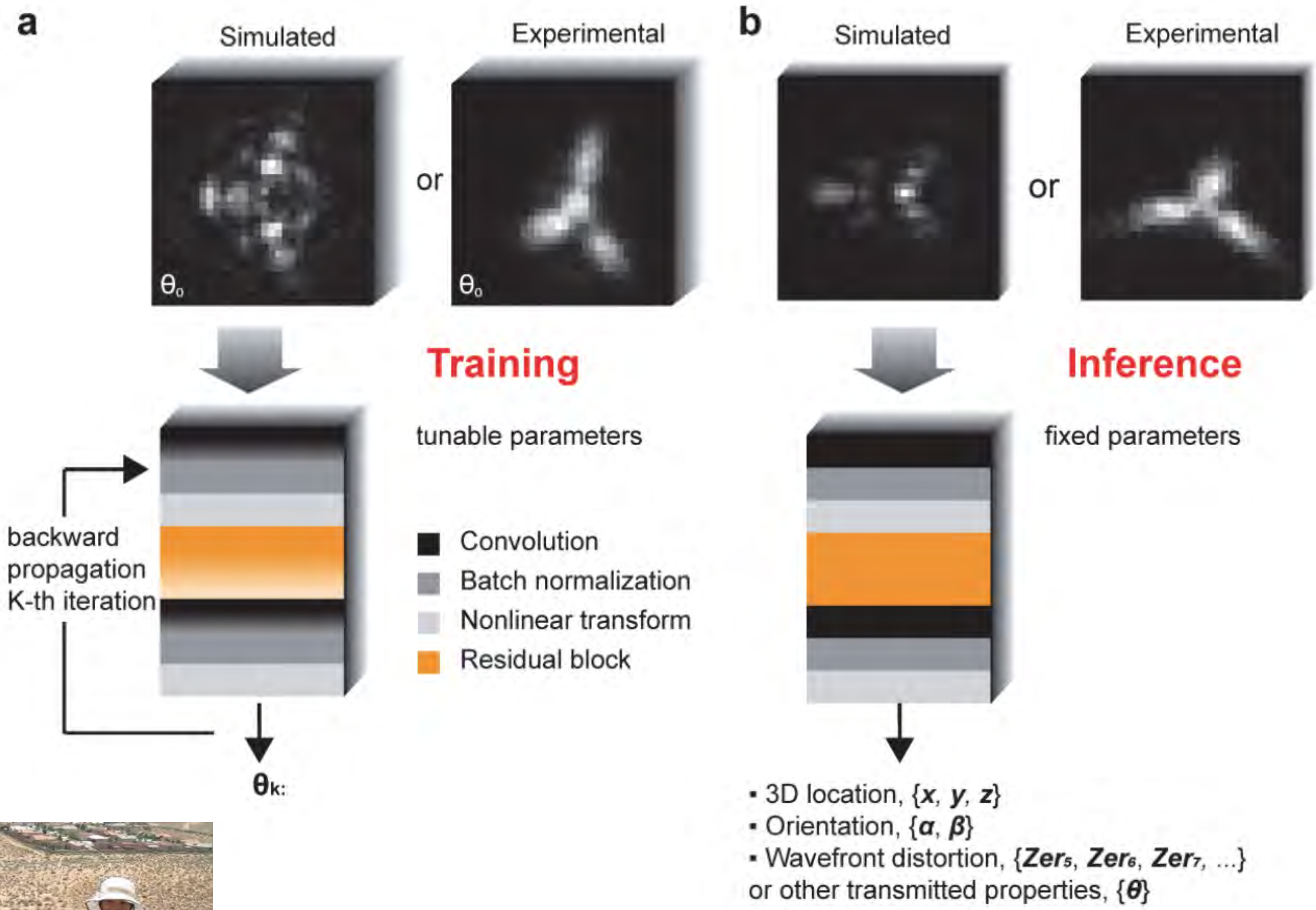
Let's design some measurements:

1. Peak offset from the center
2. Number of peaks
3. Peak distances
4. Width of this pattern
5. Number of peaks that pass the certain threshold intensity?

…

1. Is this an exhaustive list of features?
2. How should we weight them?

High dimensional analysis of single molecule emission pattern remains difficult limited by abilities to extract complex features and computation power



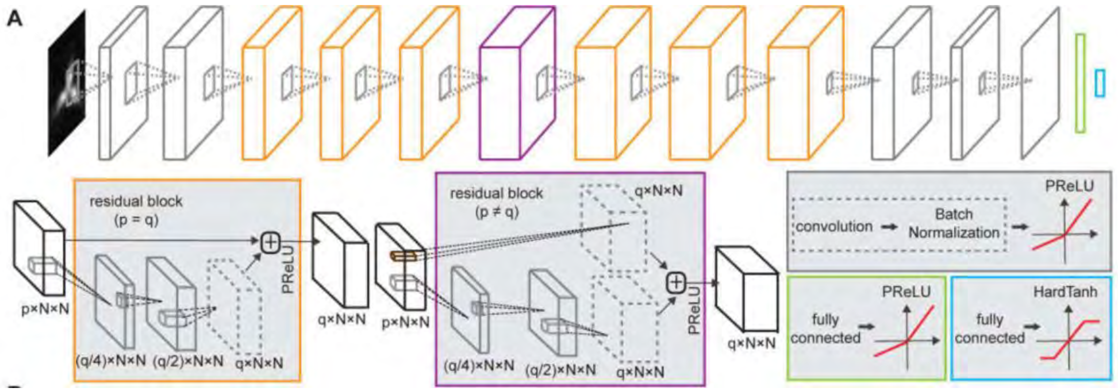
Peiyi Zhang

Sheng Liu
Ph.D.

Zhang* and Liu*, *et al.*, *Nature Methods*, 2018, 15,7, 583–586, *co-first author

- Deep neural network architecture

smNet architecture

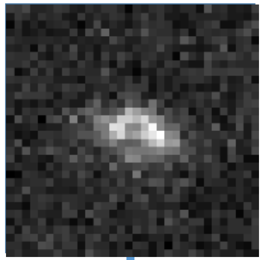


- Learning algorithm: Adam, an optimizer in Torch packages (http://torch.ch/).

How do we know if the optimizer converge to the right point?

cost function: MSE

$$E_{\hat{\theta}} = \frac{1}{NT}\sum_{n=1}^{N}\sum_{t=1}^{T}\left(\hat{\theta}_{tn} - \theta_{tn}\right)^2,$$



Expected precision

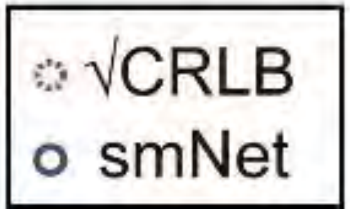
Fisher information

Cramér–Rao lower bound

$$\mathrm{var}(\theta_i) \geq [F(\theta)^{-1}]_{ii},$$

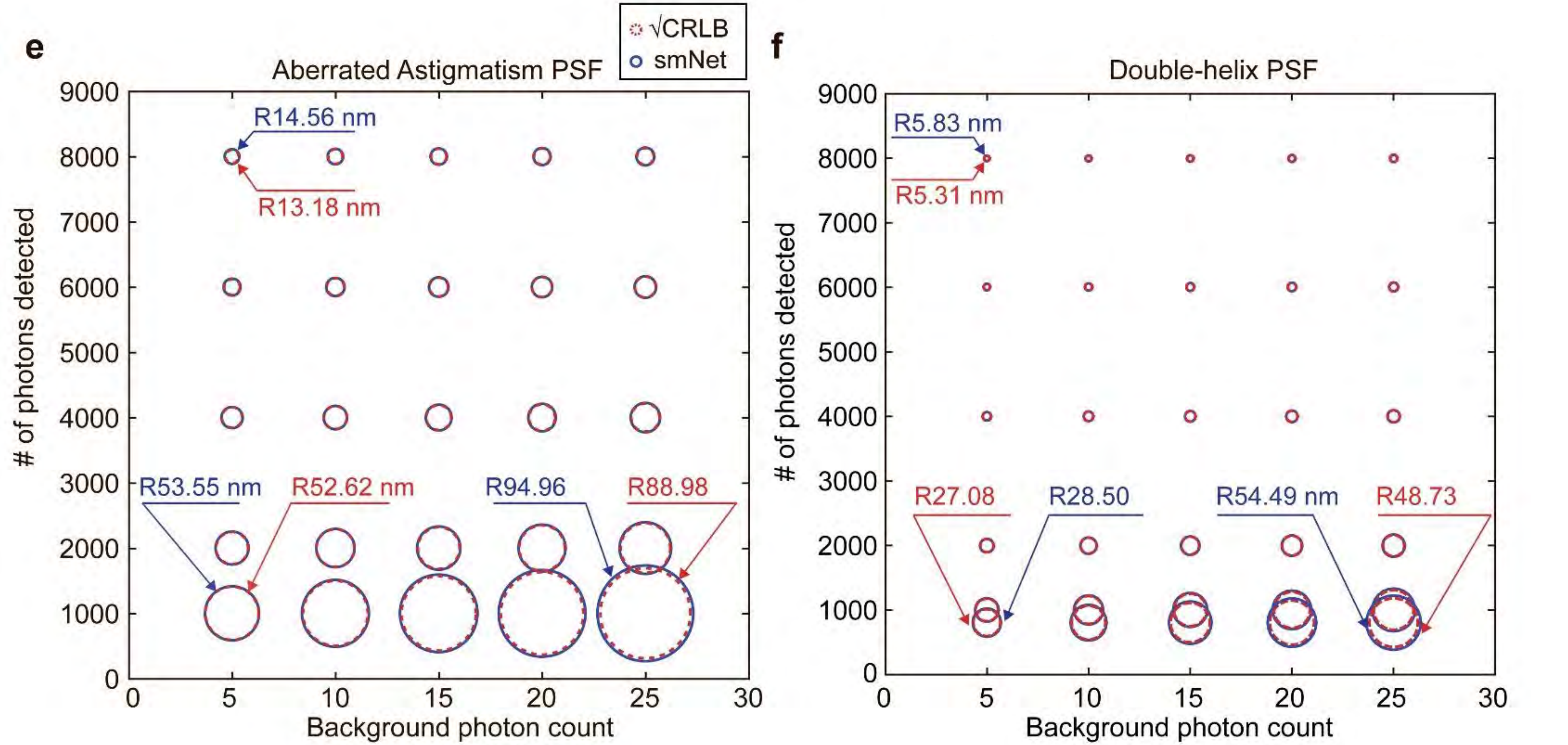
$$F_{i,j} = \mathrm{E}\left[\frac{\mathrm{d}ln(L(\theta|D))}{\mathrm{d}\theta_i}\frac{\mathrm{d}ln(L(\theta|D))}{\mathrm{d}\theta_j}\right],$$

Fisher Information based training

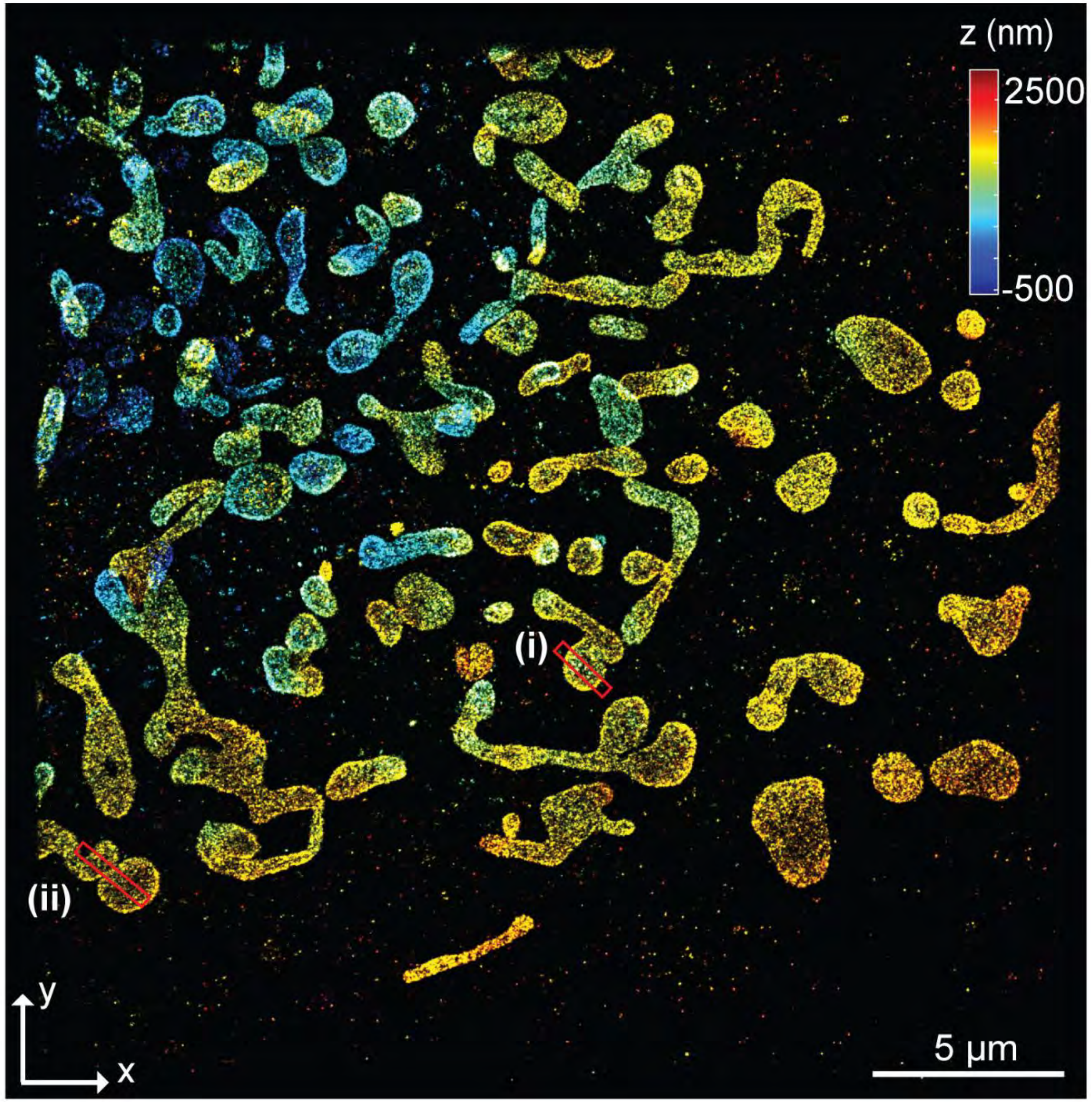


Accuracy from theoretical information limit

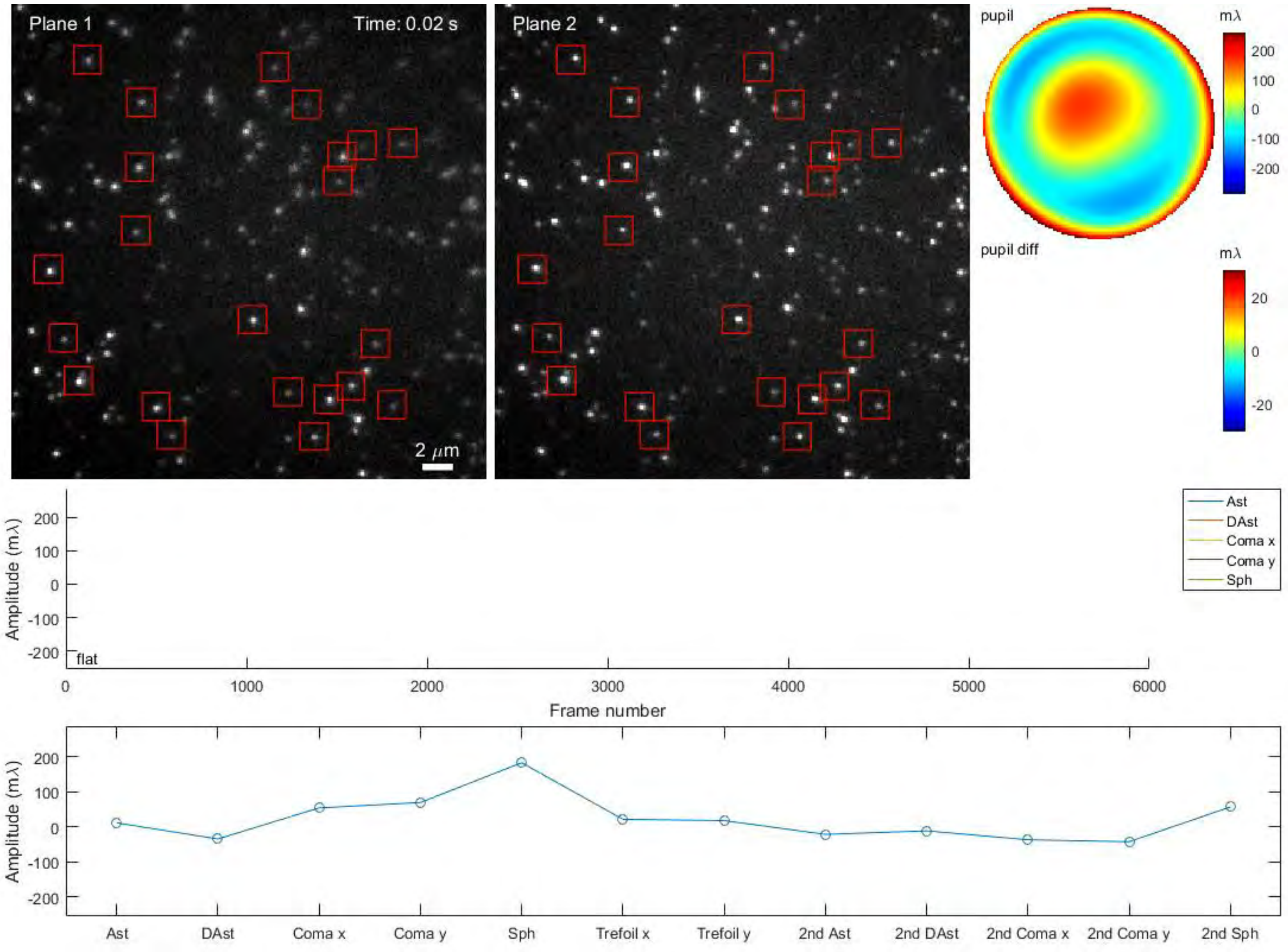
Accuracy of smNet



smNet super-resolution reconstruction of TOM20 in COS-7 cells

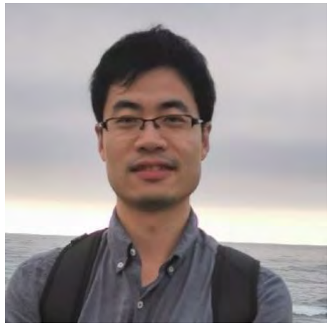


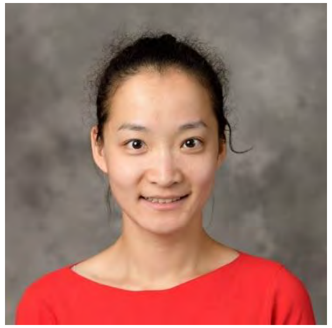
Zhang* and Liu*, et al., *Nature Methods*, 2018, 15,7, 583–586, *co-first author



Zhang* and Liu*, et al., *Nature Methods*, 2018, 15,7, 583–586, *co-first author

3D super-resolution imaging with ***INSPR*** (*In situ PSF retrieval*) in cells and tissues





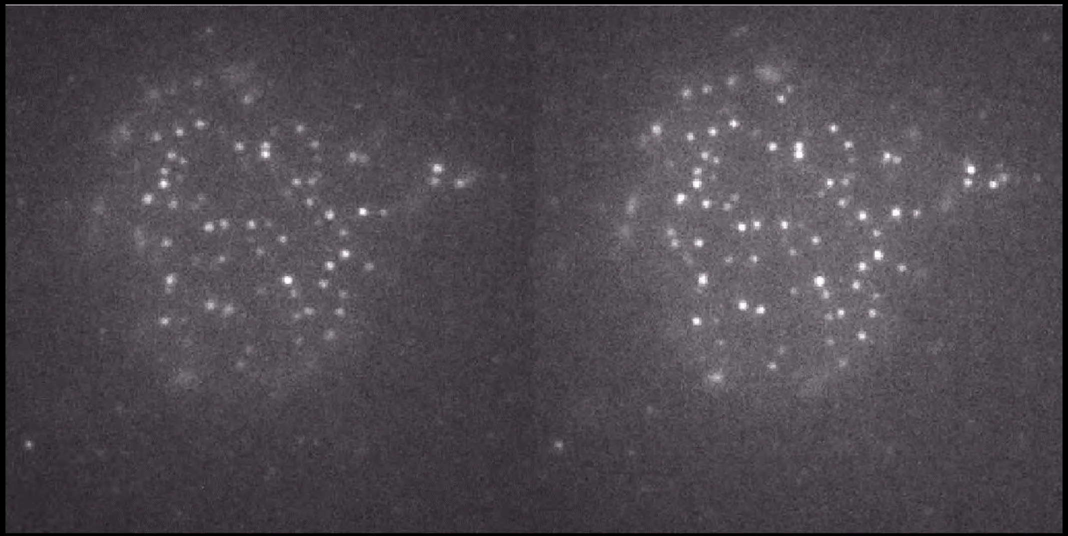
Fan Xu
(Ph.D)

Donghan Ma
(Ph.D)

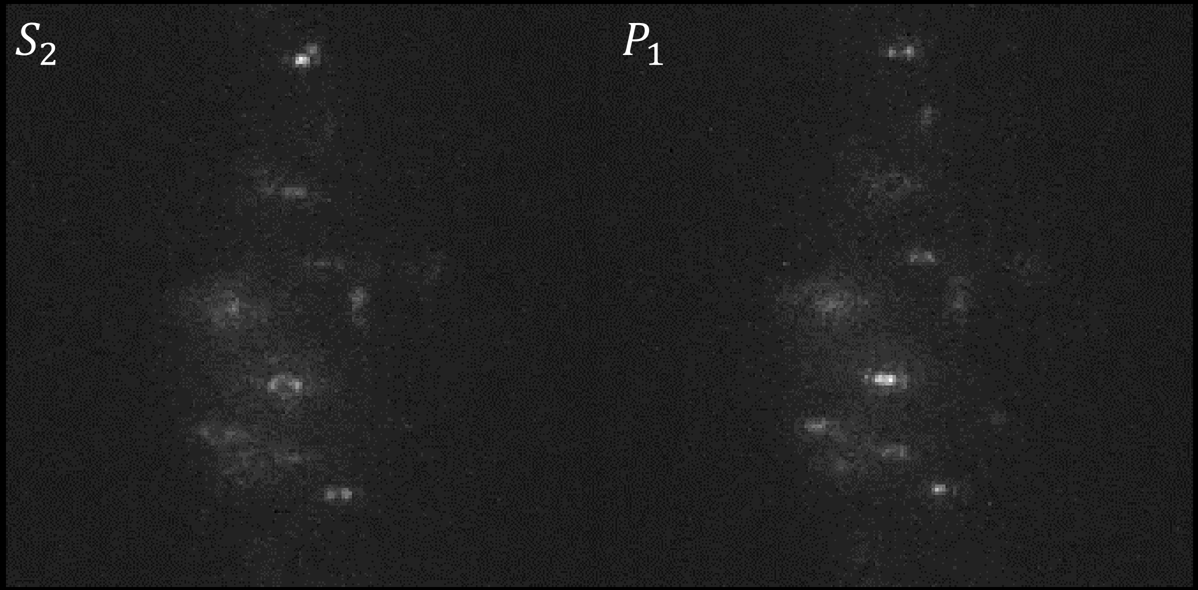
Fan Xu* and Donghan Ma* et al., (2020) *Nat. Methods*, 17, 531–540, *co-first author

Astigmatism

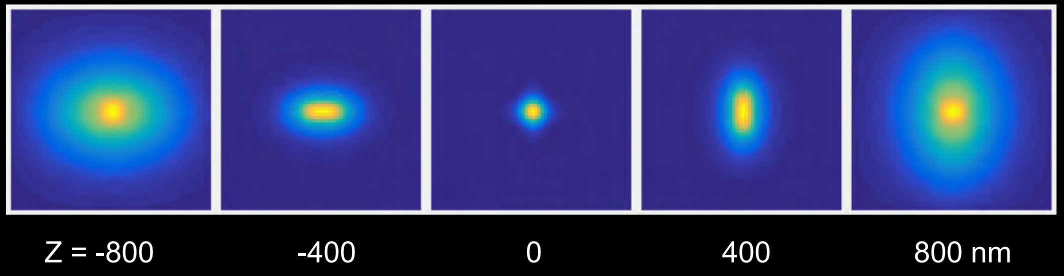
Biplane



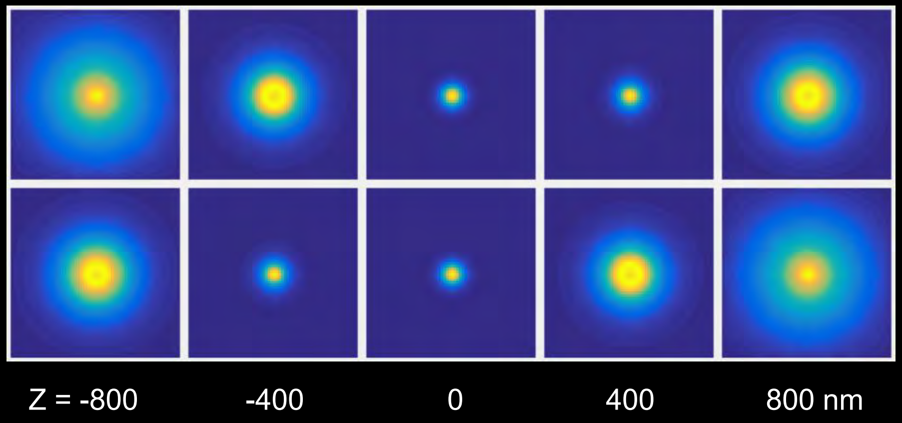
4Pi based single molecule imaging



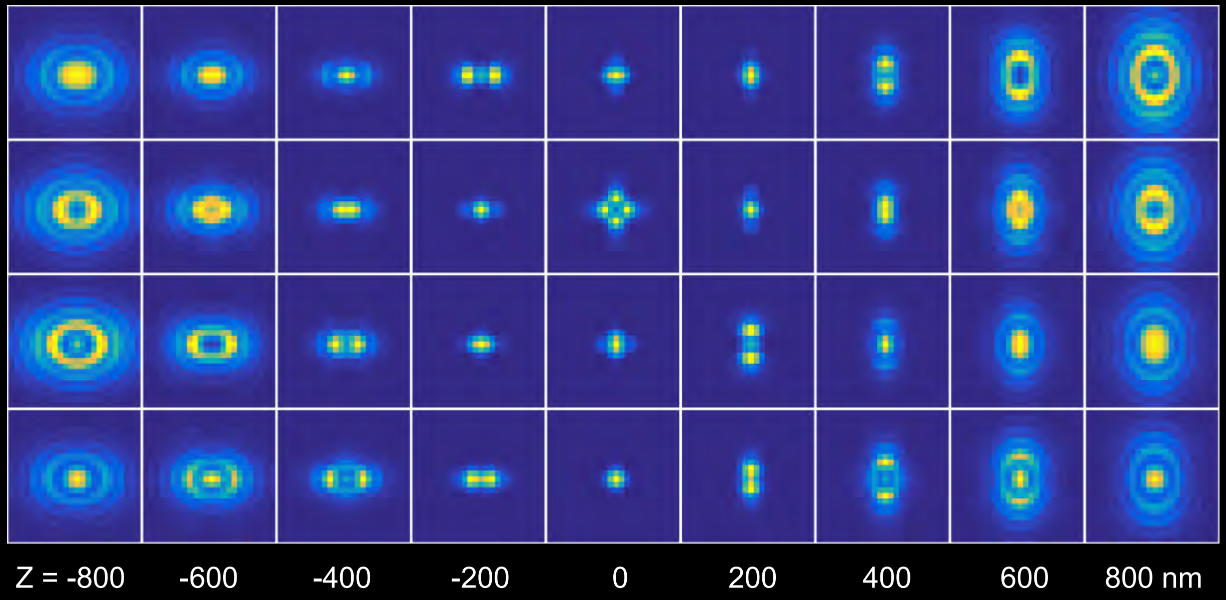
Astigmatism

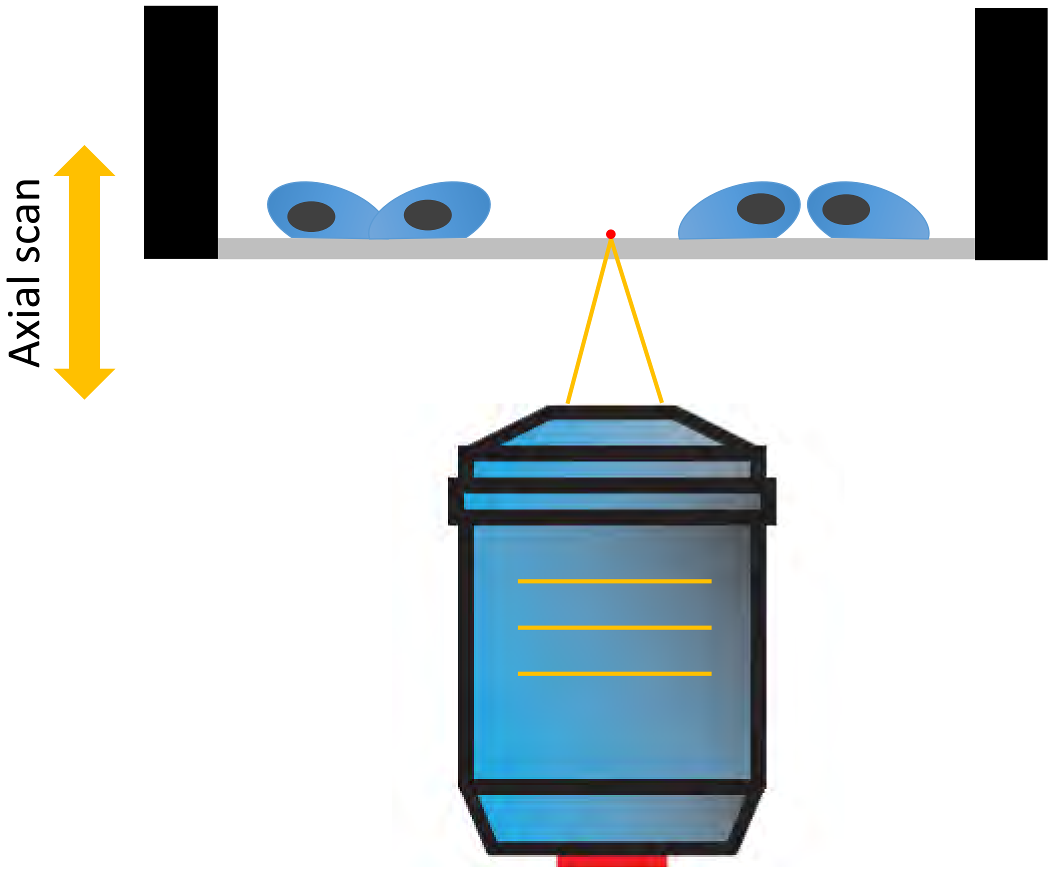


Biplane

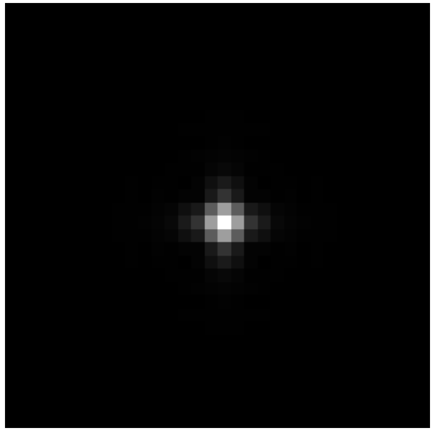


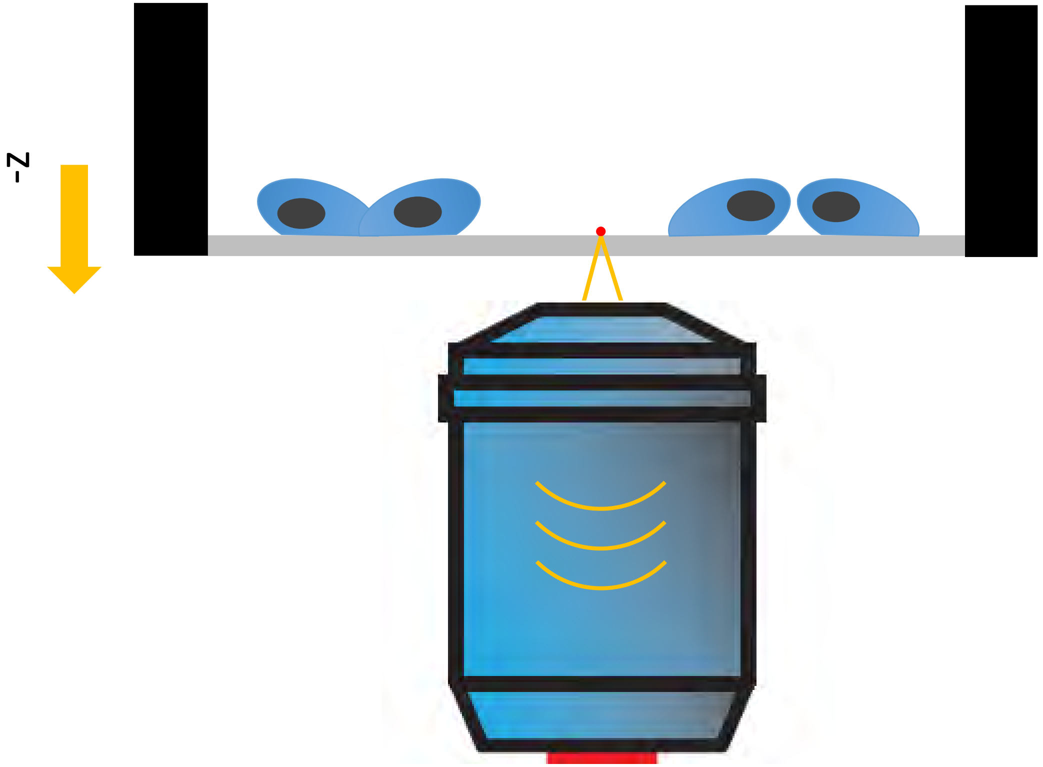
4Pi based single molecule imaging



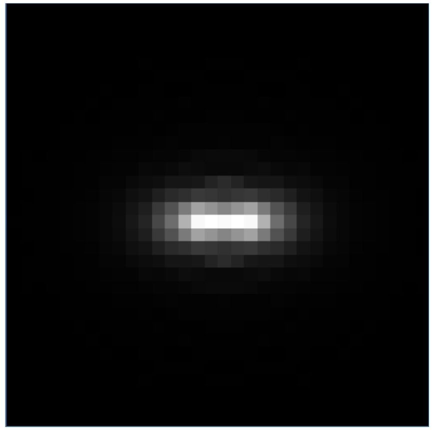


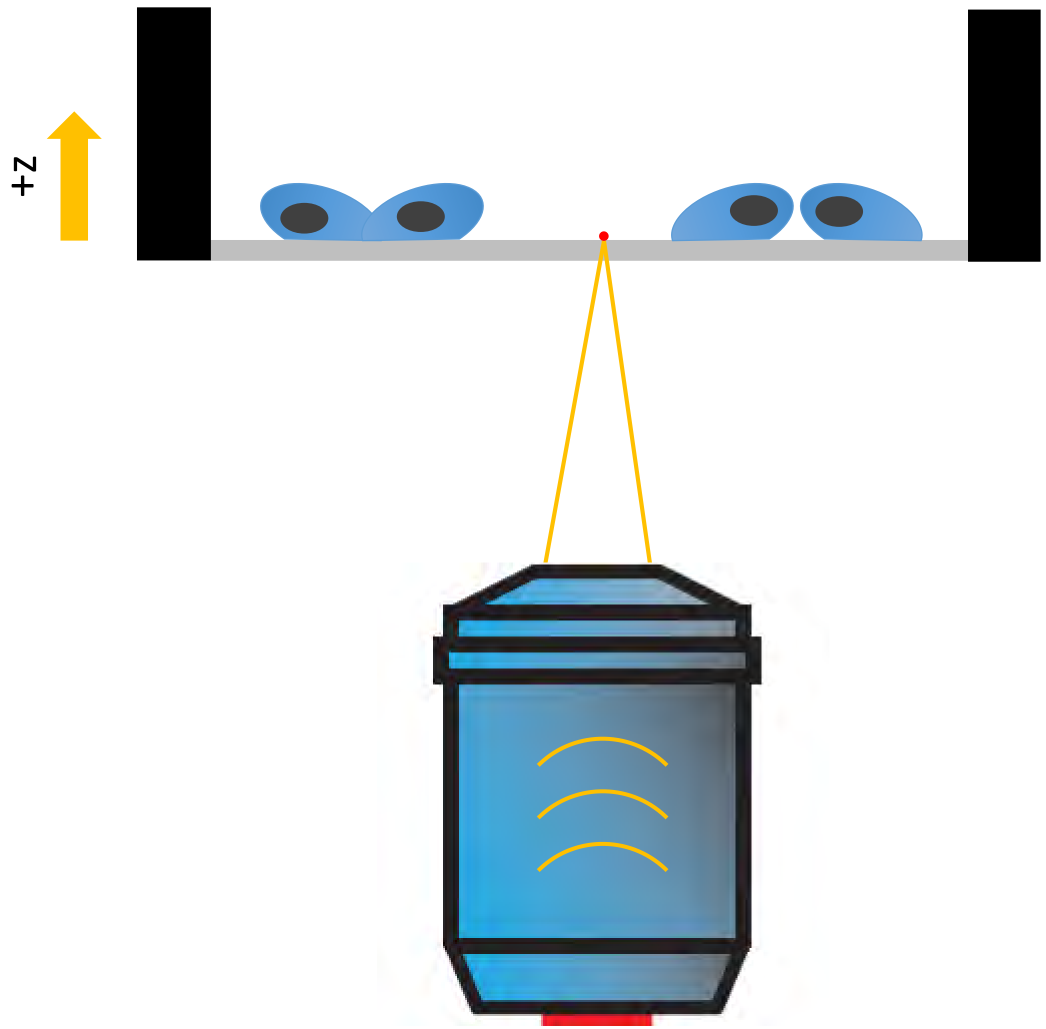
Z = 0 nm



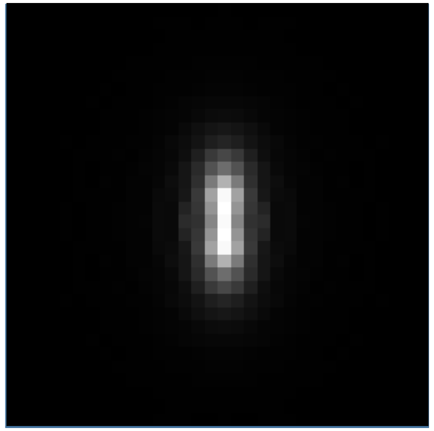


Z = -500 nm





Z = 500 nm

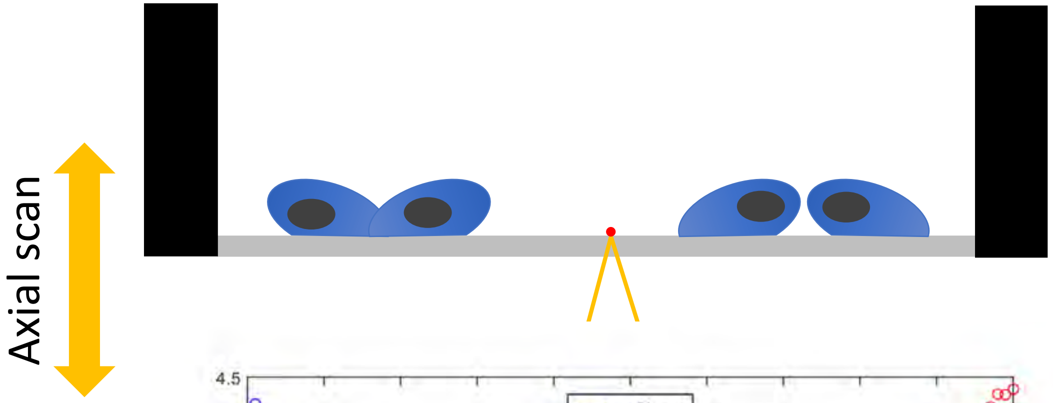


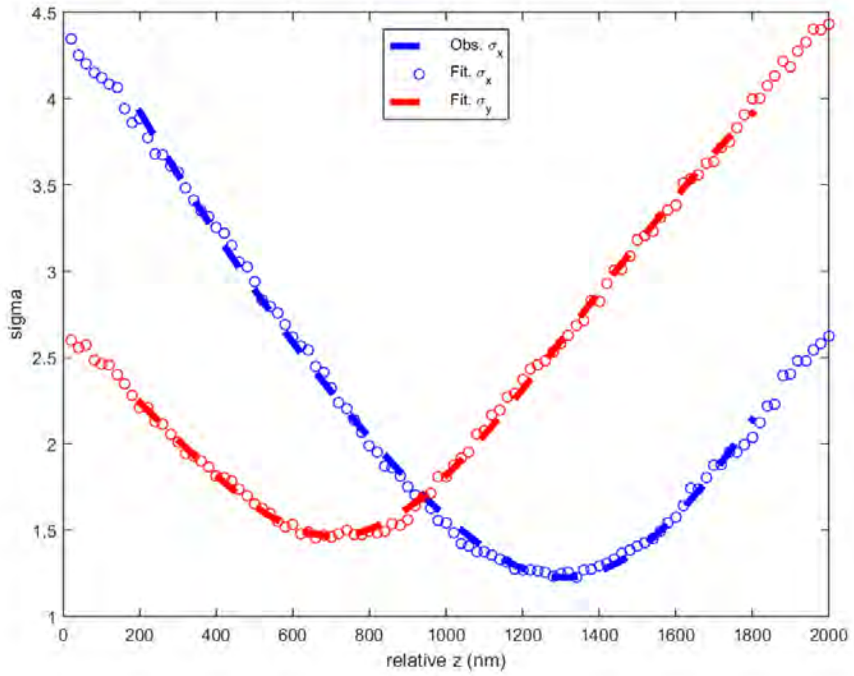
in vitro calibration

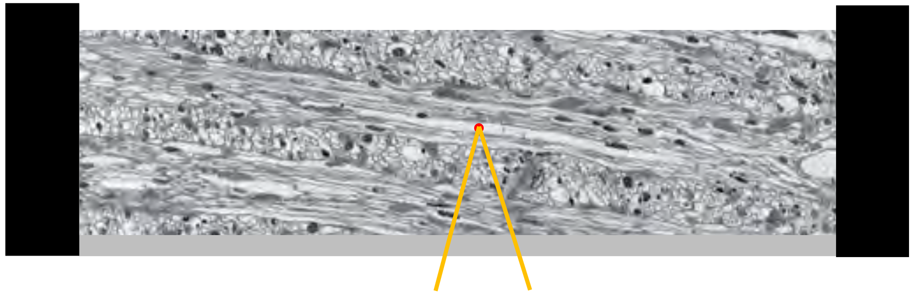
in situ PSF

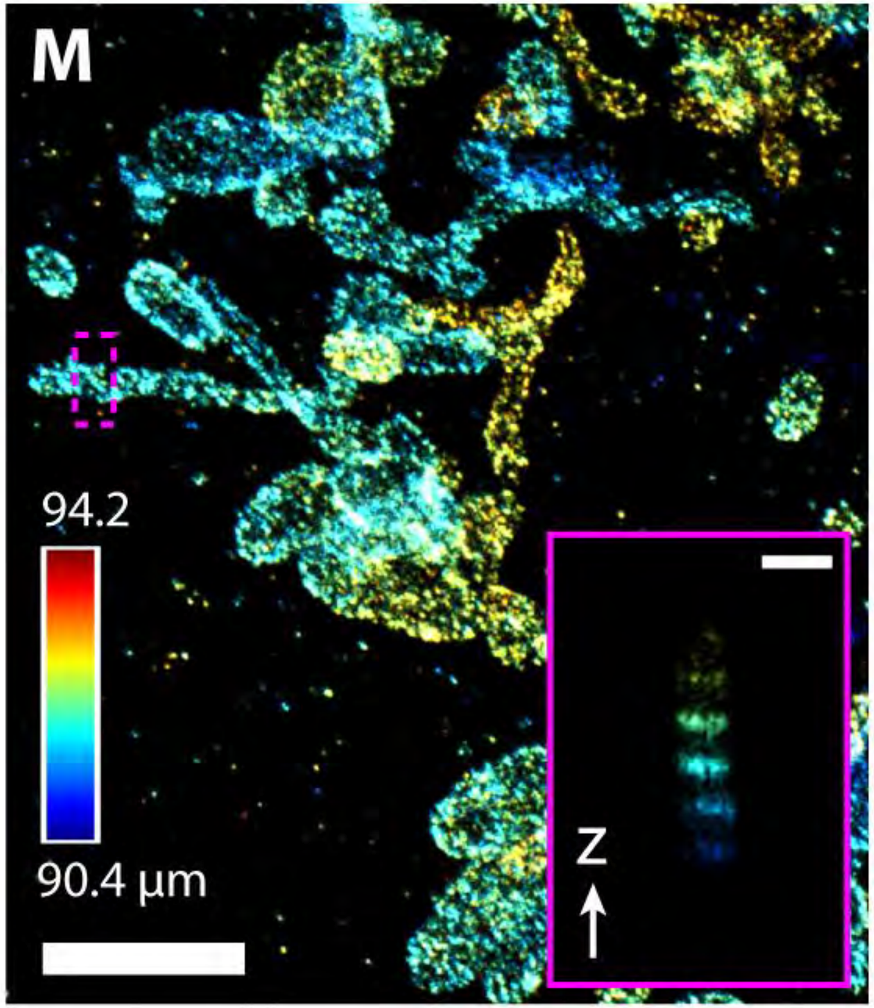
Photons never go through the sample

Photons experience sample aberration









Attenuation of PSF shape change along z leads significant artifacts in the axial cross section and deteriorates resolution

Mlodzianoski et al., *Nat. Methods*, 2018

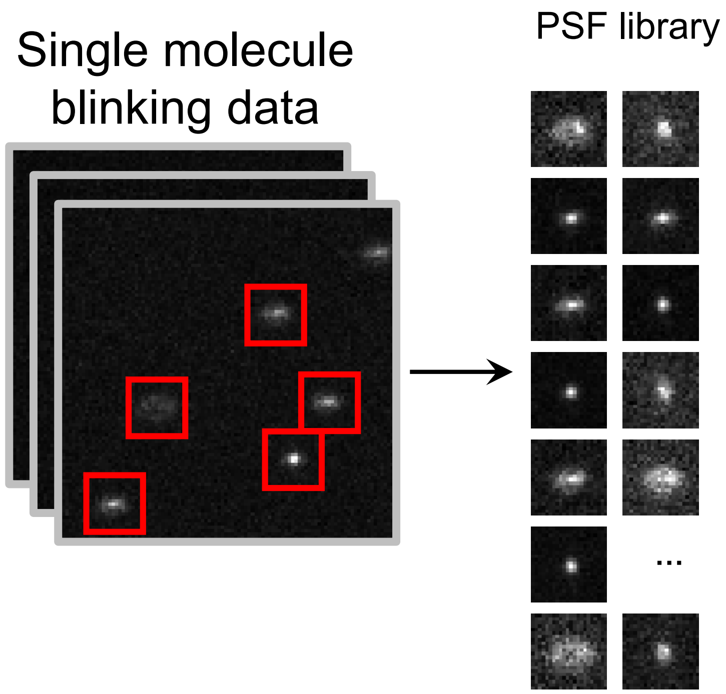
- PSF calibration using beads is *in vitro*
- Accurate single molecule localization requires *in situ* PSF especially in thick specimens (tissues and whole cells)

Question: How can we obtain an *in situ* PSF?

<u>If only</u> we can assign each emission pattern to its correct position.

INSPR: *in situ* PSF retrieval based on blinking data in whole cell/tissue

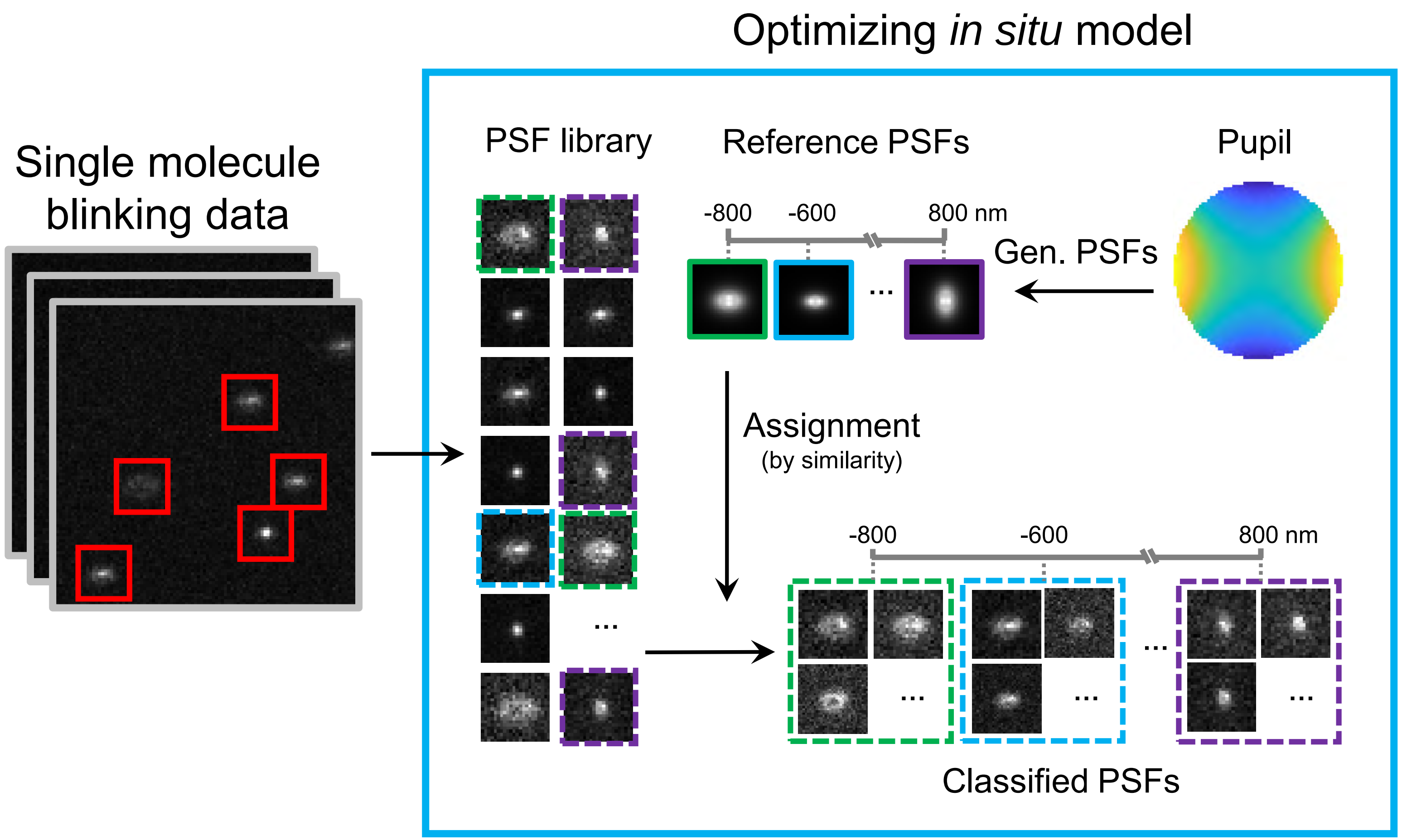
1. PSF library construction



Fan Xu* and Donghan Ma* et al., (2020) *Nat. Methods*, 17, 531–540, *co-first author

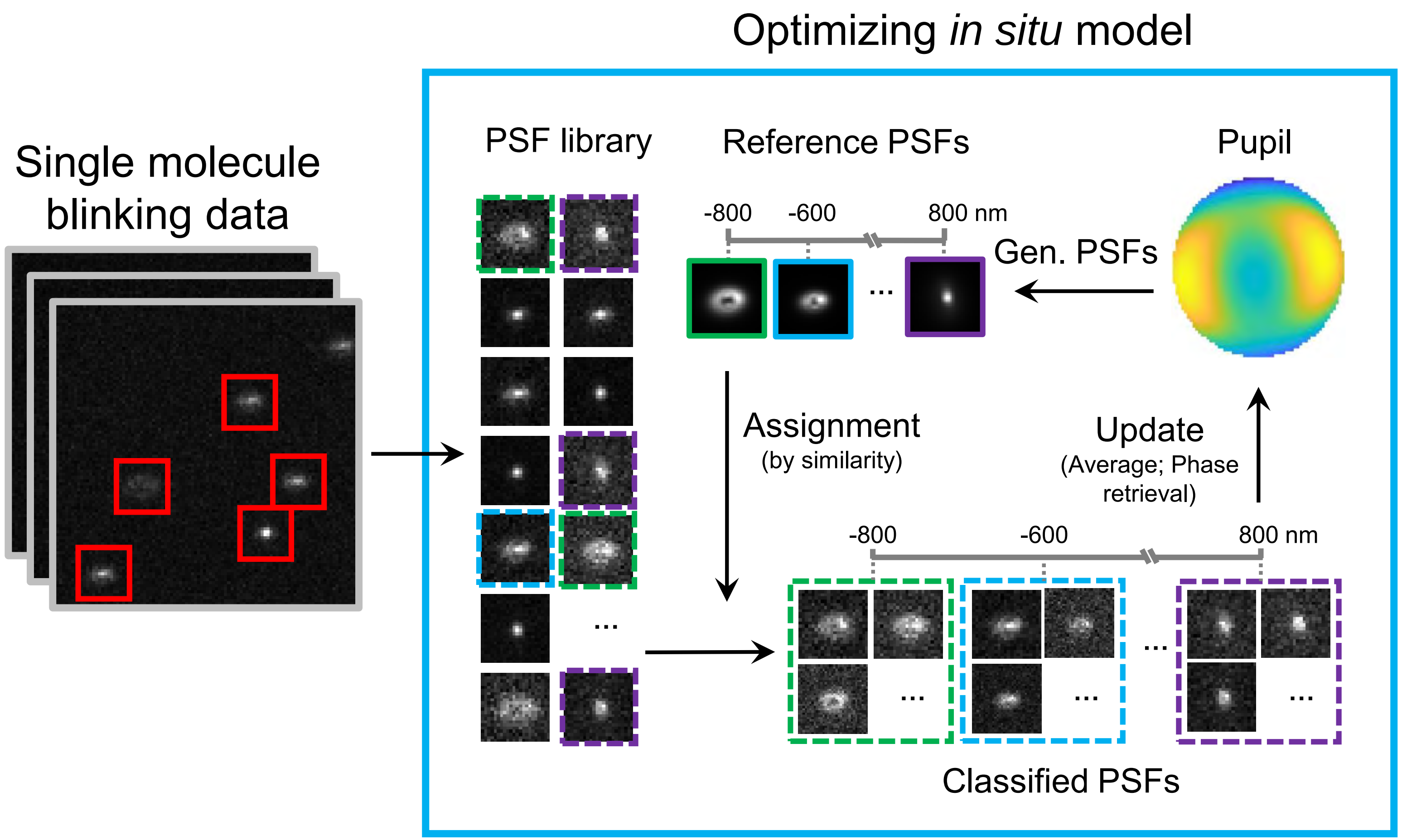
INSPR: *in situ* PSF retrieval based on blinking data in whole cell/tissue

2. *in situ* model optimization



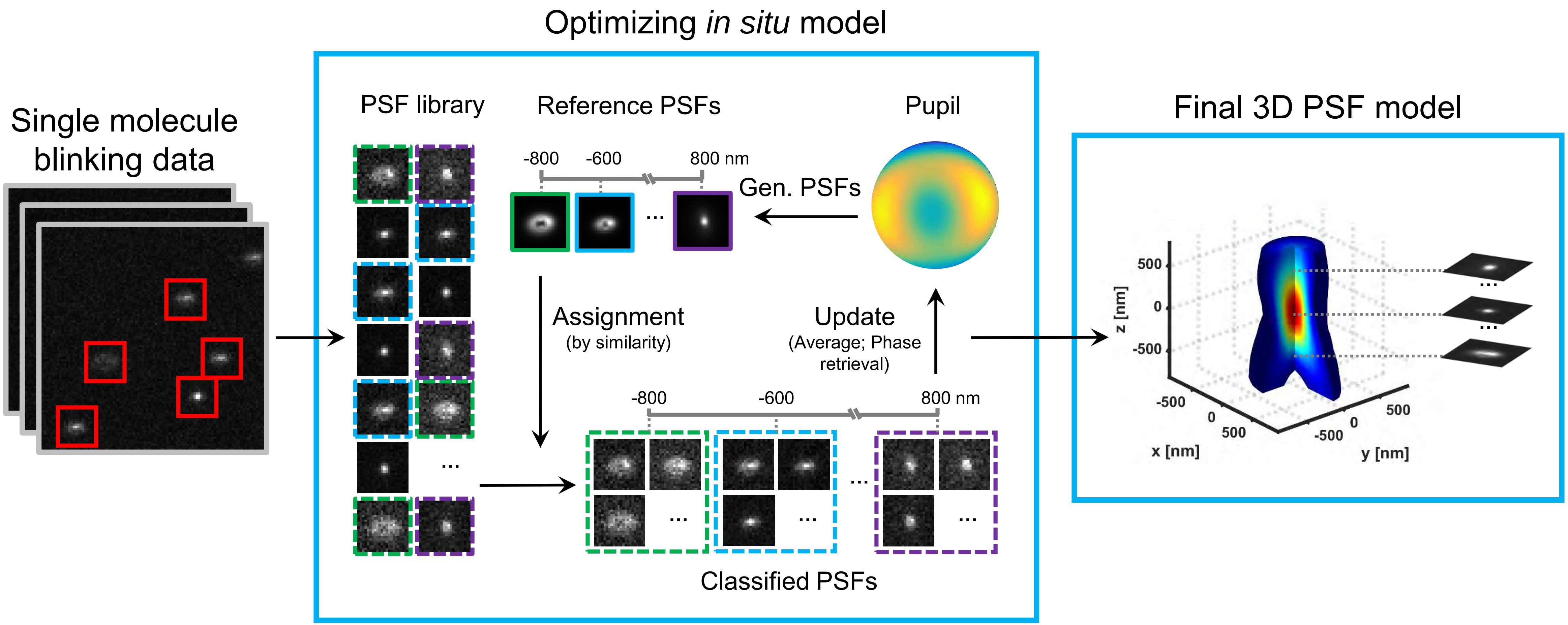
INSPR: *in situ* PSF retrieval based on blinking data in whole cell/tissue

2. *in situ* model optimization



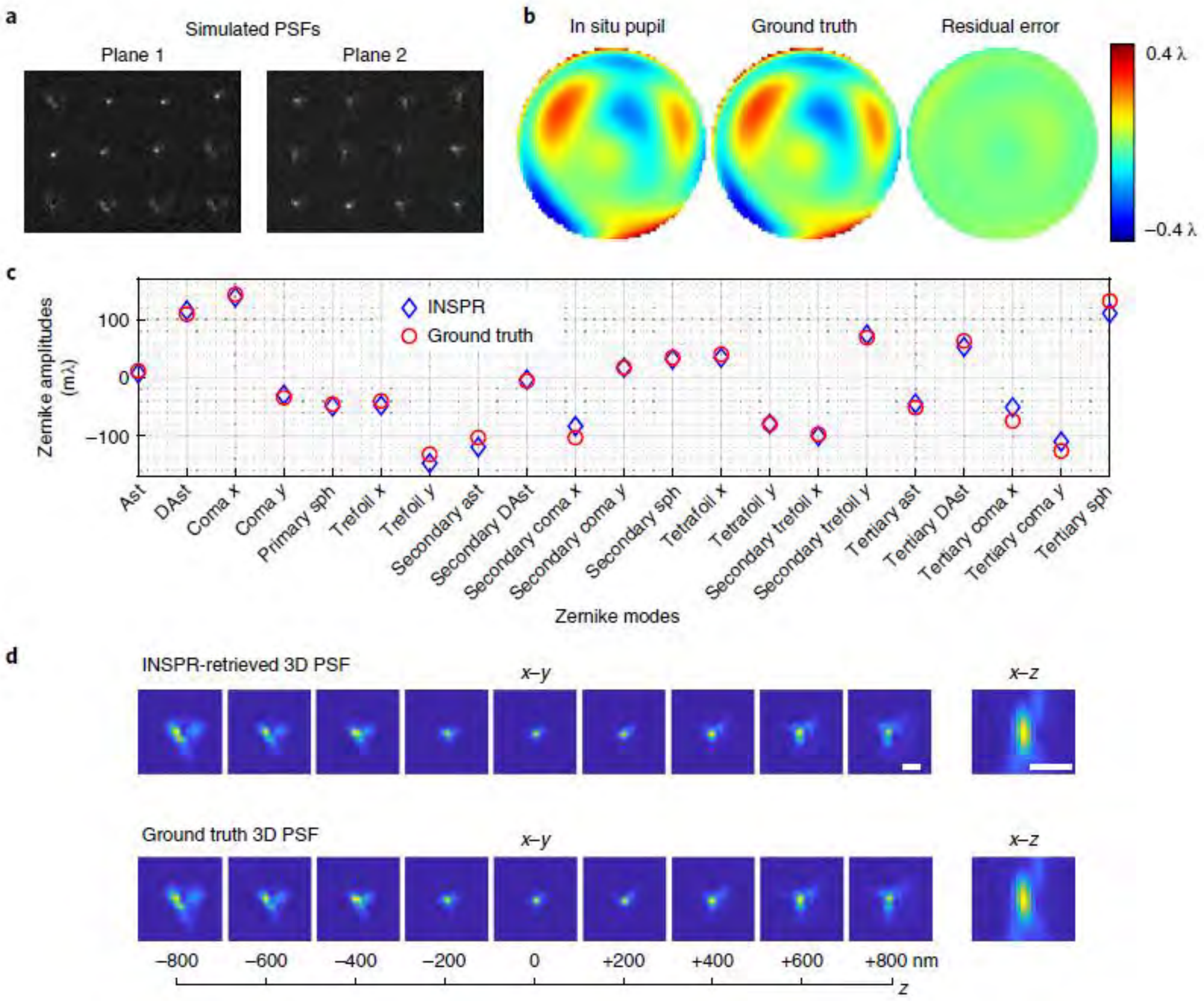
INSPR: *in situ* PSF retrieval based on blinking data in whole cell/tissue

3. Final 3D PSF model generation



- Construct an *in situ* 3D response directly from single-molecule datasets
- Pin-point their locations with high resolution through whole cells and tissues

Fan Xu* and Donghan Ma* et al., (2020) *Nat. Methods*, 17, 531–540, *co-first author



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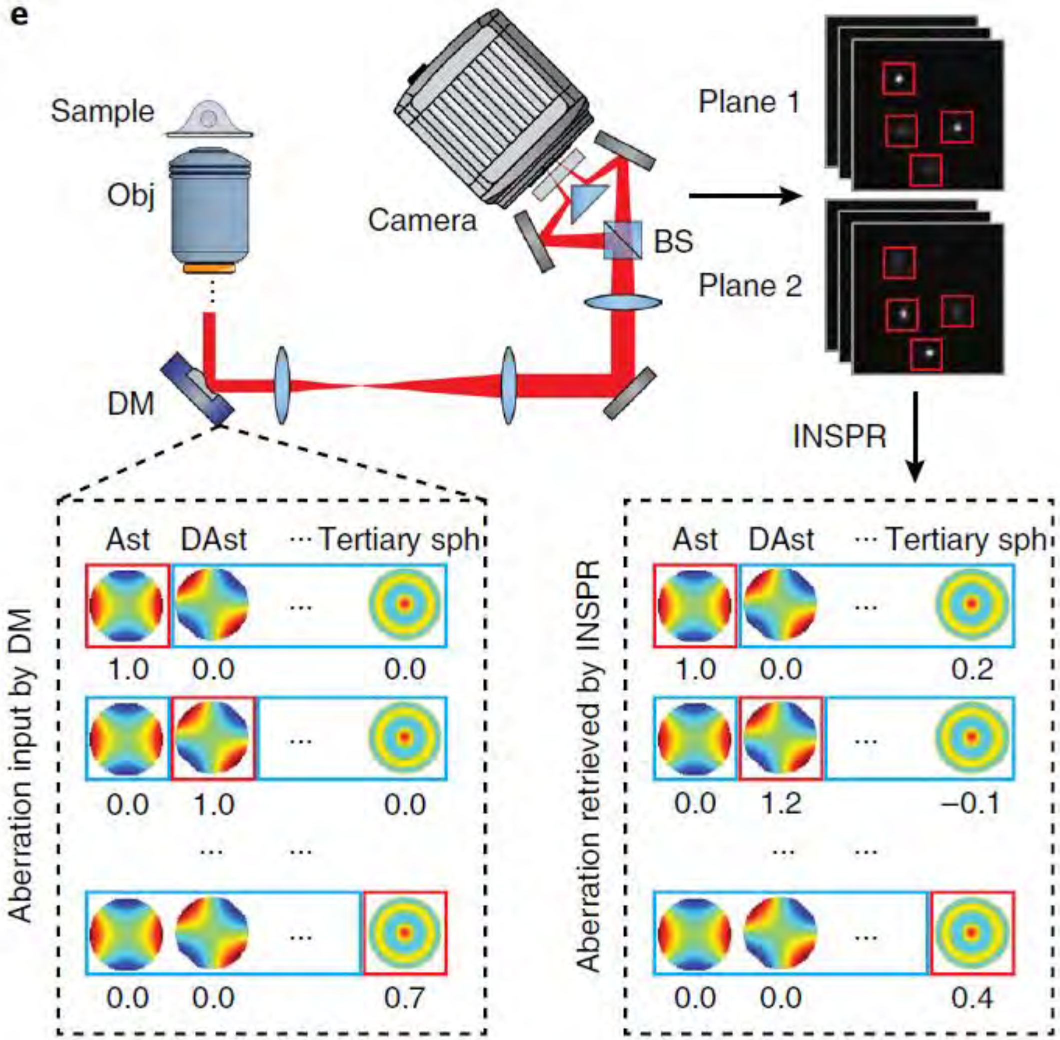
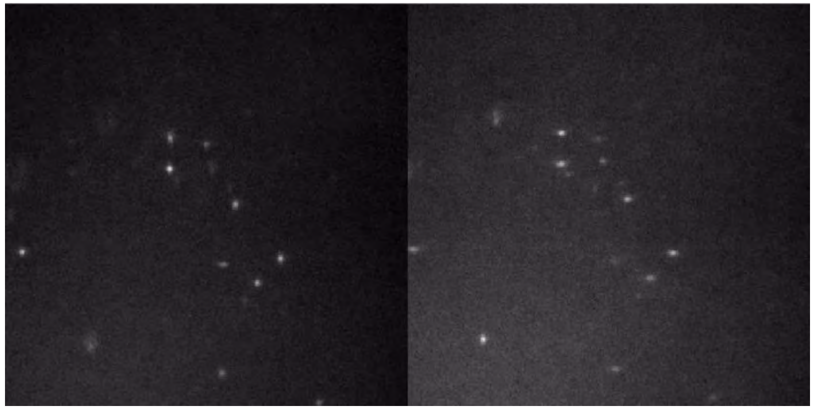
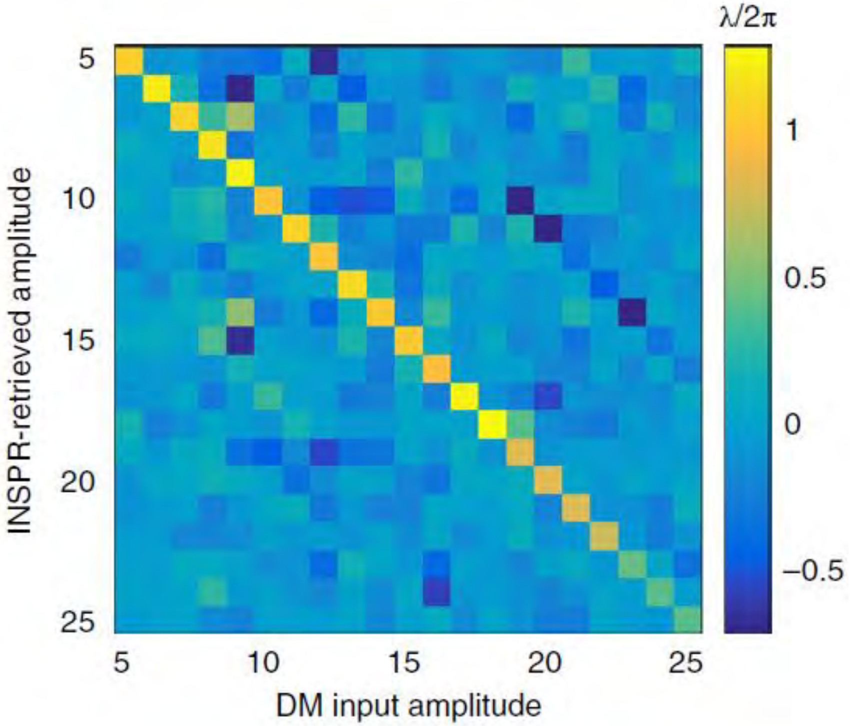
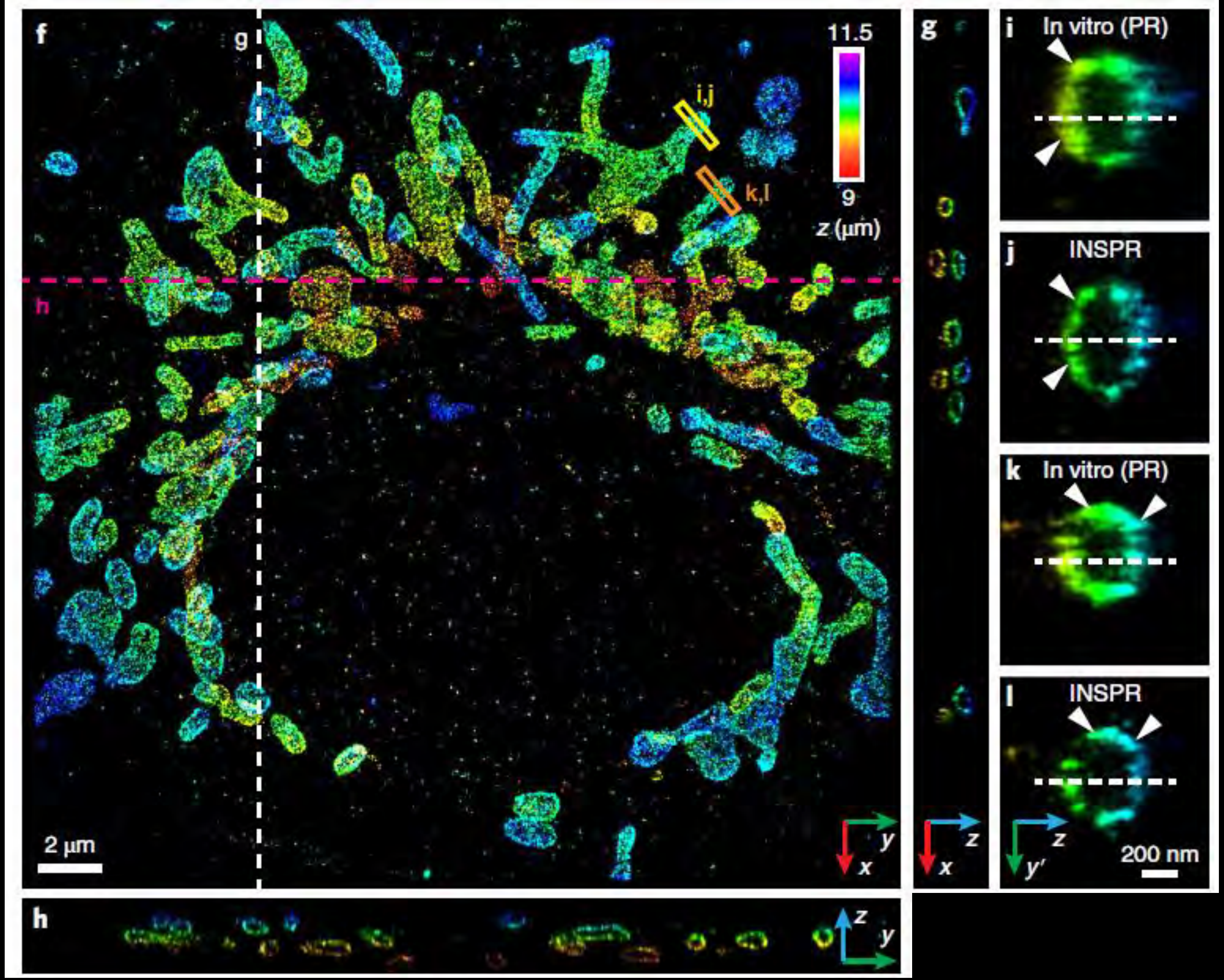
e


Sample
Obj
Camera
BS
DM
Plane 1
Plane 2
INSPR
Ast
DAst
··· Tertiary sph
Aberration input by DM
1.0
0.0
0.0
0.0
1.0
0.0
0.0
0.0
0.7
Aberration retrieved by INSPR
1.0
0.0
0.2
0.0
1.2
−0.1
0.0
0.0
0.4



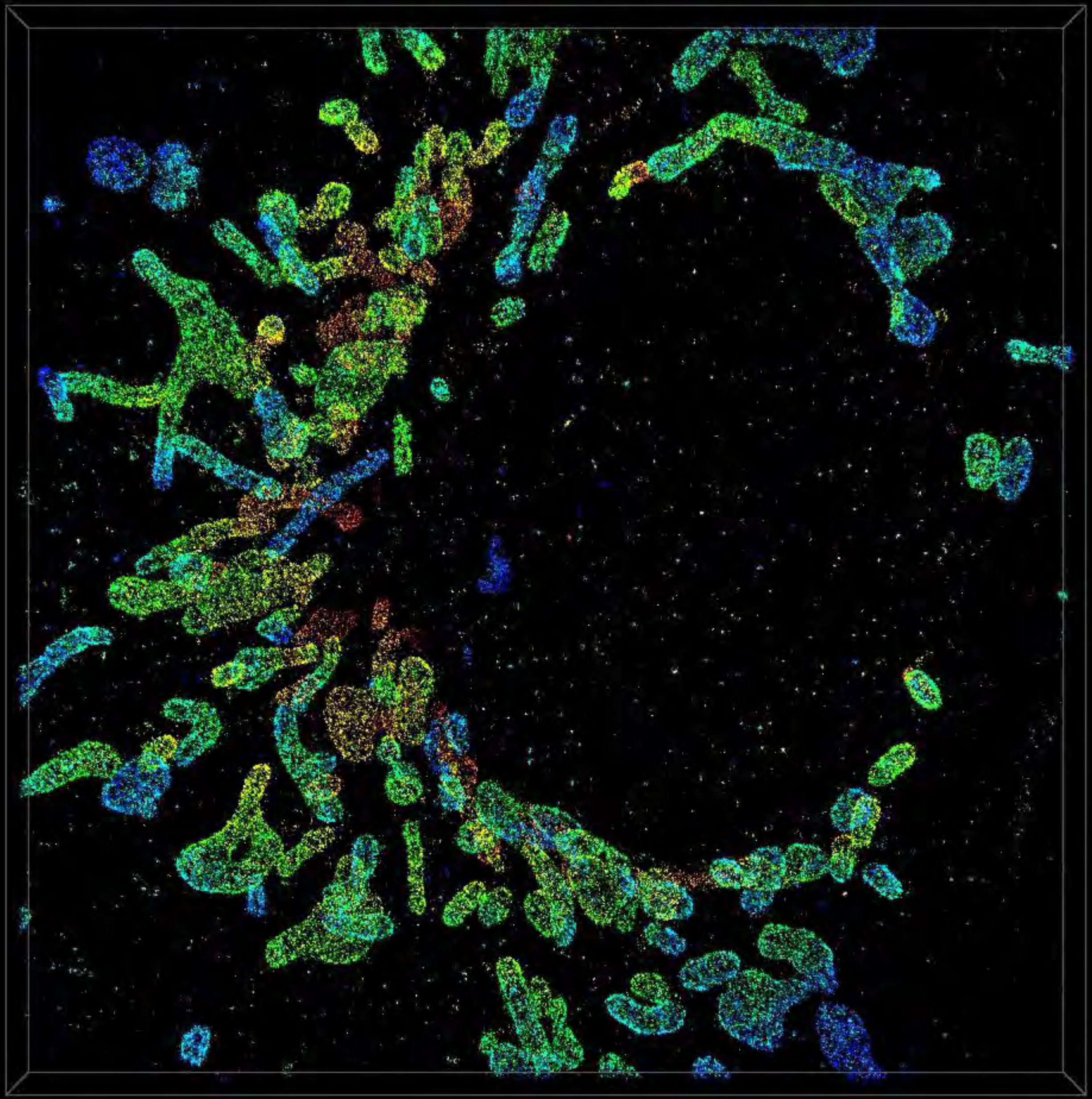

λ/2π
1
0.5
0
−0.5
INSPR-retrieved amplitude
5
10
15
20
25
DM input amplitude



f
g
h
i,j
k,l
11.5
9
z (μm)
2 μm
y
x
g
z
x
i
In vitro (PR)
j
INSPR
k
In vitro (PR)
l
INSPR
z
y′
200 nm
h
z
y

COS-7 cell
immune-labeled TOM20 with Alexa 647

# of localization: 1.1 Million
X-y precision: 8 nm
Z precision: 21 nm



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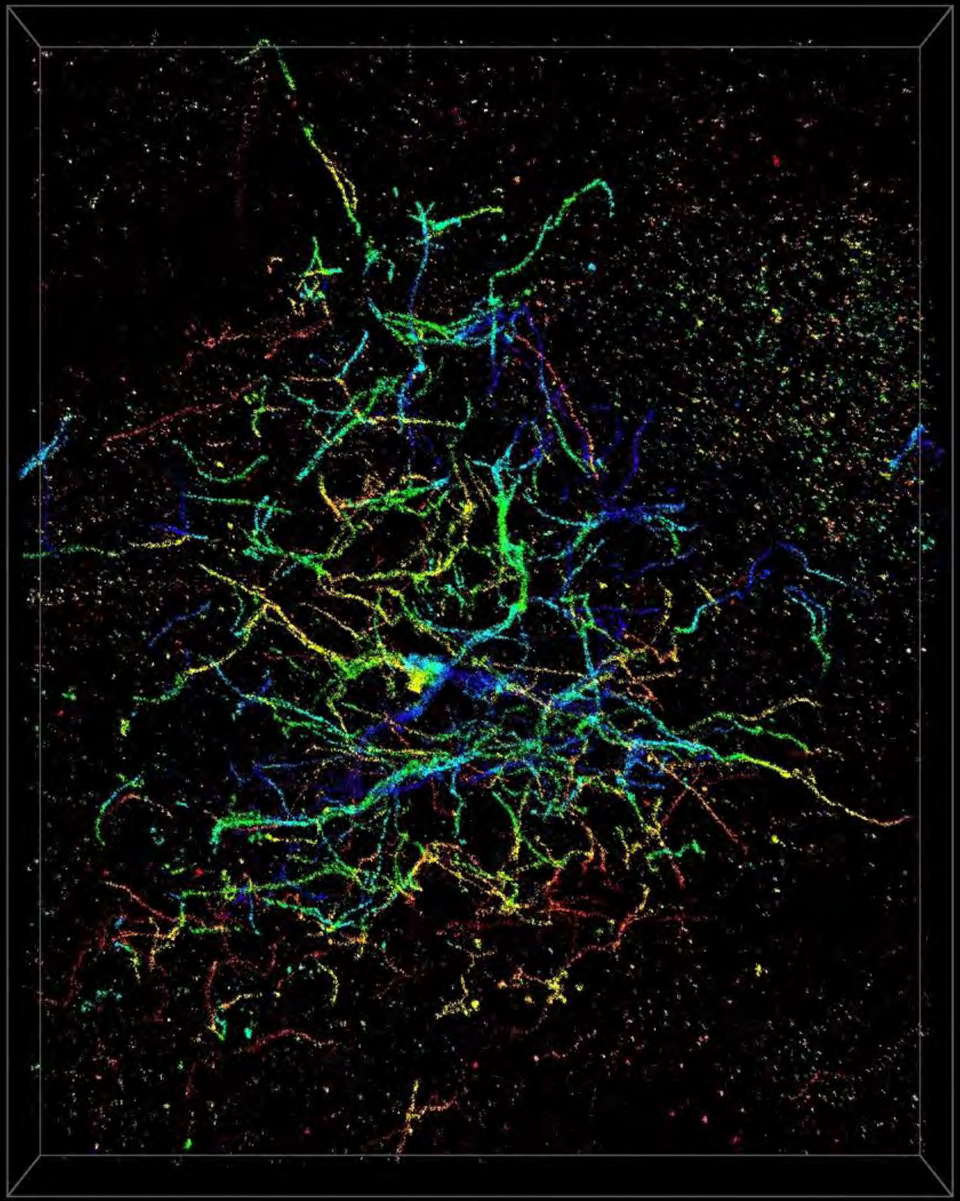
30 µm brain section
(8-month-old 5XFAD mouse)
immune-labeled Amyloid beta

# of localization: 586,109
X-y precision: 7 nm
Z precision: 28 nm



Fan Xu* and Donghan Ma* et al., (2020) *Nat. Methods*, 17, 531–540, *co-first author

Acknowledgements

Lab members:
Dr. Donghan Ma
Dr. Fan Xu
Dr. Sheng Liu (former)
Peiyi Zhang
Hao-Cheng Gao
Bi Cheng
Li Fang
Yilun Li

Acknowledgements

Thomas Pollard, Yale
Joerg Bewersdorf, Yale
Alexander Chubykin, Purdue
Gary Landreth, IUSOM
Daniel Suter, Purdue
Jing Liu, IUPUI
Peng Yin, Harvard

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Basic Discoveries for Better Health

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BRAIN INITIATIVE BRAIN RESEARCH THROUGH ADVANCING INNOVATIVE NEUROTECHNOLOGIES

PURDUE INSTITUTE FOR INFLAMMATION, IMMUNOLOGY AND INFECTIOUS DISEASE

Disclaimer: F.H. has significant financial interest in Hamamatsu Photonics K.K.

https://github.com/HuanglabPurdue/