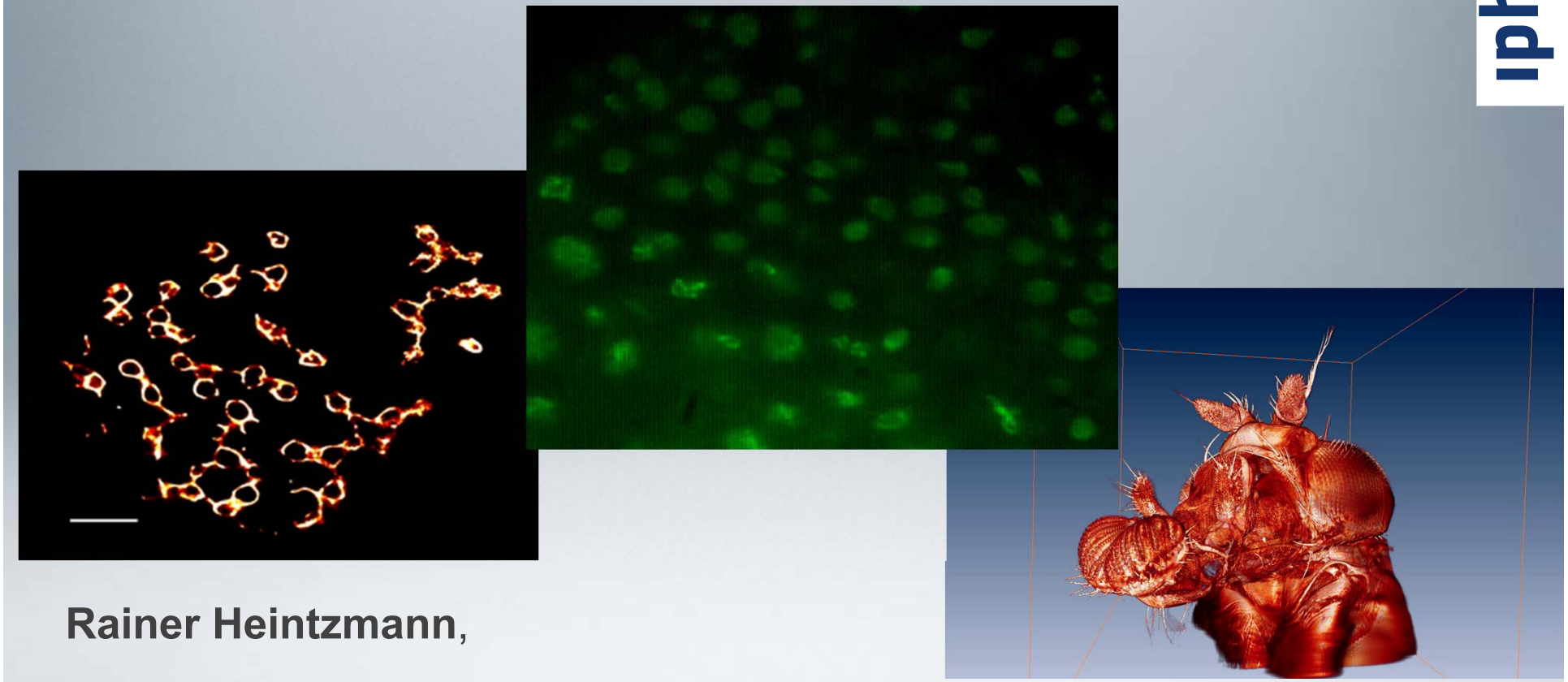
Computational aspects of SIM

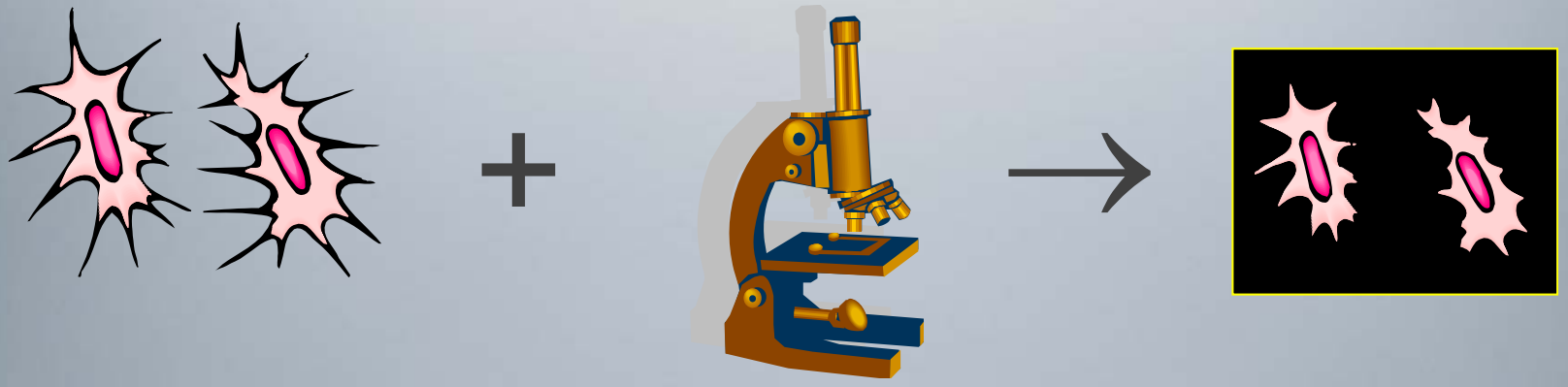


Rainer Heintzmann,

- Leibniz Institute of Photonic Technology (IPHT),
- Friedrich Schiller University of Jena

Trieste, 23/02/2017

Paradigm: Optimize for direct visibility

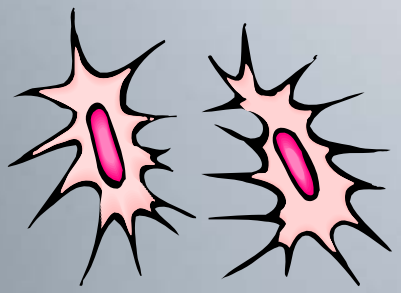
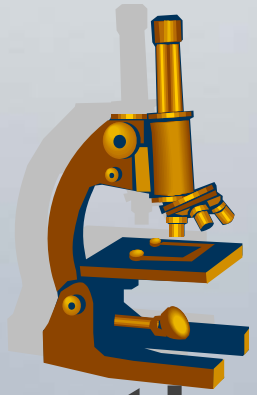
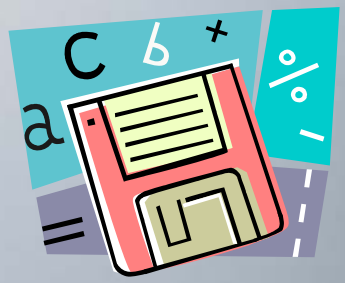


Object Optics Image

E.g.: Widefield, Confocal, STED

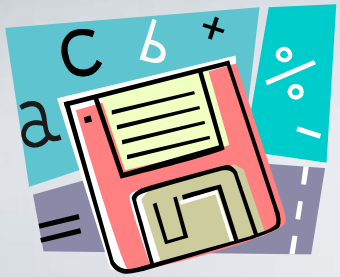
Does not necessarily optimize information content!

Paradigm: Optimize for information content

Object Optics Data

Data Computation Image



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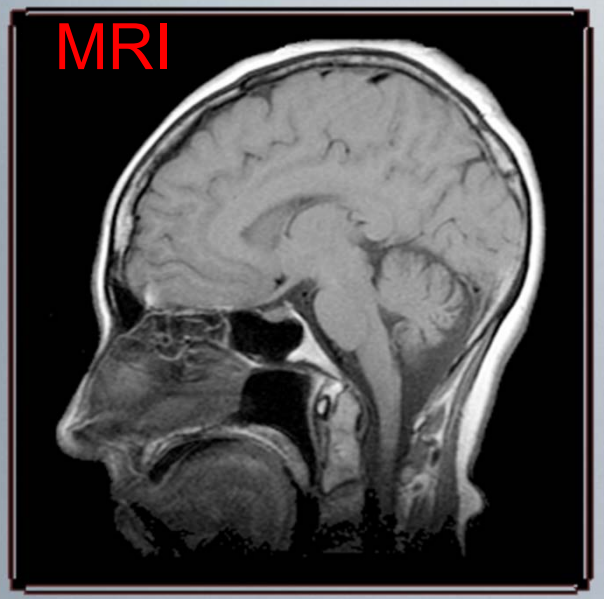
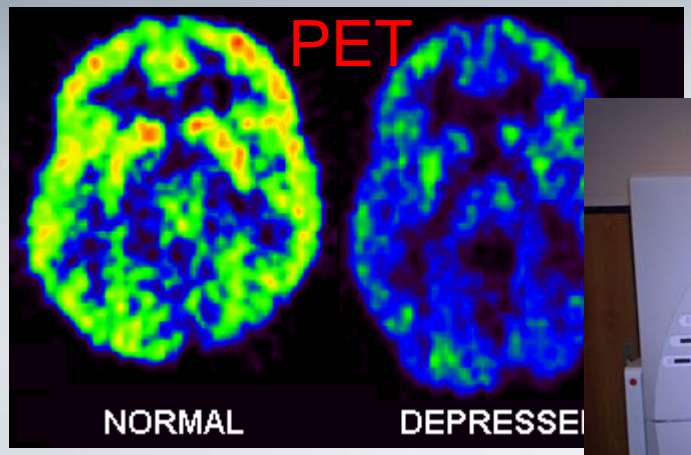
Leibniz-Gemeinschaft

Examples in Medical Imaging



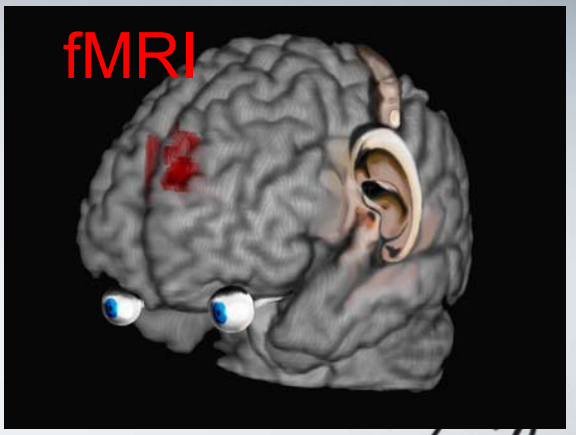
http://www.cis.rit.edu/htbooks/mri/images/head.gif

http://www.physics.ubc.ca/research/images/spect.gif



http://www.cerebromente.org.br/n01/pet/petdep.gif

http://www.vetmed.lsu.edu/vth&c/Orthopedics/Images/Computed%20Tomography%20(CT)%20Scanner.RV.jpg

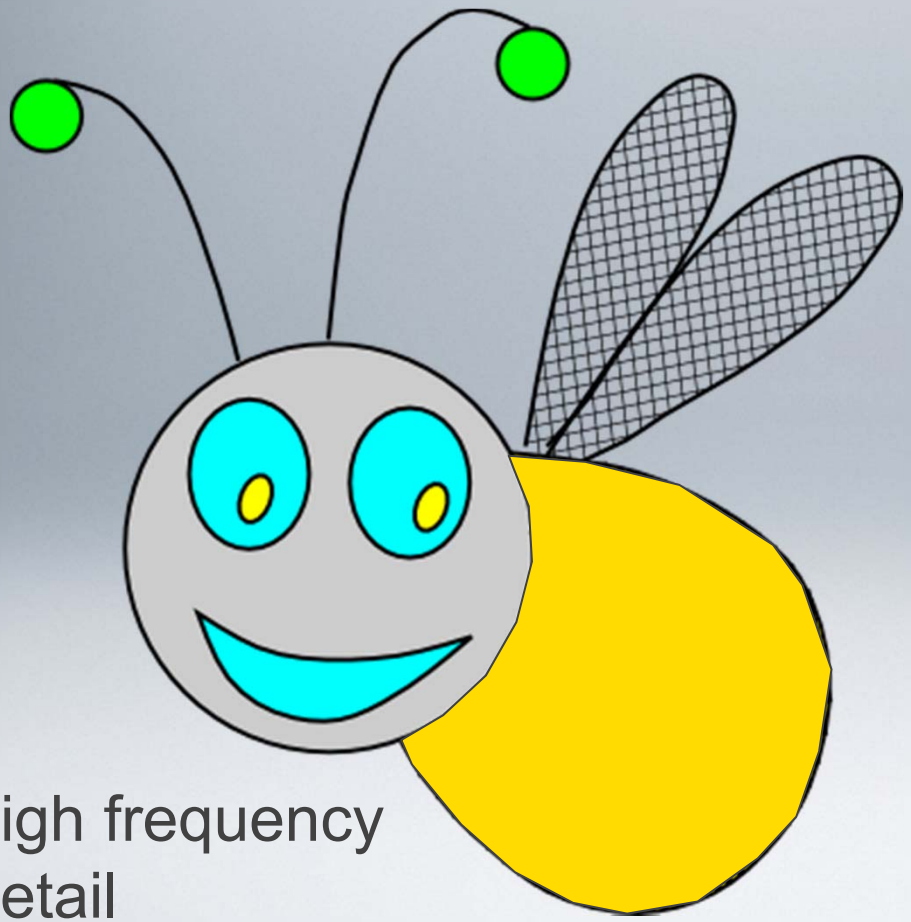


http://www.fmri.wfubmc.edu/other%20pics/lab_brain_logo.JPG

Structured Illumination (SIM)

Moiré Demonstration

Moiré effect



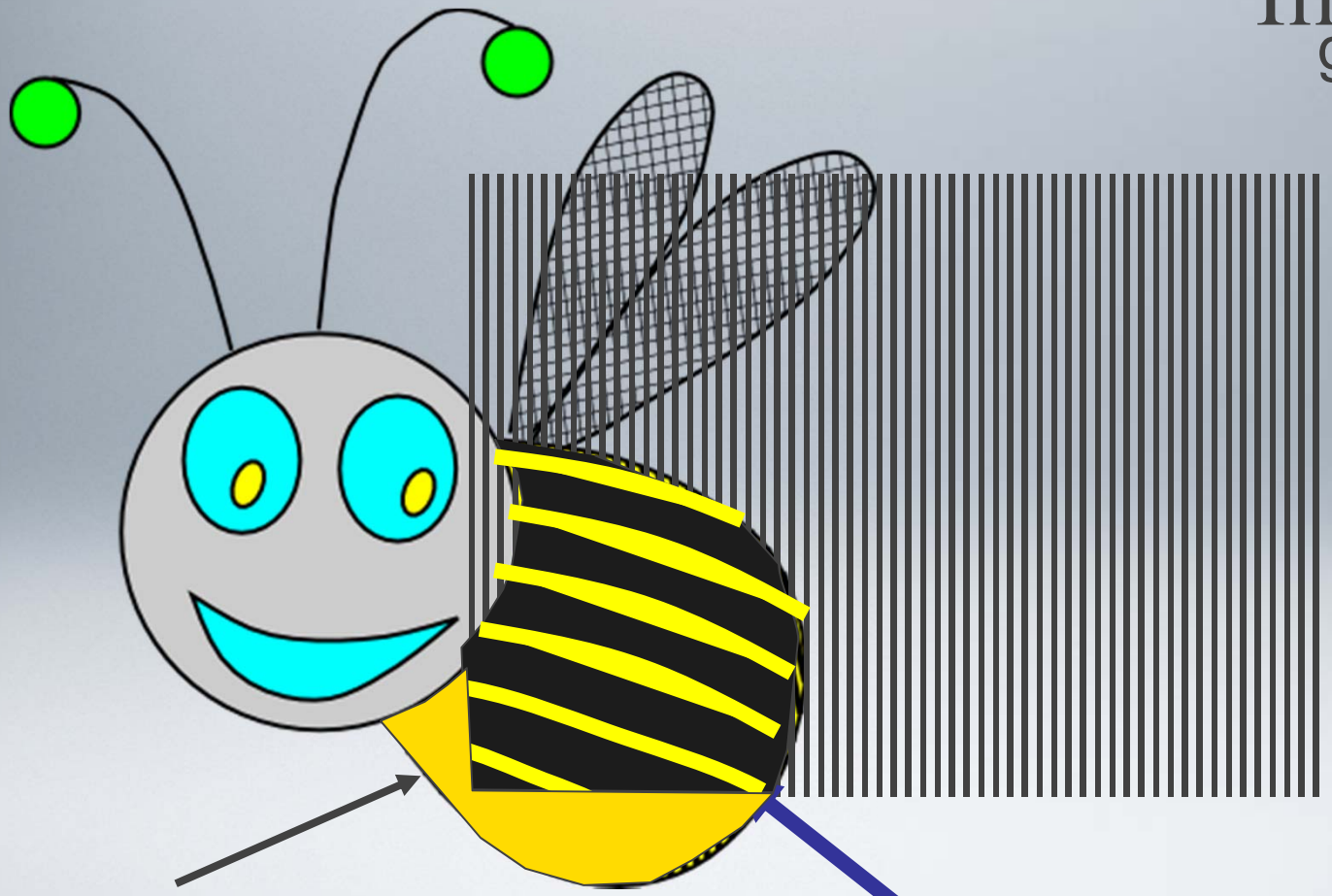
high frequency detail

... is lost

Sample

Aurélie Jost

Moiré effect



Aurélie Jost

The Moiré effect

Moiré fringes

Image: Wikipedia (author:Ildar Sagdejev)

Image formation in FLUORESCENCE

$$I_{em}(\mathbf{x}) = Obj(\mathbf{x}) \cdot I_{ex}(\mathbf{x})$$

Multiplication in real space

↕

Convolution in Fourier space

$$\tilde{I}_{em}(\mathbf{k}) = \tilde{Obj}(\mathbf{k}) \otimes \tilde{I}_{ex}(\mathbf{k})$$

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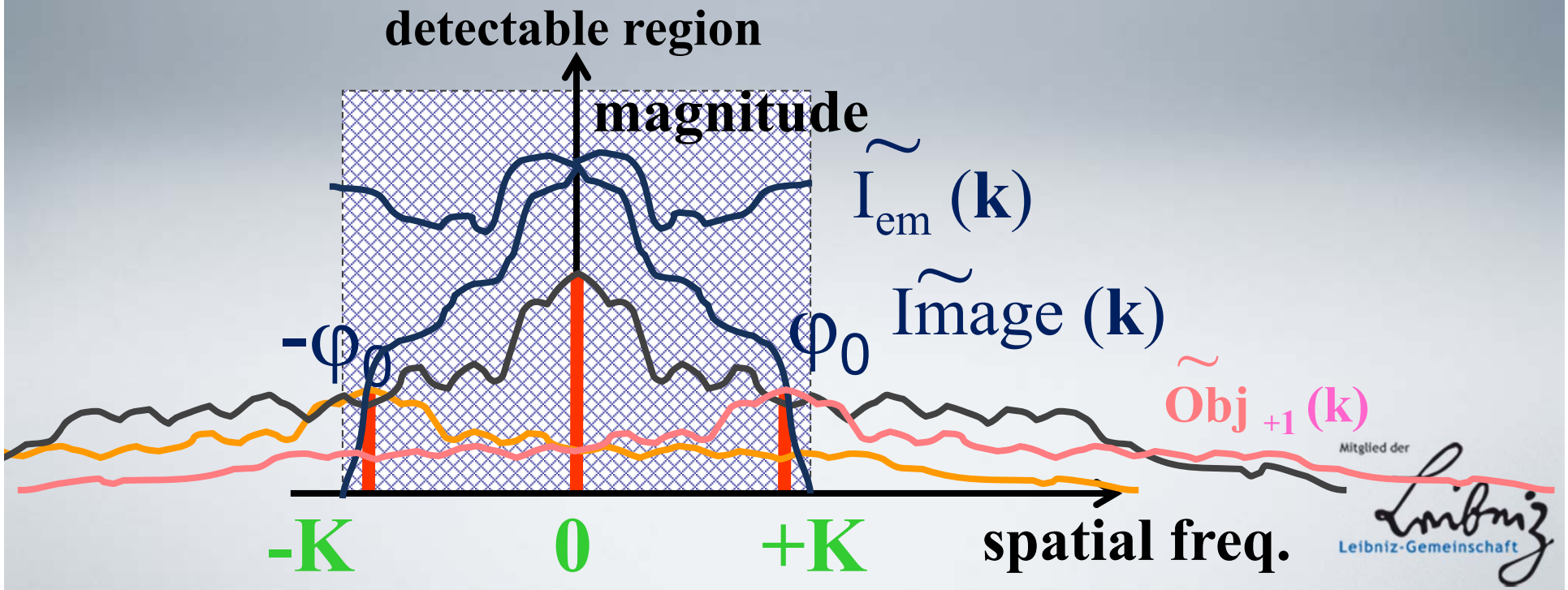
Object Fluorescence Distribution

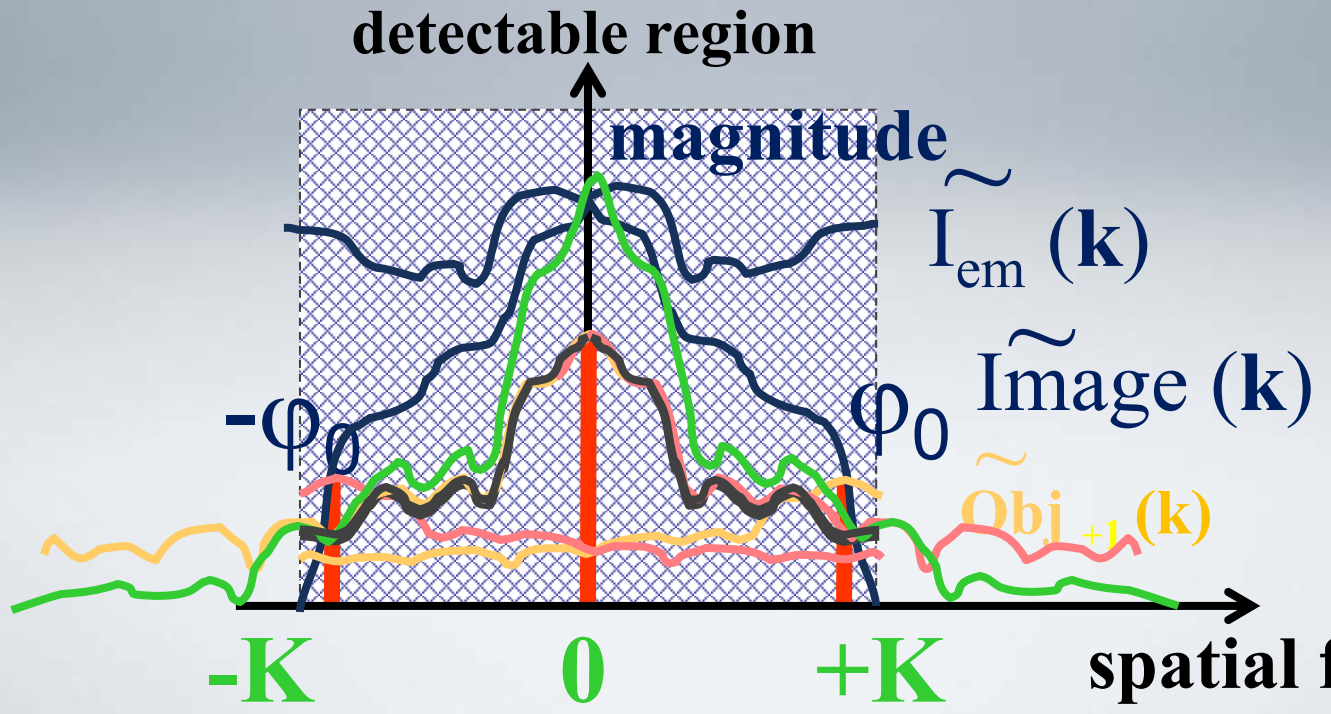
$$I_{em}(\mathbf{x}) = Obj(\mathbf{x}) \cdot I_{ex}(\mathbf{x})$$

$$\tilde{I}_{em}(\mathbf{k}) = \tilde{Obj}(\mathbf{k}) \otimes \tilde{I}_{ex}(\mathbf{k})$$



Piecing Parts Together

- **Correct for OTF**
- **Extract components**
- **Shift into place**
- **Weighted average**
- **Apodize**



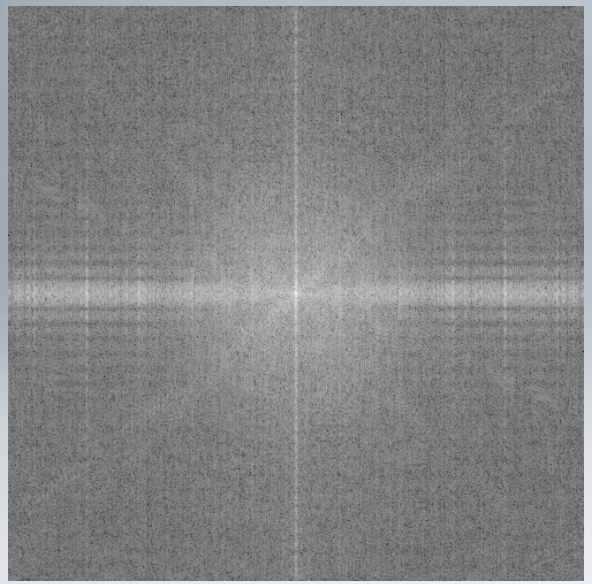
Structured Illumination Micropscopy

Sample Sample with structured illumination Illumination

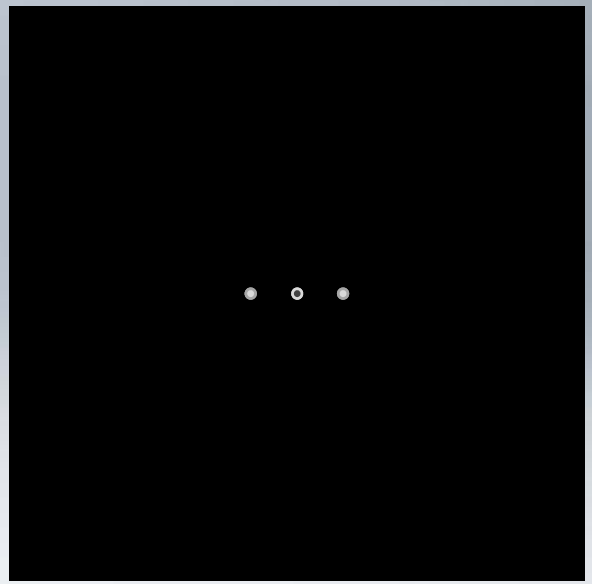
Multiplication of sample and illumination

Structured Illumination Micropscopy

Sample

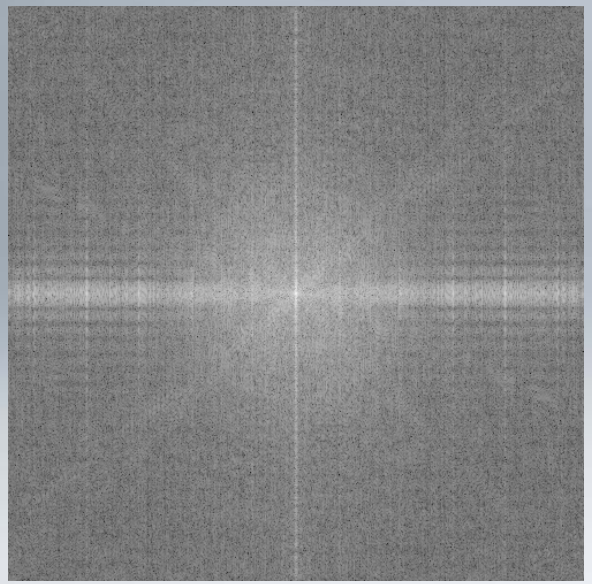


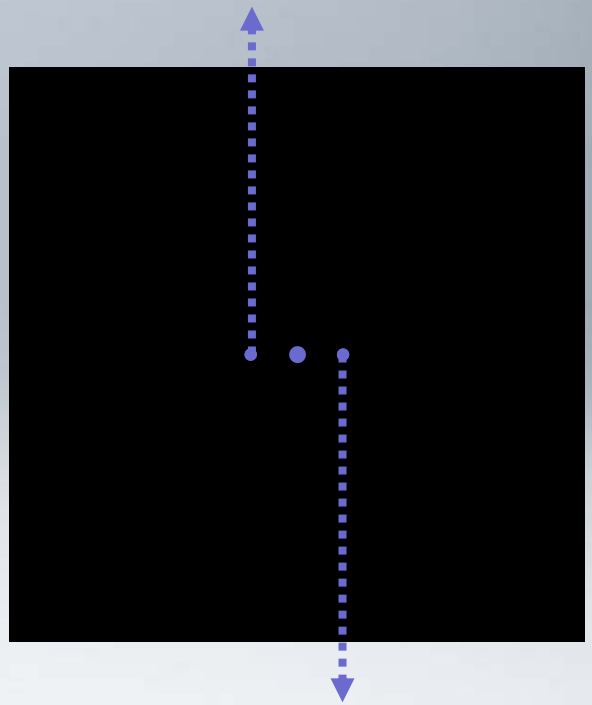
Illumination



Structured Illumination Micropscopy

Sample

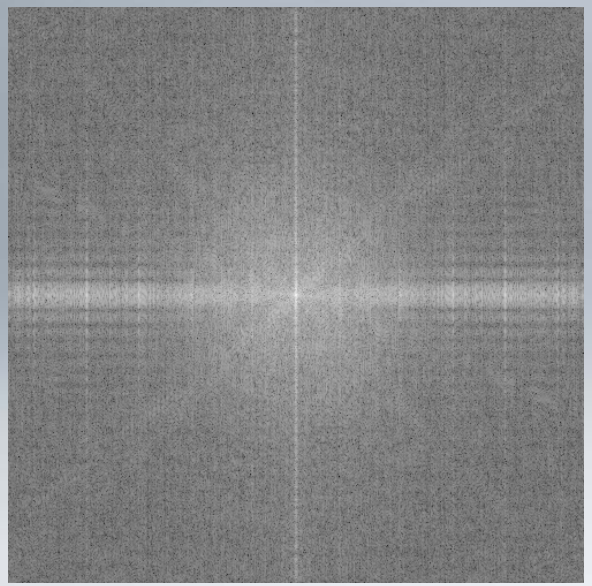




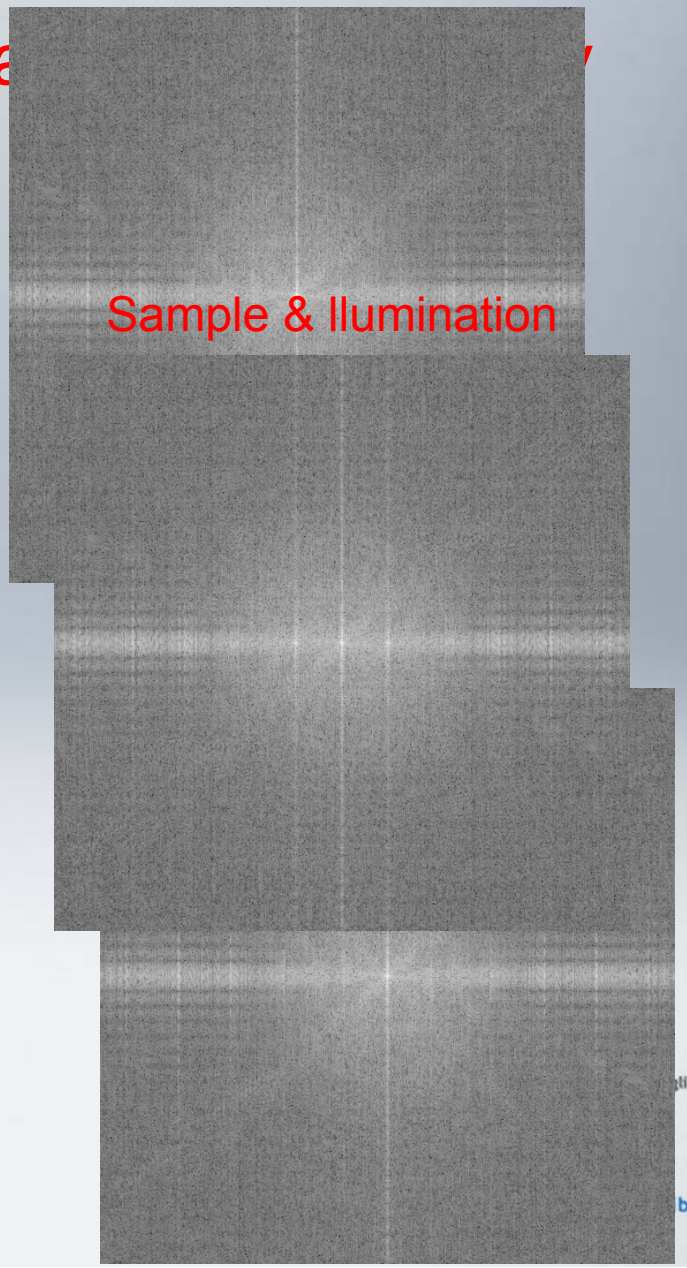
Mitglied der Leibniz-Gemeinschaft

Structured Illumina

Sample



Sample & Illumination



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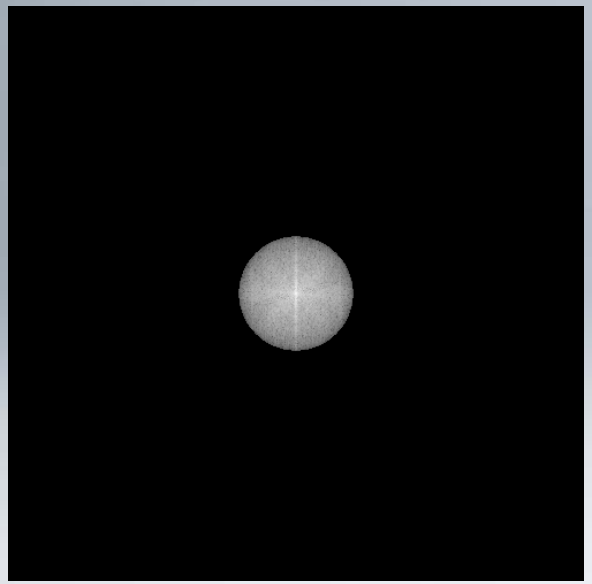
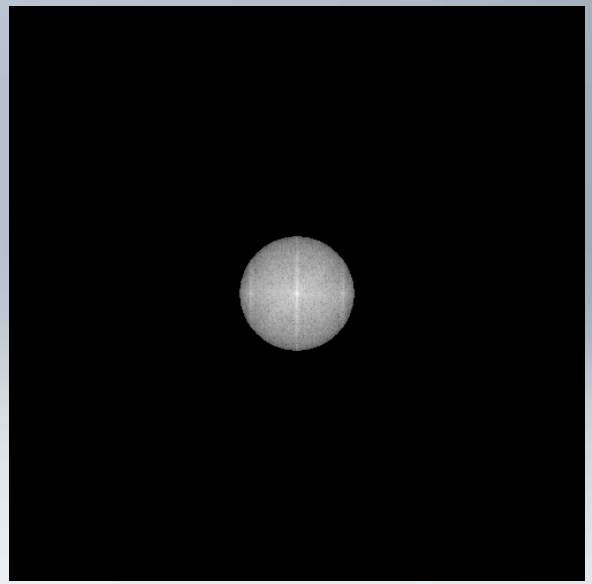
bniz-Gemeinschaft

Sample

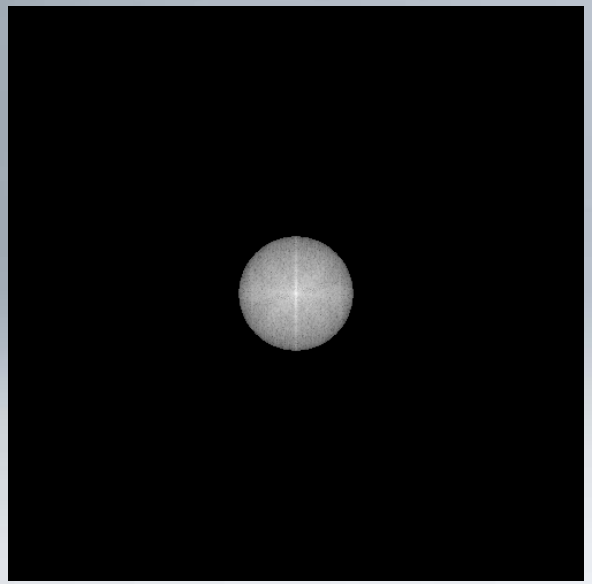


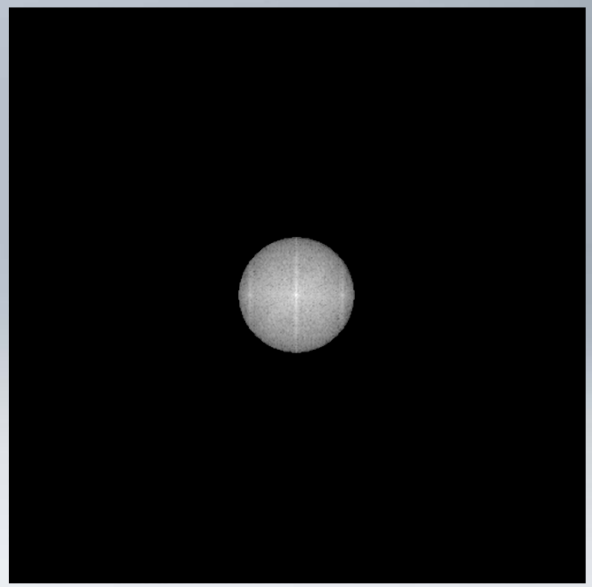
Sample & Illumination



Imaging leads to loss of
high frequencies (OTF)

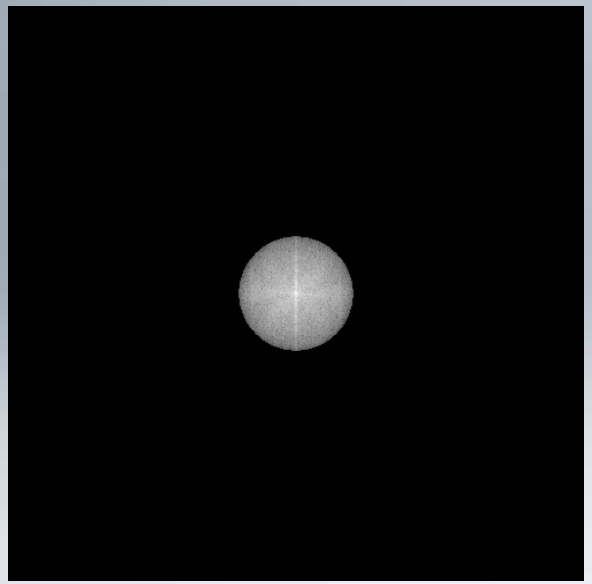
Sample

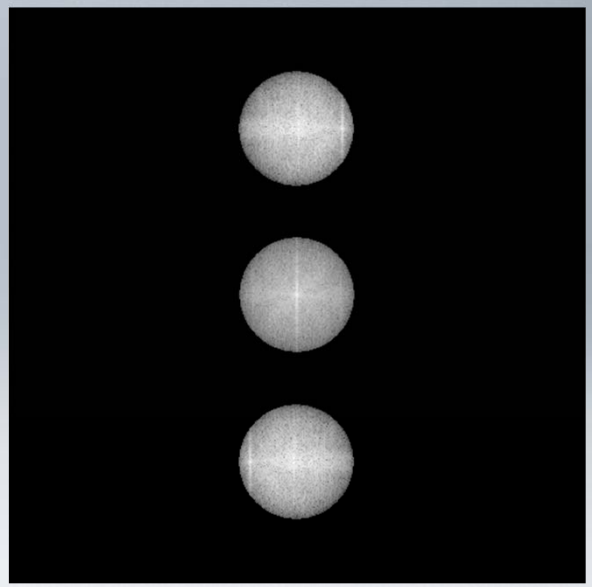




Separating the components…

Sample

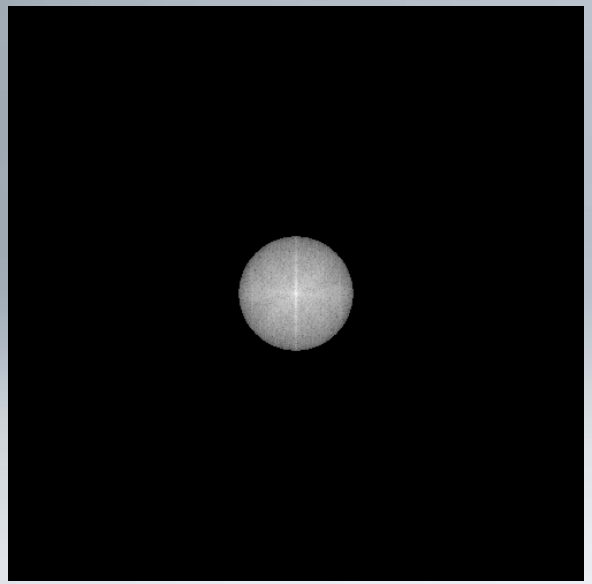


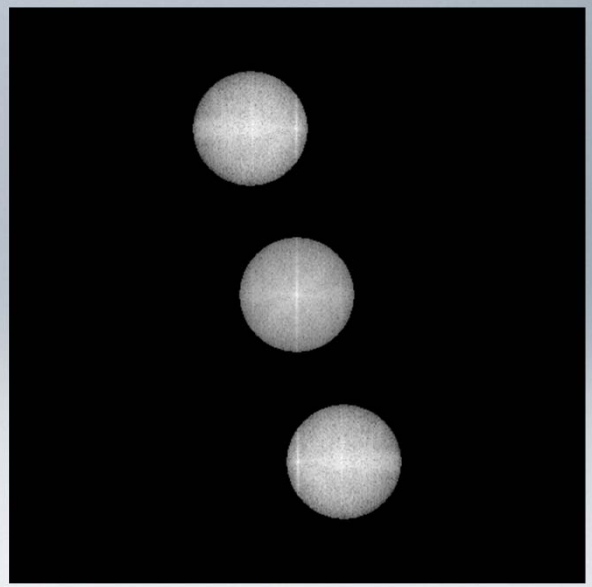


Separating the components…

Shifting the components…

Sample





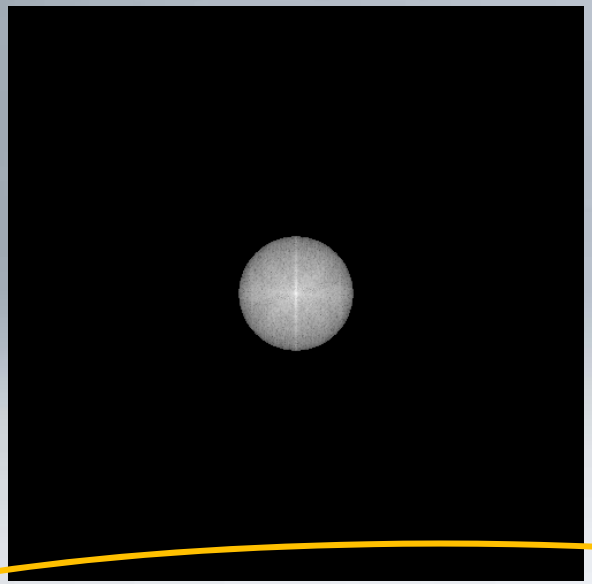
Separating the components…

Shifting the components…

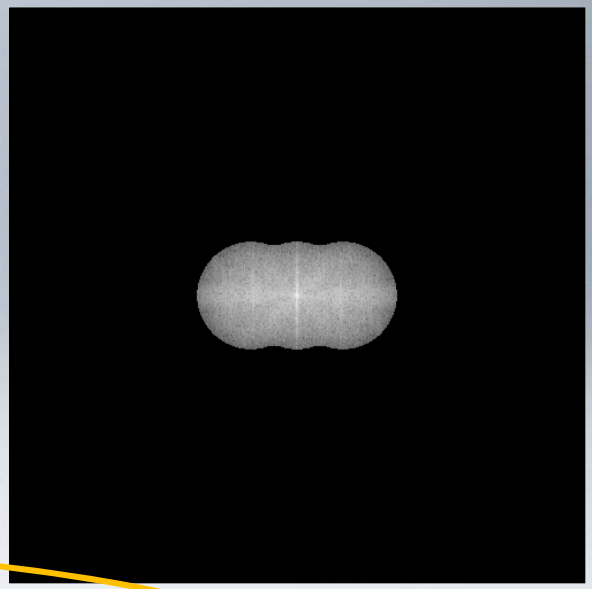
Recombining the components…

Mitglied der Leibniz-Gemeinschaft

Sample



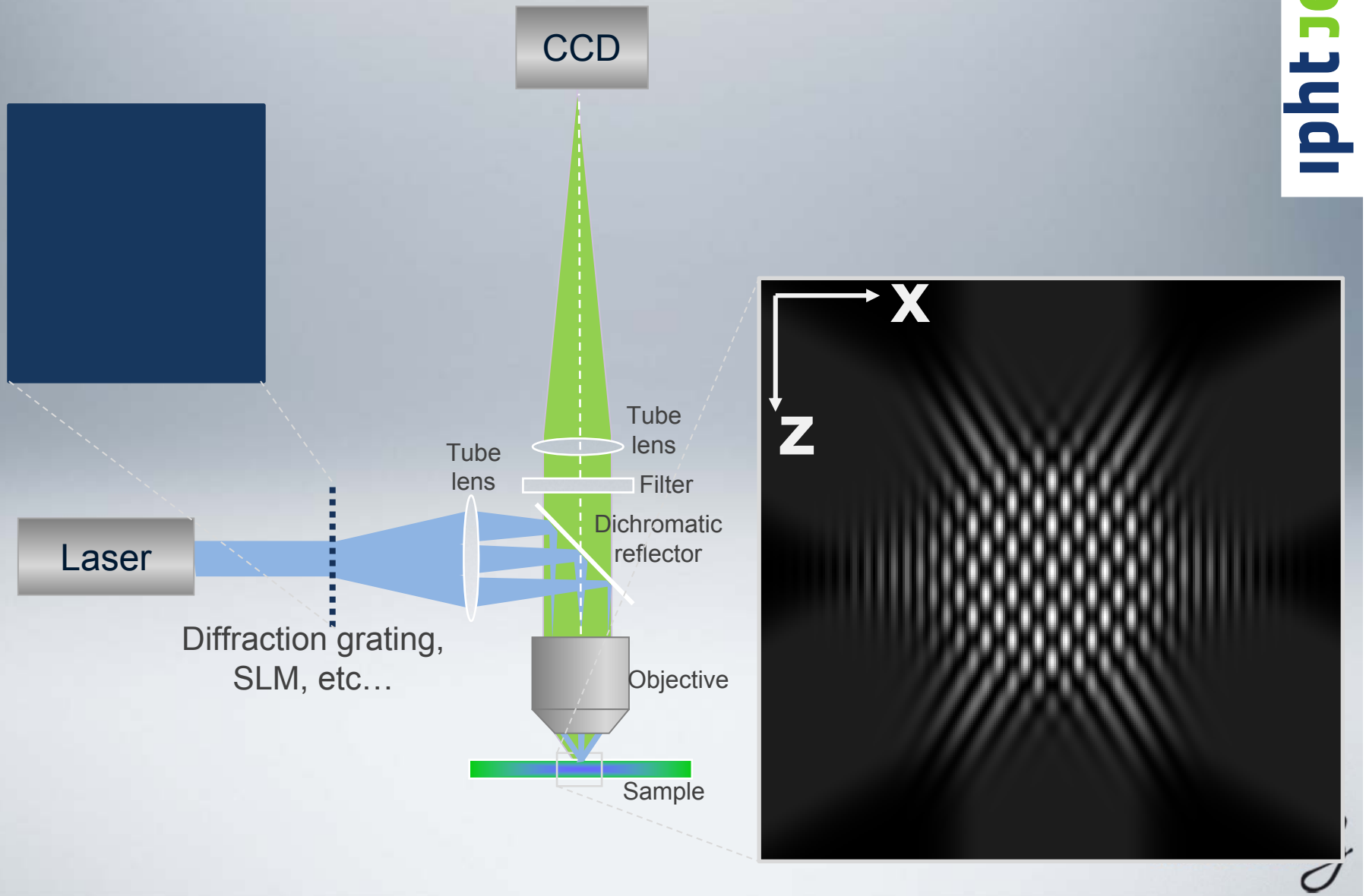
Reconstructed sample



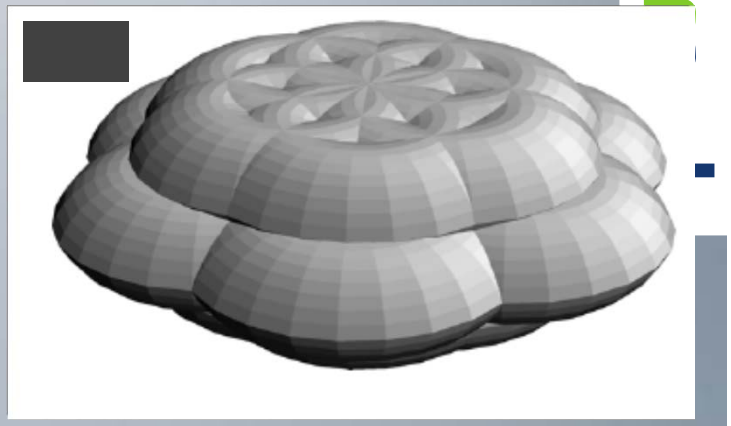
Separating the components…
Shifting the components…
Recombining the components… using the correct weights.

Image processing !

Multiple images for order separation



3D Structured Illumination



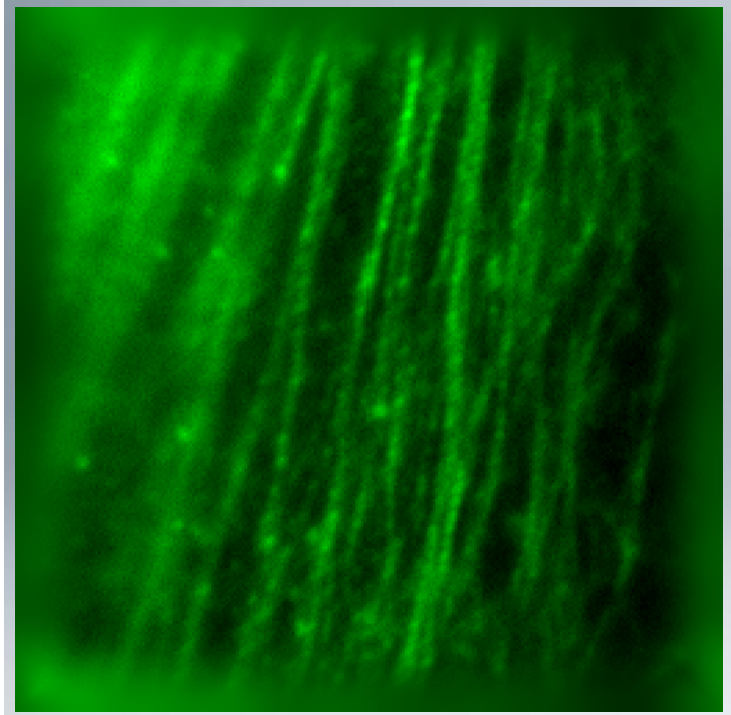
Microtubule cytoskeleton in HeLa cells

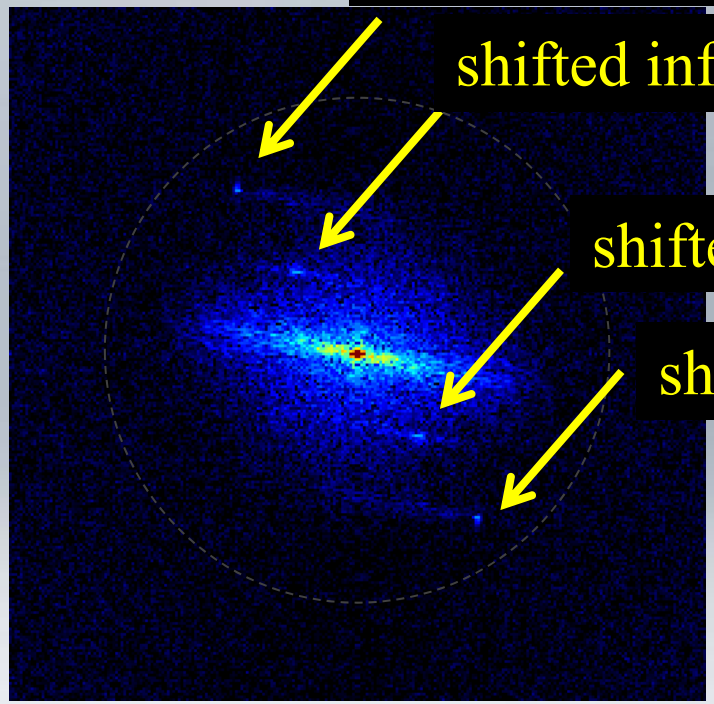
M.G.L Gustafsson *et al*., Three-dimensional Resolution Doubling in Widefield Fluorescence Microscopy by Structured Illumination, *Biophys. J. (BioFAST), 2008*

Mitglied der Leibniz-Gemeinschaft

Microscopy image

Resolution map





Reconstruct high resolution image like a puzzle

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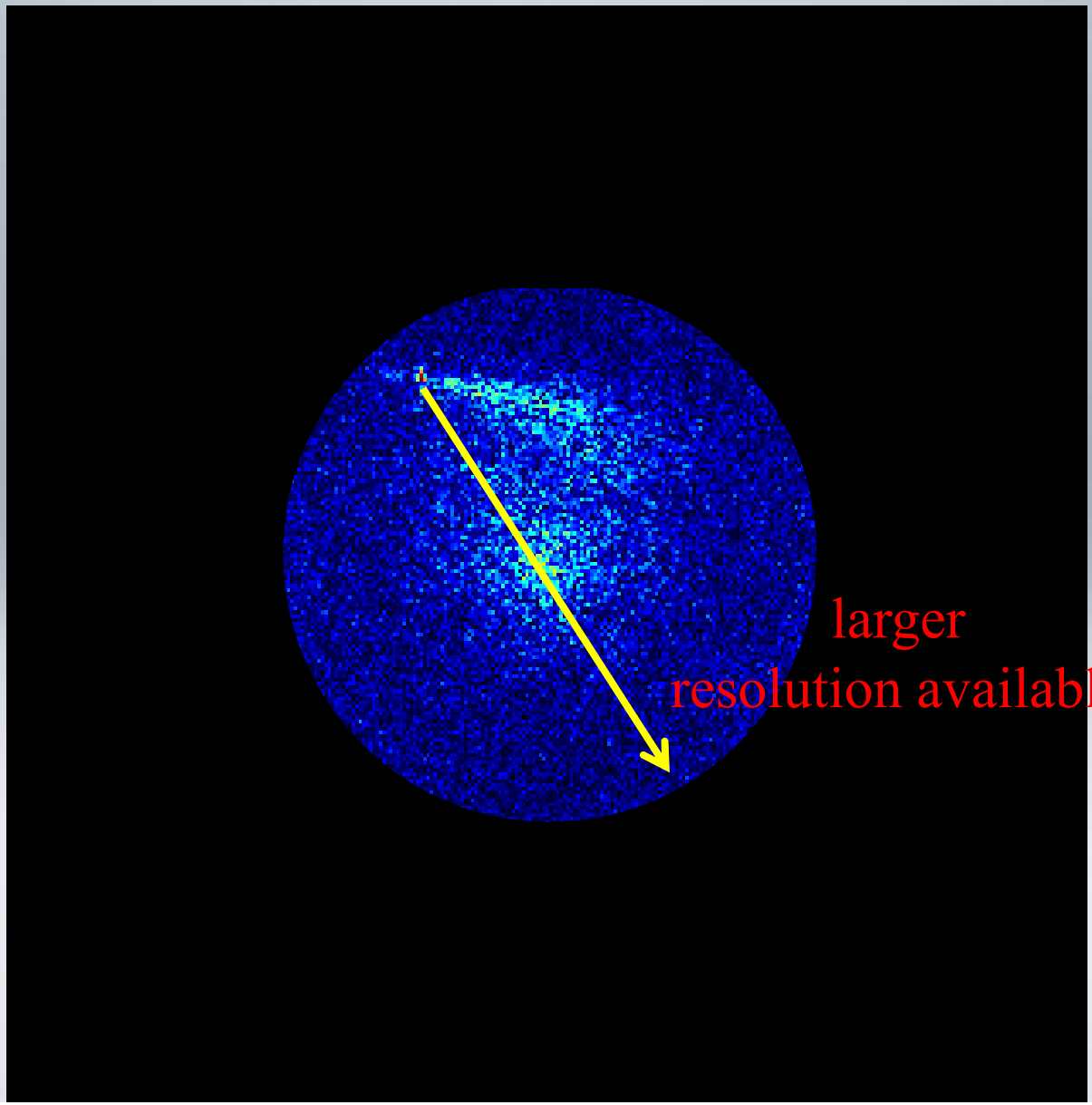
Separated puzzle pieces



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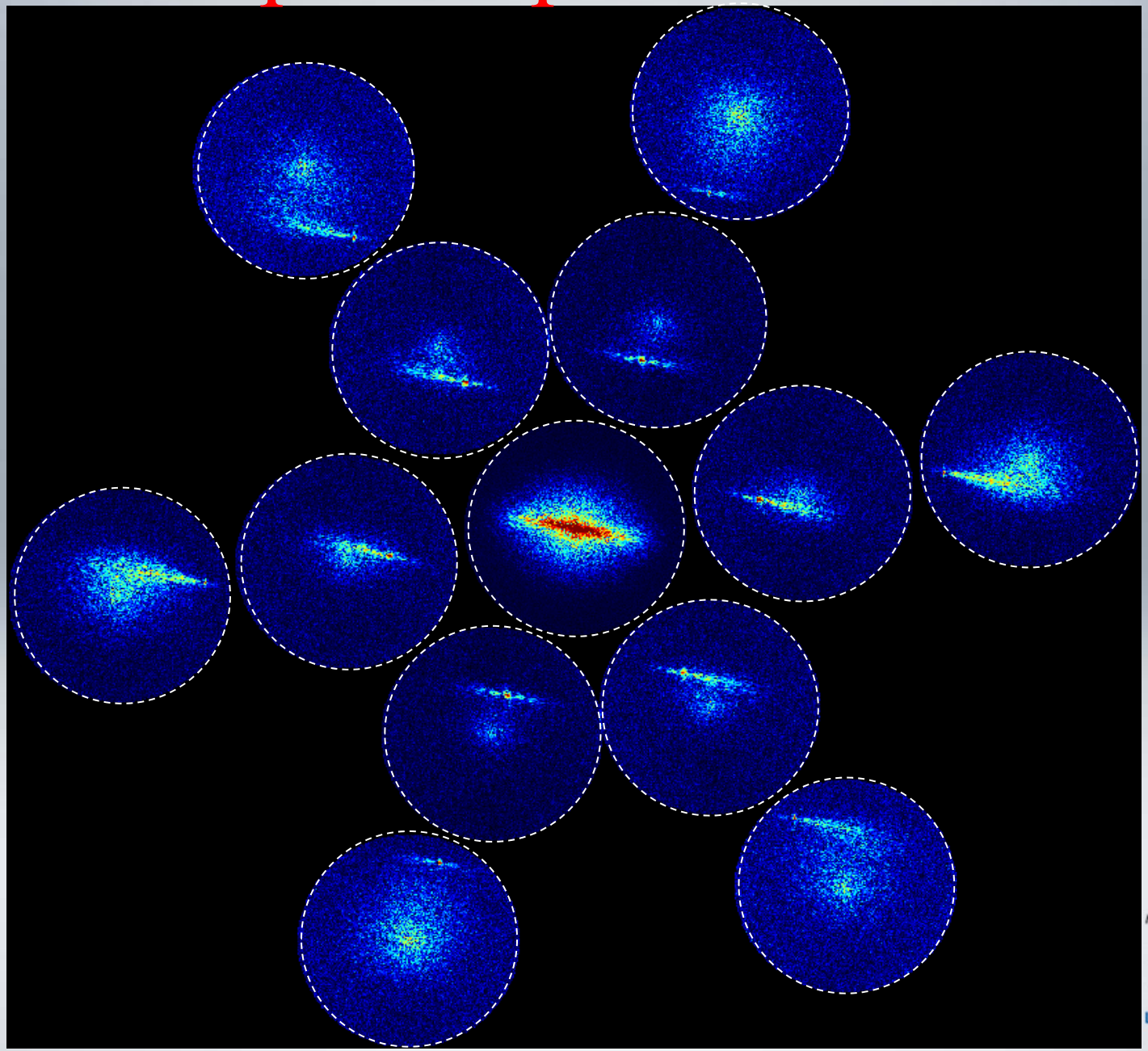
Mitglied der Leibniz-Gemeinschaft

Separated puzzle pieces



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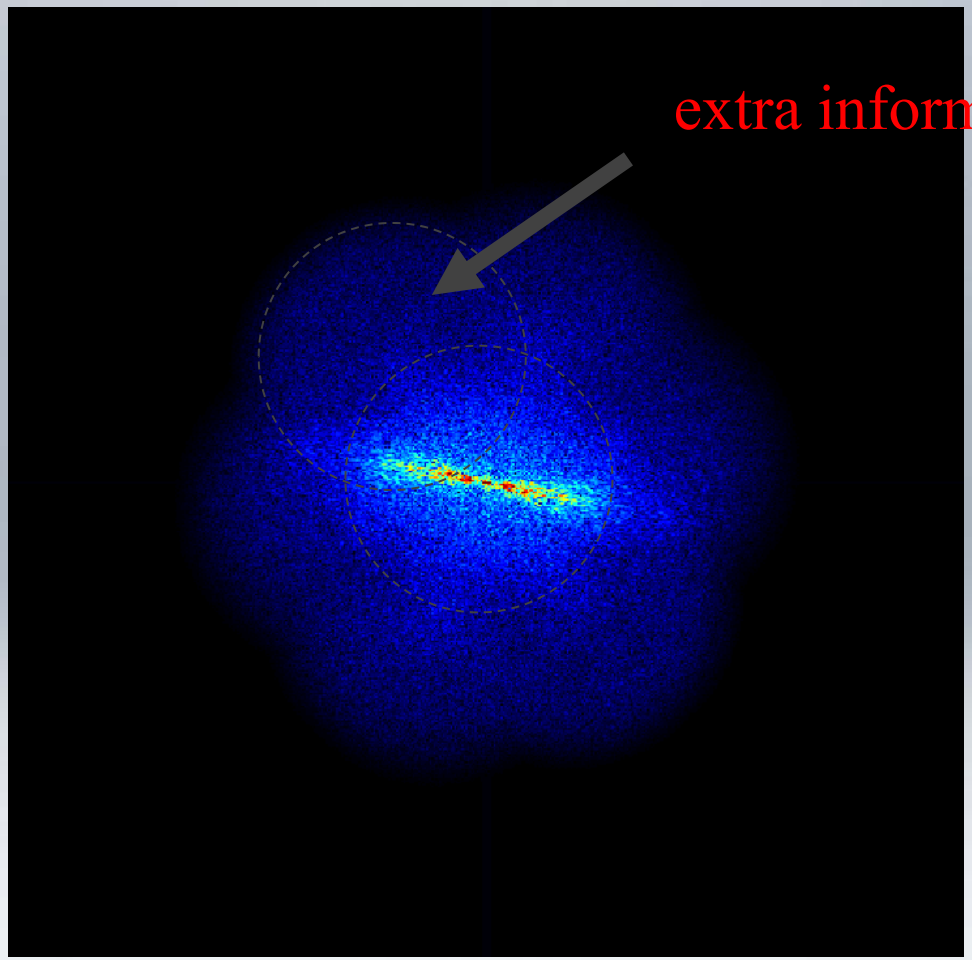
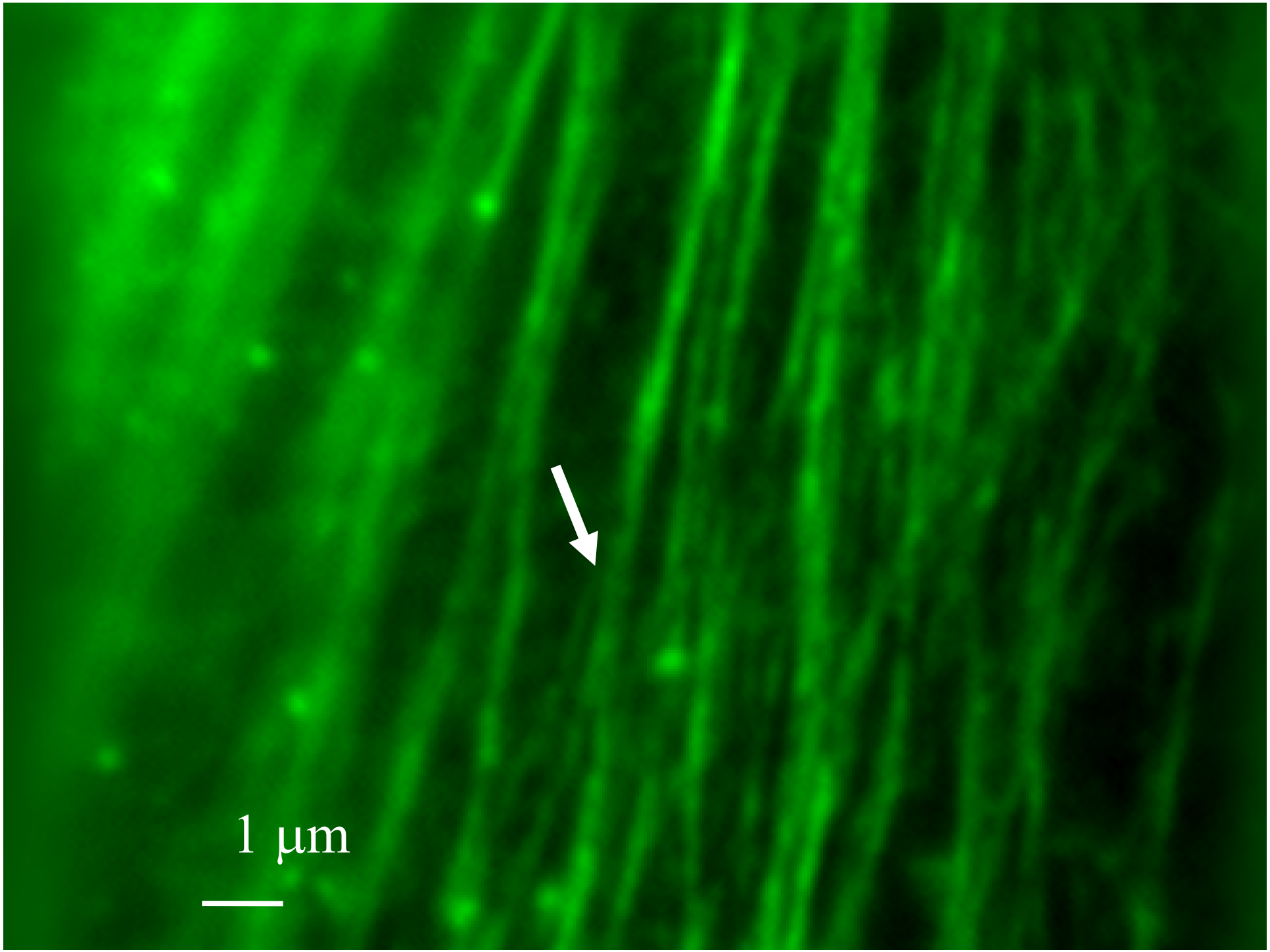
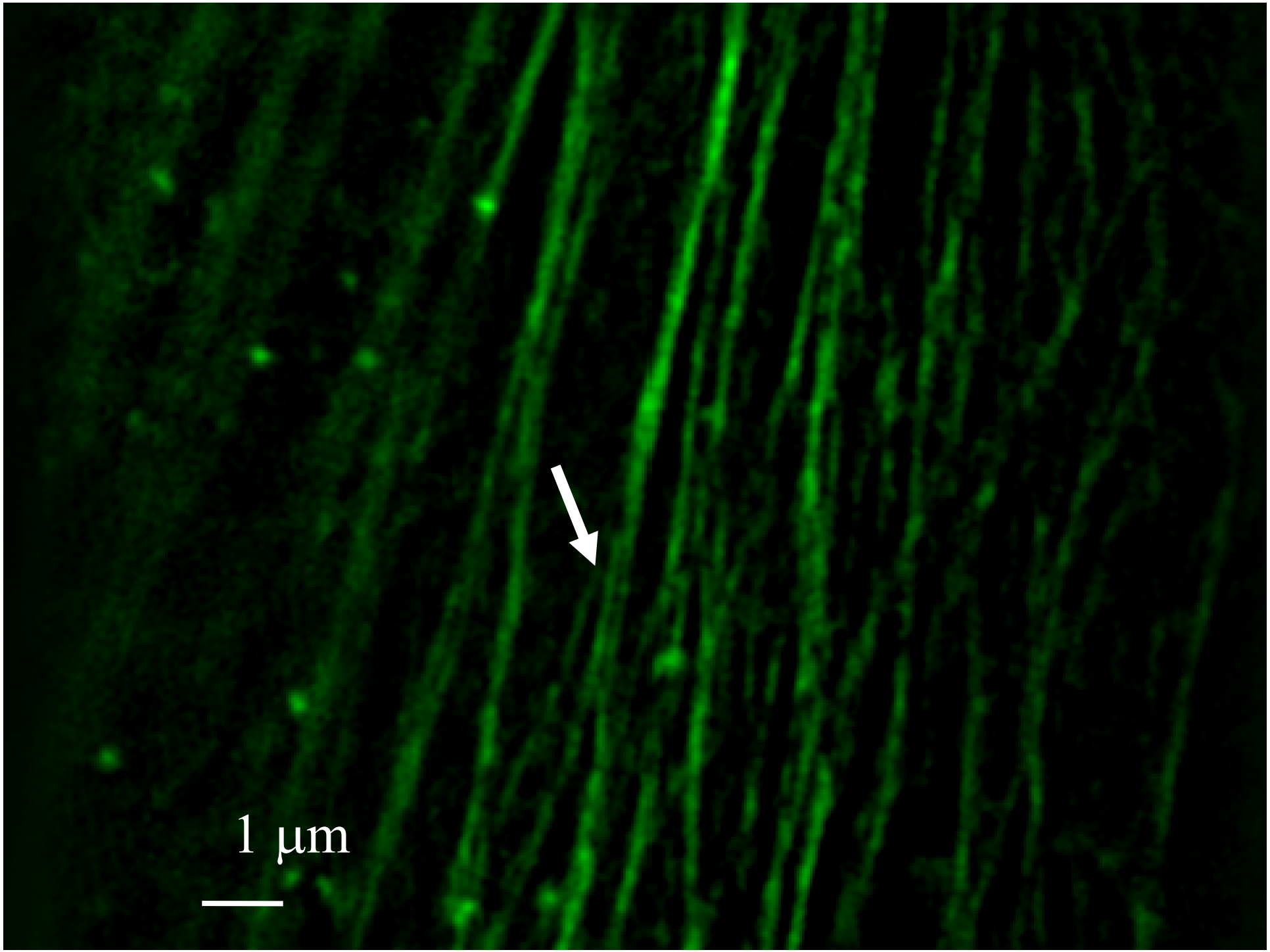
Mitglied der Leibniz-Gemeinschaft

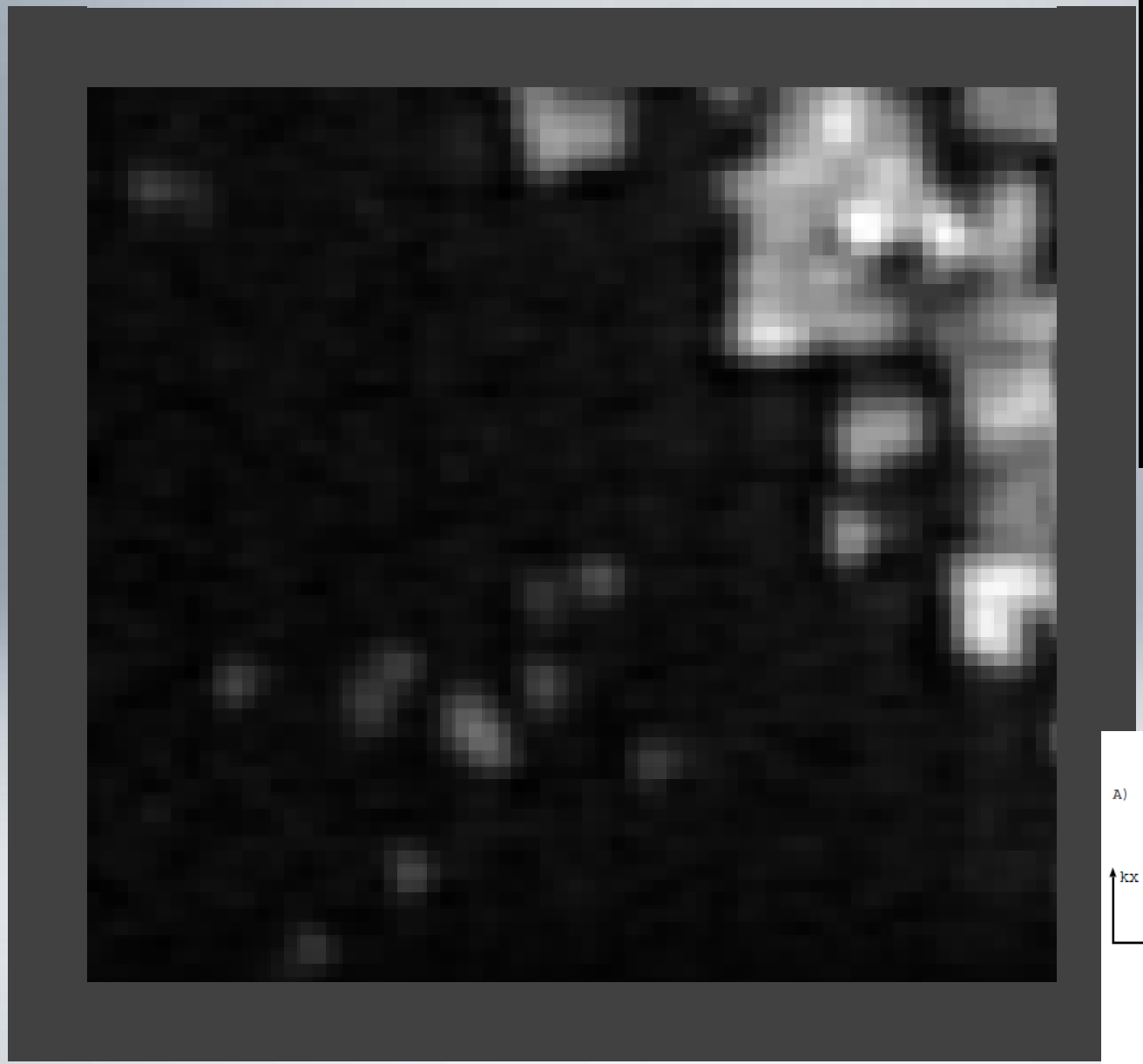
Joined puzzle

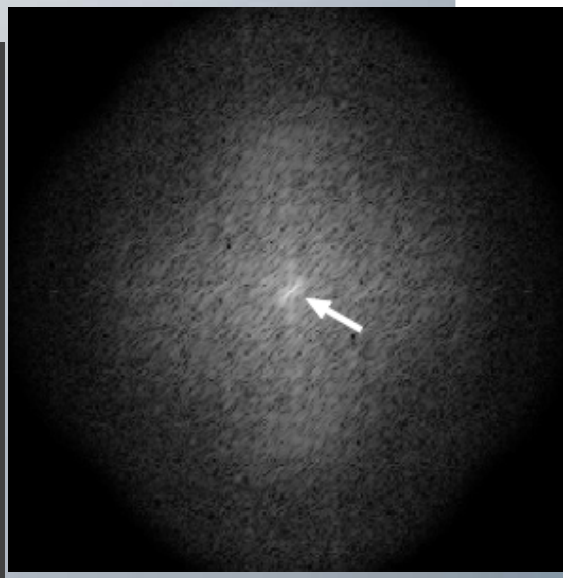


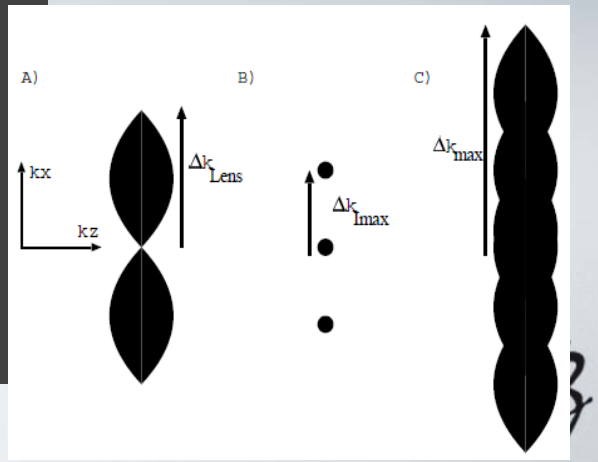

1 µm


1 μm

Proof of Principle 1999



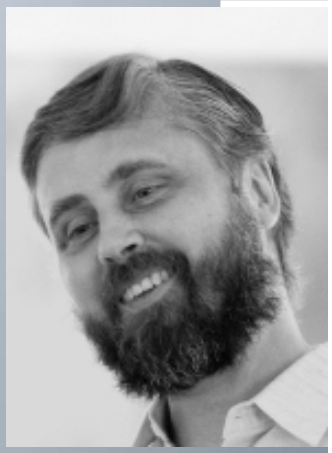


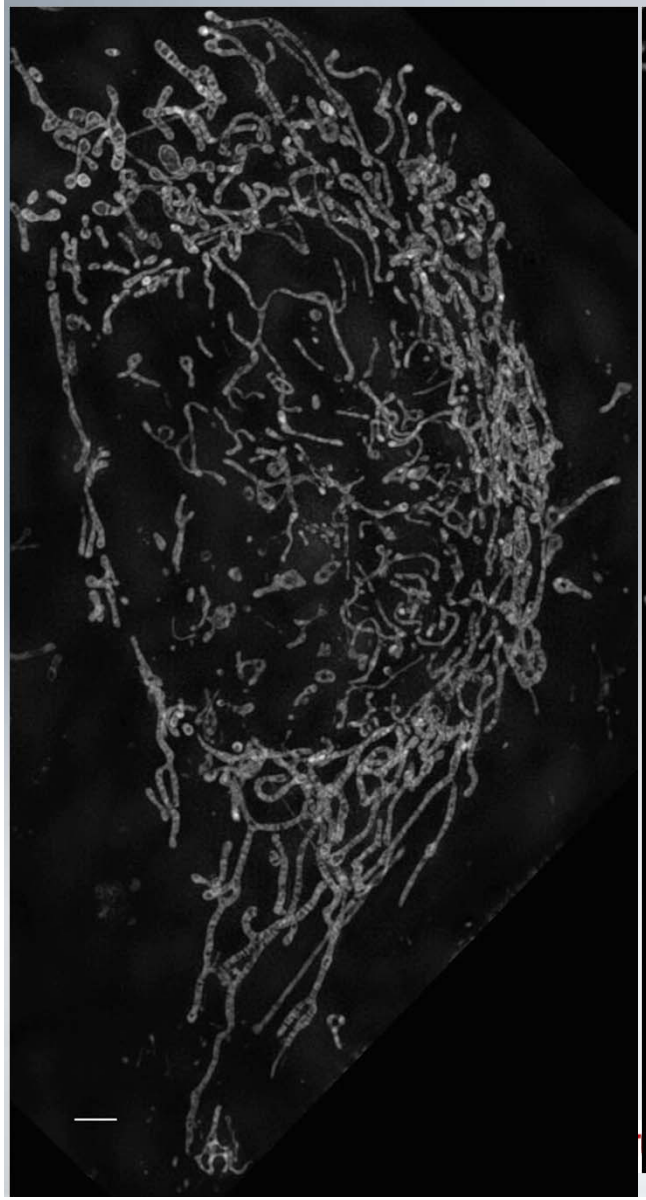


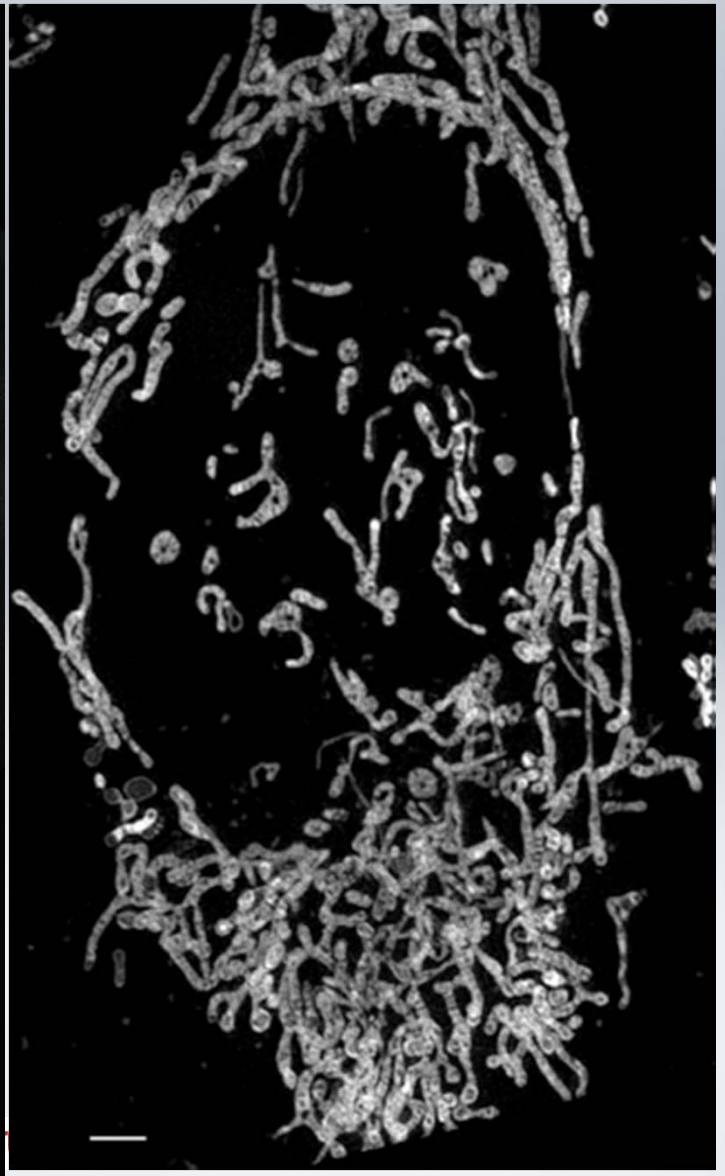
Heintzmann & Cremer 1999 Proc. SPIE **3568**, 185-196

Structured Illumination

2011







3D live video

Super-resolution 3D microscopy of live whole cells using structured illumination

Lin Shao[1], Peter Kner[2], E Hesper Rego[1,3] & Mats G L Gustafsson[1,4]

Mitglied der Leibniz-Gemeinschaft

Time-lapse two-color 3D imaging of live cells with doubled resolution using structured illumination

Reto Fiolka[a,1], Lin Shao[a], E. Hesper Rego[a,b,2], Michael W. Davidson[c], and Mats G. L. Gustafsson[a,3]

PNAS | April 3, 2012 | vol. 109 | no. 14 | 5311–5315

3d live cell SIM

cytosol (red), actin (green)

Images by Reto Fiolka,

Janelia Farm Research Campus, HHMI, Ashburn, VA, USA

The nitty gritty details

Unknowns:

- grating constant (precise value)
- grating orientation
- local phase
- global phase
- order contrast
- illumination intensity
- sample position (drift)

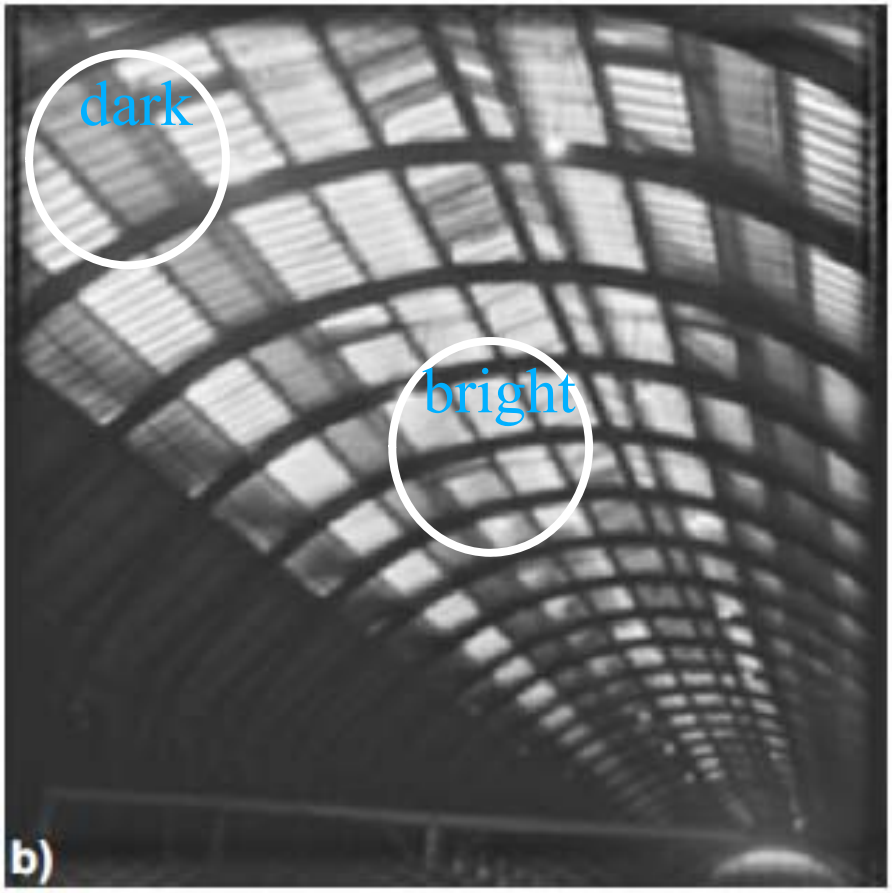
The nitty gritty details

Unknowns:

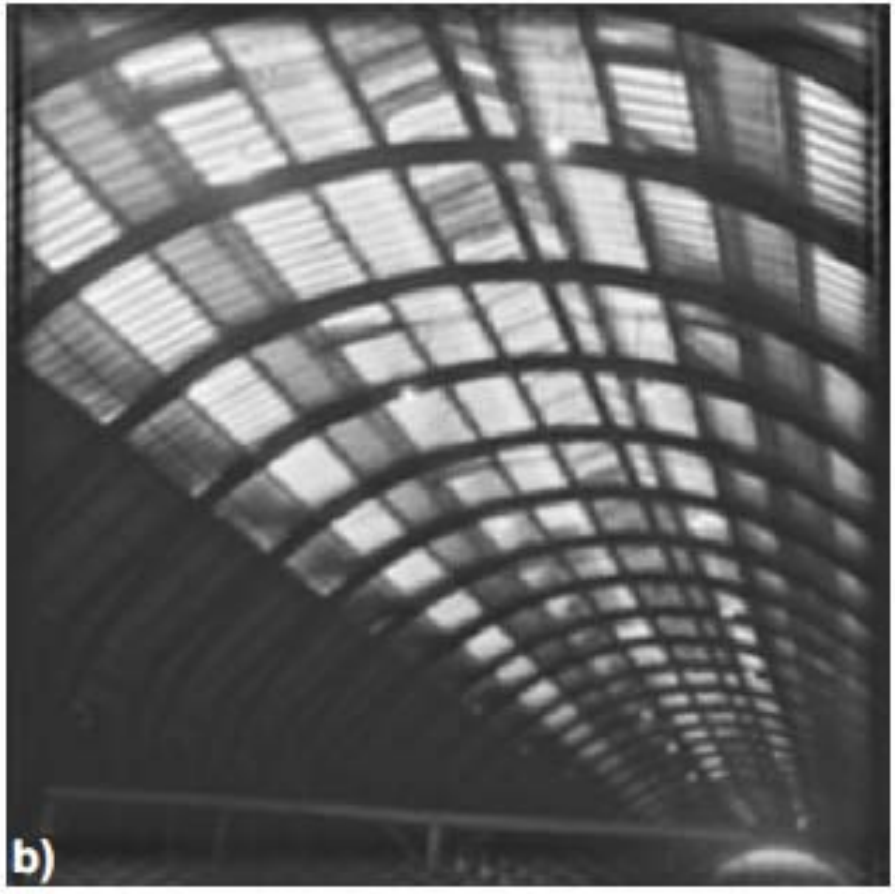
- grating constant (precise value)
- grating orientation
- local phase
- global phase
- order contrast
- illumination intensity
- sample position (drift)

The nitty gritty details

wrong grating constant



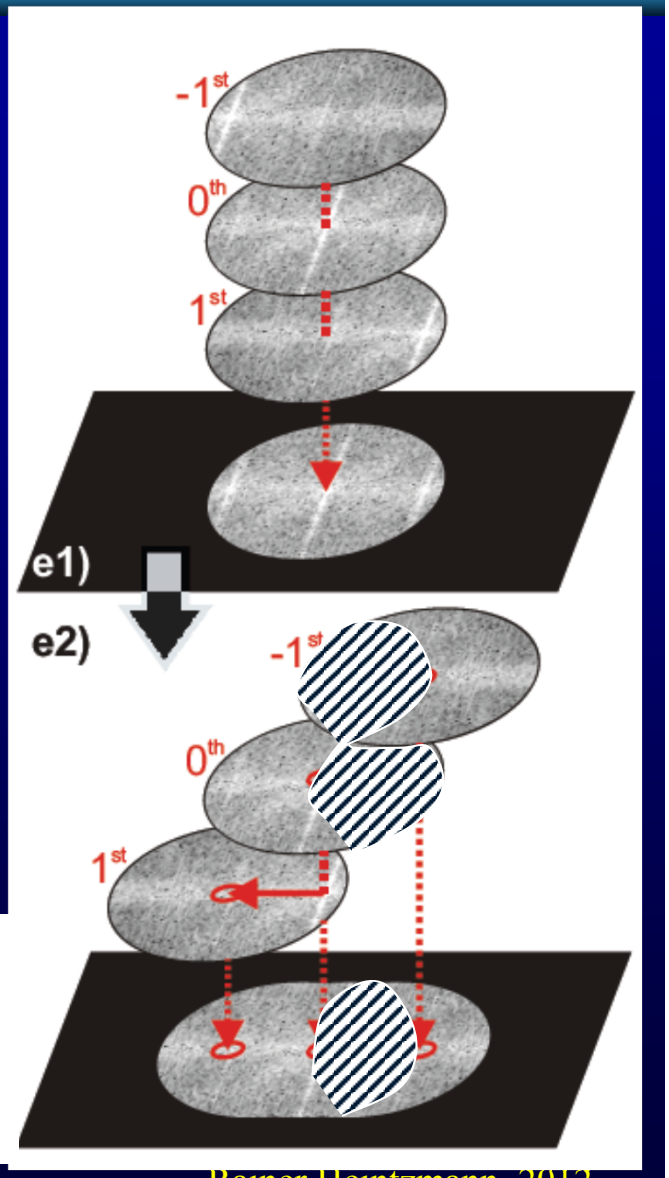
correct grating constant



intensity beating, splitting of structures
Cave! Hard to distinguish from real data

The nitty gritty details

Same information:
Use overlap and cross correlation



SNR-weighted cross correlation for best results
(assume contant variance in Fourer space)
typically iterative (3 iterations)

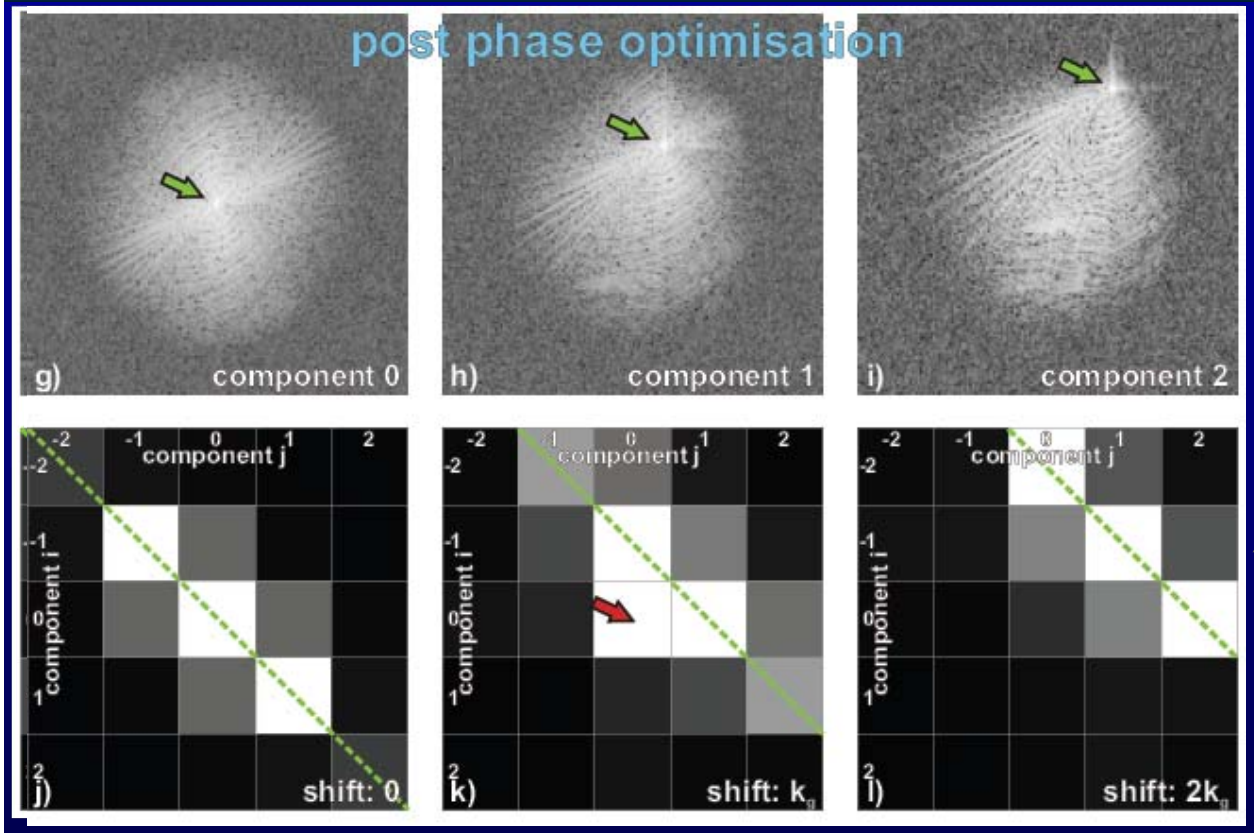
The nitty gritty details

Unknowns:

- grating constant (precise value)
- grating orientation
- **local phase**
- **global phase**
- **order contrast**
- illumination intensity
- sample position (drift)

The nitty gritty details

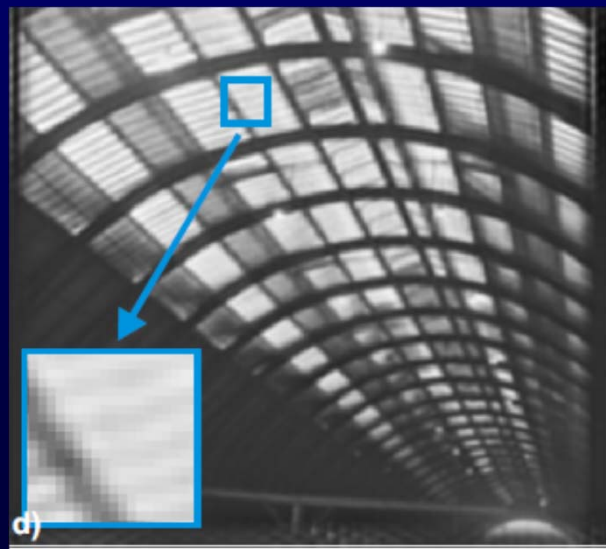
$$c_{ij} = [\tilde{\Omega}_i(\vec{k}) \circledast_w \tilde{\Omega}_j(\vec{k})](0)$$



separated components matrix

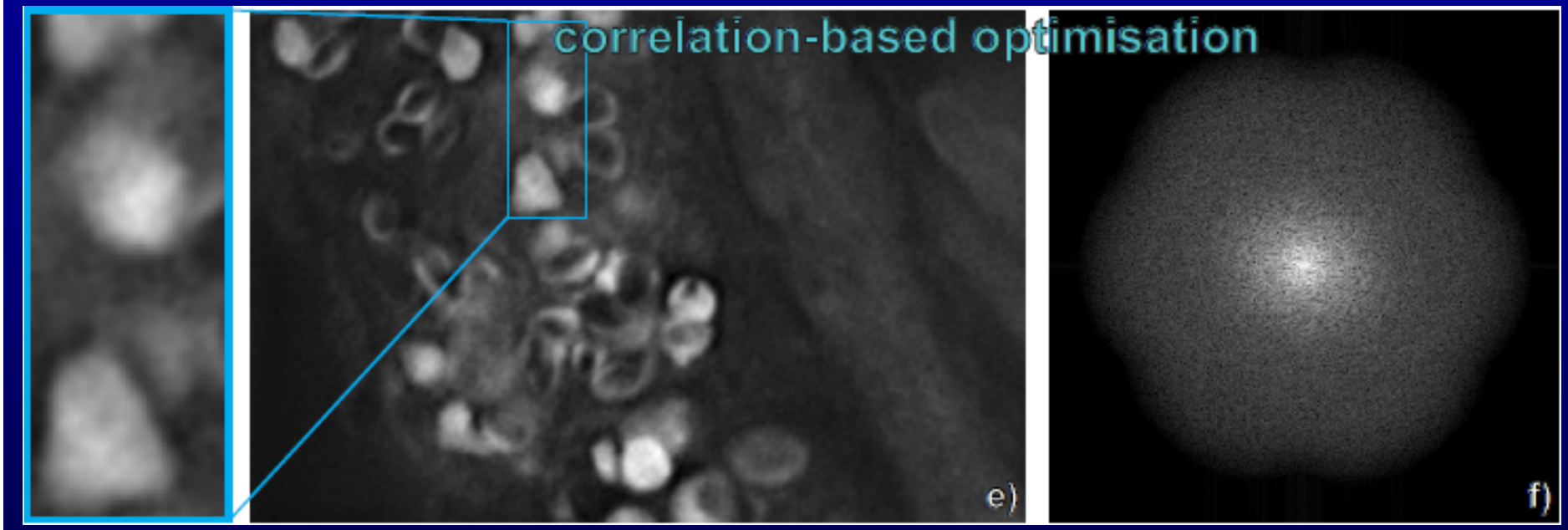
wrong phases

Global phase: Correlation needs to be real valued



The nitty gritty details

$$\mathscr{C}_{i,j}^{(l)} = [\tilde{C}_i(\vec{k}) \circledast_w \tilde{C}_j(\vec{k} - l\vec{p})]|_{\vec{k}=0}$$

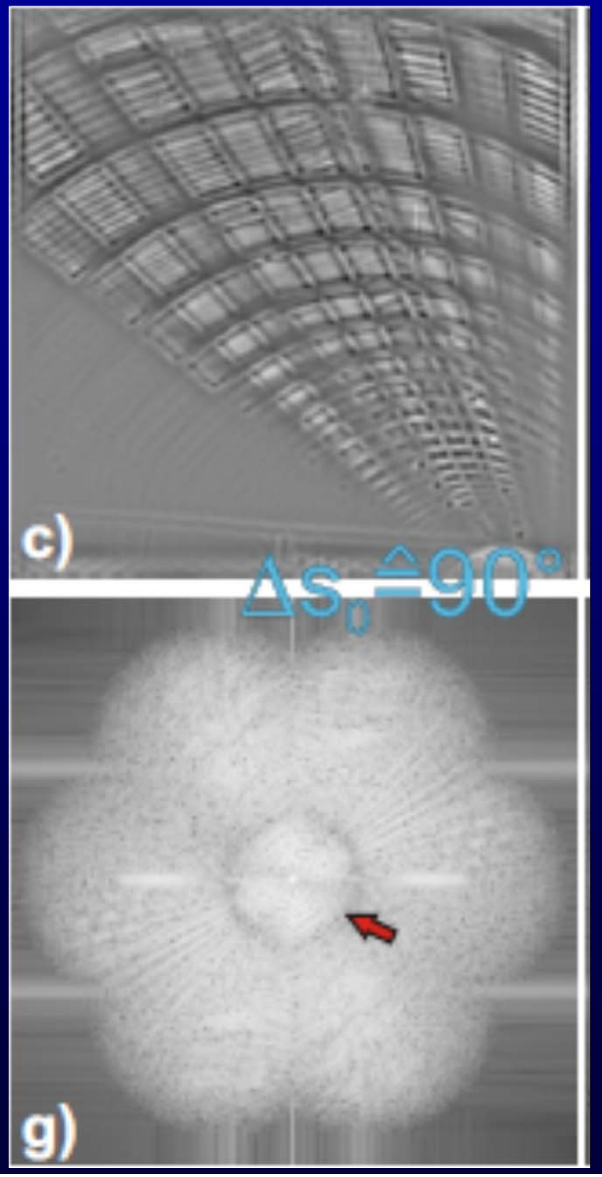


Collaboration: Dithmar, Ach, Best, Cremer (Heidelberg University)
Algorithm: Kai Wicker

The nitty gritty details

Global phase errors:

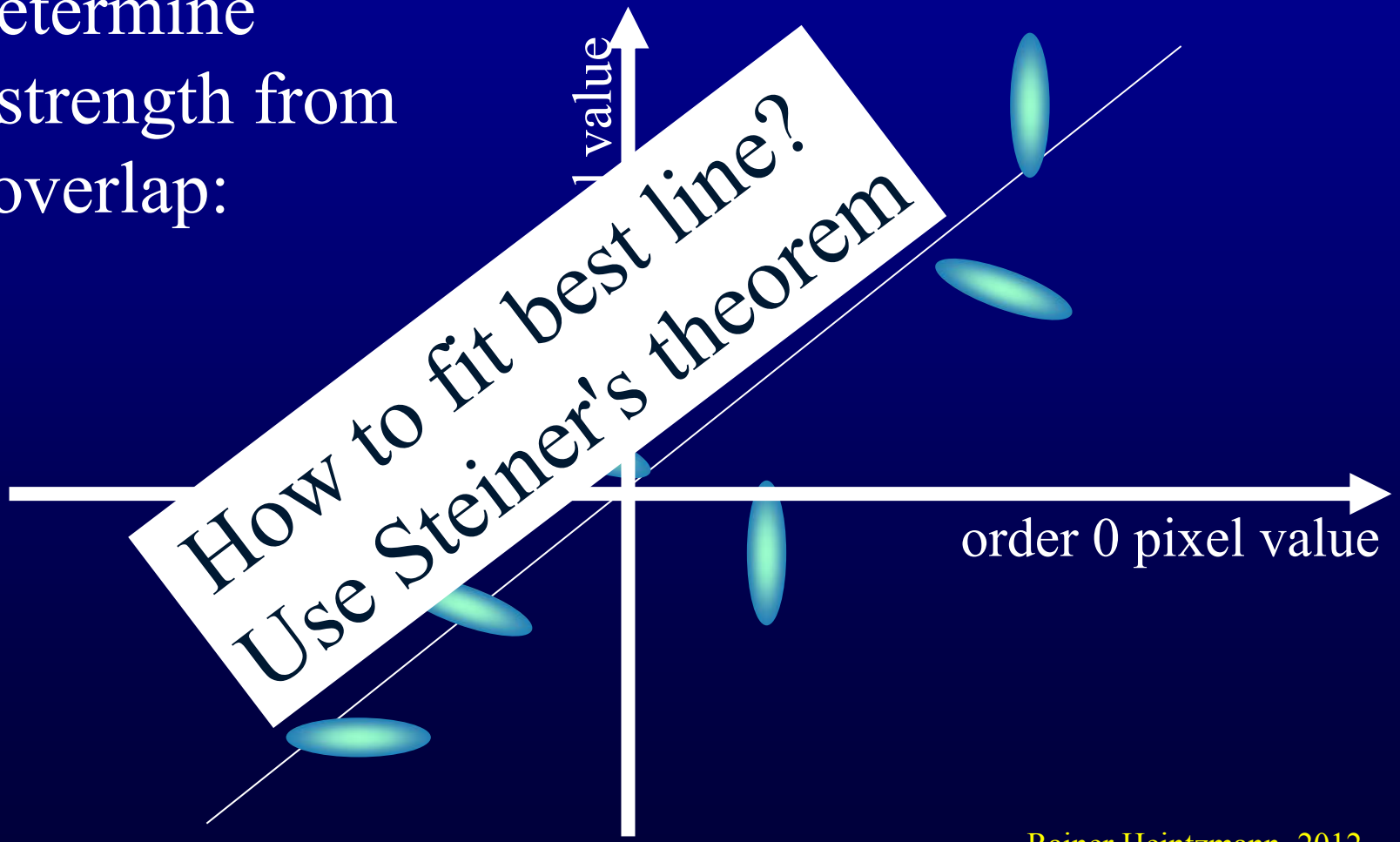
destructive "interference"
in Fourier space



The nitty gritty details

Order contrast errors: Part of the matrix M

Determine order strength from overlap:



The nitty gritty details

$$c_{ij} = [\tilde{\Omega}_i(\vec{k}) \circledast_w \tilde{\Omega}_j(\vec{k})](0)$$

Speed up: Avoid recalculation of c elations (Kai Wicker)

pre calcul correlations:

$$d_{st}^{(p)} = [\tilde{J}_s(\vec{k}) \circledast \tilde{J}_t(\vec{k} + p\vec{k}_g)](0)$$

and use

$$C^{(p)} = \bar{M}^{-1} D^{(p)} \bar{M}^{-1\dagger}$$

unmixing matrix

Analytical solution possible ???

→ correlations do not need to be recomputed

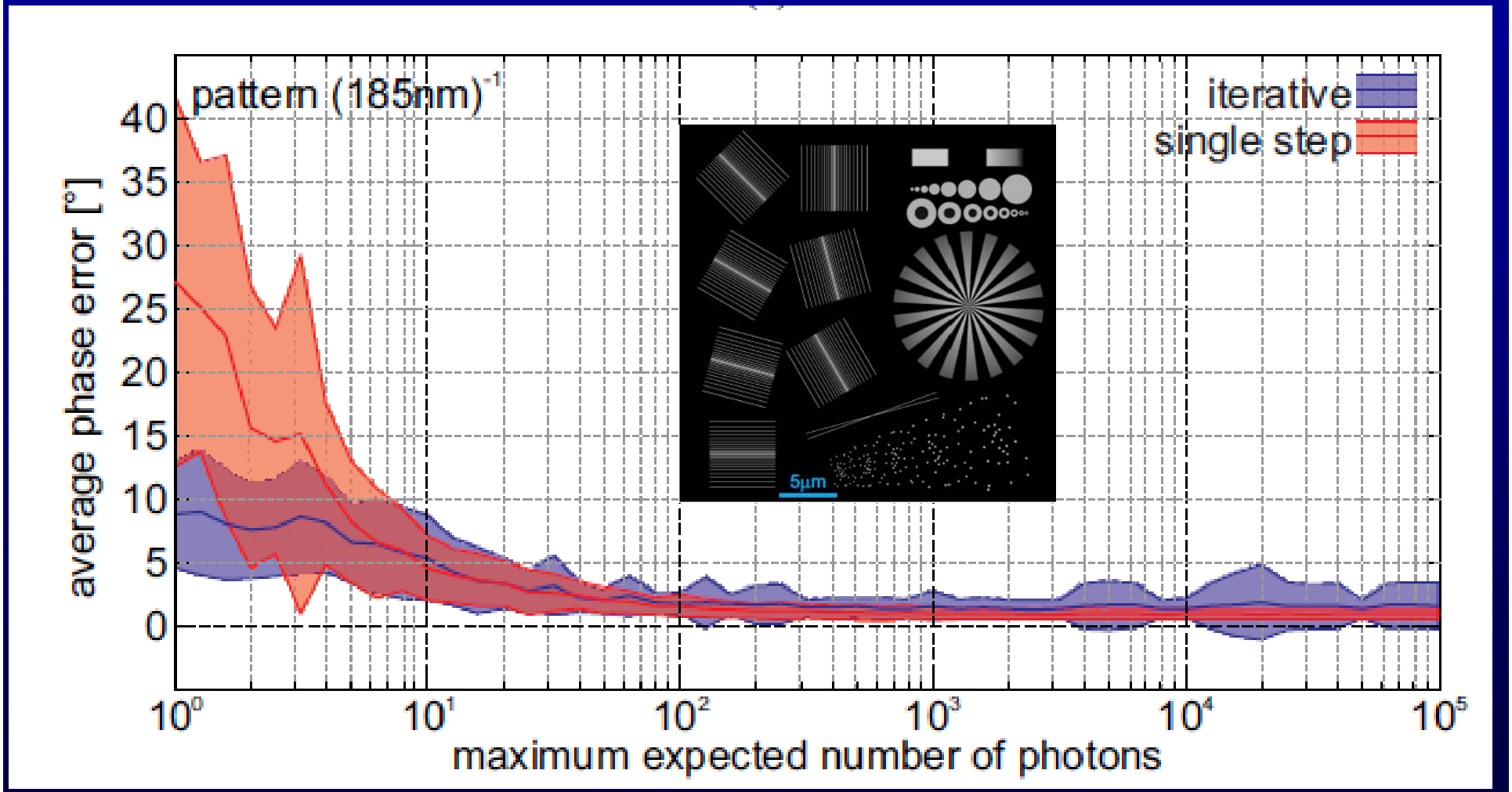
fast processing

Doing it faster?

Phase of a single image by

peaks in weighted autocorrelation

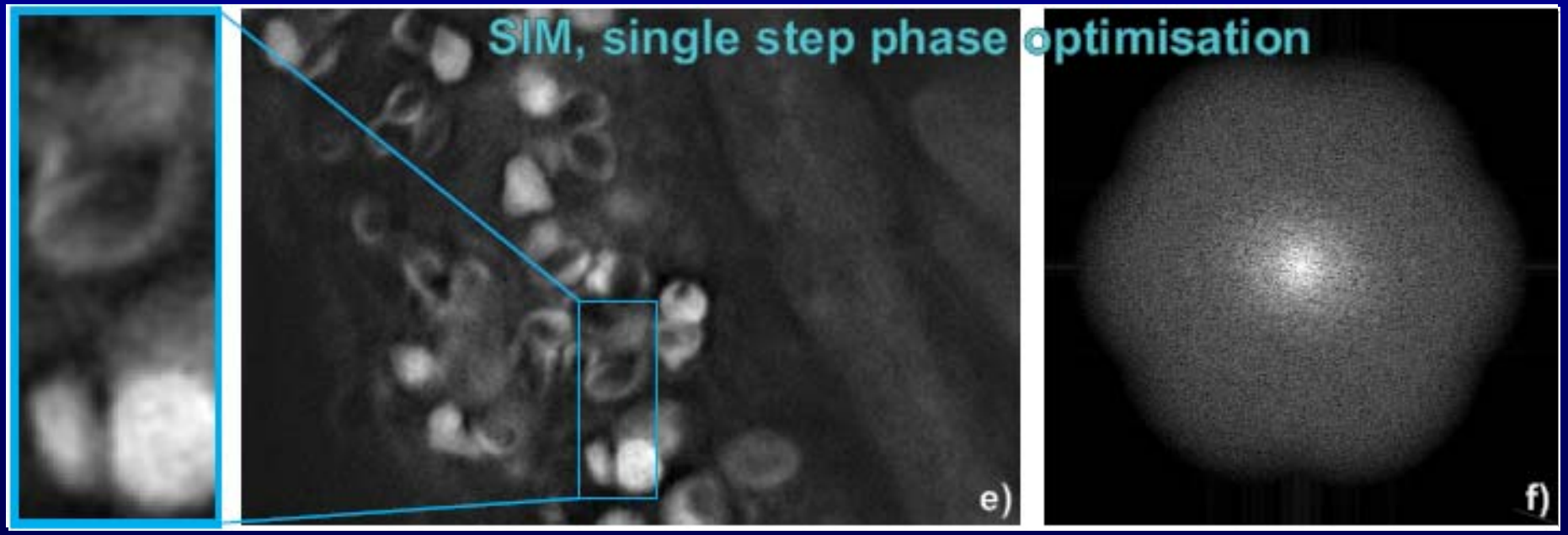
The nitty gritty details



K.Wicker, Opt. Expr. 2013

Rainer Heintzmann, 2012

Single image autocorr. optimization



Collaboration: Dithmar, Ach, Best, Cremer (Heidelberg University)
K.Wicker, Opt. Expr. 2013

The Wiener Filter Problem

Wiener Filtering assumes constant noise in image and known spectrum

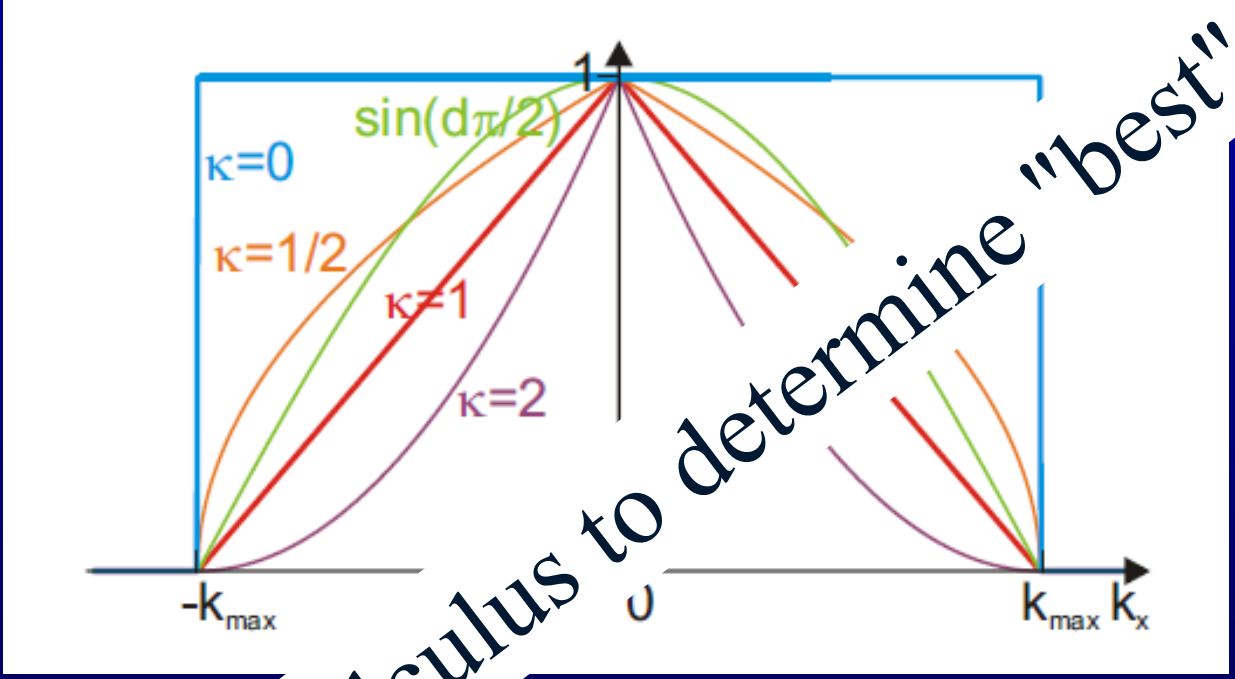
One Step Algorithm?

$$\tilde{W}(\vec{k}) = \frac{\tilde{h}_{wn}(\vec{k})\langle|\tilde{S}(\vec{k})|^2\rangle}{\tilde{h}^2_{\rm wn}(\vec{k})\langle|\tilde{S}(\vec{k})|^2\rangle + \langle|\tilde{n}(\vec{k})|^2\rangle}.$$

But

- **noise variance is proportional to signal**
- **spectrum is unknown**

The Apodization function (goal functi



Variational calculus to determine "best" filters?

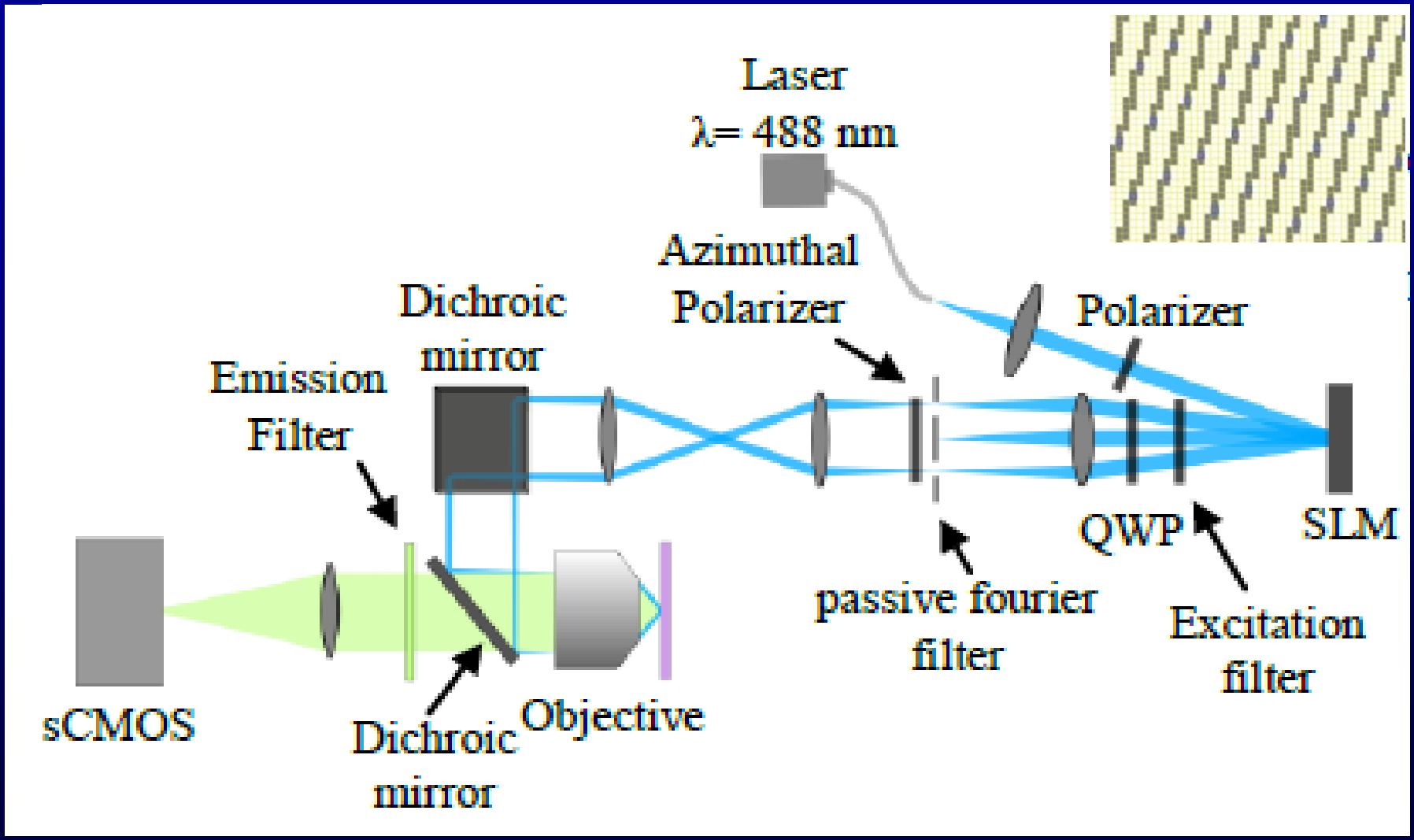
ideal: no (or s… …gative values, small sidelobes, small width

$\ldots) := [d($

Using the "Lucosz-bound"
Stalllinga et al. 2013

fast SIM

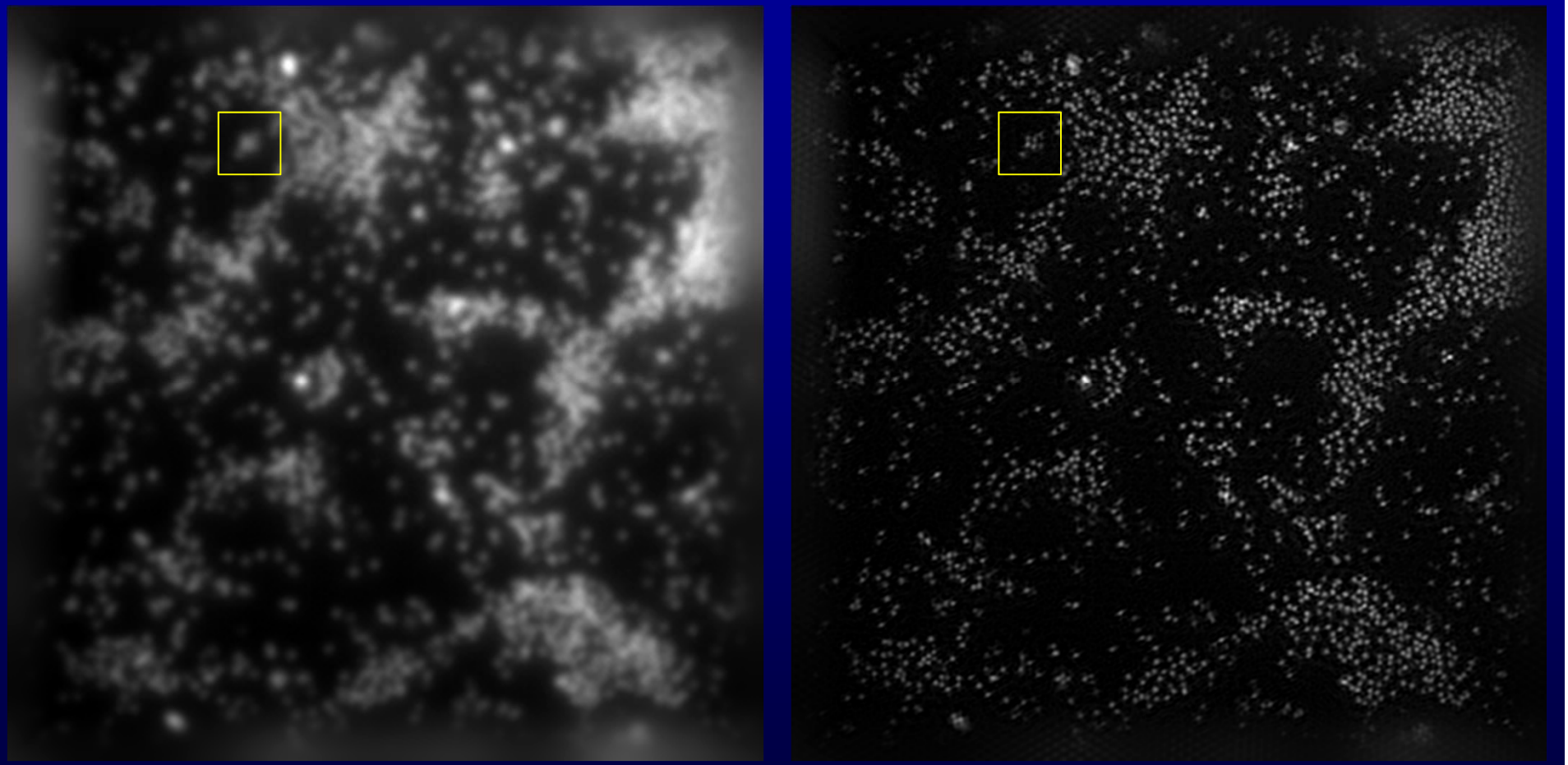
fast SIM setup



Foerster, R., et al., *Optics Express* **22**, 20663-20677 (2014)

Rainer Heintzmann, 2012

High-Speed SIM: Freely diffusing 100nm beads



162 raw frames/s, Orca FLASH 4V2

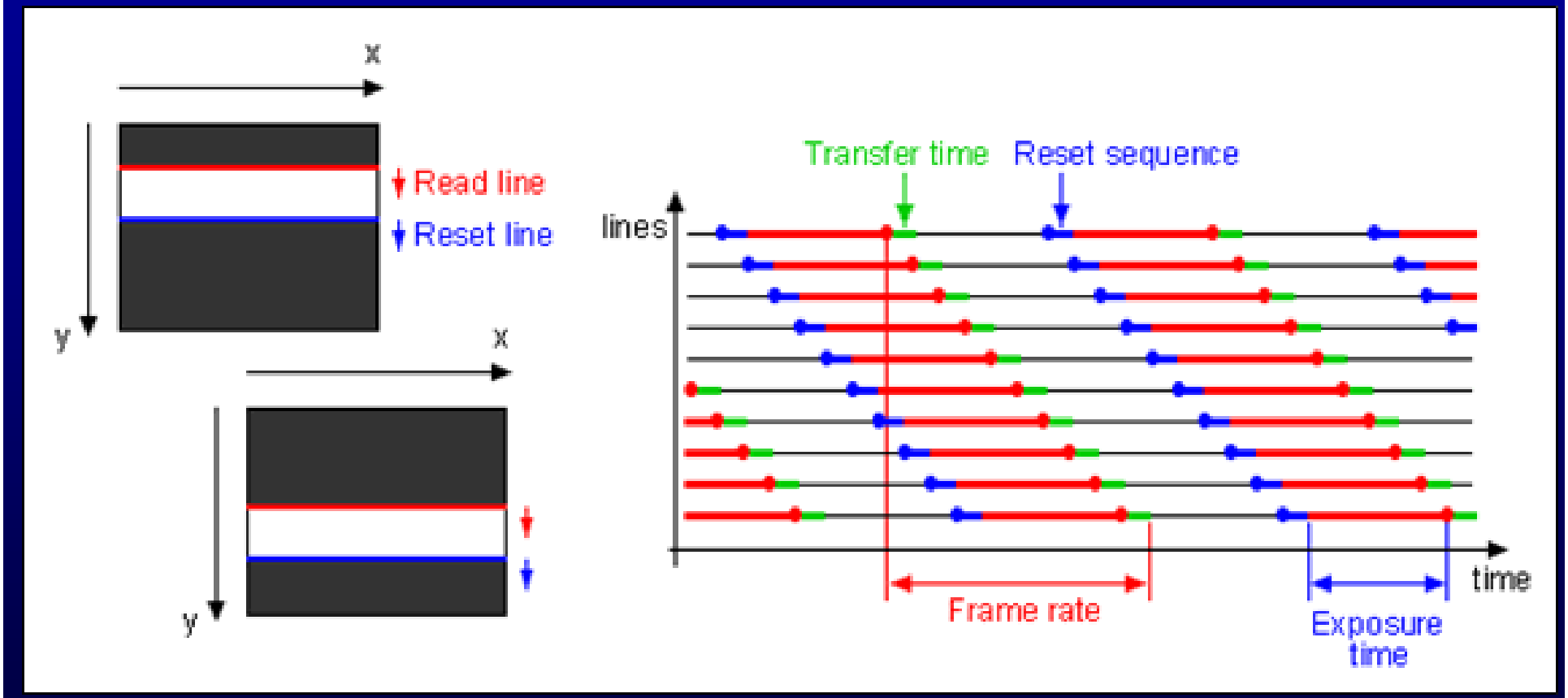
Hui-Wen Lu-Walter

Foerster, R., et al., *Optics Express* **22**, 20663-20677 (2014)

Rainer Heintzmann, 2012

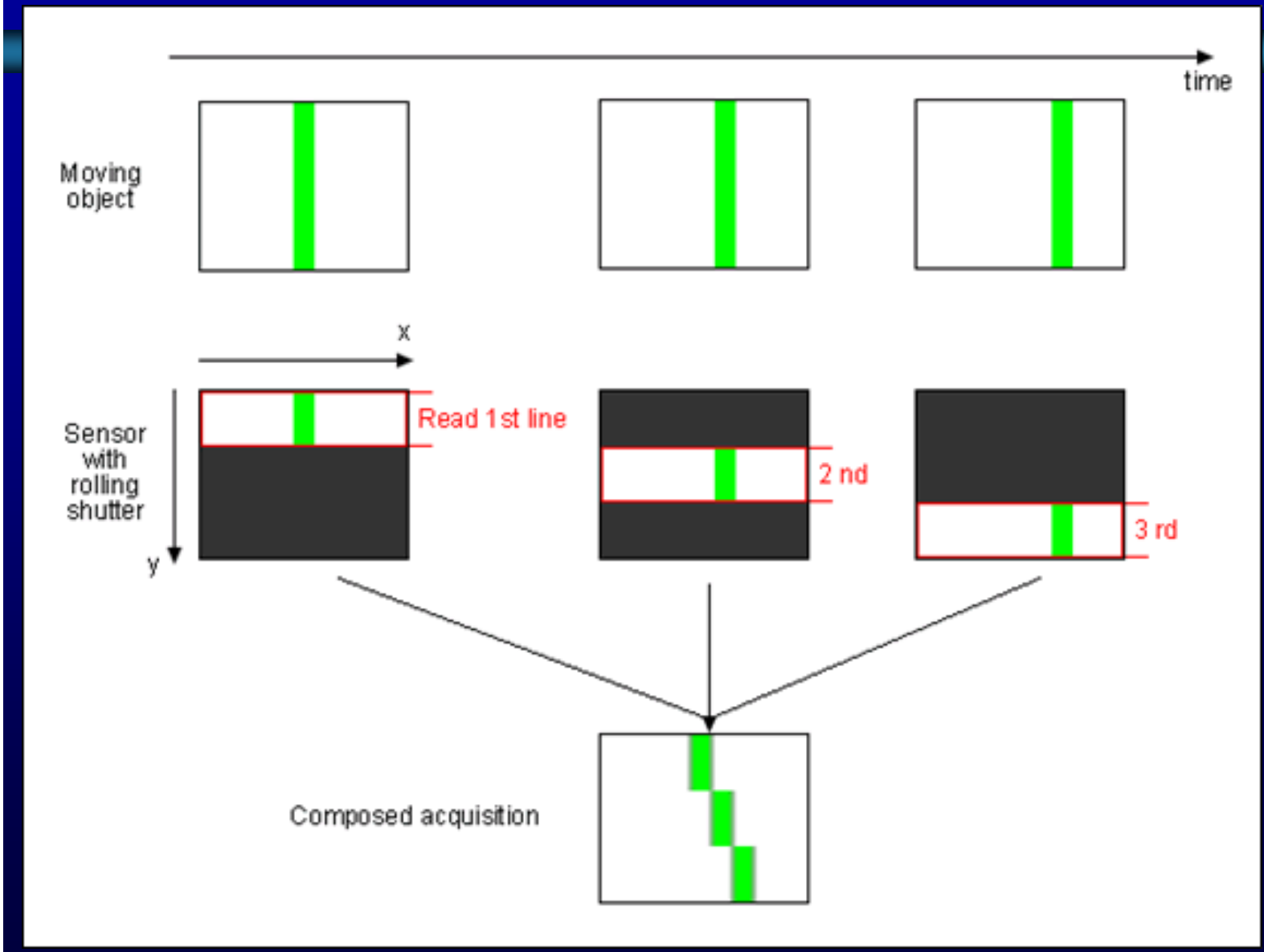
Problem: Rolling shutter readout

Typical: Two rolling shutters per camera



http://www.matrix-vision.com/glossar.html

sCMOS Cameras: rolling shutter



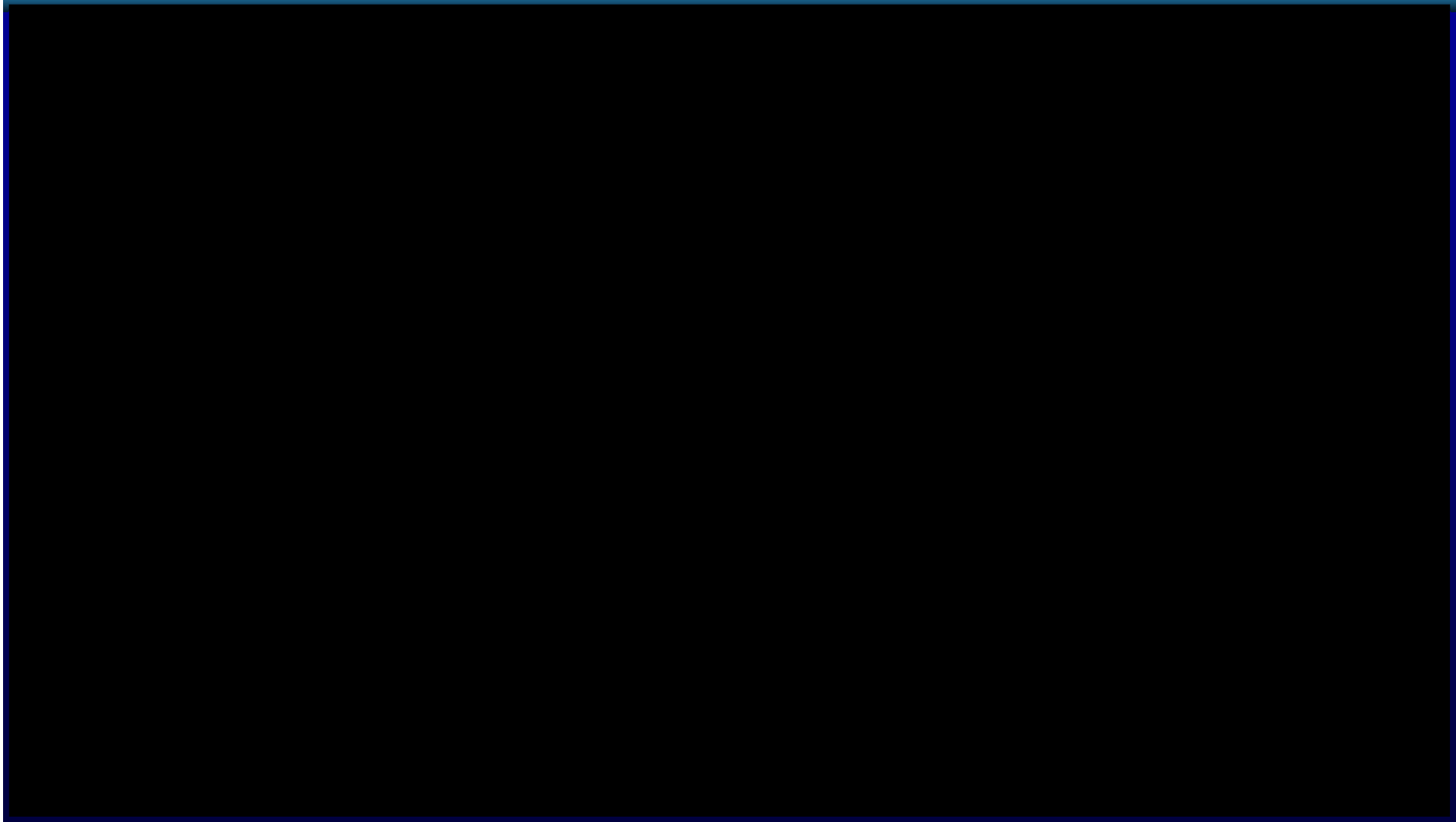
http://www.matrix-vision.com/glossar.html

sCMOS Cameras

sCMOS rolling shutters

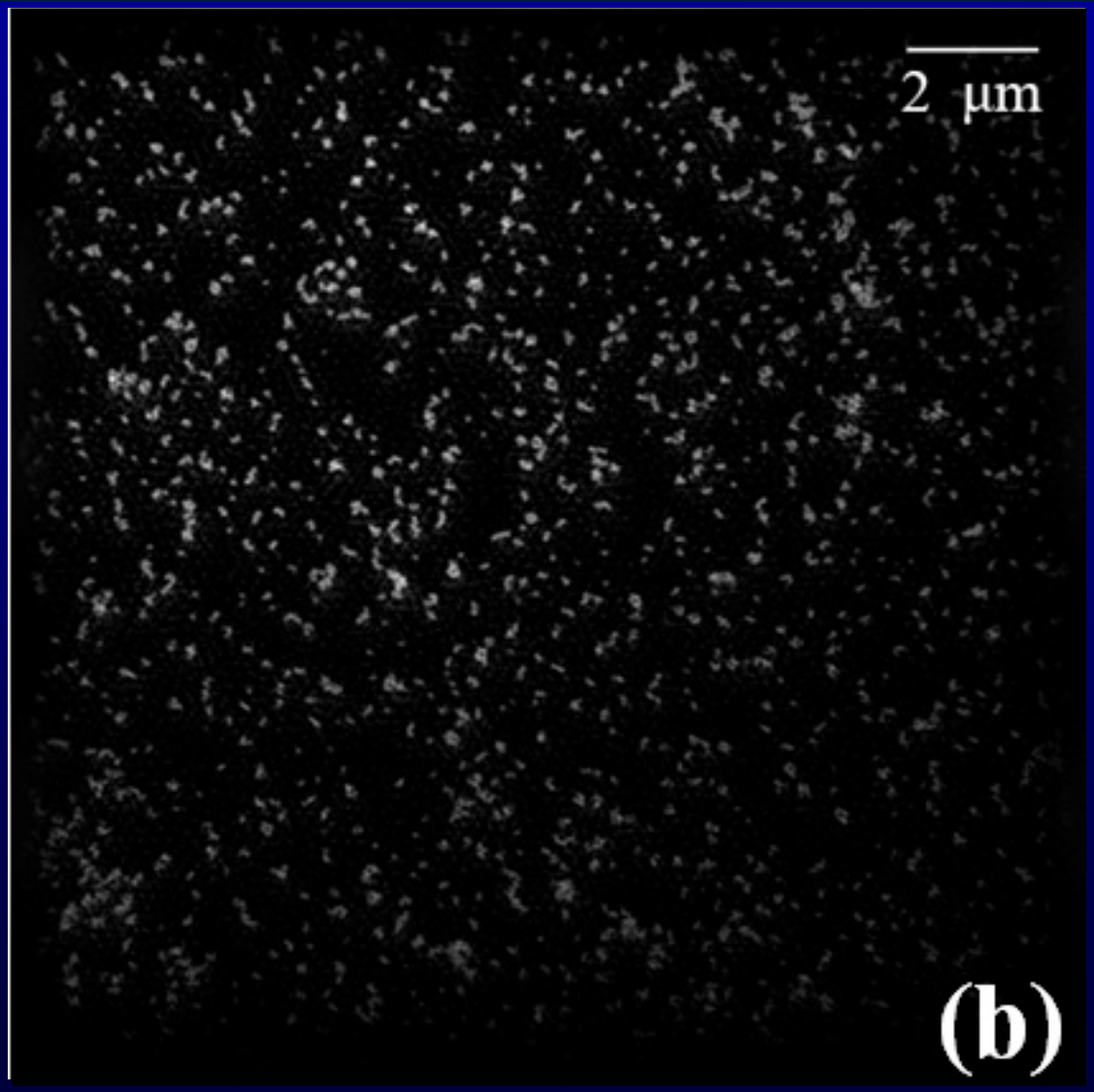
http://en.wikipedia.org/wiki/File:CMOS_rolling_shutter_distortion.jpg

Solution: Synchronised partial frames



Song et al., Measurement Science Technology 27,066401 (2016)

Solution: Synchronised partial frames



Rate:
714 fps (raw)
79 fps (SIM)

FWHM= 108nm

Song et al., Measurement Science Technology 27,066401 (2016)

Overview

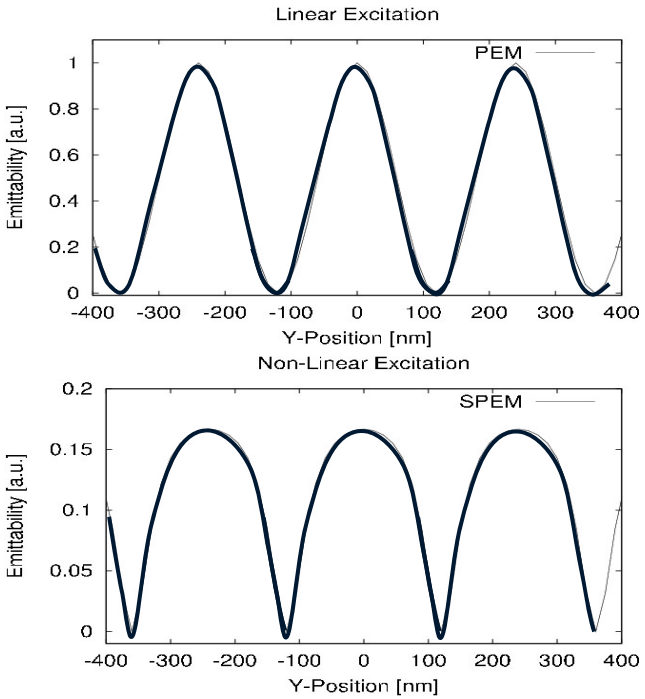
- Introduction: Resolution, Fourier and Abbe
- Superresolution
  - Structured Illumination
  - Circumventing the limit: Nonlinearity

Non-linearity

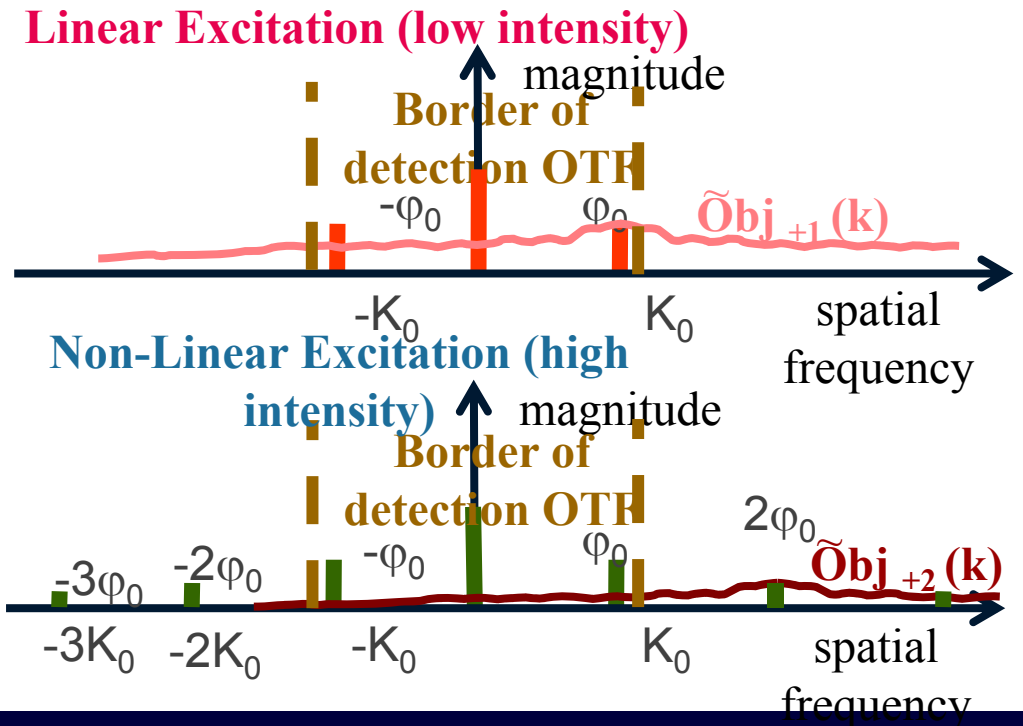
$$I_{em}(\mathbf{x}) = Obj(\mathbf{x}) \cdot I_{ex}(\mathbf{x}) \qquad \tilde{I}_{em}(\mathbf{k}) = \tilde{Obj}(\mathbf{k}) \otimes \tilde{I}_{ex}(\mathbf{k})$$

$$I_{em}(\mathbf{x}) = Obj(\mathbf{x}) \cdot f(I_{ex}(\mathbf{x})) \qquad \tilde{I}_{em}(\mathbf{k}) = \tilde{Obj}(\mathbf{k}) \otimes \tilde{f}(\tilde{I}_{ex}(\mathbf{k}))$$

In Real Space



In Reciprocal Space



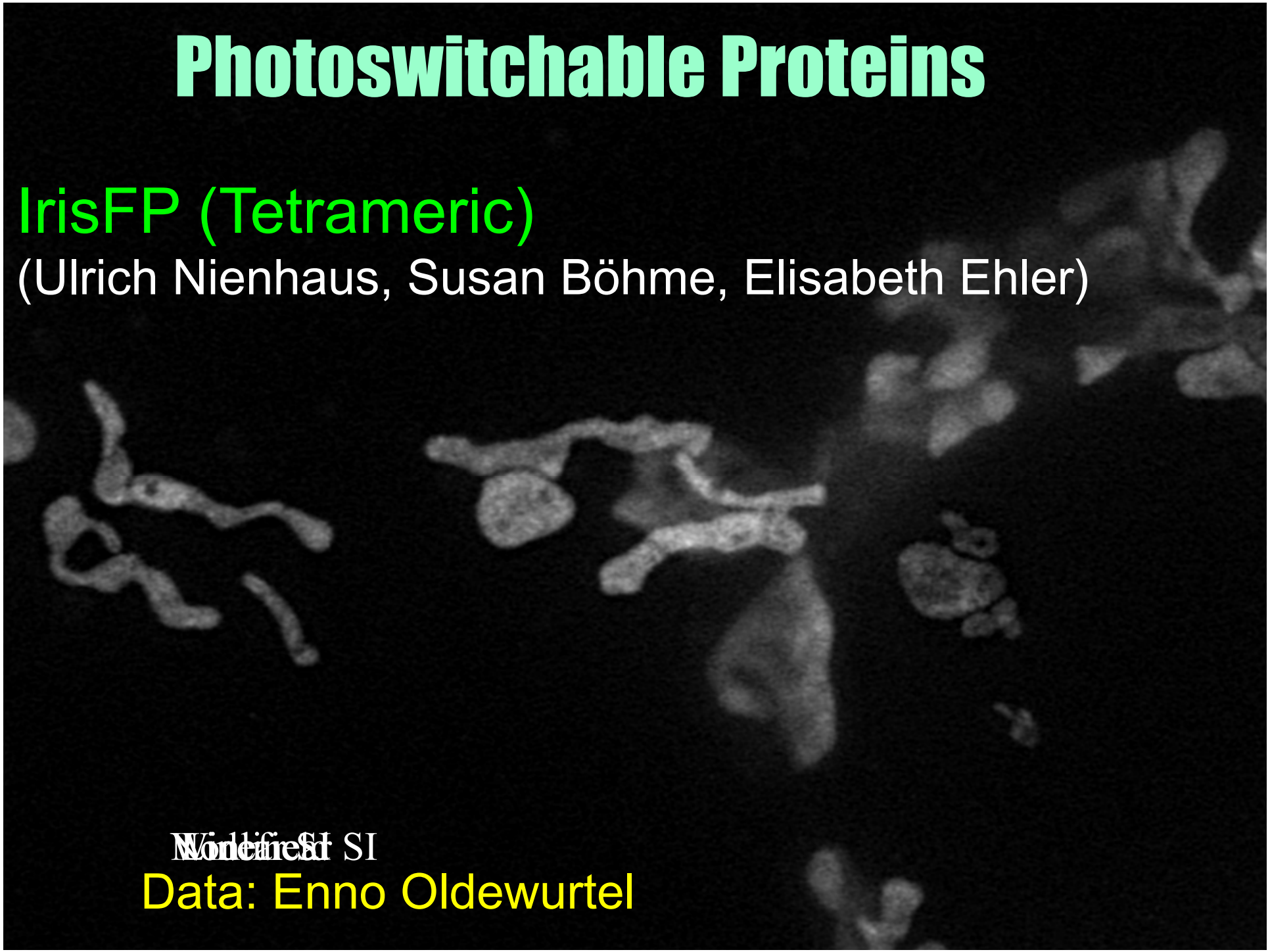
Past

R. Heintzmann, T.M. Jovin, and C. Cremer., **J. Opt. Soc. Am. A**,19 (8), 1599-160
2002

Photoswitchable Proteins

IrisFP (Tetrameric)

(Ulrich Nienhaus, Susan Böhme, Elisabeth Ehler)



Widefield SI

Data: Enno Oldewurtel

History

2011

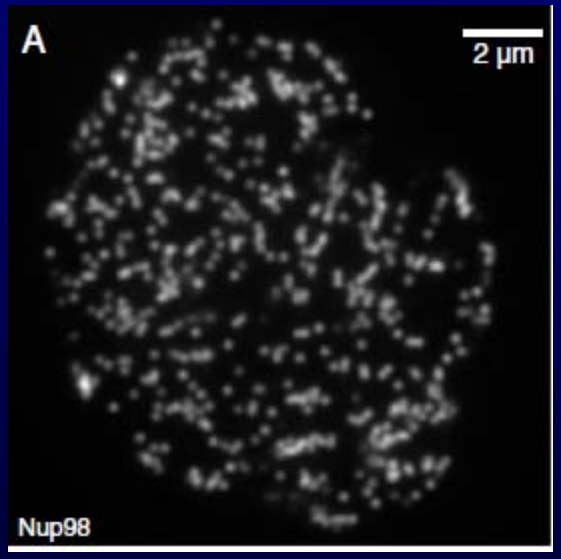
Nonlinear structured-illumination microscopy with a photoswitchable protein reveals cellular structures at 50-nm resolution

E. Hesper Rego[a,b,1,2], Lin Shao[b], John J. Macklin[b], Lukman Winoto[c], Göran A. Johansson[d], Nicholas Kamps-Hughes[d], Michael W. Davidson[e], and Mats G. L. Gustafsson[b,3]

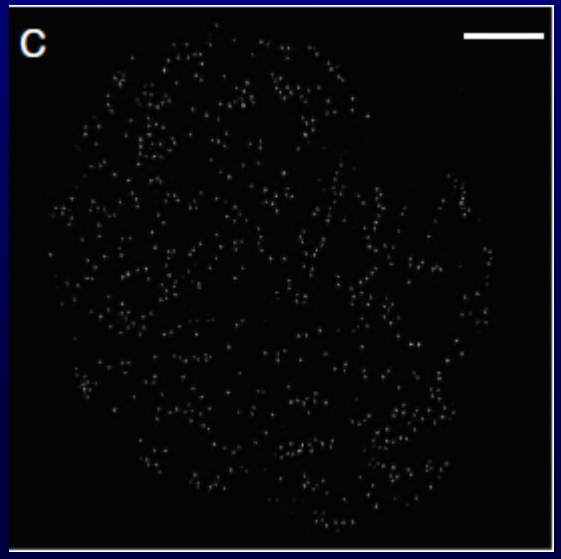
PNAS | January 17, 2012 | vol. 109 | no. 3 | E135–E143

Nuclear Pores (Nup98):

TIRF



NL-SIM TIRF

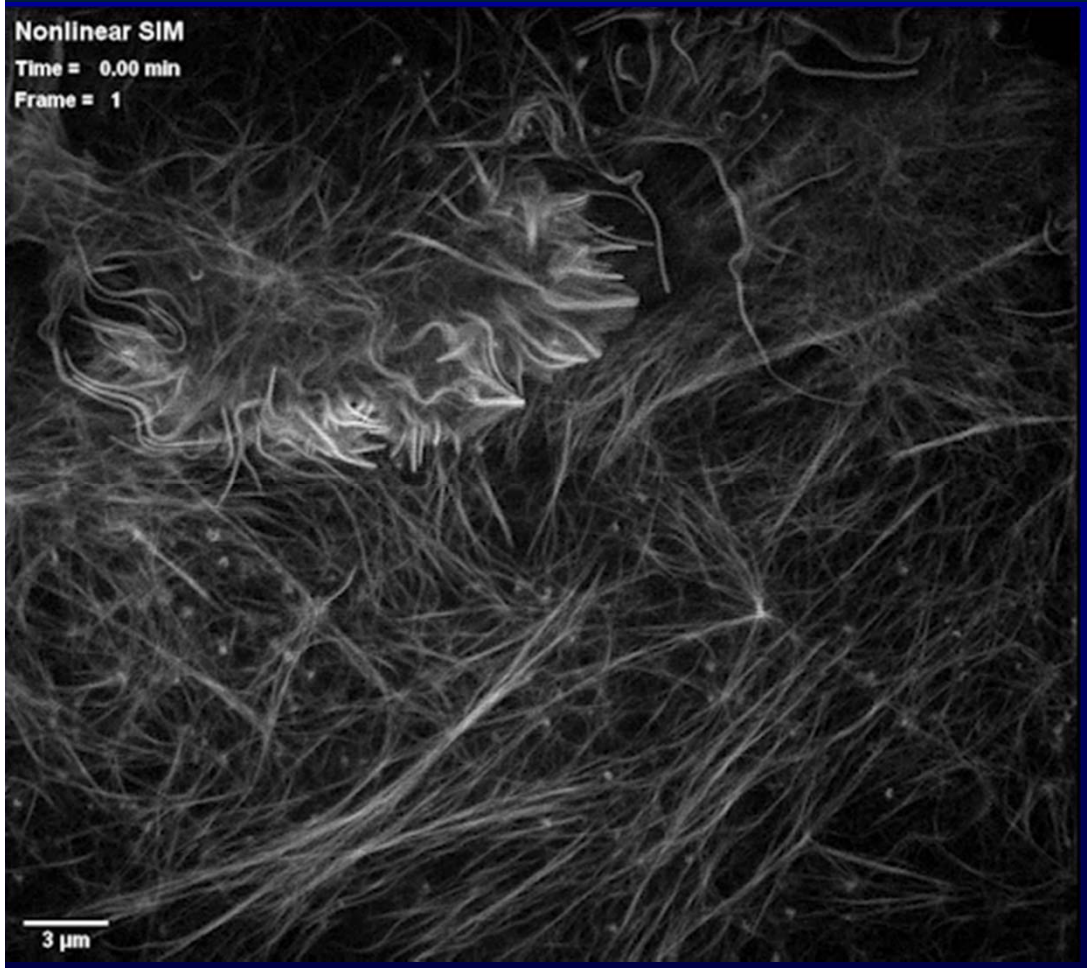


NL-SIM
on biological objects
using saturated
switching (Dronpa)

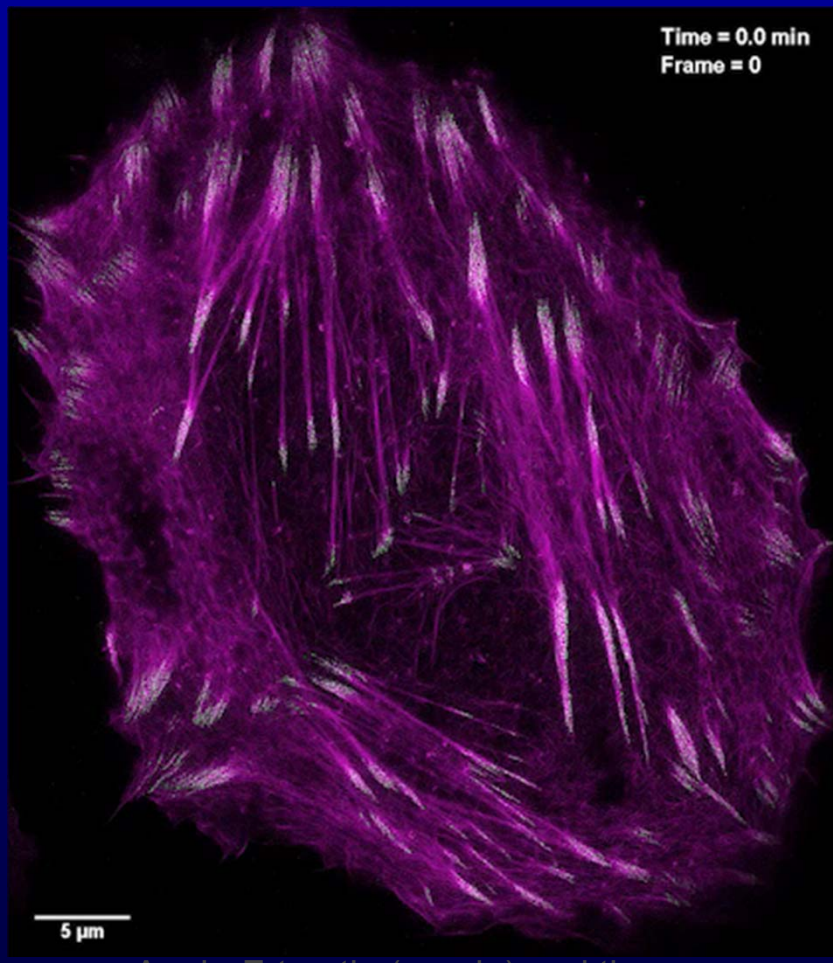
Science 28 August 2015: vol. 349 no. 6251 DOI: 10.1126/science.aab3500

Extended-resolution structured illumination imaging of endocytic and cytoskeletal dynamics

Li et al. (Betzig lab)



evolution of cortical f-actin in a COS-7 cell at 23°C transfected with Skylan-NS-Lifeact,



mApple-F-tractin (purple) and the focal
adhesion protein mEmerald-paxillin (green)
in a U2OS cell (movie S2).

Overview

- High-res modes: SIM
- Blind: PSF, illumination estimation

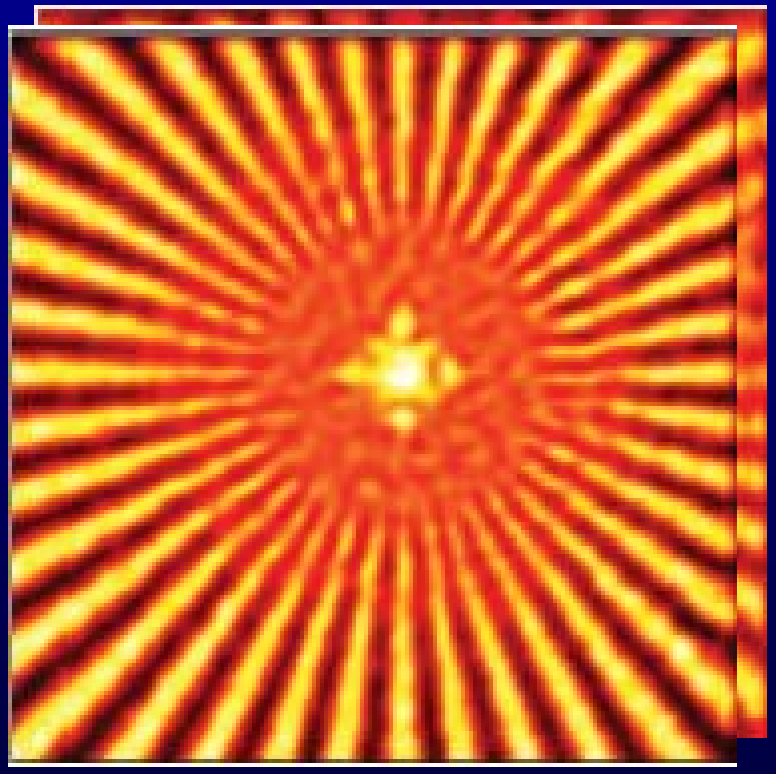
Blind deconvolution (illumination)

- always slightly underdetermined
  (like blind source separation)
- sum of all illumination is assumed constant
  (also for 200 speckle patterns)
- tiny Fourier-space support

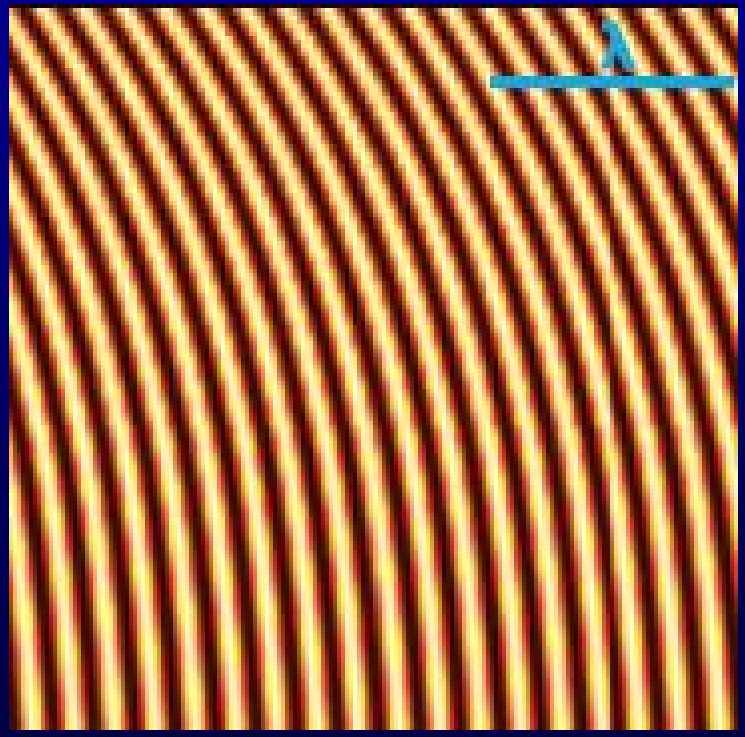
Overview

SIM reconstruction

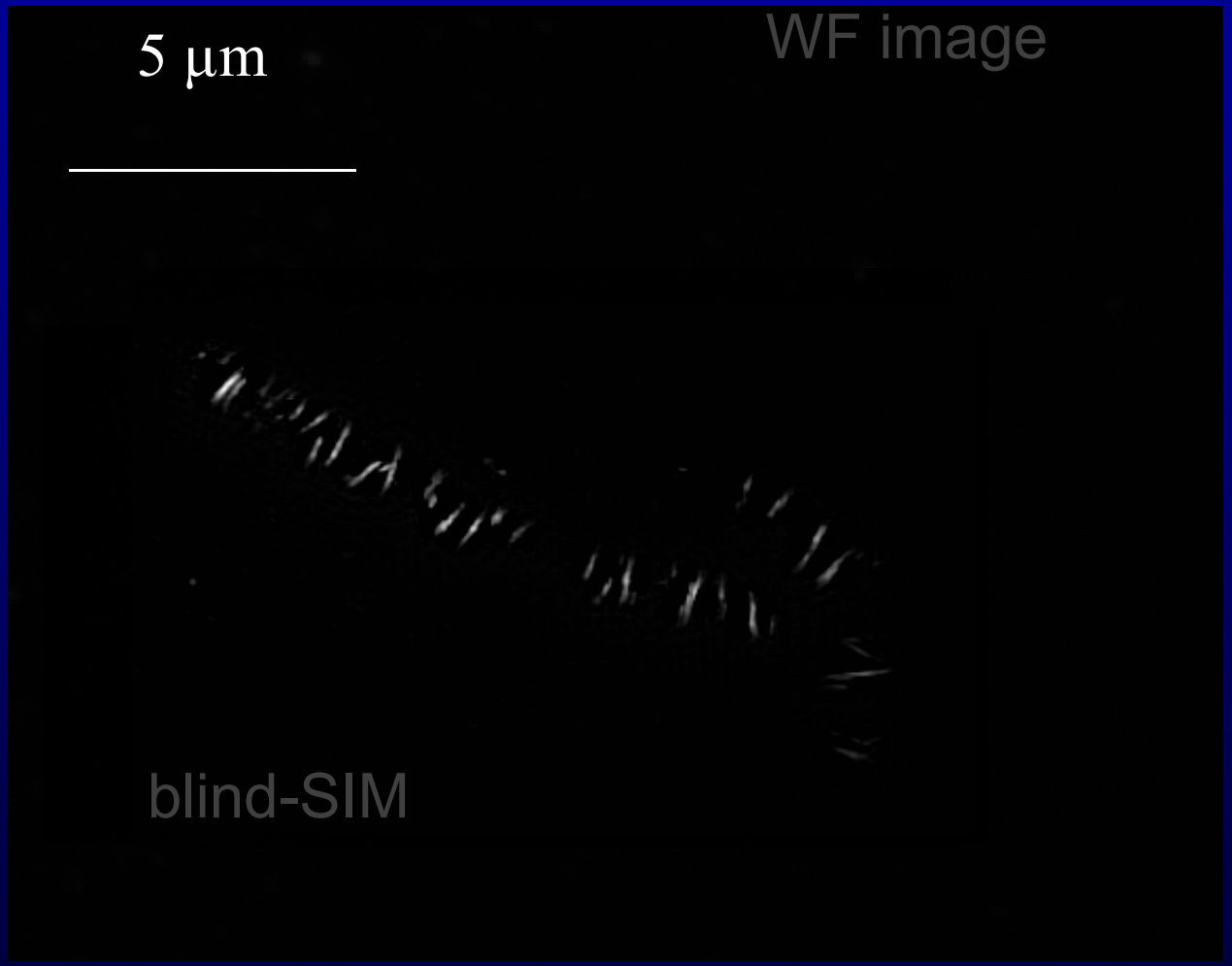
blind deconvolution



distorted Pattern



Blind-SIM: experimental TIRF-SIM data



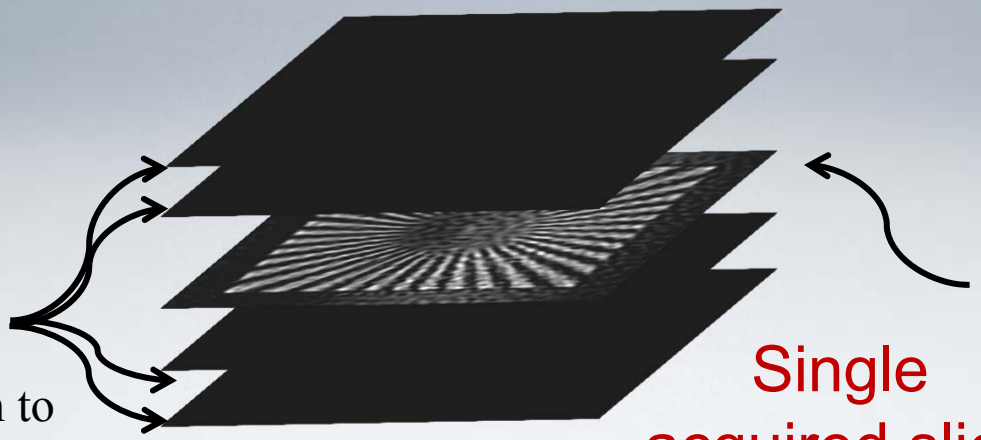
Aurélie Jost

Image courtesy Philipp von Olshausen / Alexander Rohrbach, Freiburg

Blind-SIM on thick samples

Principle of the *thick slice* deconvolution:

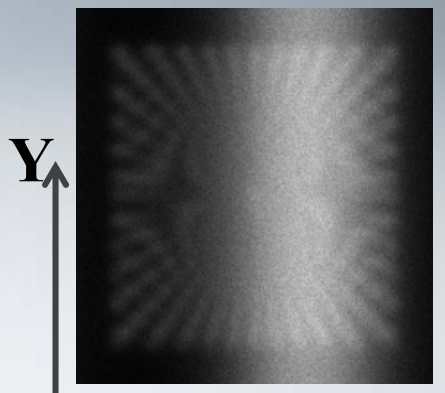
- 2-beam illumination
- Single-slice acquisition at $z = z_0$
- 3D blind-SIM deconvolution using 3D PSF and extended stack



Additional planes

No contribution to the cost functional

Single acquired slice



BlindSIM: Aurélie Jost

iphtjena

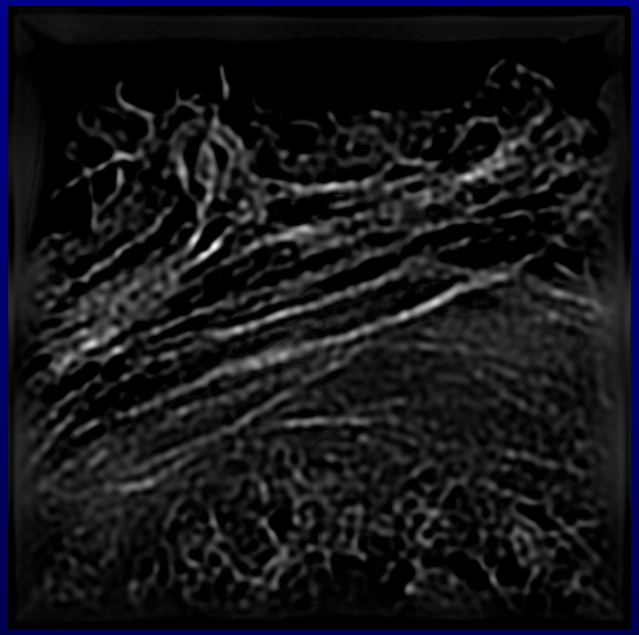
Mitglied der Leibniz-Gemeinschaft

Blind-SIM on thick samples

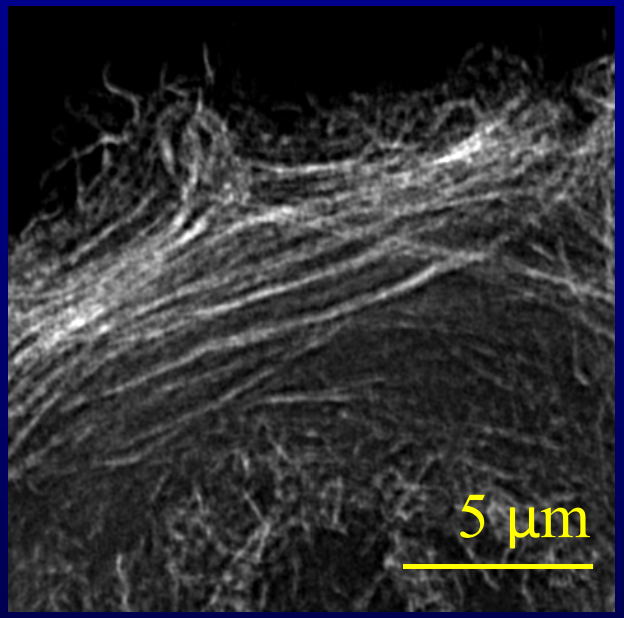
BlindSIM: Aurélie Jost

Blind-SIM on thick samples

Experimental thick samples:



WF 2D deconvolution



Elyra result

BlindSIM
Aurélie Jost

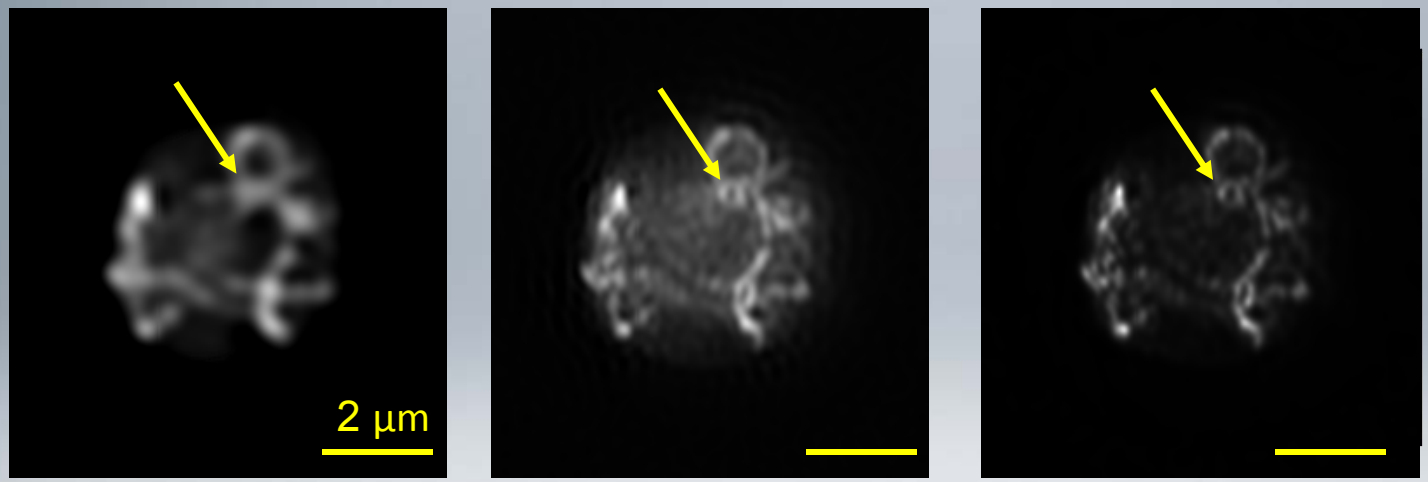
Image courtesy Elena Tolstik
Data acquired on the Elyra (3-beam)

Blind-SIM on thick samples

Experimental thick samples:

Yeast, csiLSFM set-up (SIM-SPIM)

standard SIM



3D WF deconv | 2D blind-SIM | *Thick slice* blind-SIM

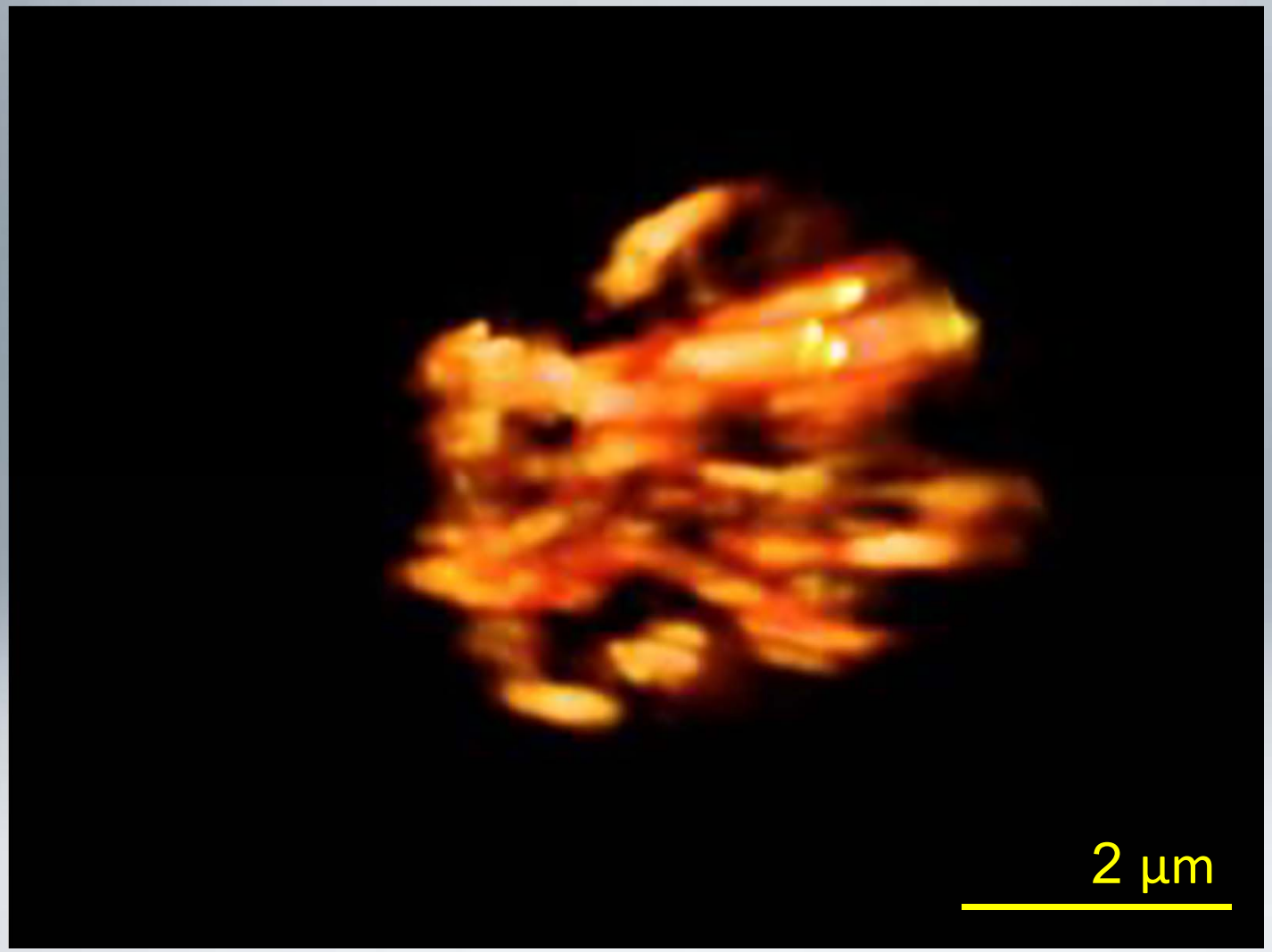
Image Data: Bo-Jui Chang
Ernst Stelzer, Frankfurt

Data information
Sample: yeast mitochondiral GFP label
Excitation: 488 nm
Emission: 509 nm
Pixel size: 57,6 nm
NA: 1,0 (water-imm.)
n: 1,33
Grating: 307,2 nm

Reconstruction parameters
Reconstructed Slices: 8
Scale z PSF: 200 nm
Good's roughness penalty
λ = 0,02
Number of iterations: 30

Mitglied der Leibniz-Gemeinschaft

Blind-SIM on thick samples



Yeast
mitochondria

Image Data: Bo-Jui Chang
Ernst Stelzer, Frankfurt

Thick slice reconstruction:
slice by slice, Aurélie Jost

iphtjena

Mitglied der Leibniz-Gemeinschaft

Summary

Linear fluorescence microscopy methods (structured illumination) can

- Enhance resolution (2x limit frequency)
- Increase HF detection

Non-linear methods are unlimited in resolution (NL-SIM, STED)

Collaborations

- **Research:** Ondrej Mandula, Susan Cox, Rolf Beutel, Y. Matsumura
- **Ideas:** Anne Sentenac
- **Images:** Mats Gustafsson, Alexander Rohrbach, Ernst Stelzer, Bo-Jui Chang
- **Samples:** Christopher Williams, James Moneypenny, Gareth Jones, Jürgen Rybak, Rolf Beutel, Y. Matsumura
- **Probes:** Ullrich Nienhaus, Susan Böhme
- **Airy Scan Slides:** Allex Sossic, Uros Krzic, Chris Power

+ DFG, JSMC, KCL, Zeiss

iphtjena

Mitglied der Leibniz-Gemeinschaft

Acknowledgement

+ Collaborators, DFG, JSMC, KCL

Summary

Many modes of microscopy exist

Linear methods yield a factor of 2

Light-sheet microscopy makes cool images

Computer-based imaging has great potential