

Outline

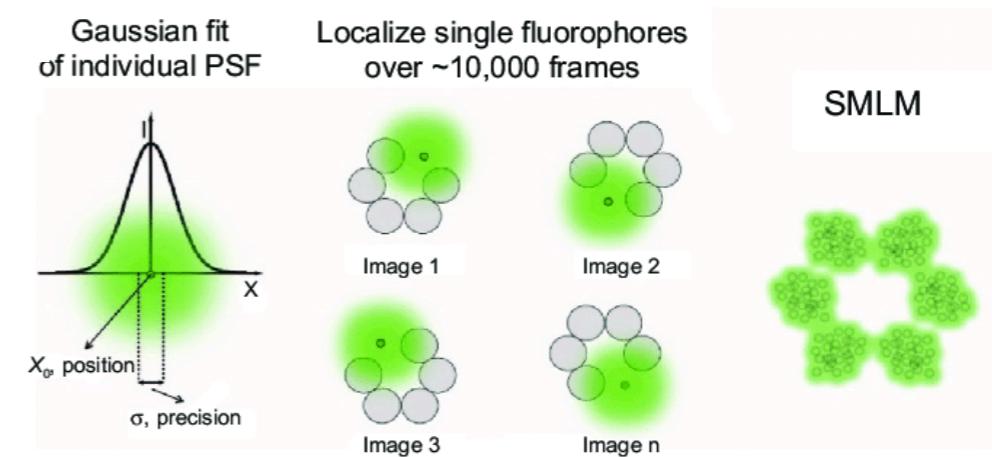
1. Introduction
2. Single molecule super resolution microscopy
3. Structured illumination super resolution microscopy
4. Case study

Outline

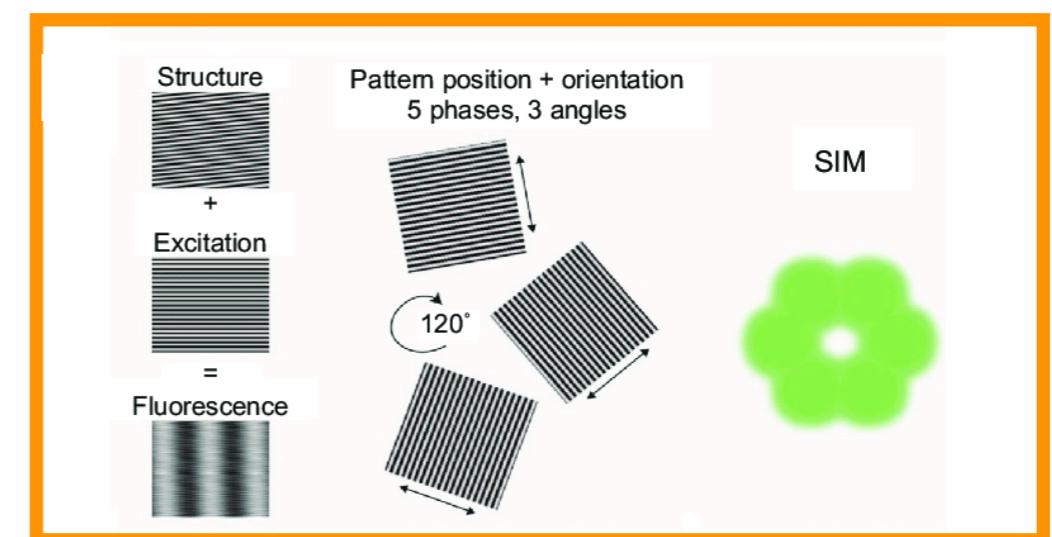
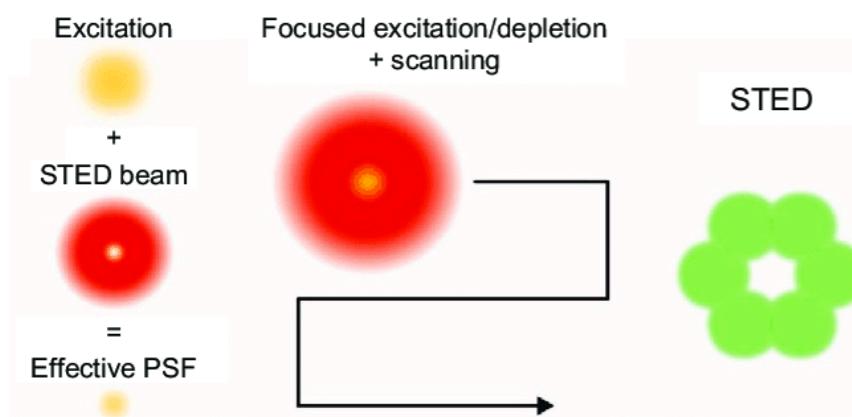
Problem : molecules within 200nm are not discernable

Solution : Keep some of them dark

SMLM → Stochastic



Structured illumination → Deterministic

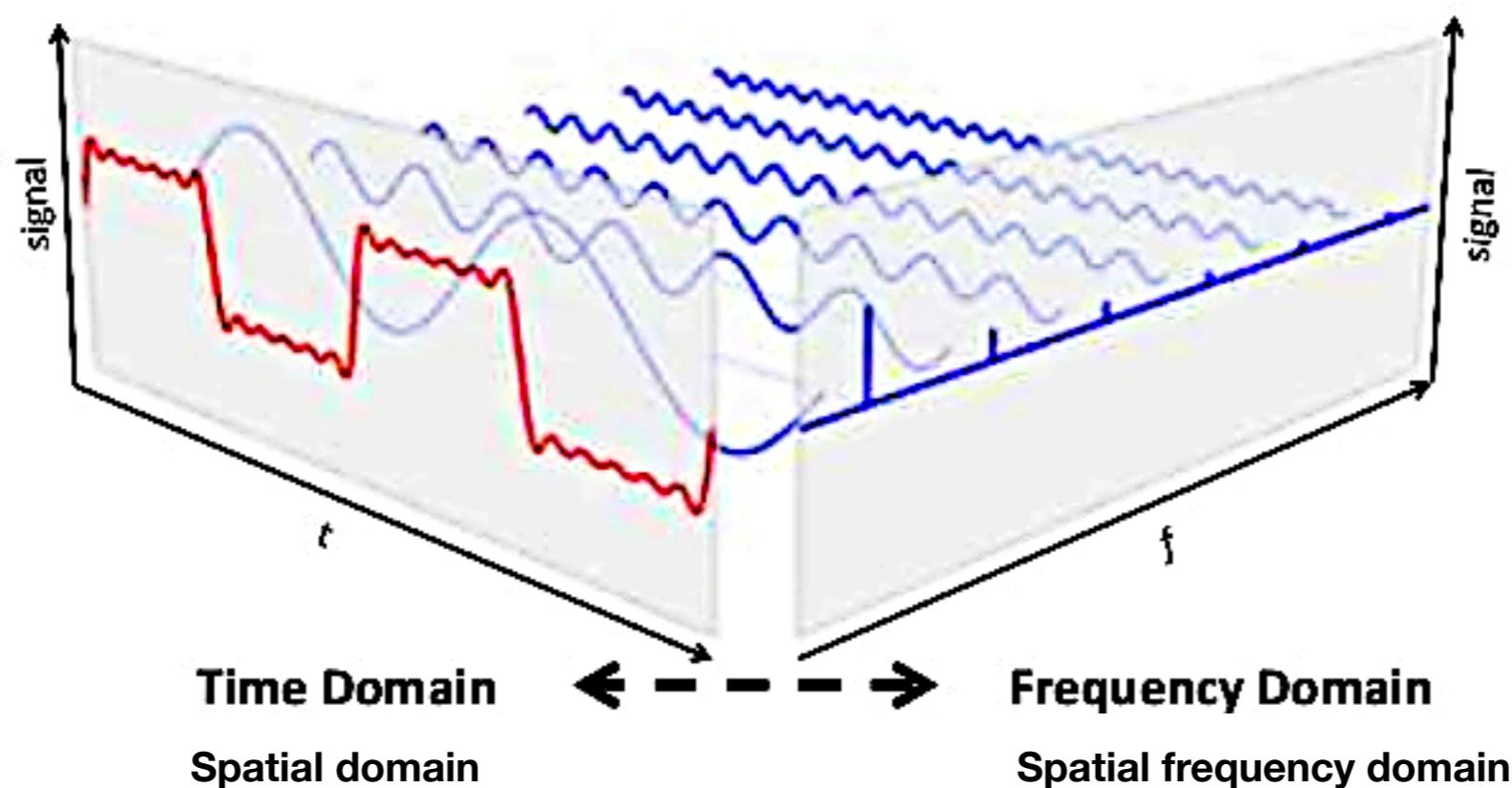


Structured Illumination Microscopy

1. Fourier transform
2. Structured Illumination Microscopy
3. Optical setup
- 4.

Fourier Transform (maths)

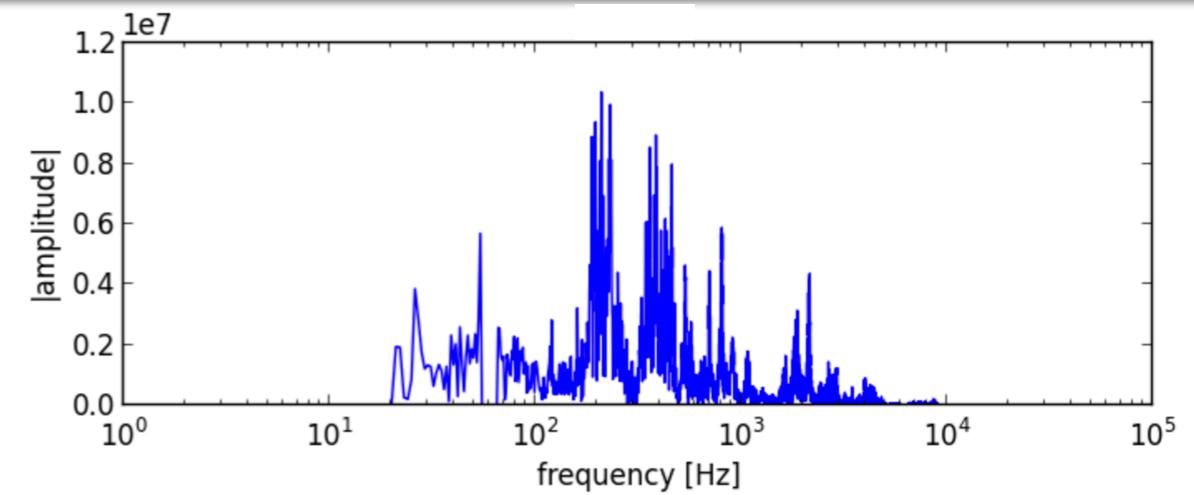
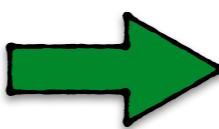
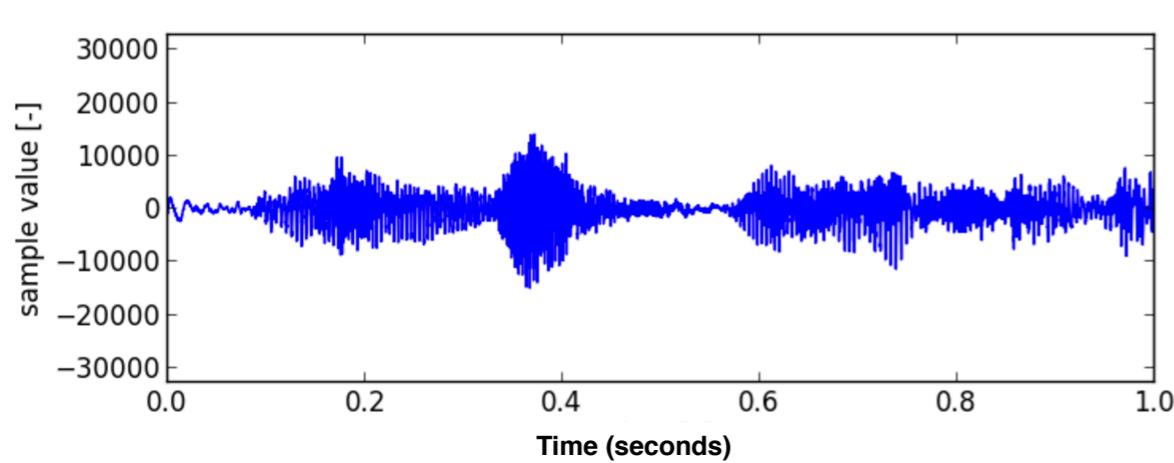
The Fourier transform is a representation of a signal as a **sum of periodic functions of varying magnitudes, frequencies and phases.**



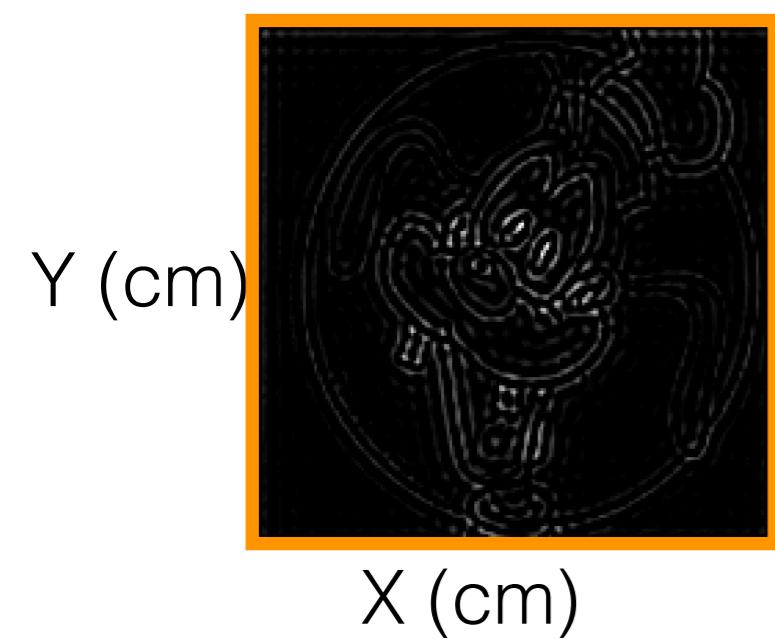
$$X(f) = \int_{-\infty}^{\infty} x(t) \times e^{-i2\pi ft} dt$$

→ decomposition of the frequency components of a signal

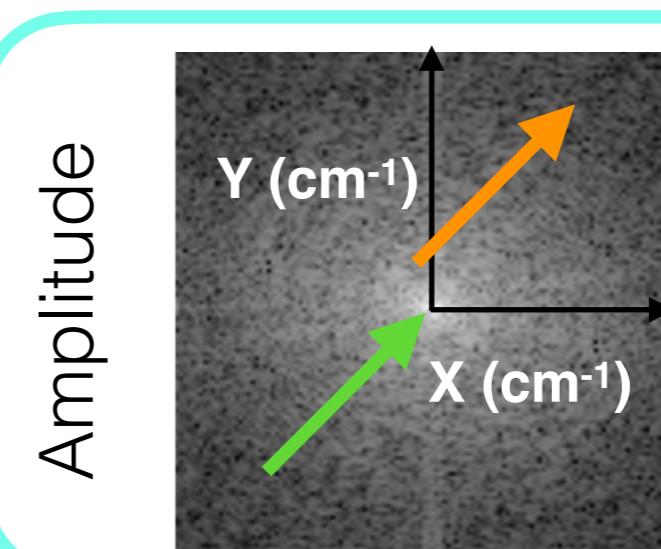
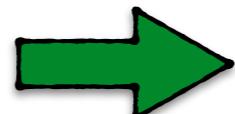
Fourier Transform



Fourier Transform (1D in time)



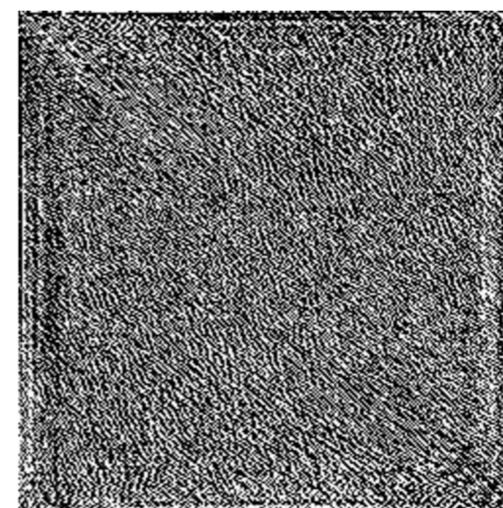
Fourier
Transform (2D
in space)



Amplitude

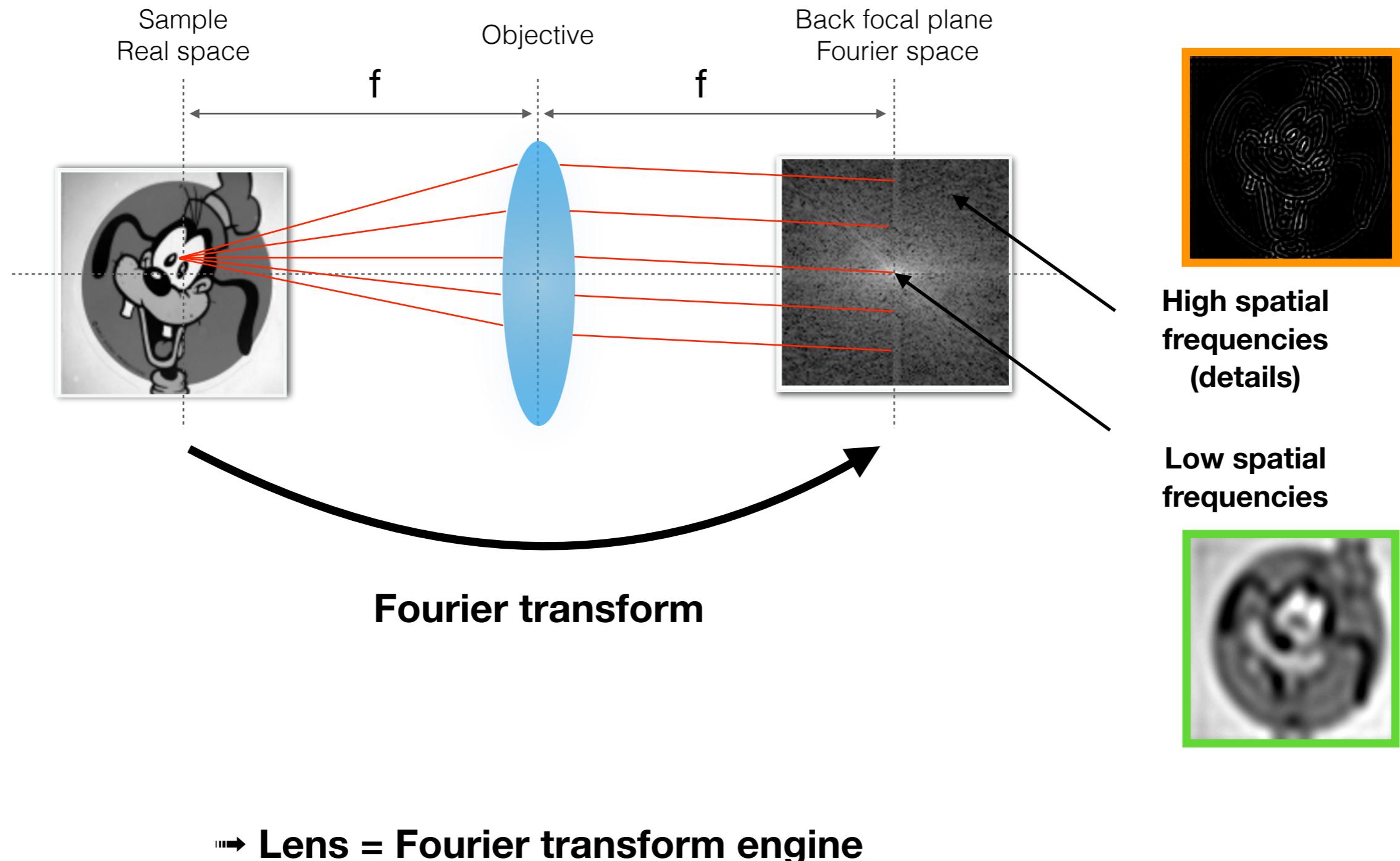
How much spatial
information in
image

Phase



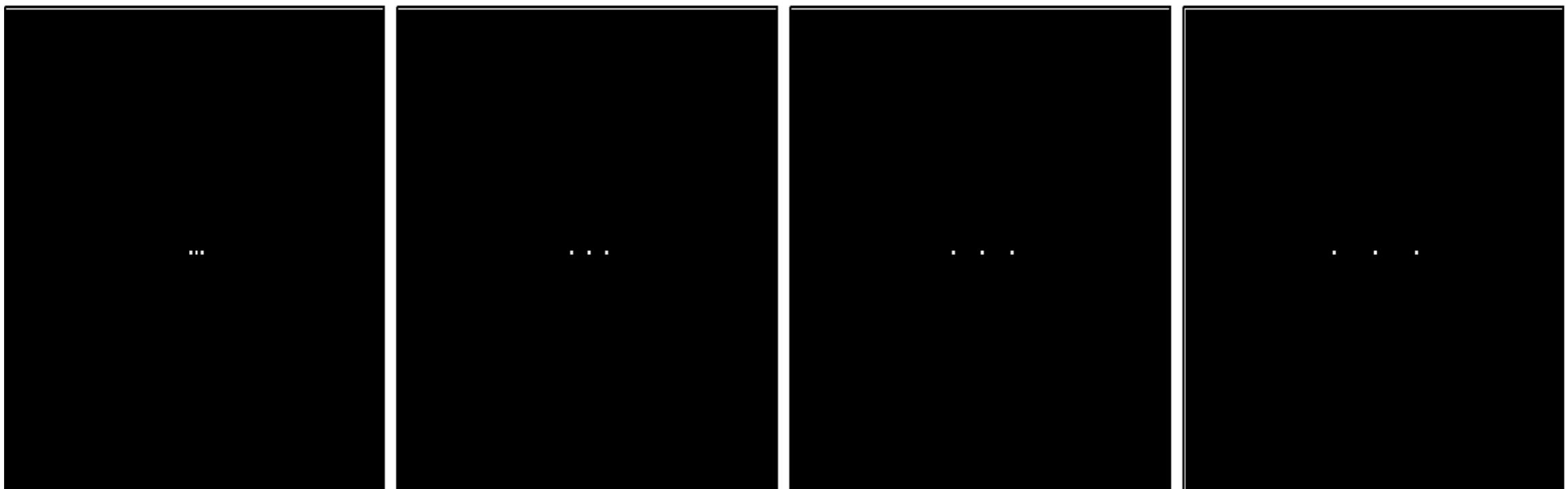
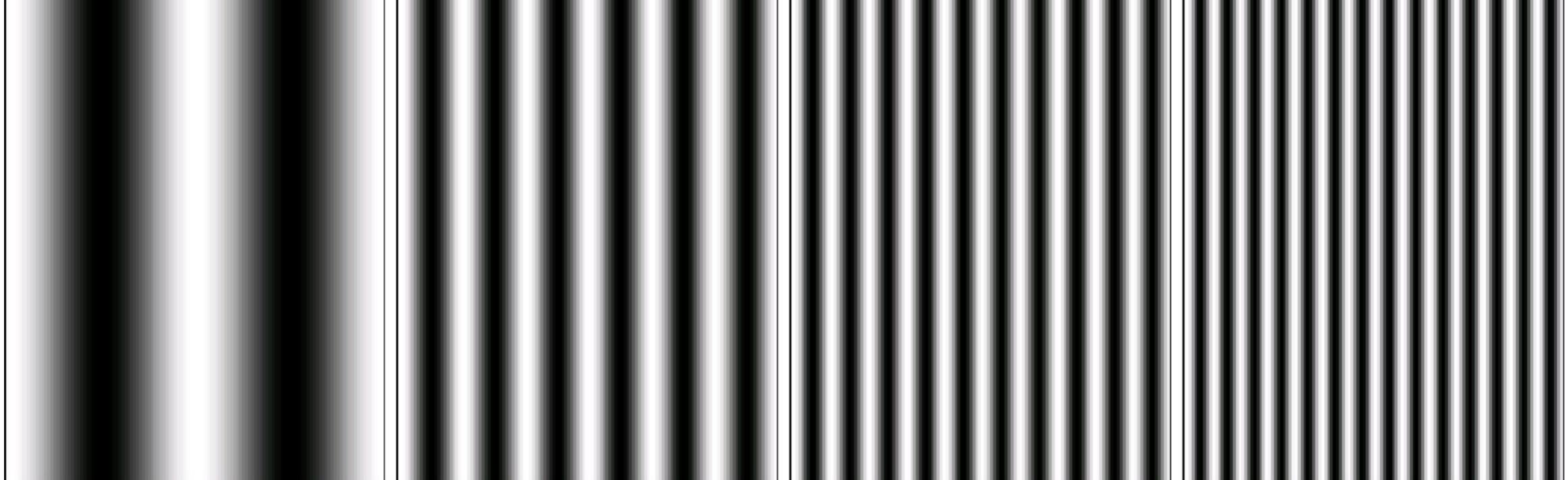
Where spatial
information is in
image

Fourier Transform (optics)



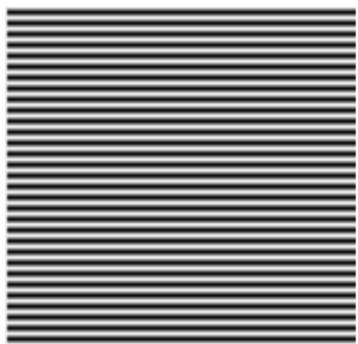
⇒ **Lens = Fourier transform engine**

Fourier Transform



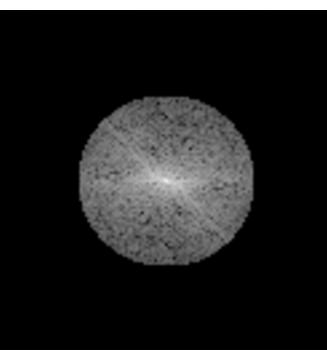
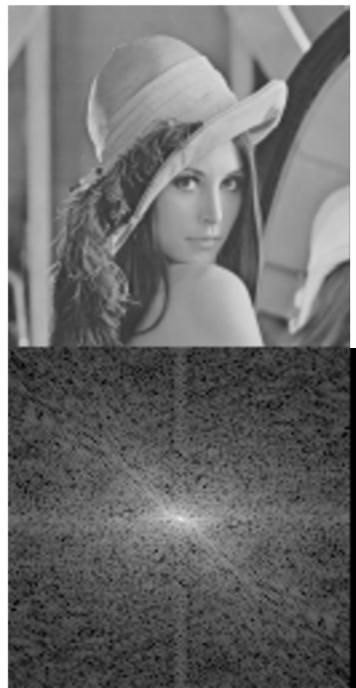
Fourier Transform : Examples

1 vertical frequency



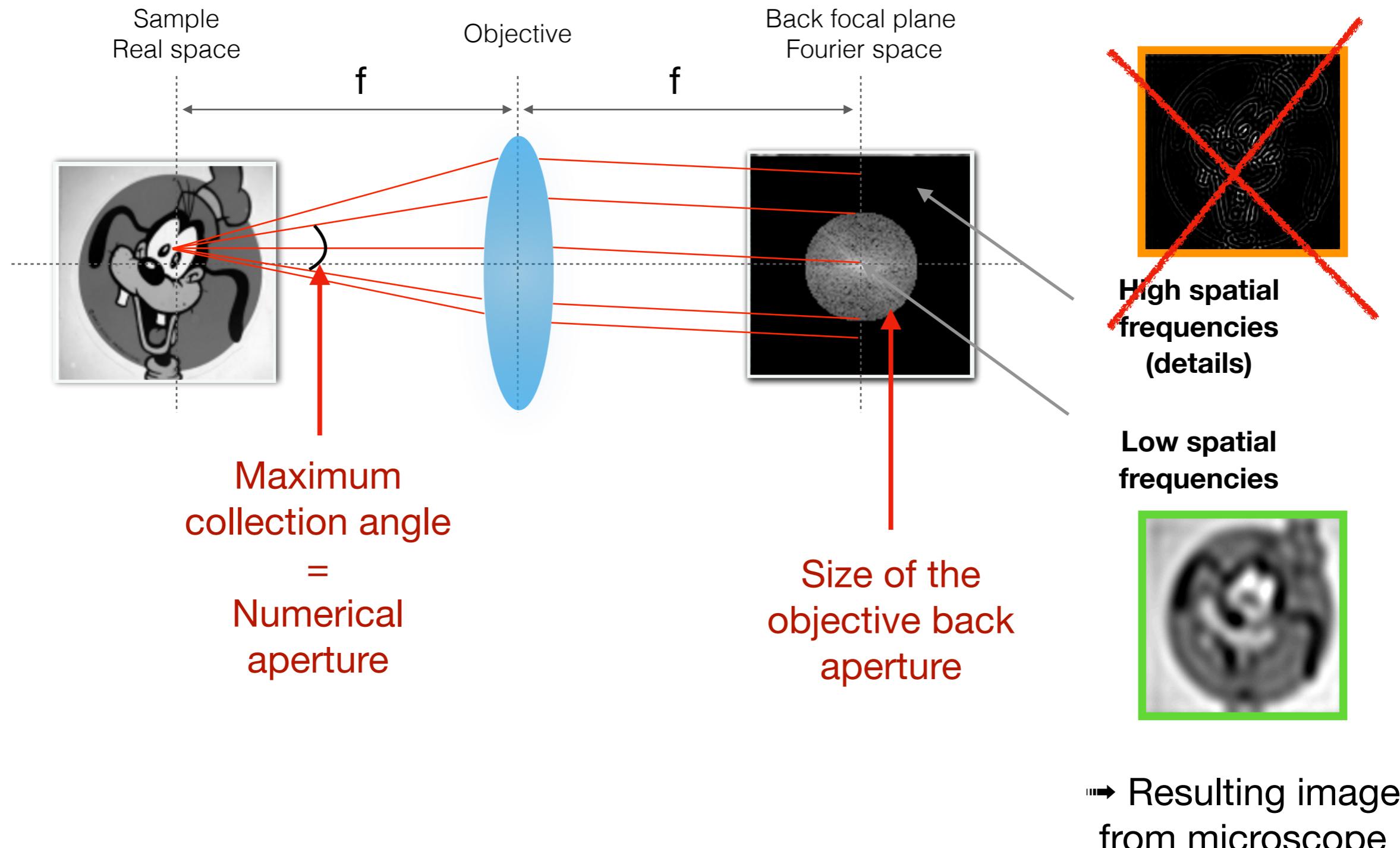
Fourier Transform : Image processing

Low pass filter



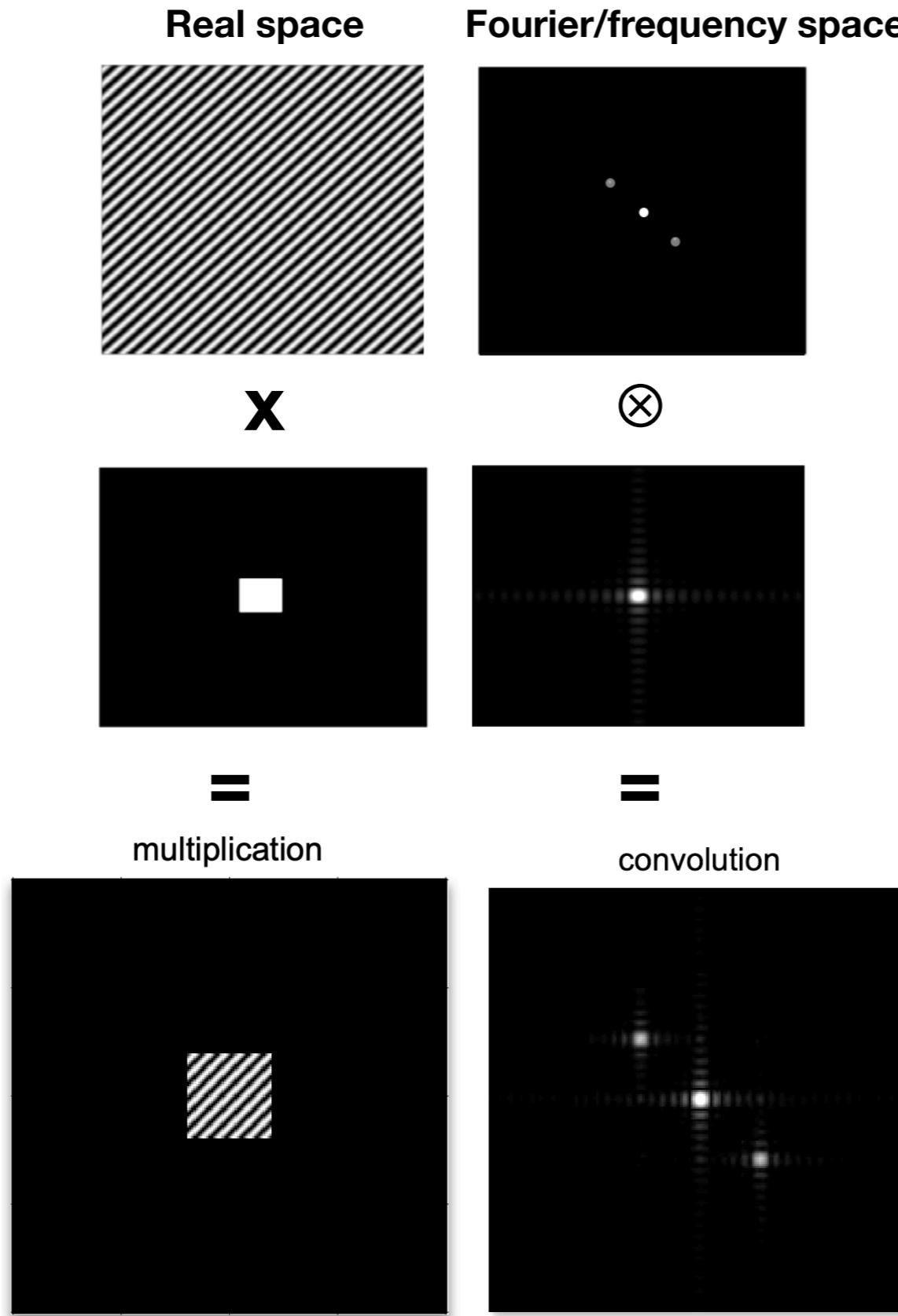
→ Denoising

Fourier Transform (optics)



How to get back high spatial frequency information?

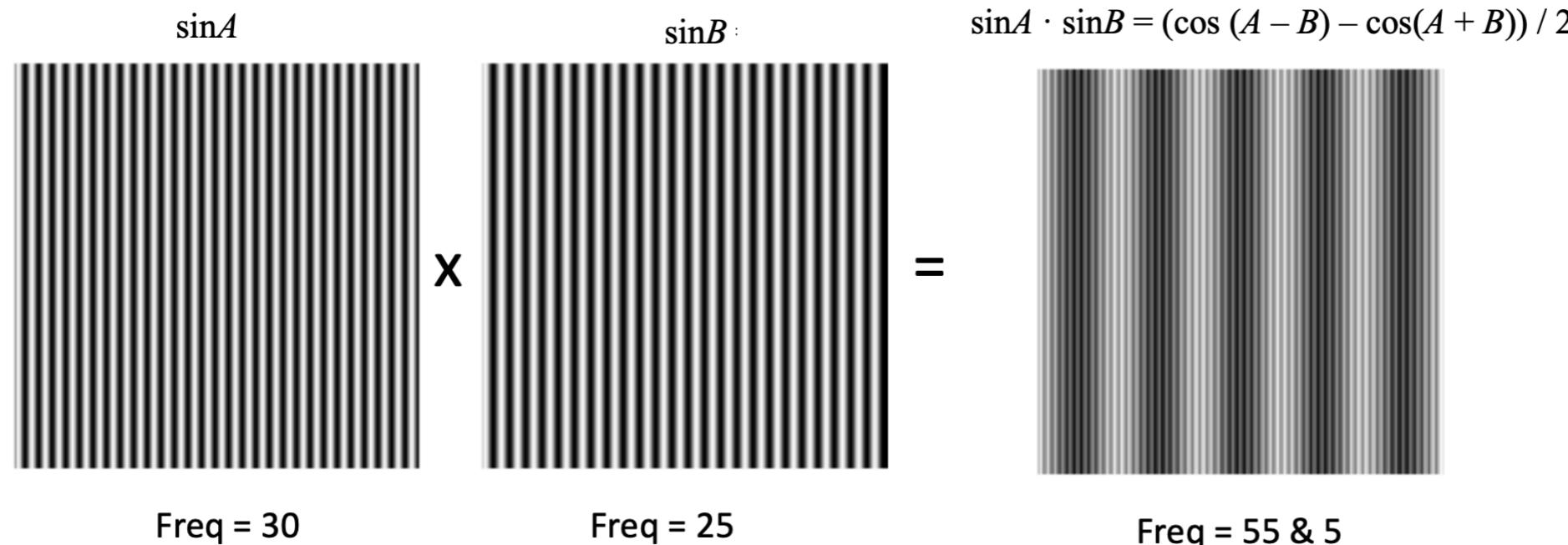
Convolution theorem



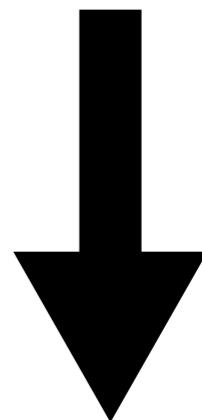
→ **Spatial frequencies
may be “transported” in
the Fourier space**

Convolution theorem

Real Space



Multiplication

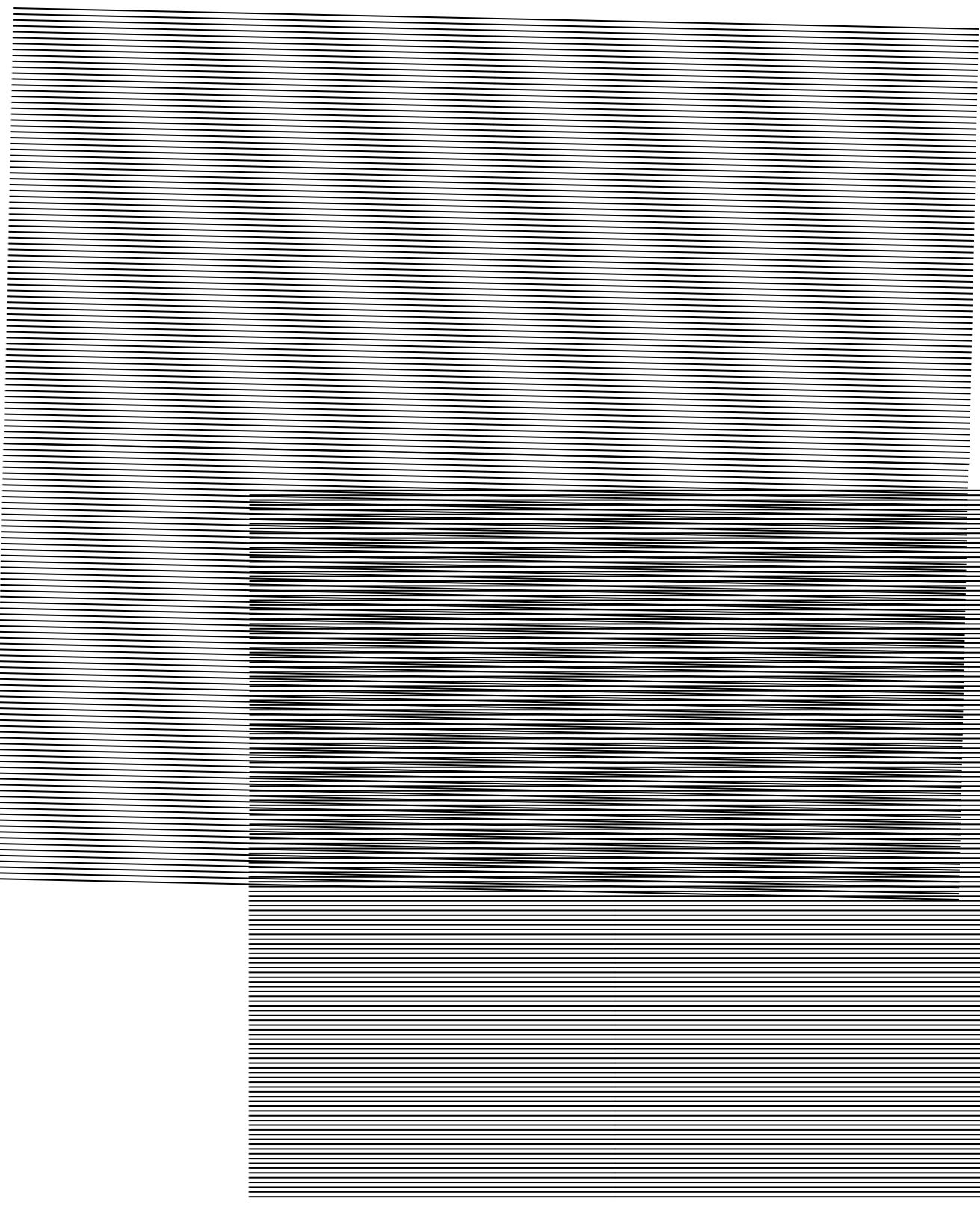
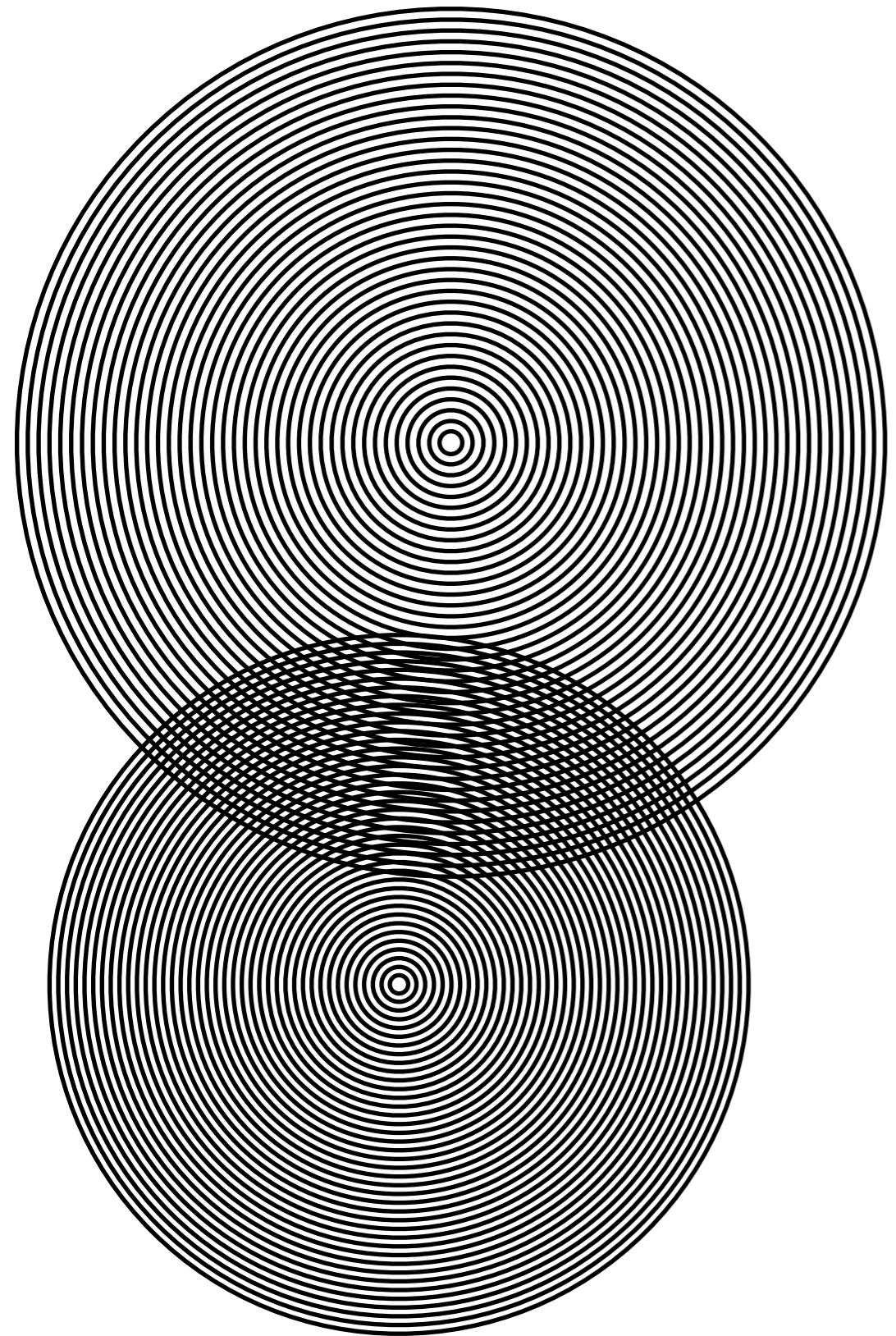


Convolution

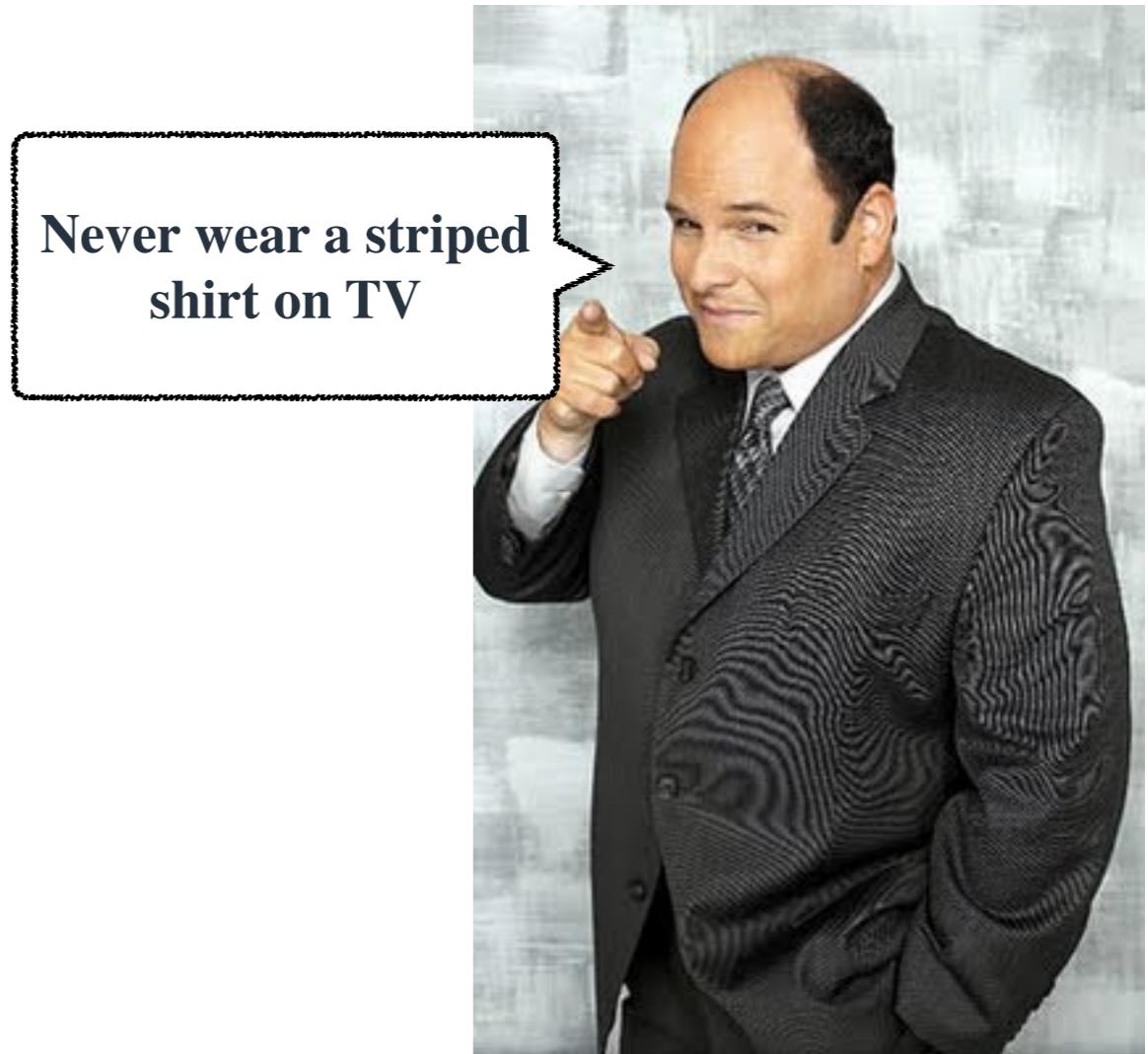
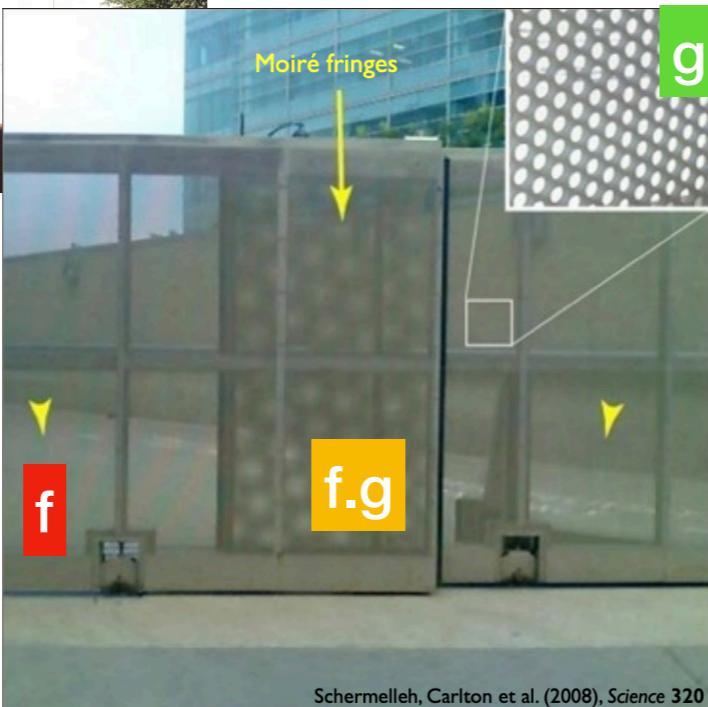
Frequencies were
“transported” in the
Fourier space...

➡ New pattern arises!

Moiré pattern



Moiré pattern



Unknown structure

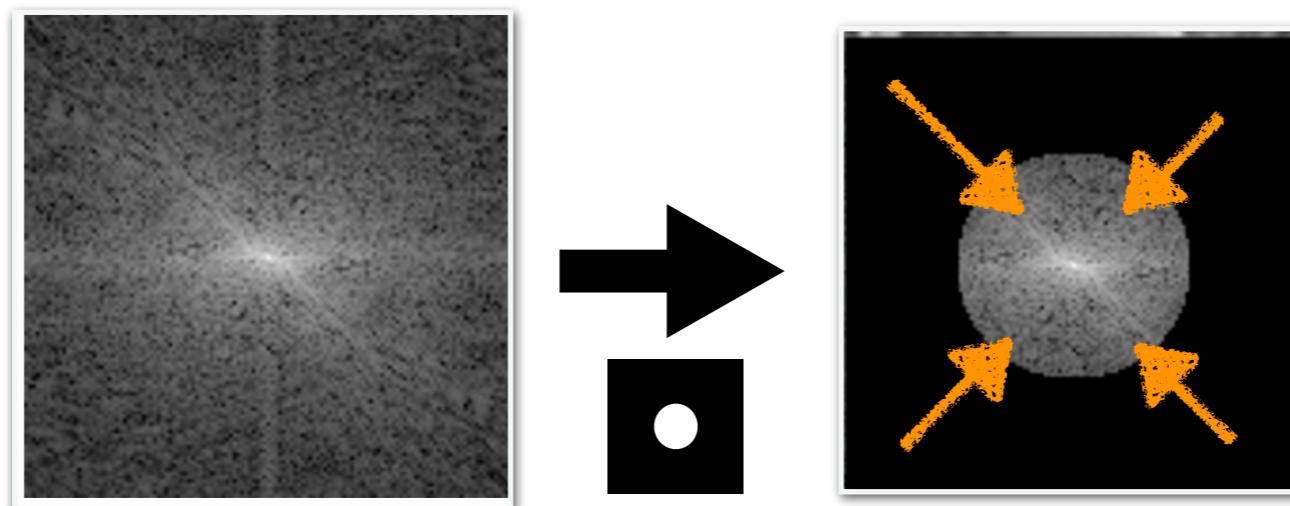
$$F\{f.g\} = \textcircled{F\{f\}} \otimes \textcircled{F\{g\}}$$

Known illumination function

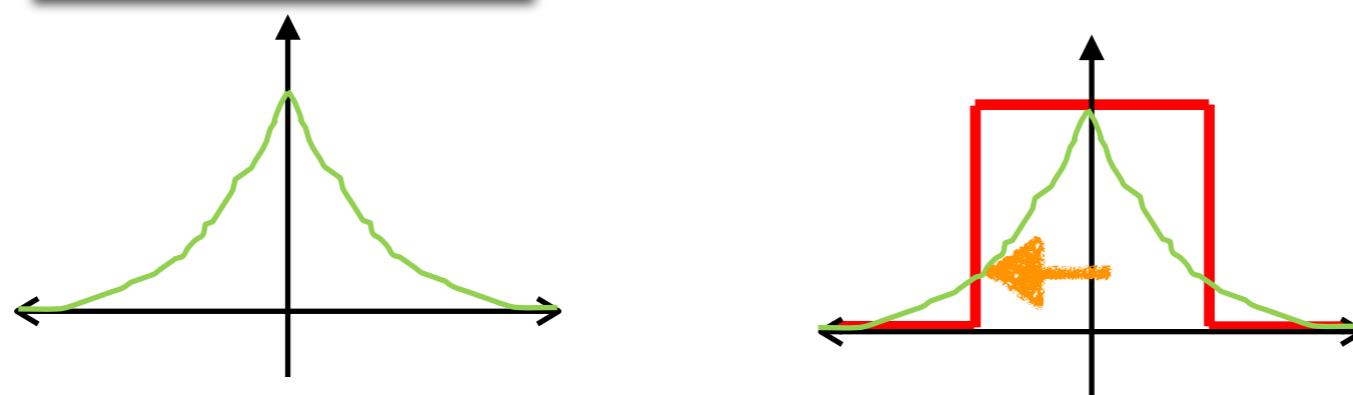
$$\rightarrow F\{f\} = F\{f.g\} \otimes^{-1} F\{g\}$$

Unknown structure may be retrieved from Moiré pattern if the second structure is known

Structured Illumination Microscopy

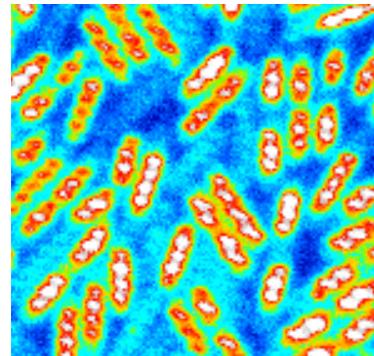
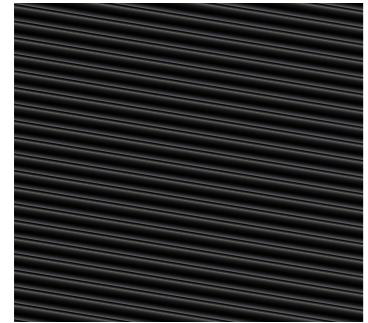
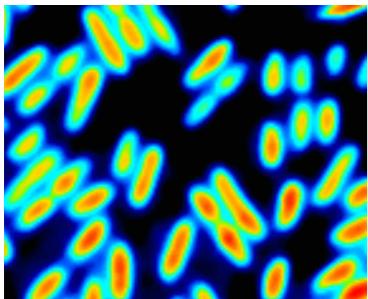


Bring high spatial frequencies back into the objective back aperture

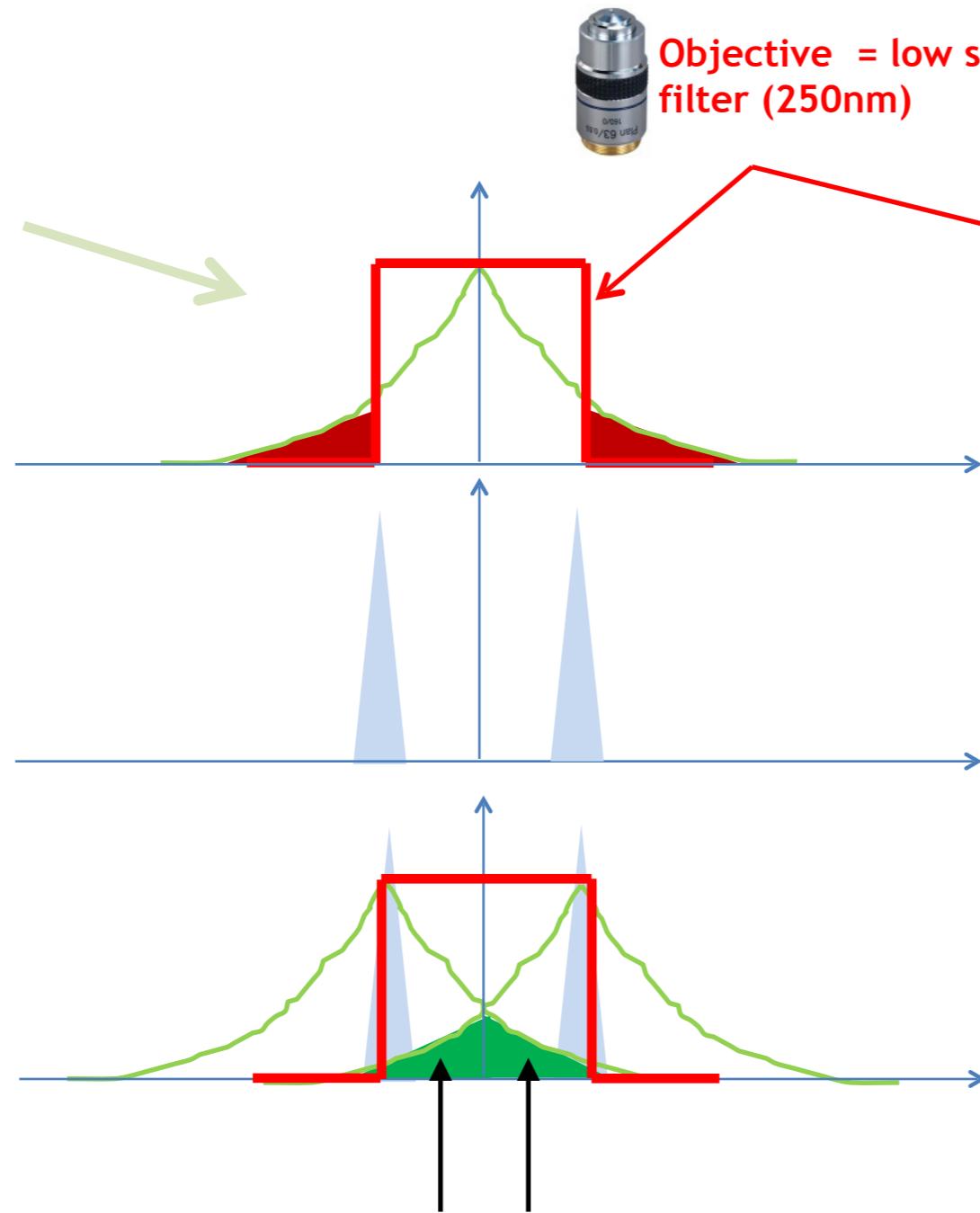


➡ May be done using Moiré effect!

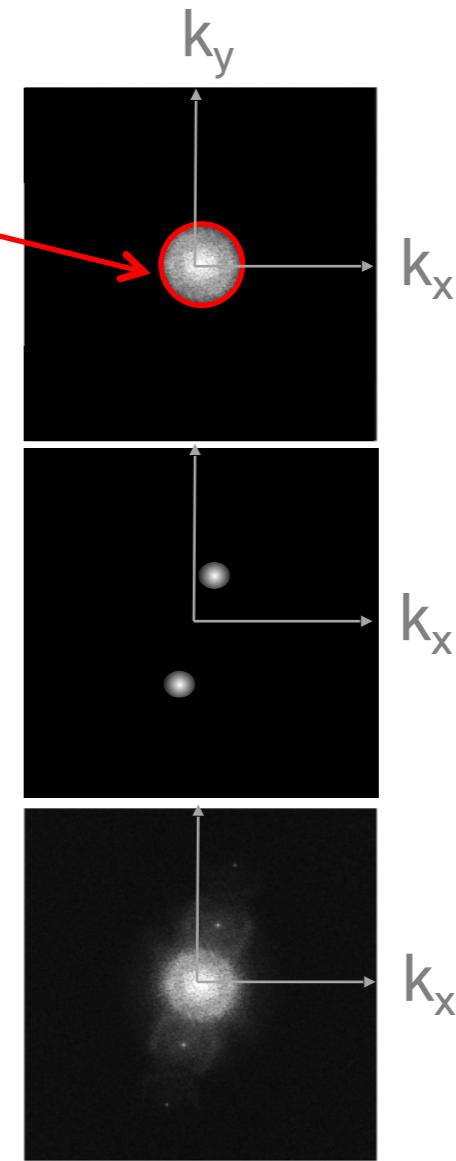
Real space



Fourier space (1D)



Fourier space (2D)

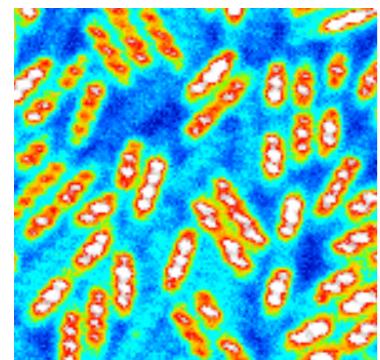
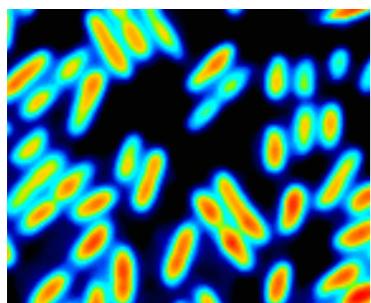


→ **High spatial frequencies are now transmitted through the objective!**

Resolution is increased along one direction only
→ Change orientation of the illumination grid

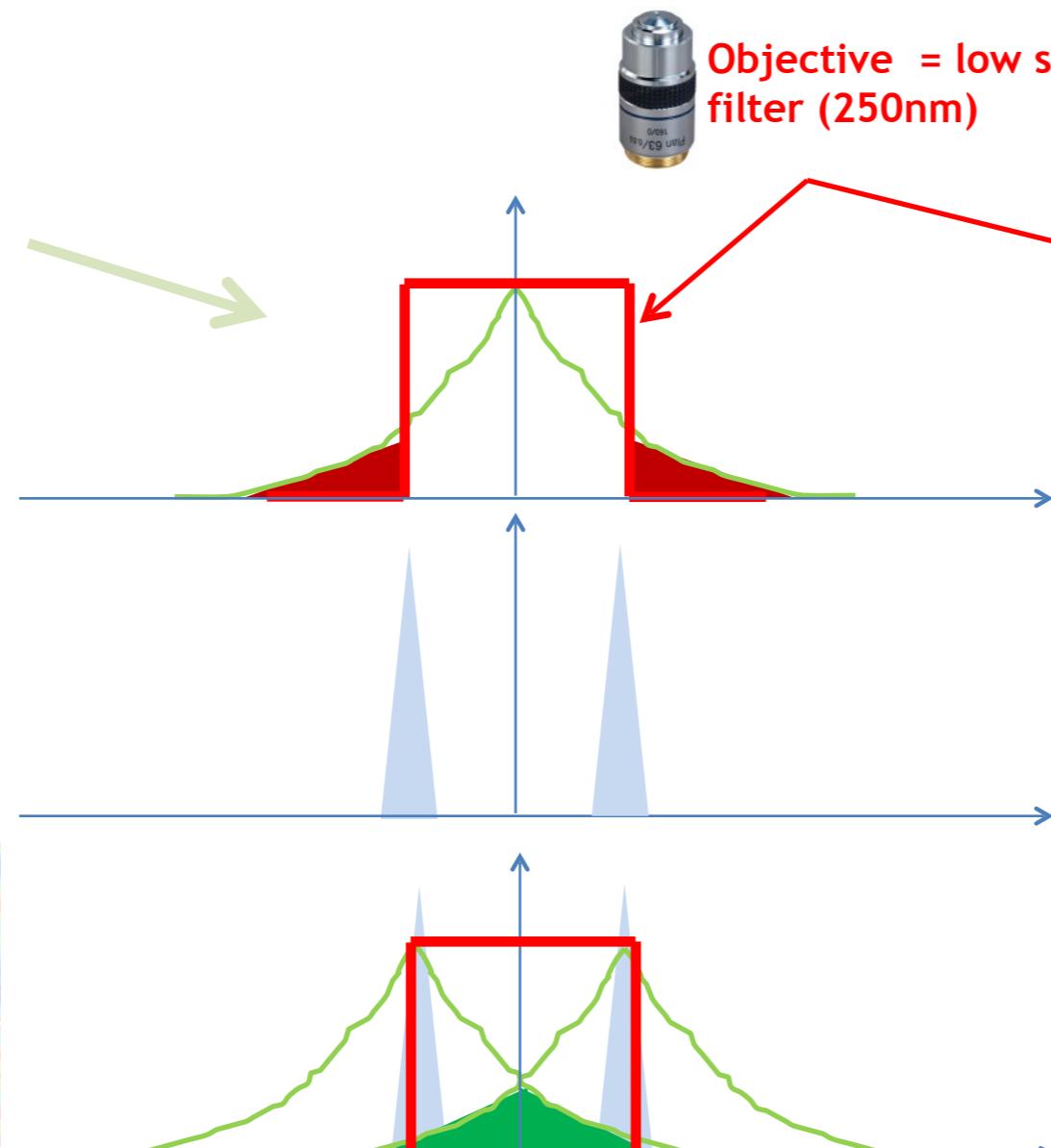
Non illuminated stripes of the sample will be missed
→ Change phase of the illumination grid

Real space

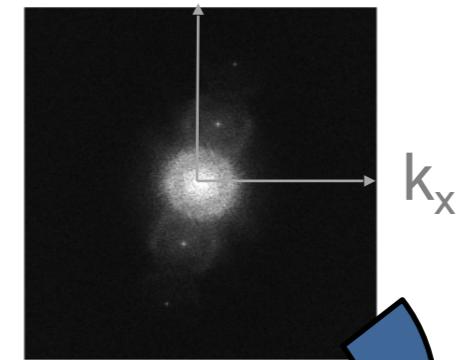
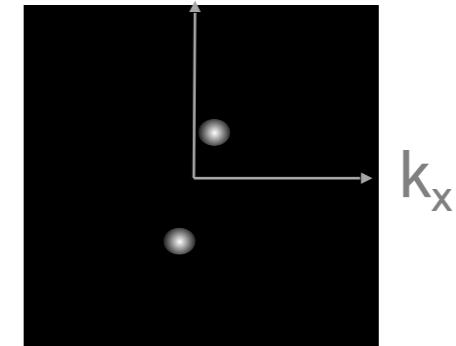
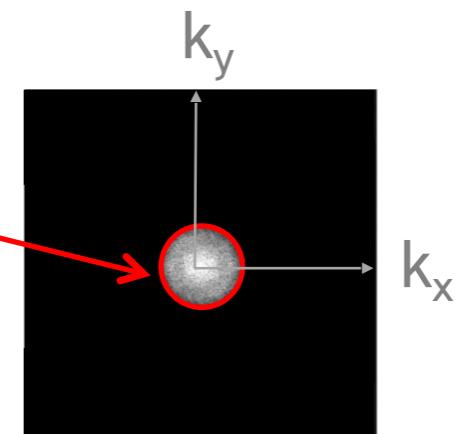


Post-processing

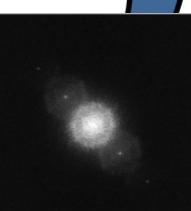
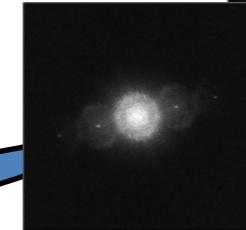
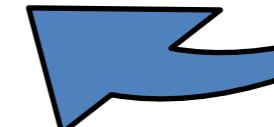
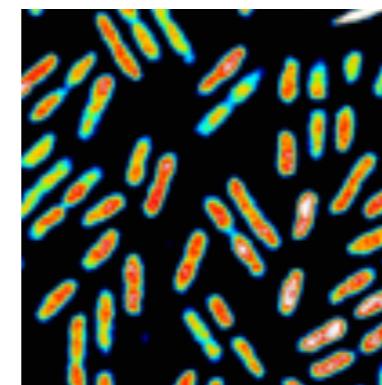
Fourier space (1D)



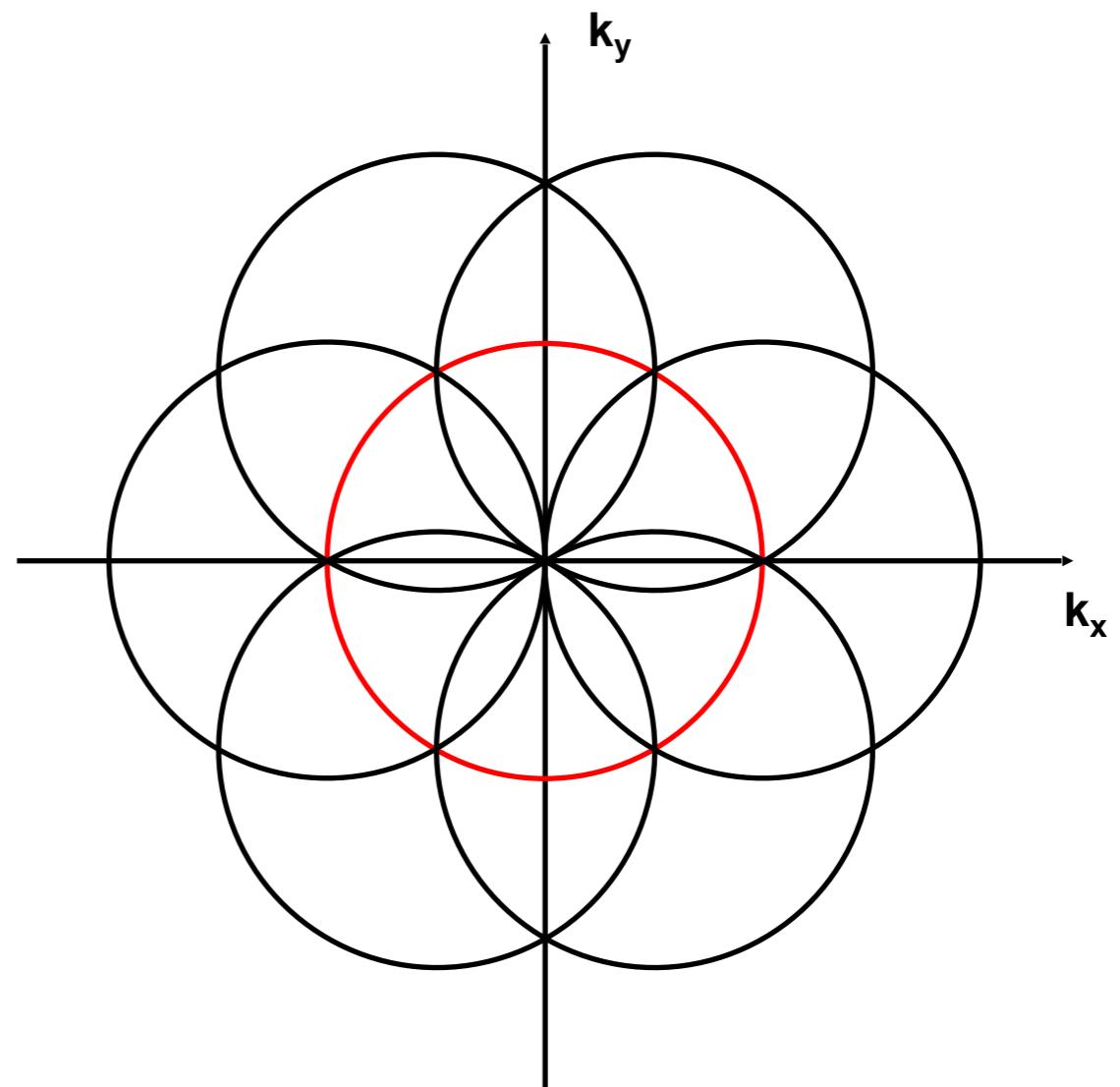
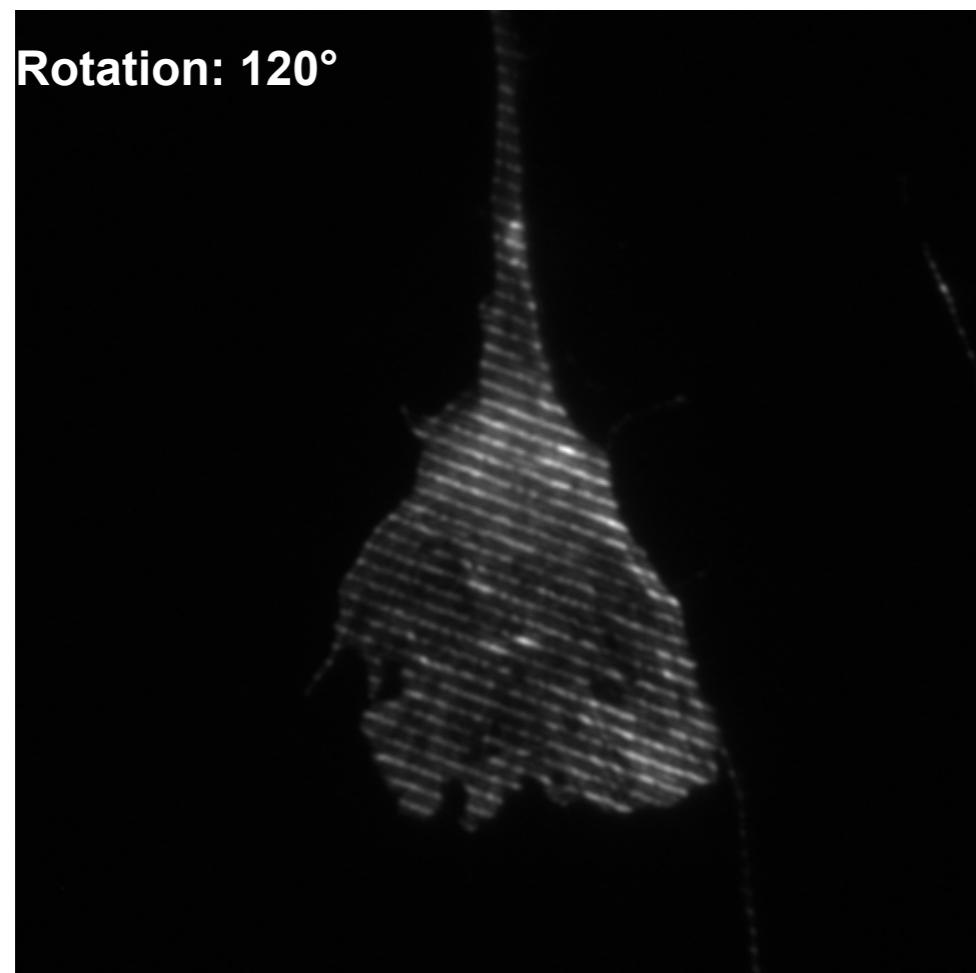
Fourier space (2D)



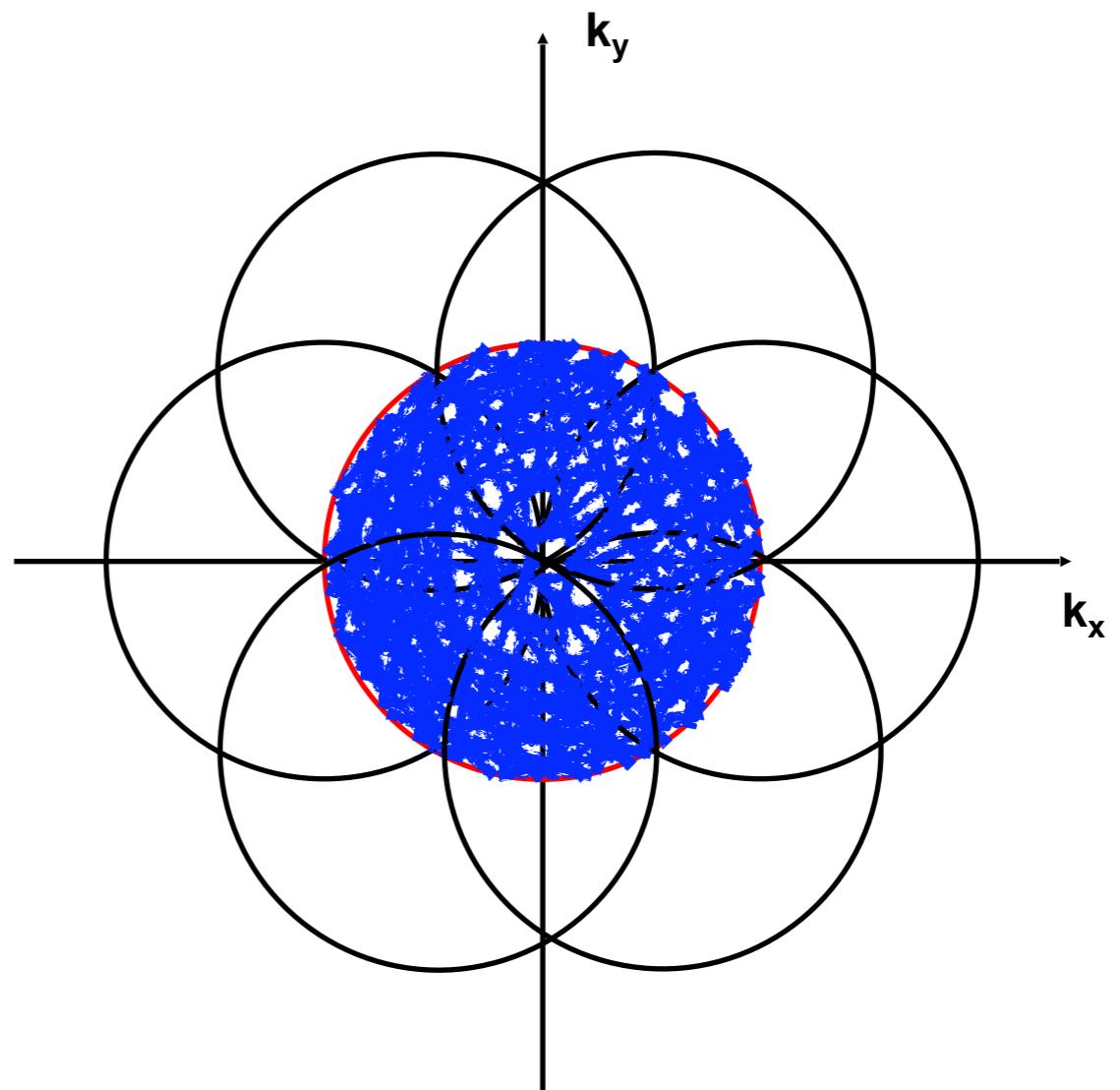
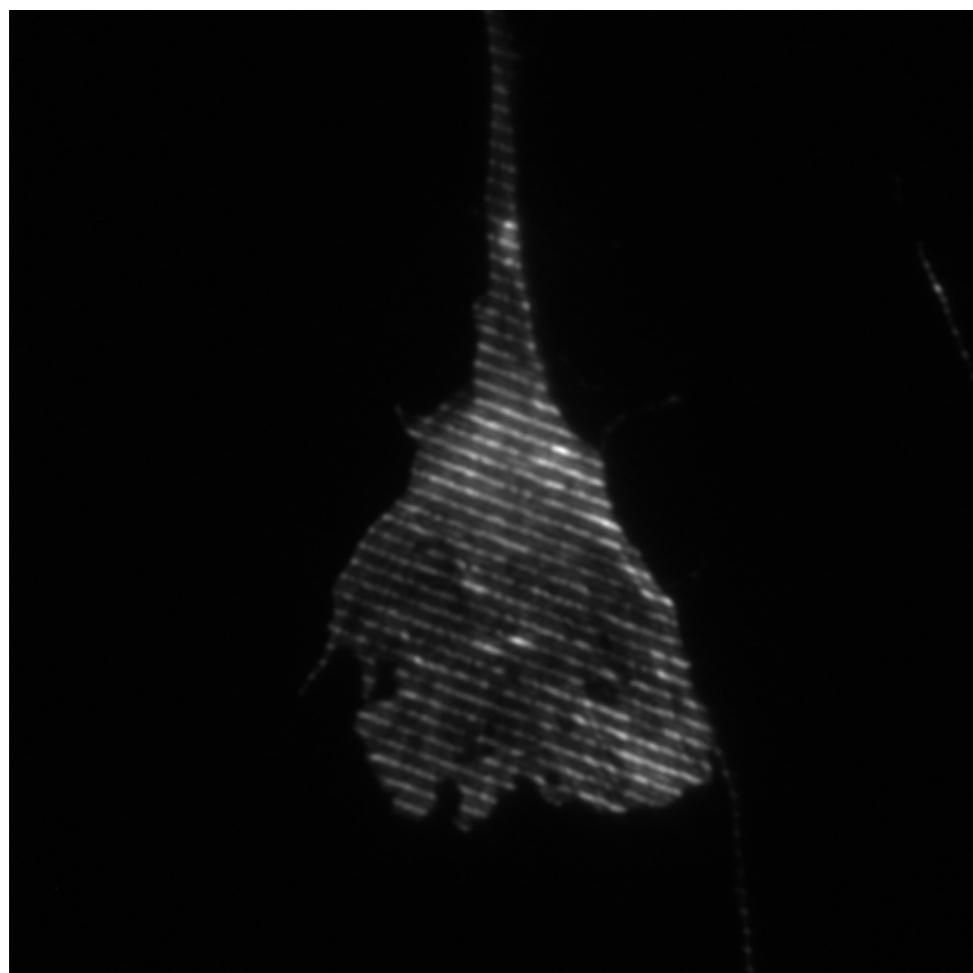
Repeat for 3 grid orientations and 5 phases



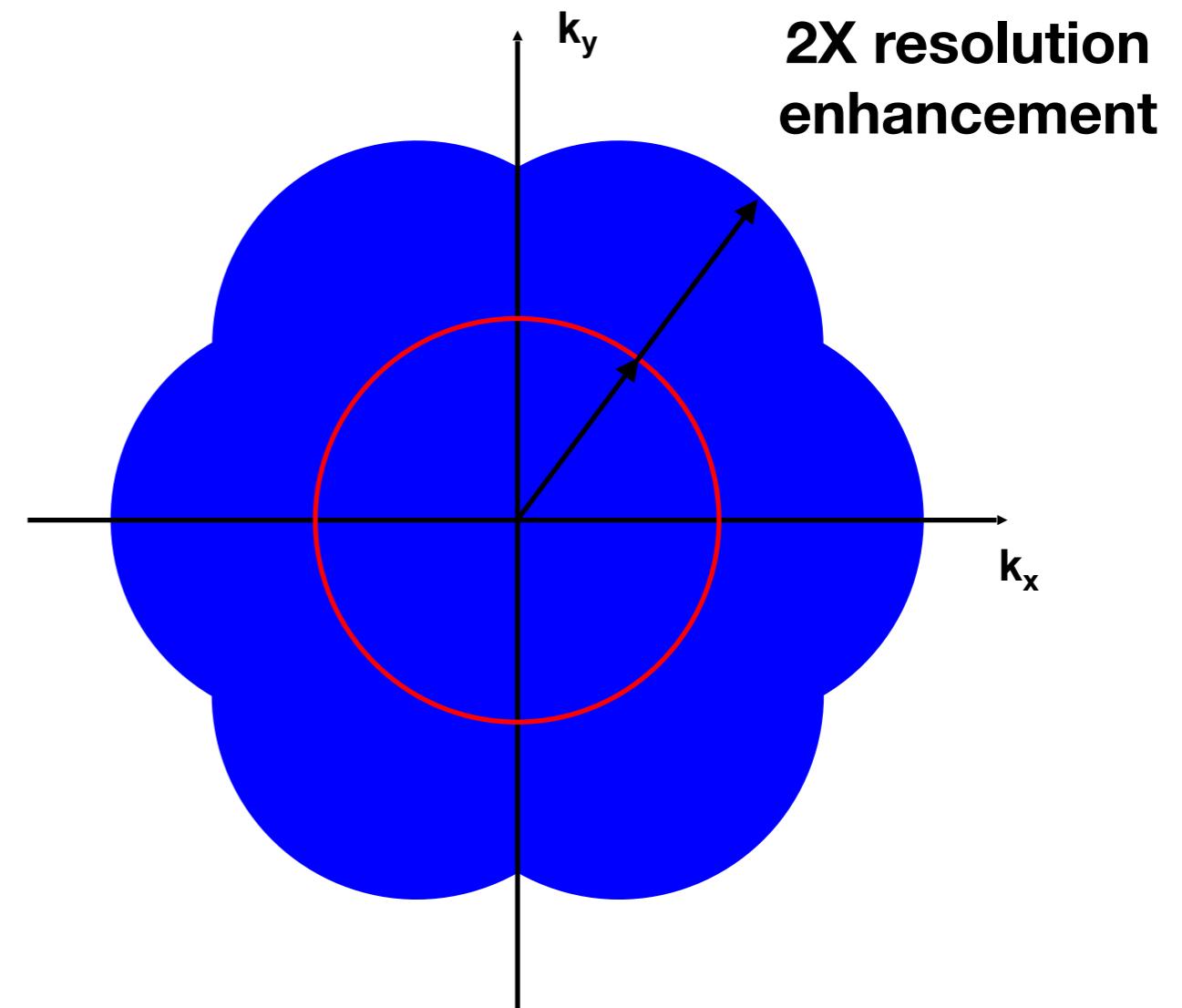
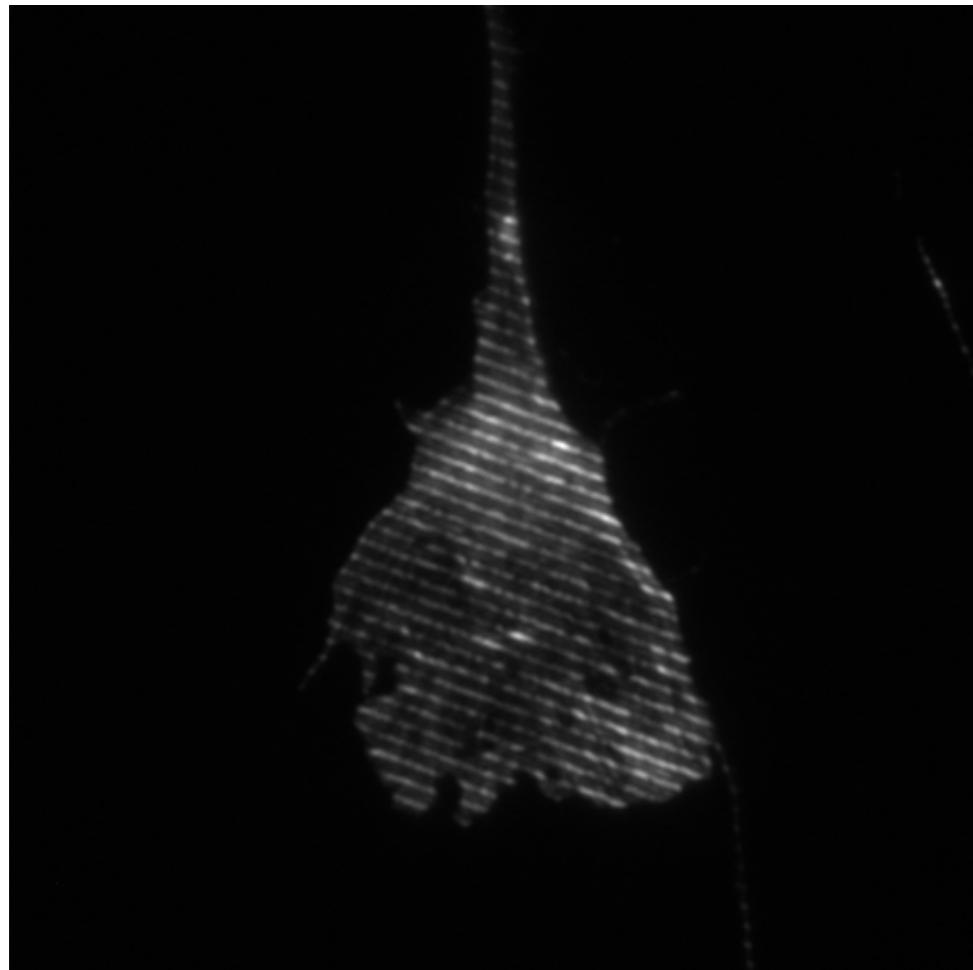
Structured Illumination Microscopy



Reattribution of each modulation



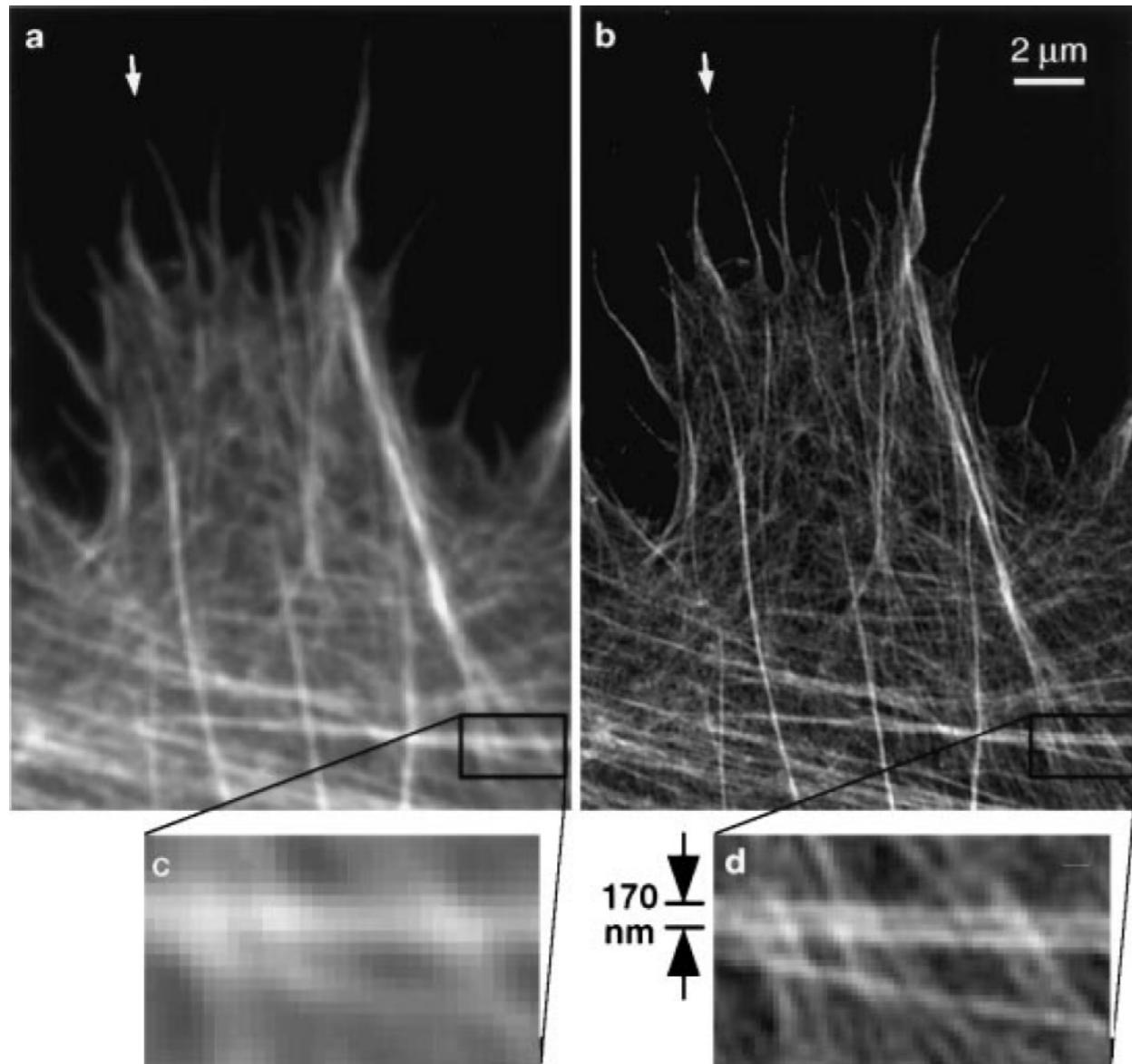
Doubling resolution



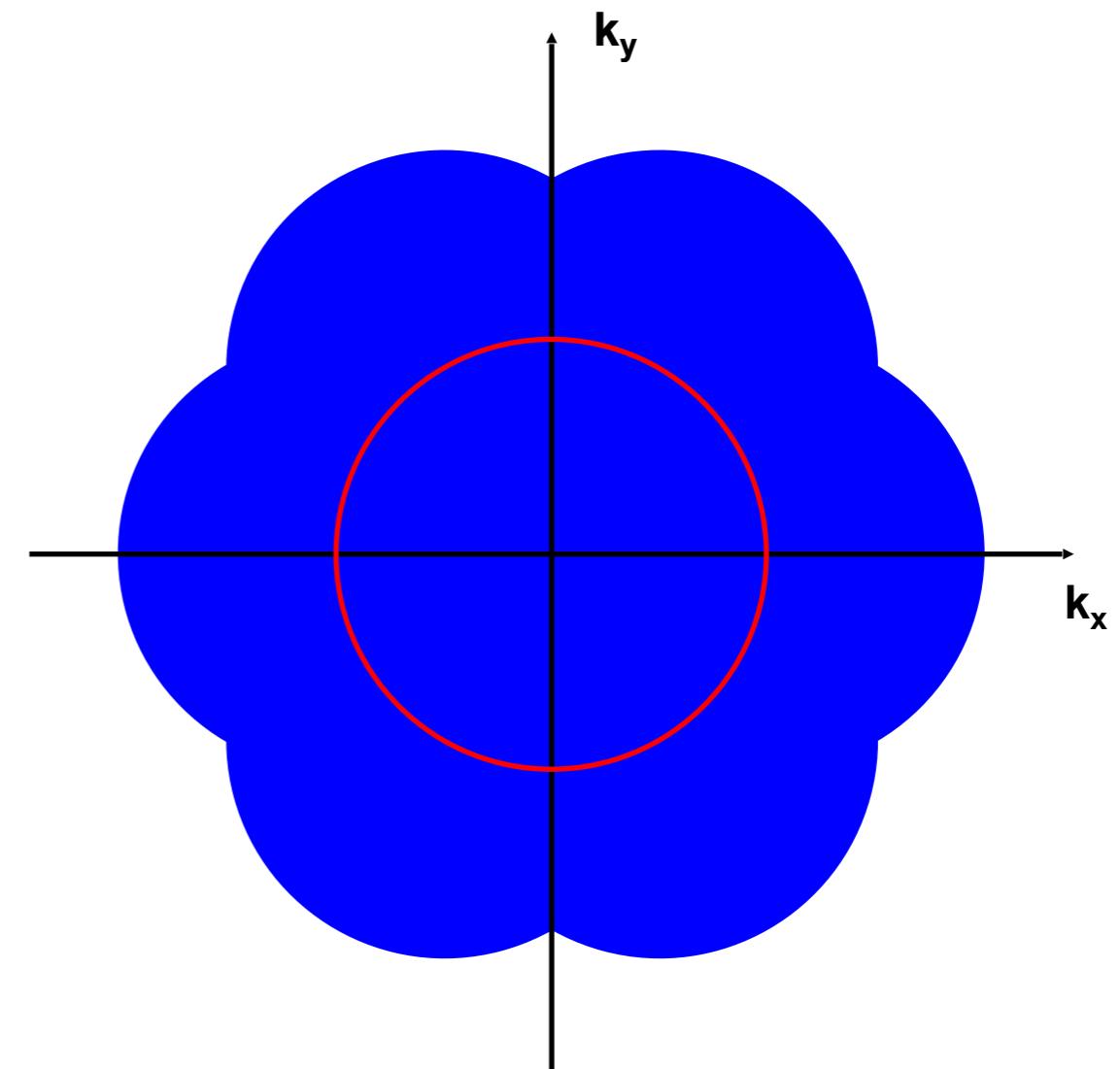
**5 shifts
3 rotations**

→ 15 images to get a **2X resolution enhancement**

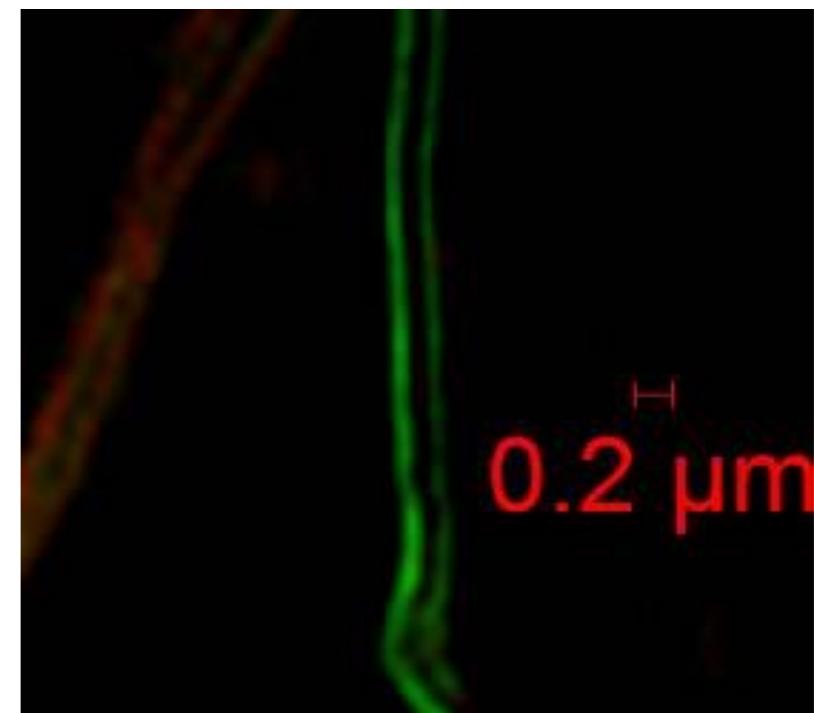
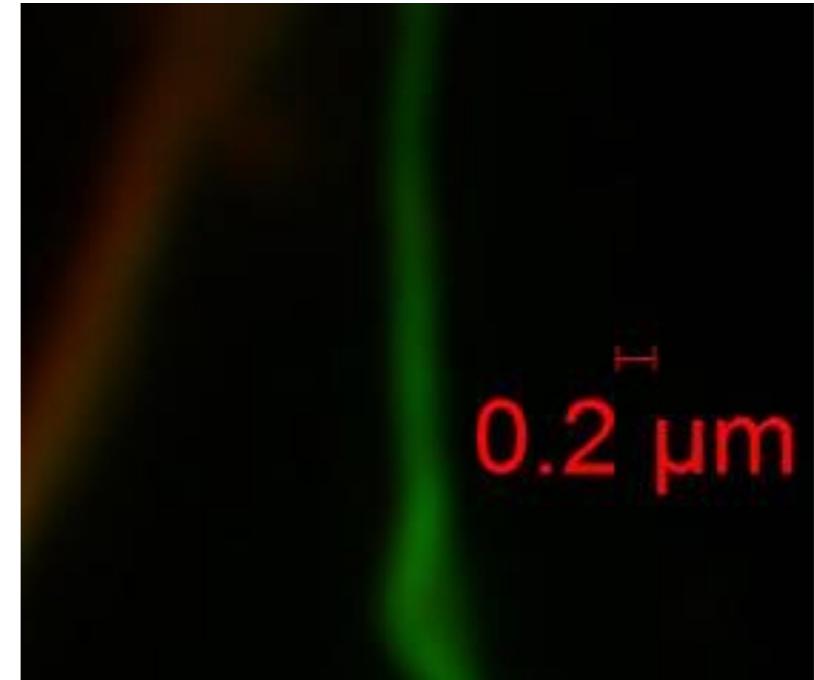
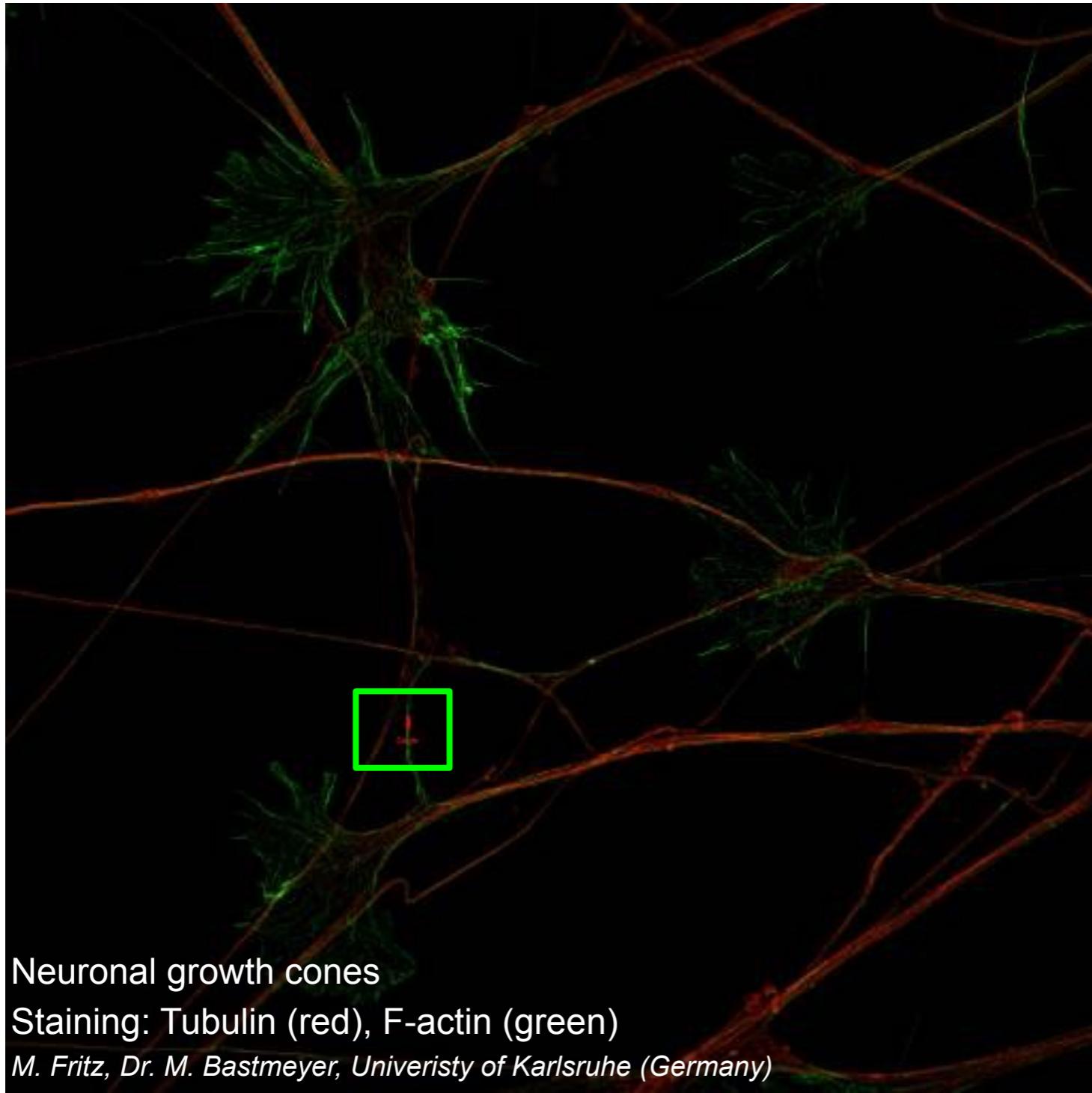
Structured Illumination Microscopy



Lateral resolution enhancement

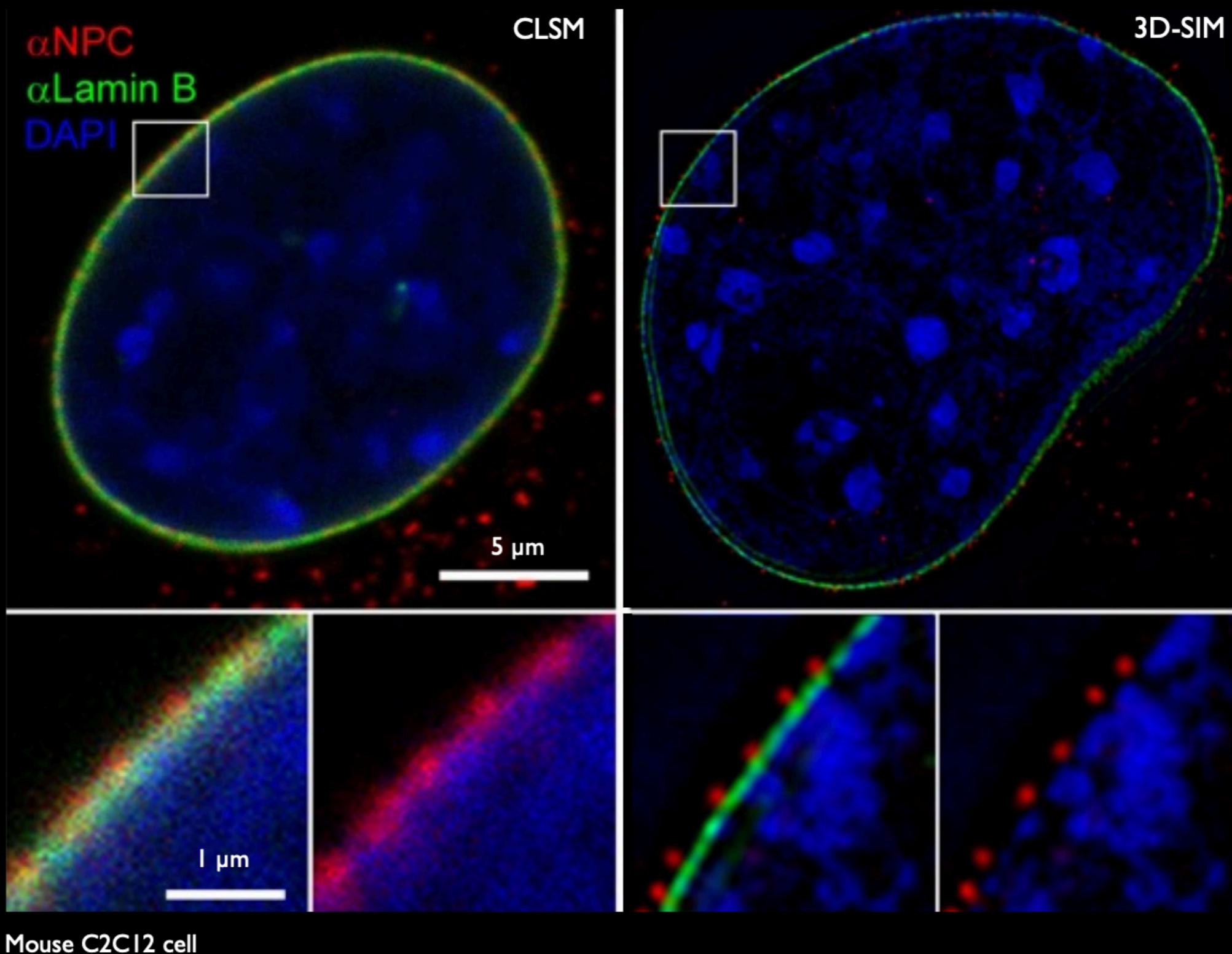


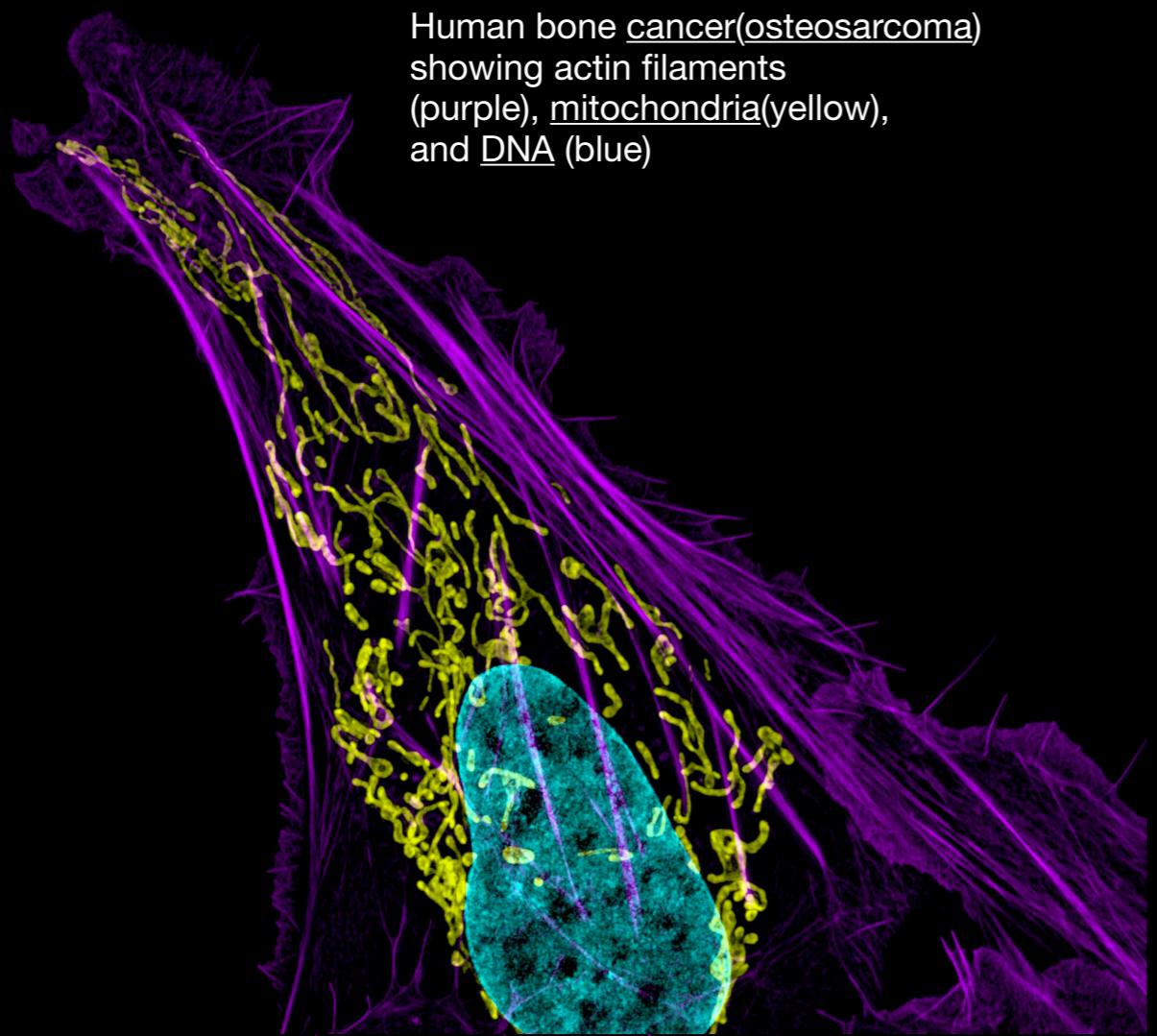
Example



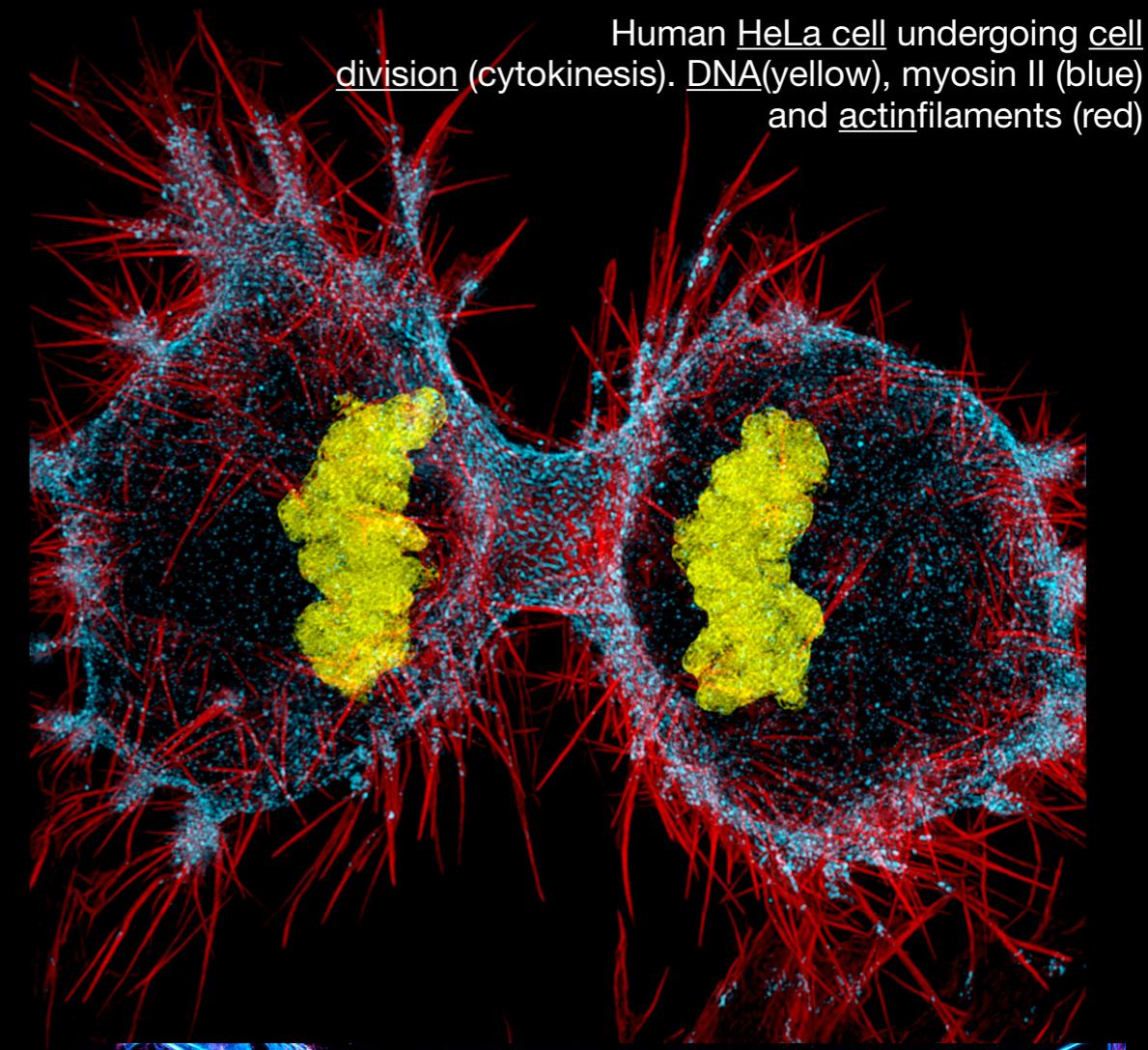
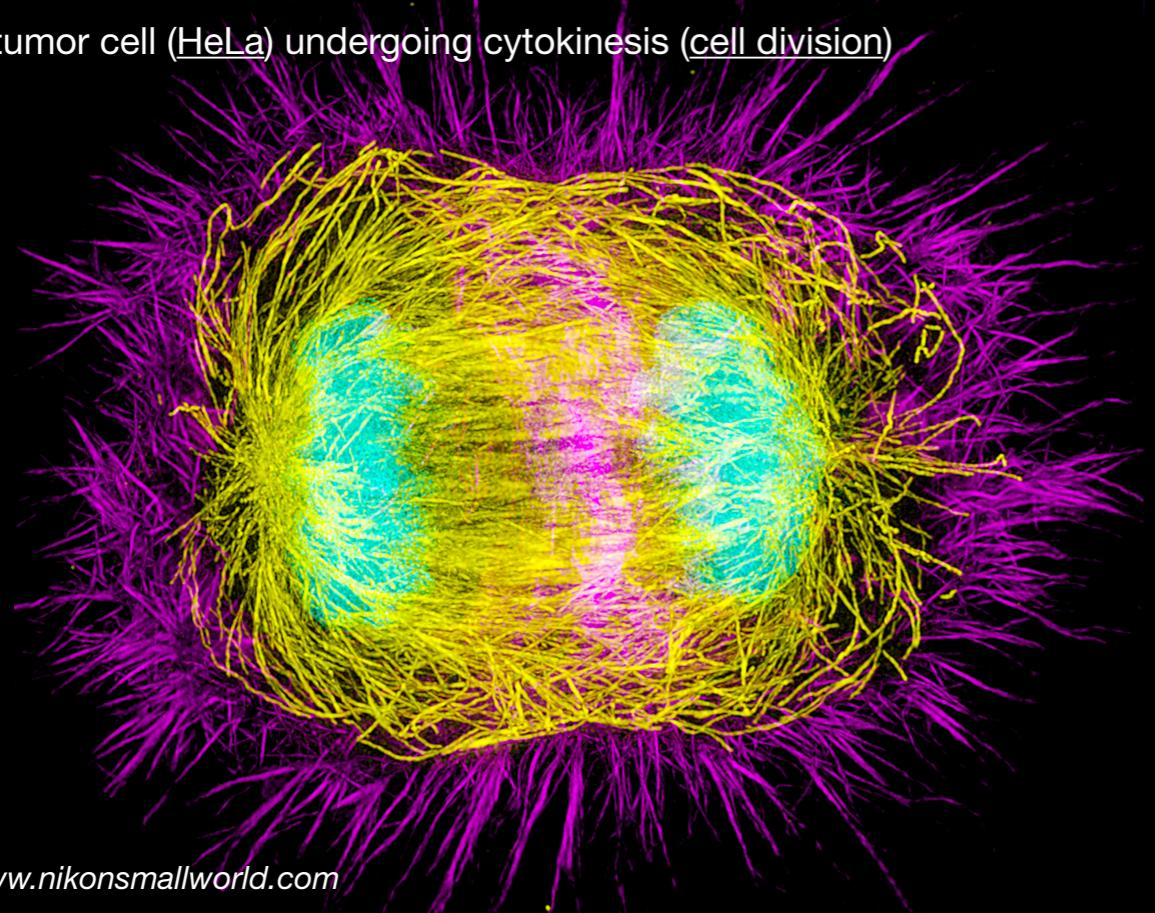
Fluorophore requirements

3D-SIM resolves chromatin domains and interchromatin channels, leading towards nuclear pores

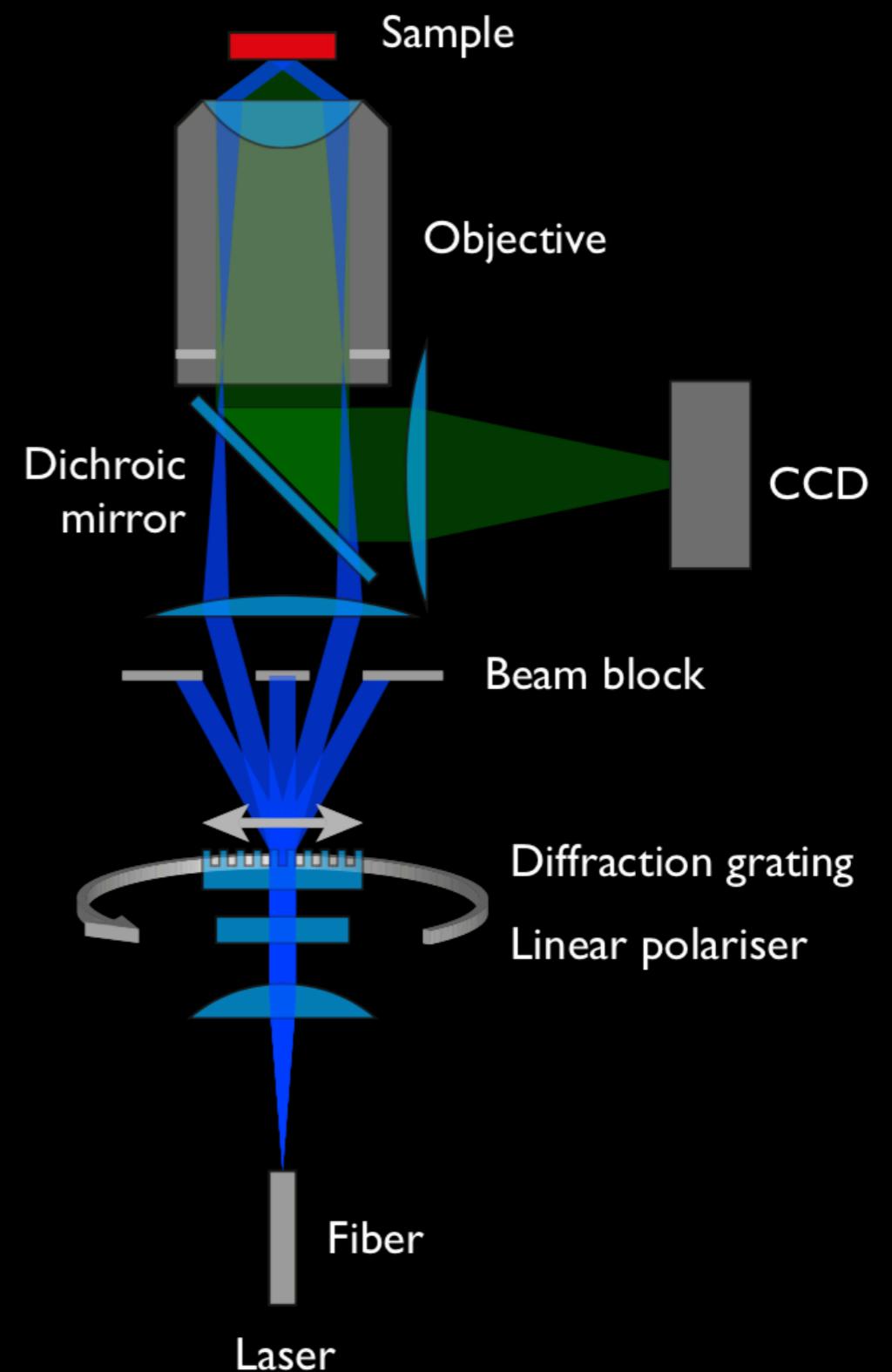




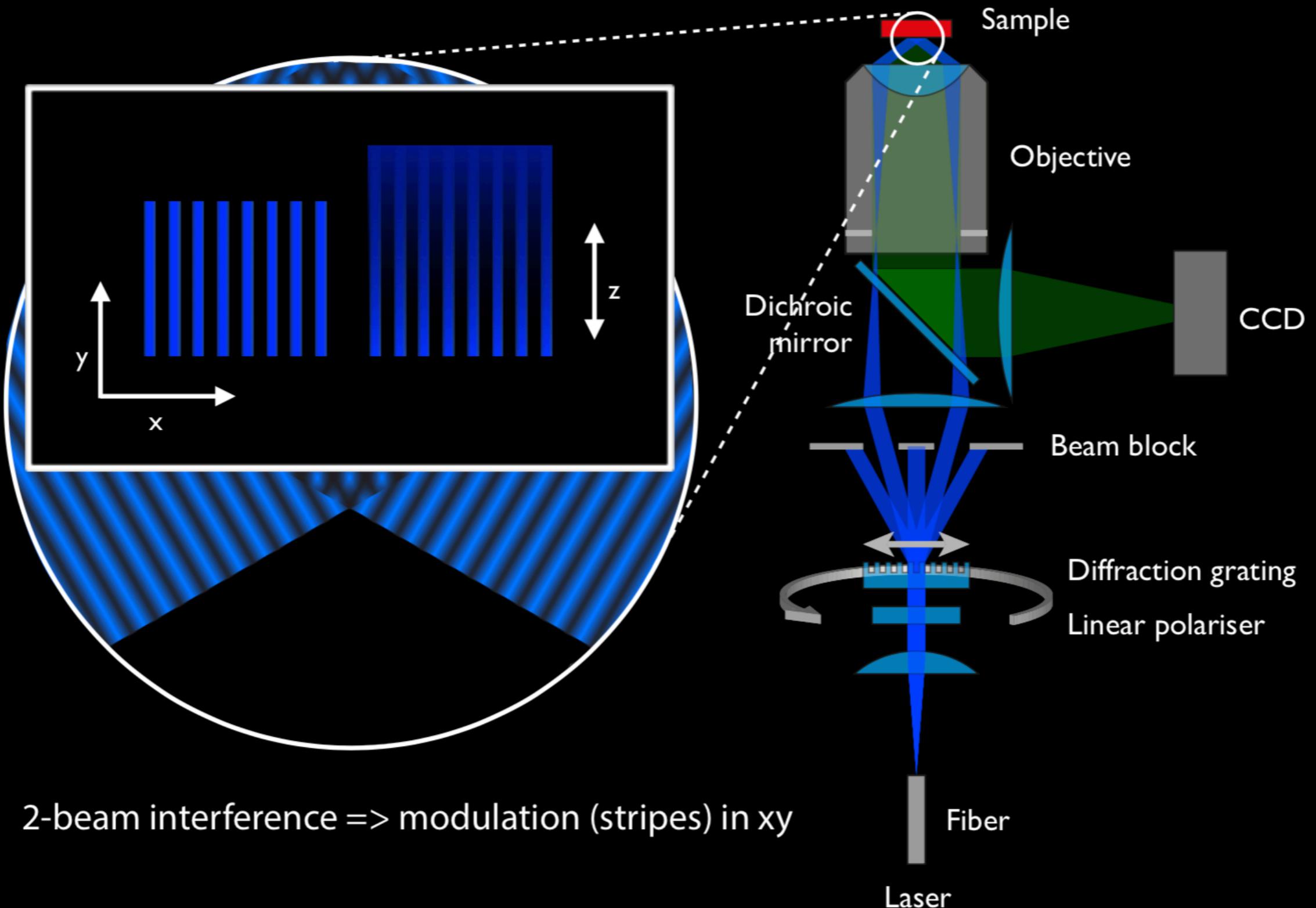
Human tumor cell (HeLa) undergoing cytokinesis (cell division)



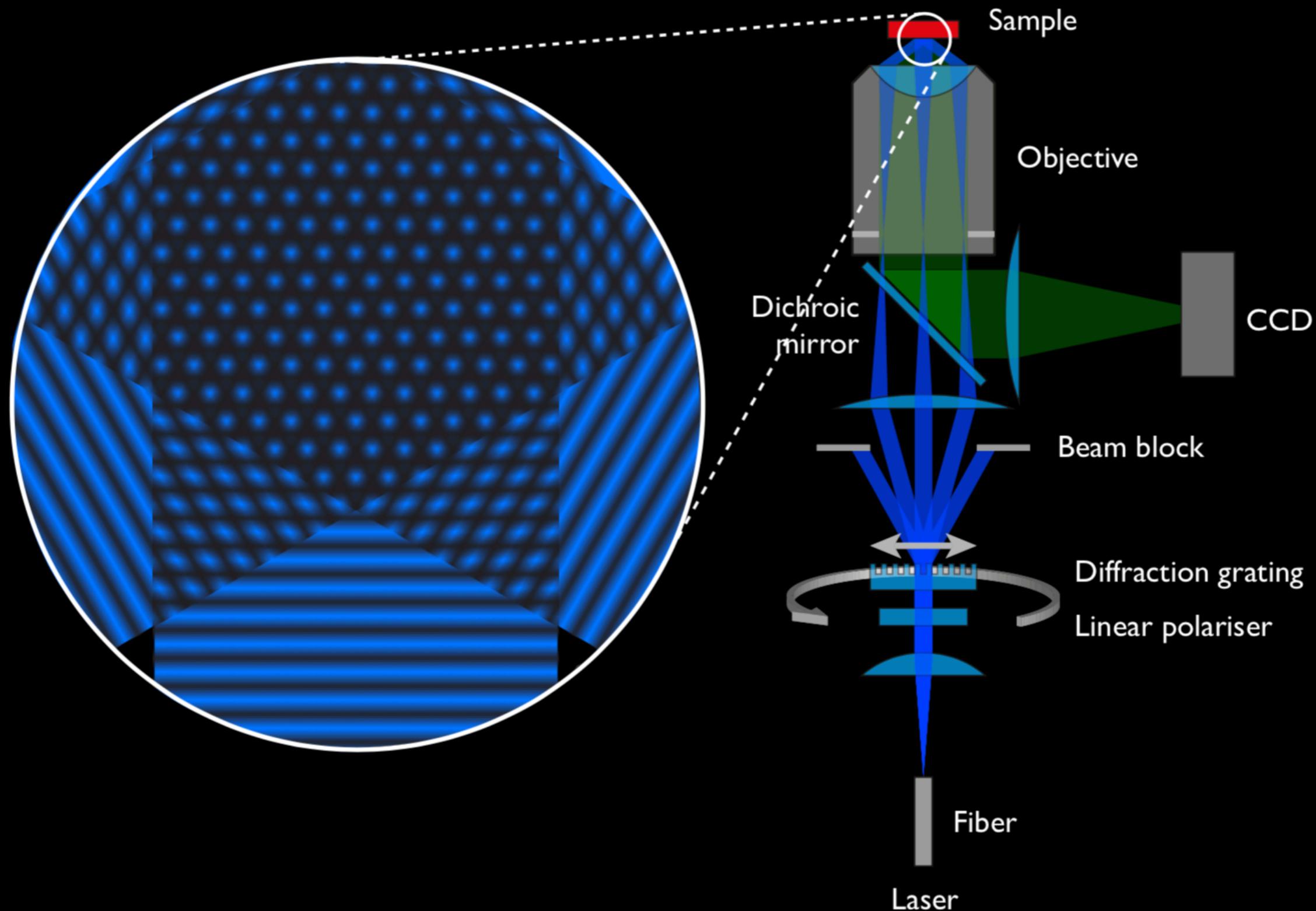
2D-SIM: microscope design



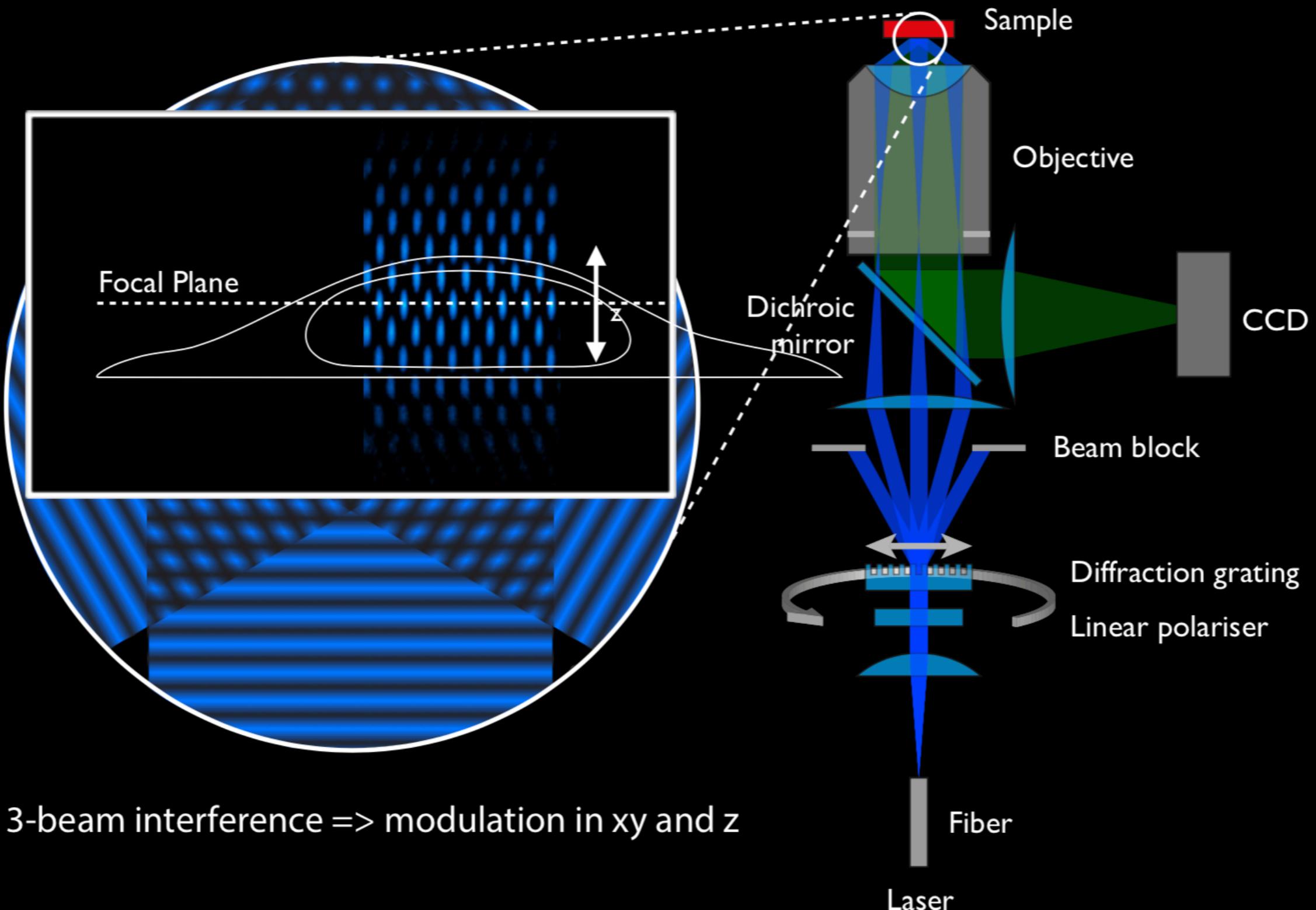
2D-SIM: microscope design



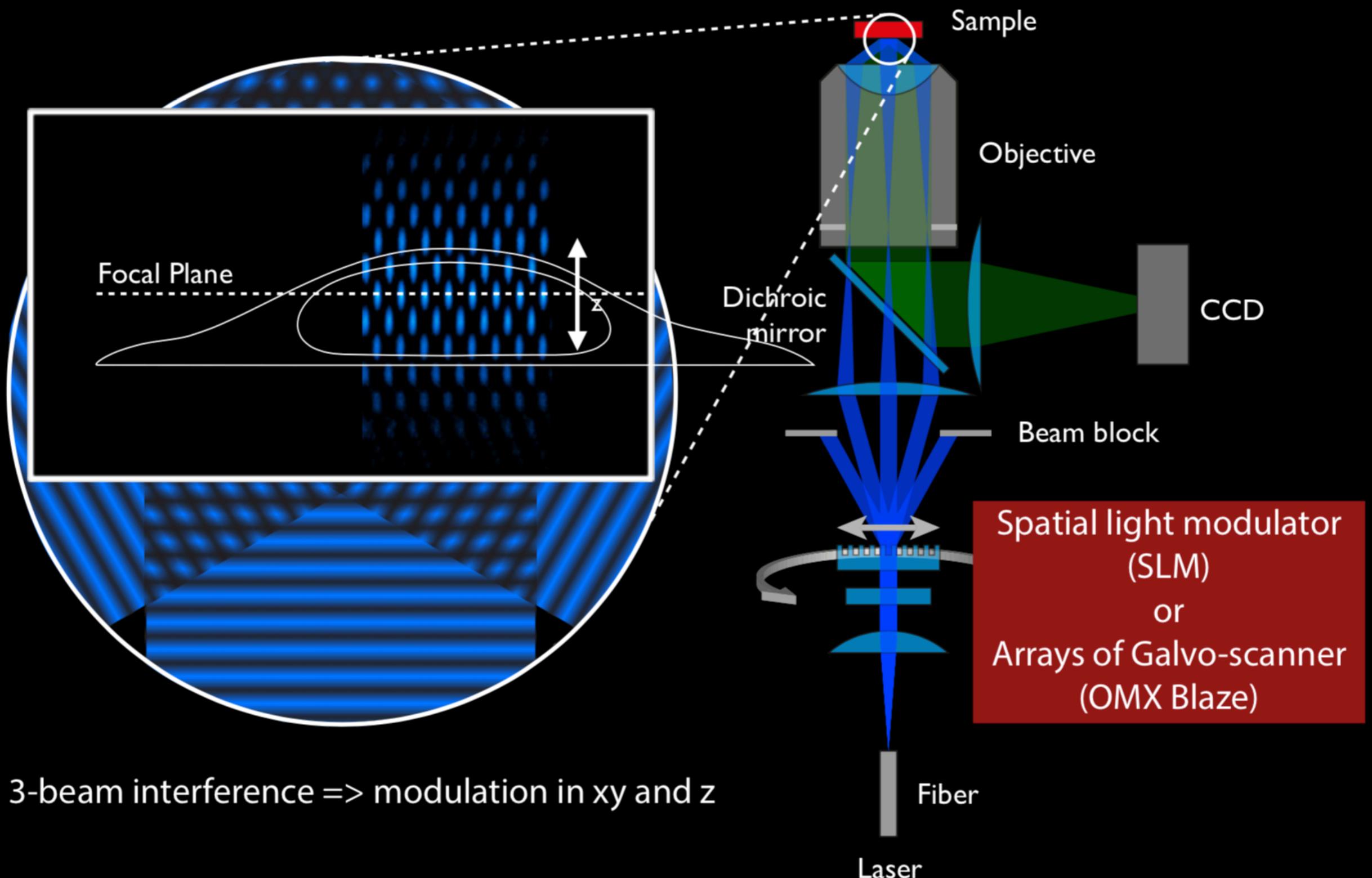
3D-SIM: microscope design



3D-SIM: microscope design



3D-SIM: microscope design

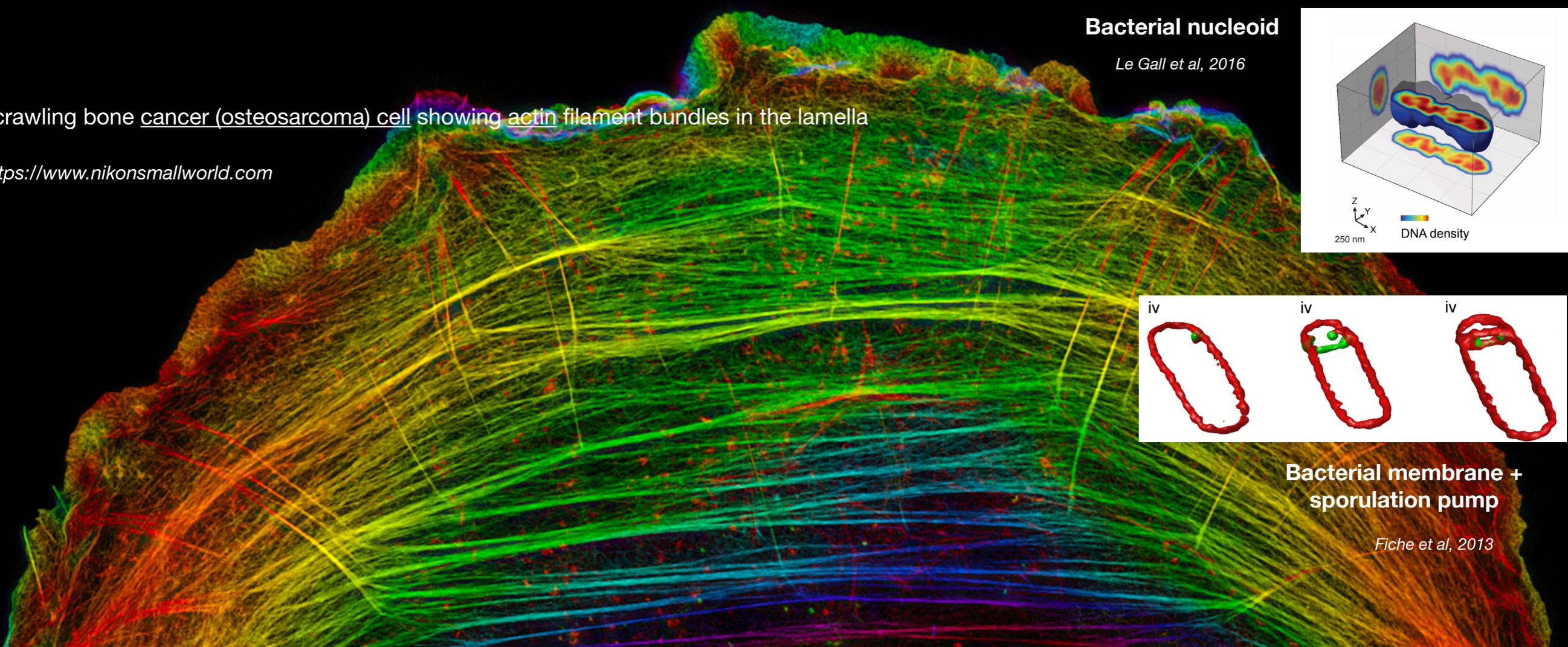


3-beam interference => modulation in xy and z

3D

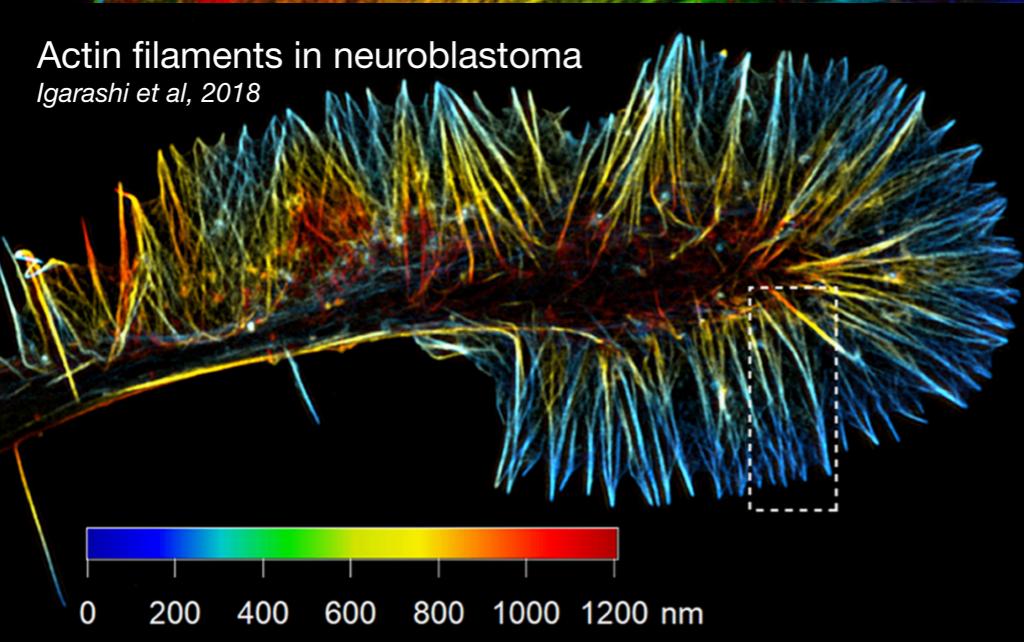
A crawling bone cancer (osteosarcoma) cell showing actin filament bundles in the lamella

<https://www.nikonsmallworld.com>



Actin filaments in neuroblastoma

Igarashi et al, 2018



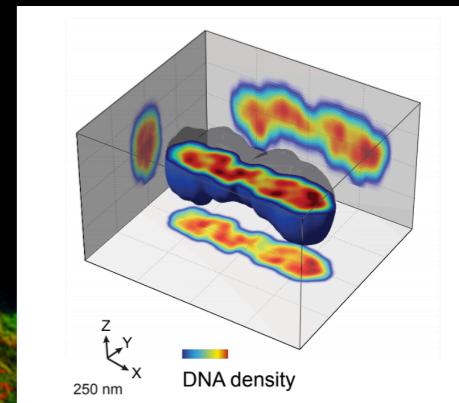
0 200 400 600 800 1000 1200 nm

→ 2X resolution
enhancement in Z

→ Don't forget Nyquist sampling!

Bacterial nucleoid

Le Gall et al, 2016

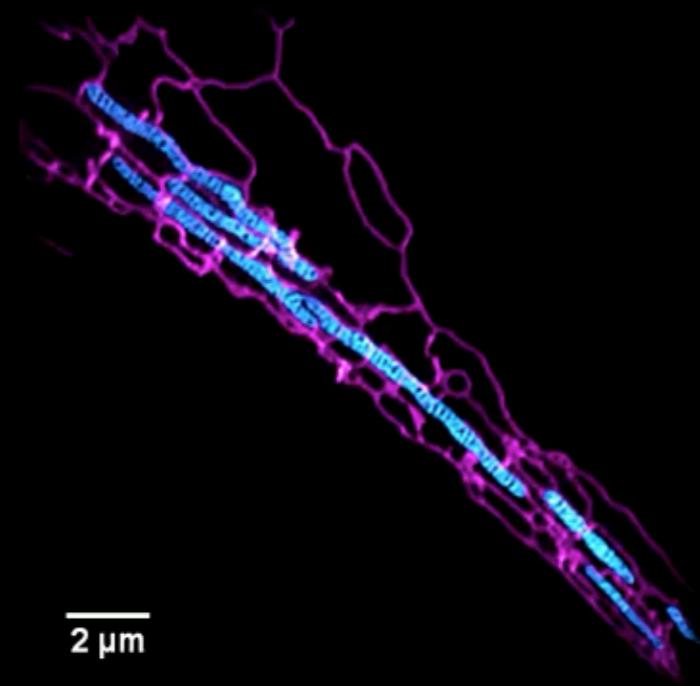


Bacterial membrane +
sporulation pump

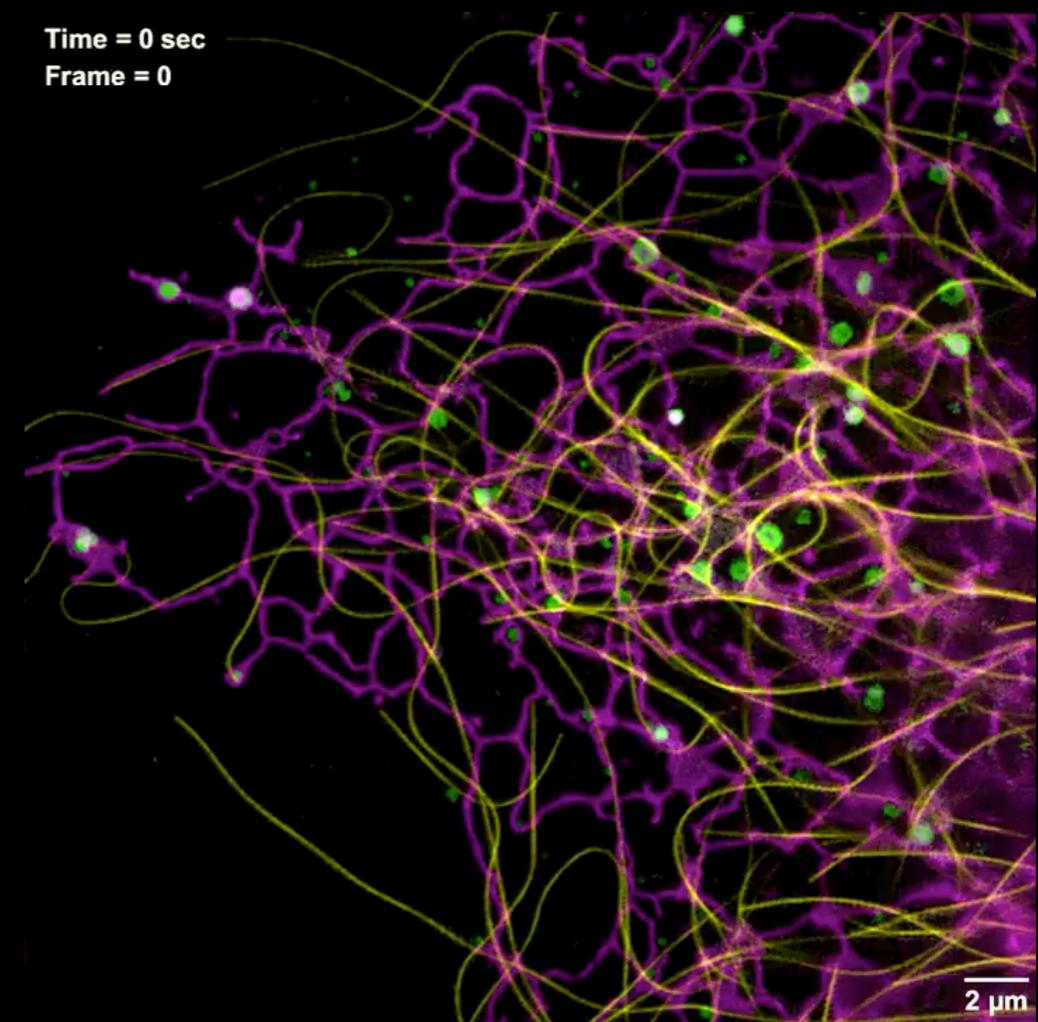
Fiche et al, 2013

Multicolor live imaging

COS7 labelled with endoplasmic reticulum (ER)-mcherry and MitoTracker Green



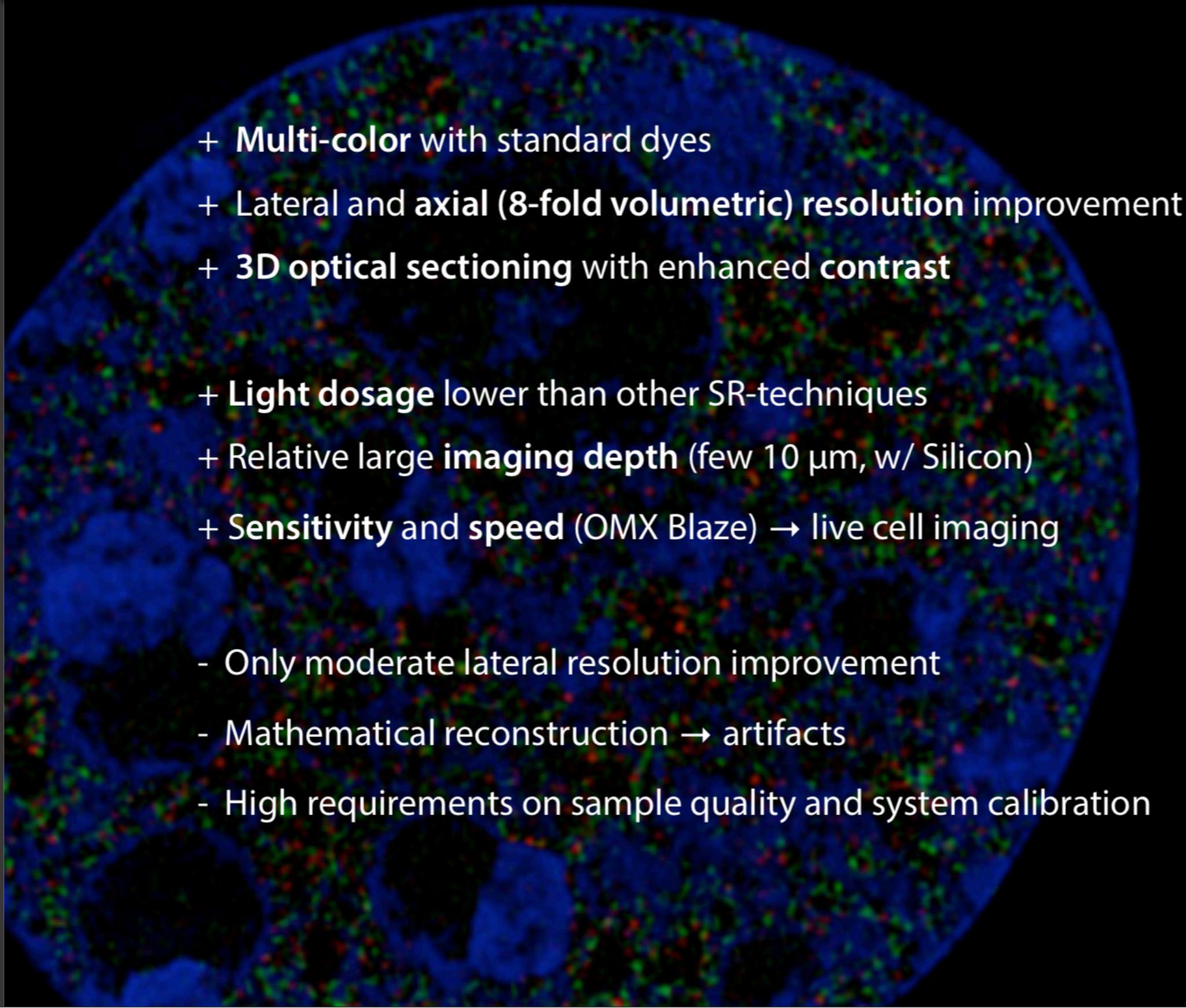
ER, with microtubules and lysosomes



Huang et al, *Nat Biotech*, 2018

Guo et al, *Cell*, 2018

Summary

- 
- + Multi-color with standard dyes
 - + Lateral and **axial (8-fold volumetric) resolution improvement**
 - + **3D optical sectioning** with enhanced **contrast**
 - + Light dosage lower than other SR-techniques
 - + Relative large **imaging depth** (few 10 µm, w/ Silicon)
 - + **Sensitivity** and **speed** (OMX Blaze) → live cell imaging
 - Only moderate lateral resolution improvement
 - Mathematical reconstruction → artifacts
 - High requirements on sample quality and system calibration

Context

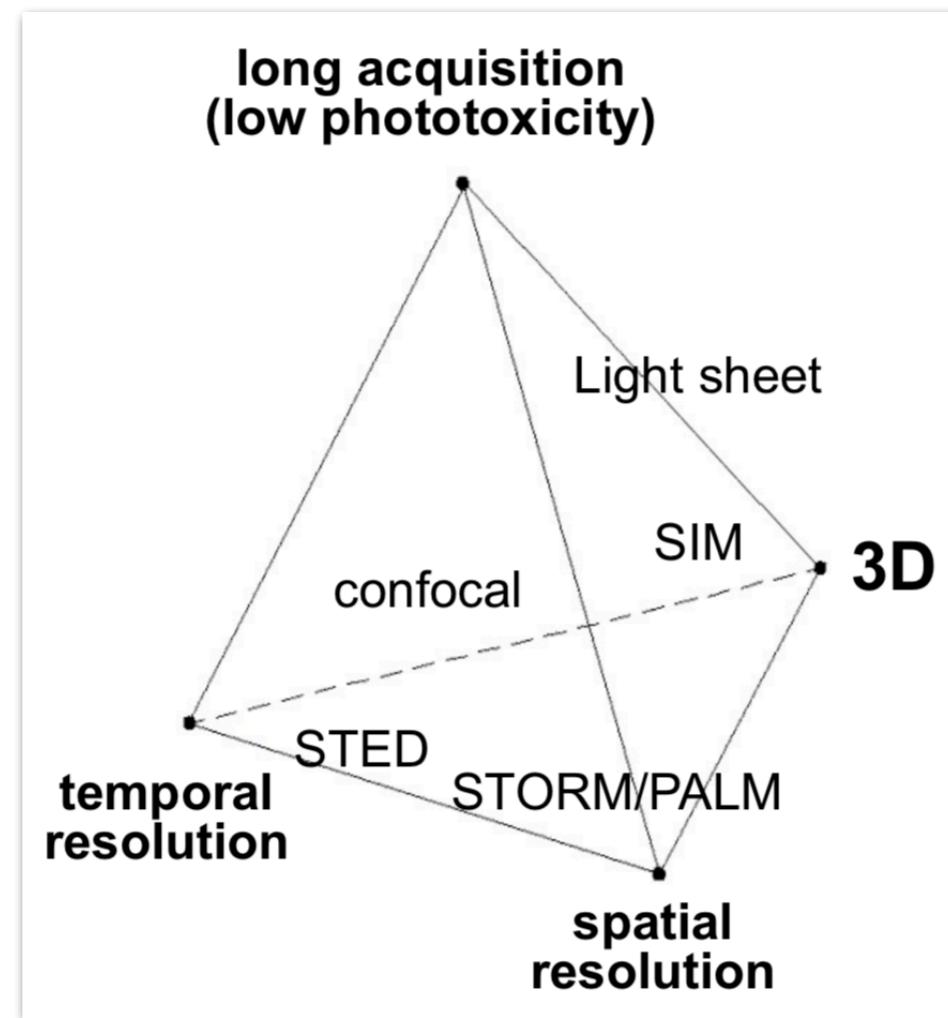
Versatility

Challenges

Comparison of methods

	Structured Illumination Microscopy (SIM)	Stimulated Emission Depletion Microscopy (STED)	Photo-Activated Localization Microscopy (PALM) and Stochastic Optical Reconstruction Microscopy (STORM)
Principle	Uses interference-generated light patterns to create a Moire effect from which higher-resolution information can be extracted	Reduces the effective excitation volume with a depletion laser	Stochastically activates a subset of photoswitchable probes at a time, and then determines the centroid position of each point spread function
Microscopy type	Wide-field	Laser scanning confocal	Wide-field
xy Resolution	100–130 nm	20–70 nm	10–30 nm
Axial resolution	~300 nm	40–150 nm	10–75 nm
Probes	Common photostable organic dyes and fluorescent proteins	Particular photostable organic dyes and fluorescent proteins	PALM: photoswitchable fluorescent proteins STORM: photoswitchable organic dyes
Temporal resolution	Milliseconds to seconds	Milliseconds to seconds	Seconds to minutes
Photodamage	Low to moderate	Moderate to high	Moderate
Photobleaching	Moderate to high	Moderate to high	High for single fluorophores, low overall
Live imaging?	Yes	Yes	Yes
Post-image processing required?	Yes	No	Yes
Maximum number of simultaneous colors	4	3	PALM: 2 STORM: 3
Considerations	Straightforward multicolor experiments and sample preparation. Reconstruction algorithm may cause artifacts	Best temporal resolution at the highest spatial resolution; however maximal in-plane can be at the expense of axial resolution	Highest spatial resolution; however sensitive to labeling density. Crosstalk between fluorophores maybe an issue

Tradeoffs



1. Resolution
2. Speed
3. 3D
4. Phototoxicity
5. Others (complexity, analysis, versatility, etc...)