Workshop

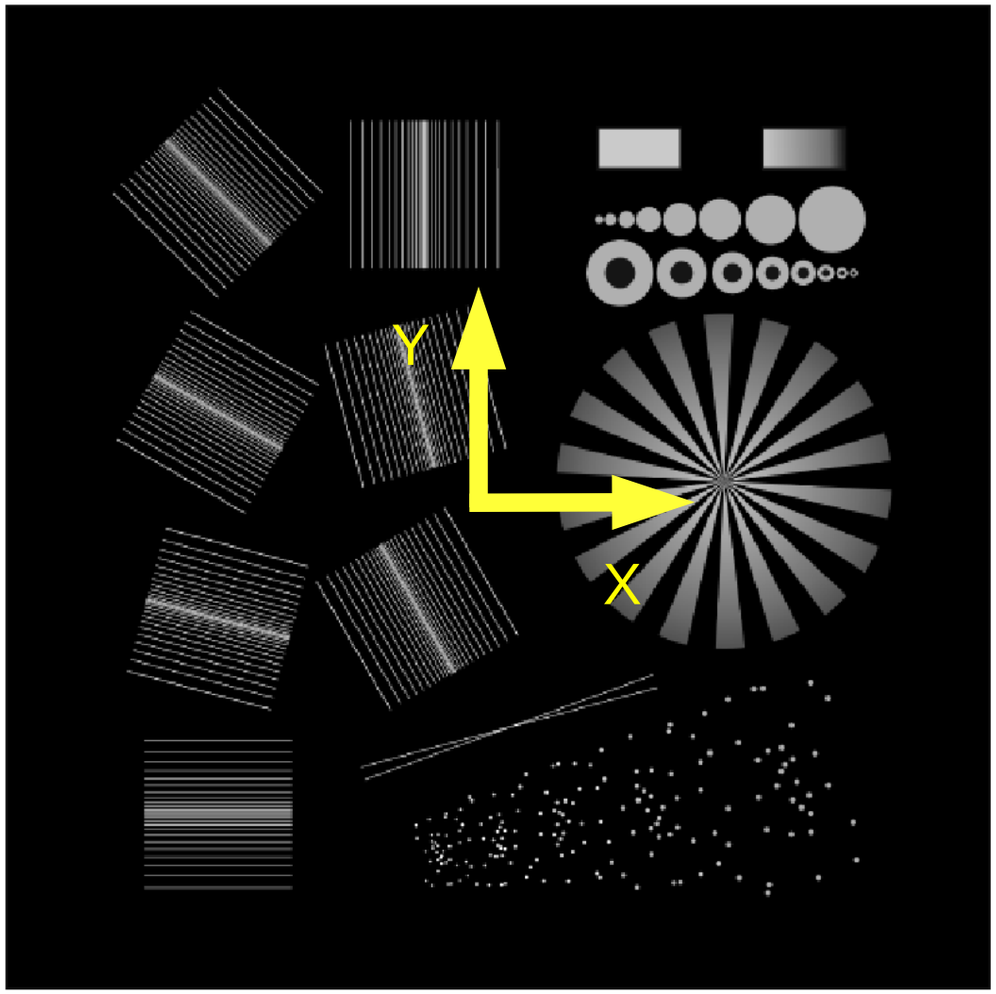
Structured Illumination Microscopy

Super-resolution microscopy based on Fourier

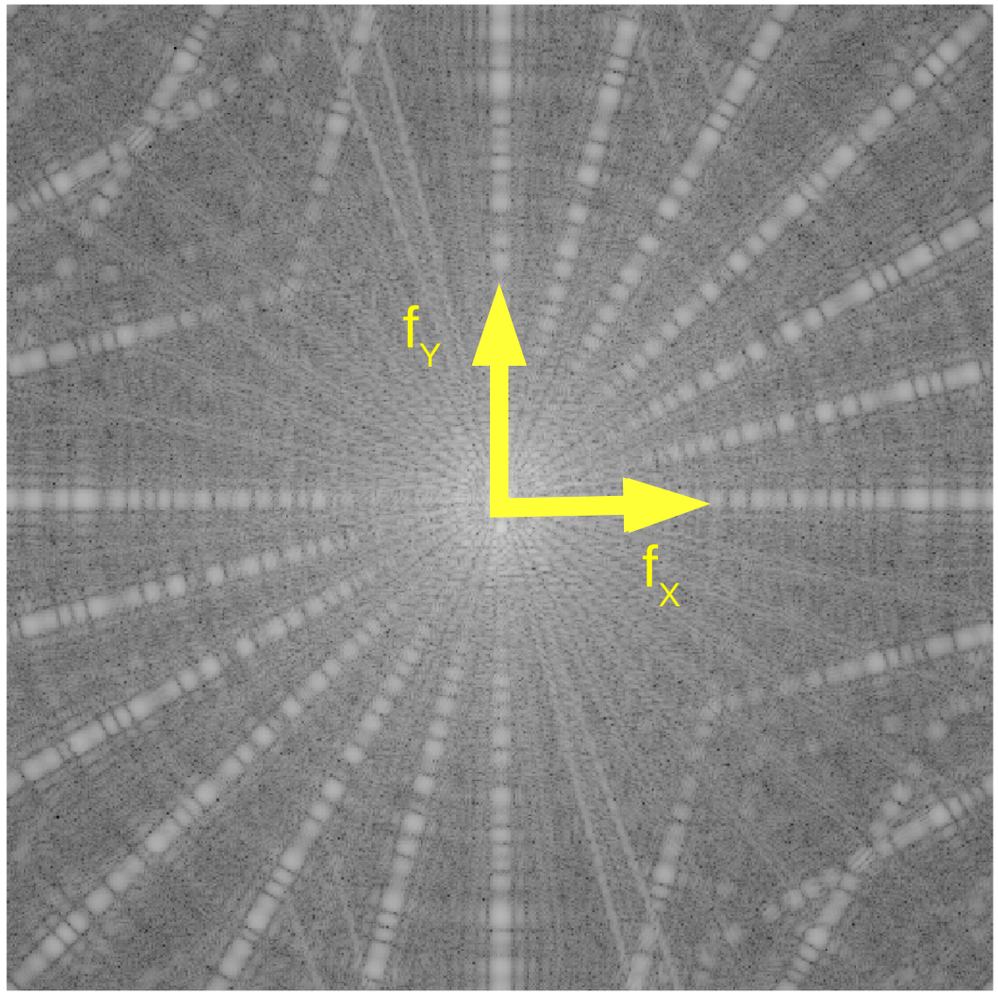
Slides of Emmanuel Soubies, IRIT Toulouse, 2024

Fourier Plane and Fourier Transform

Image



Fourier Transform



$$s(n) = \frac{1}{N} \sum_{k=0}^{N-1} S(k) \mathrm{e}^{2\mathrm{i}\pi n \frac{k}{N}}$$

Fourier Plane and Fourier Transform

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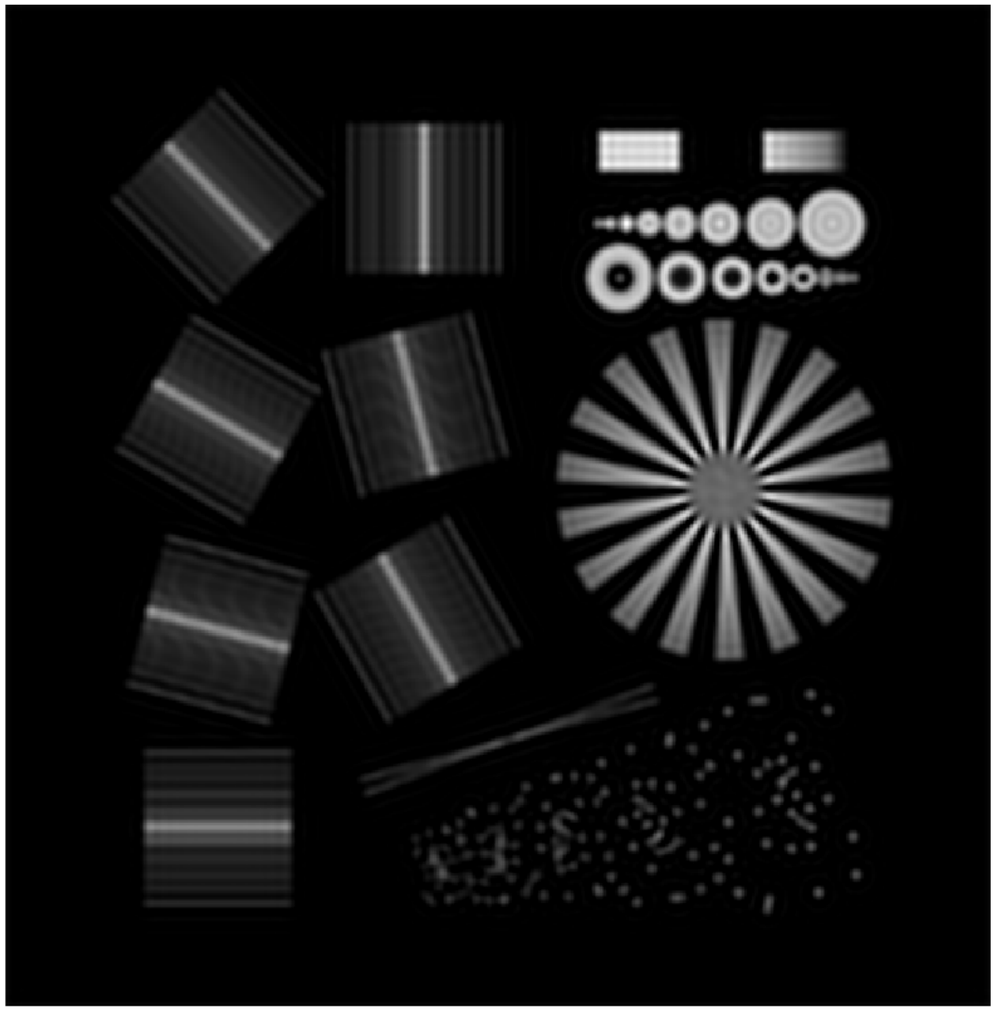

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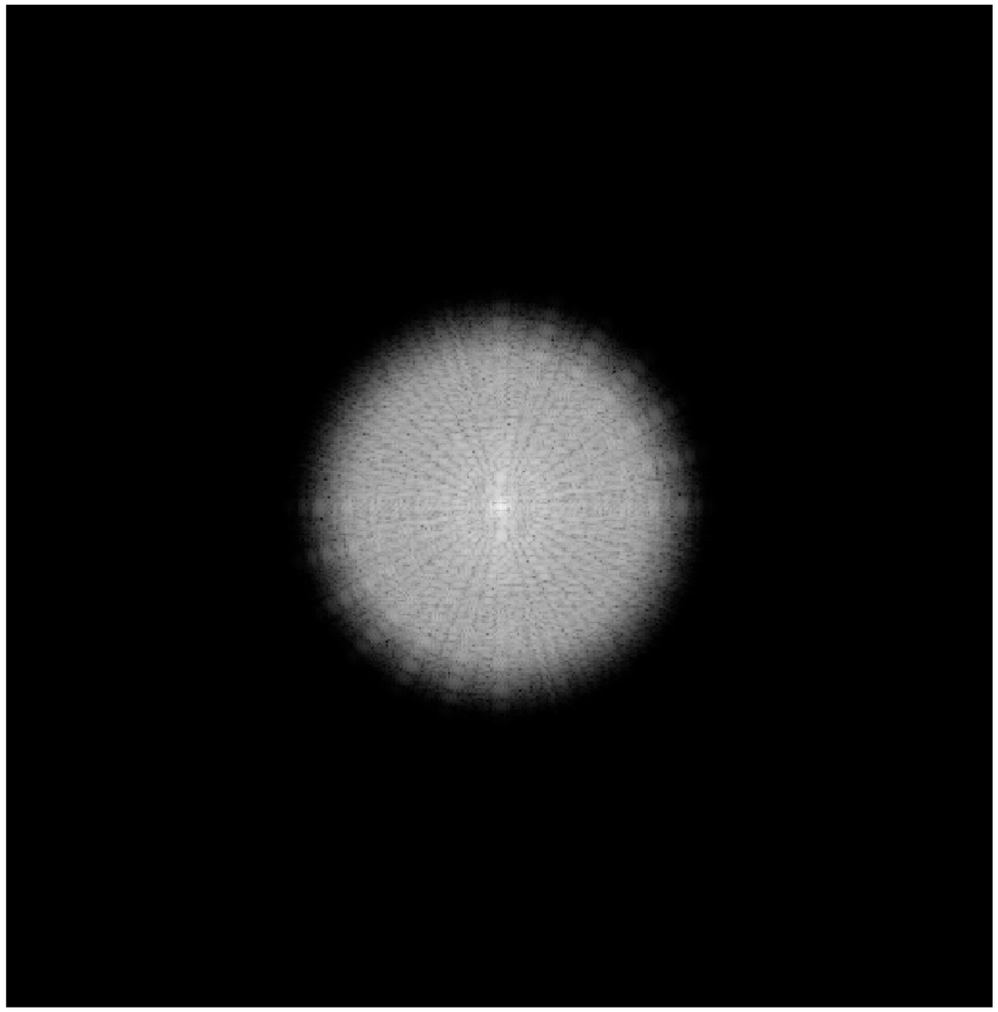

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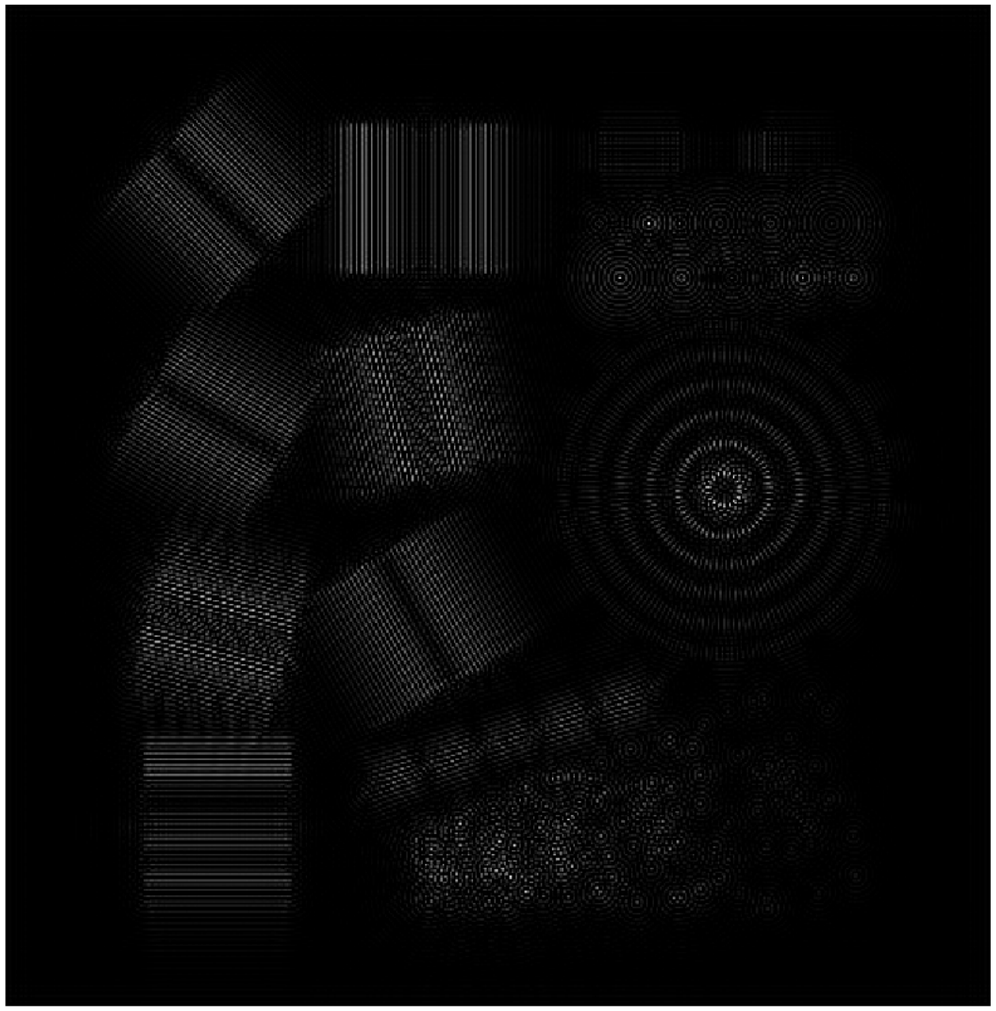
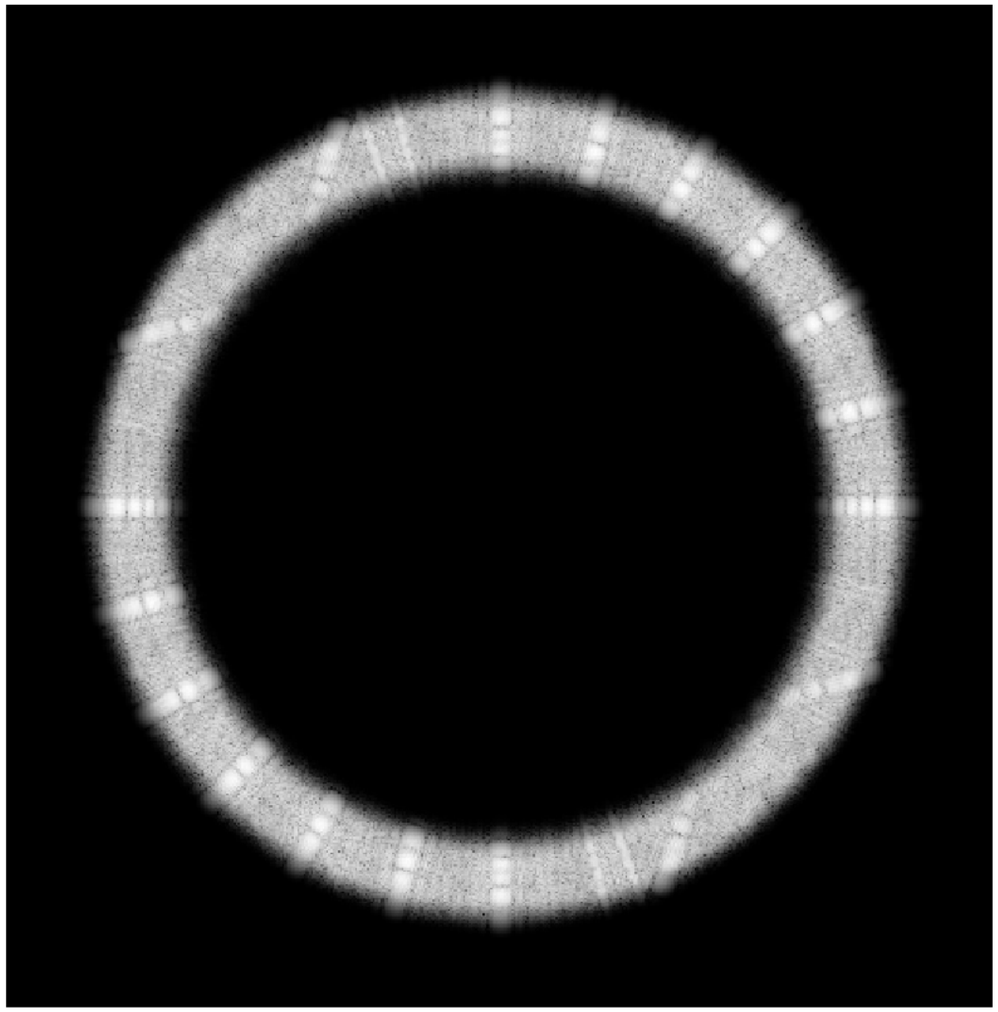


Fourier Transform



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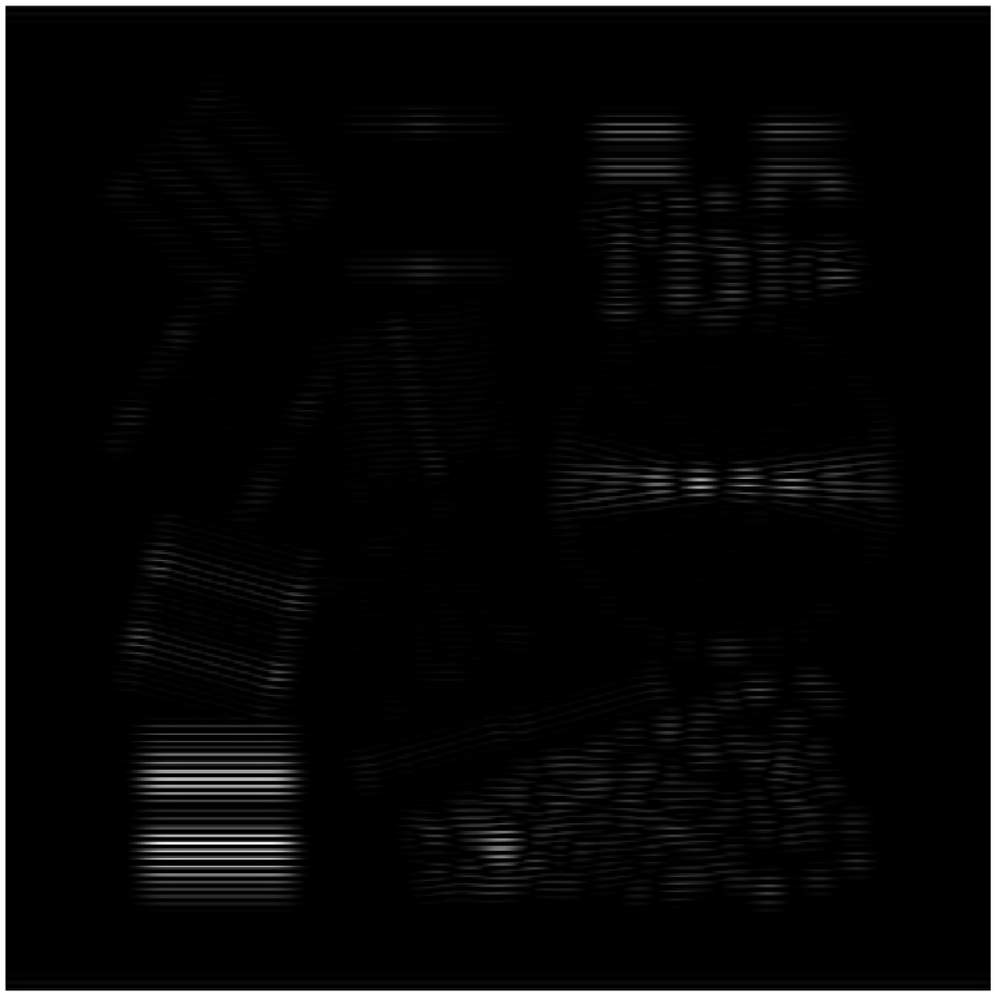
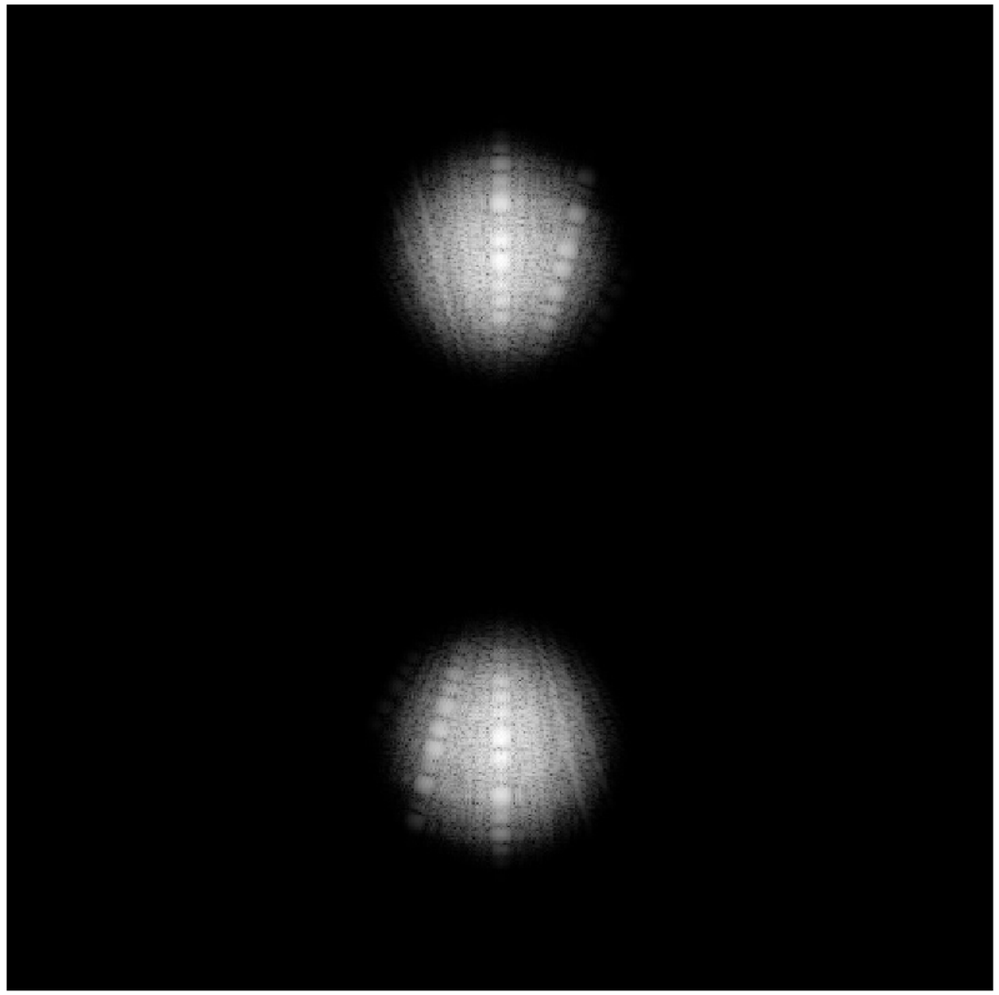


Fourier Transform

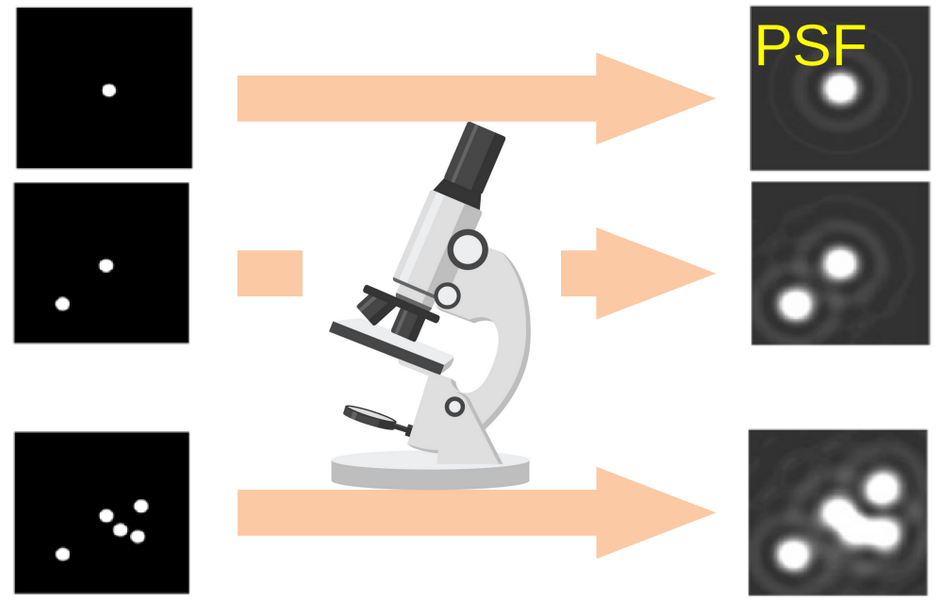


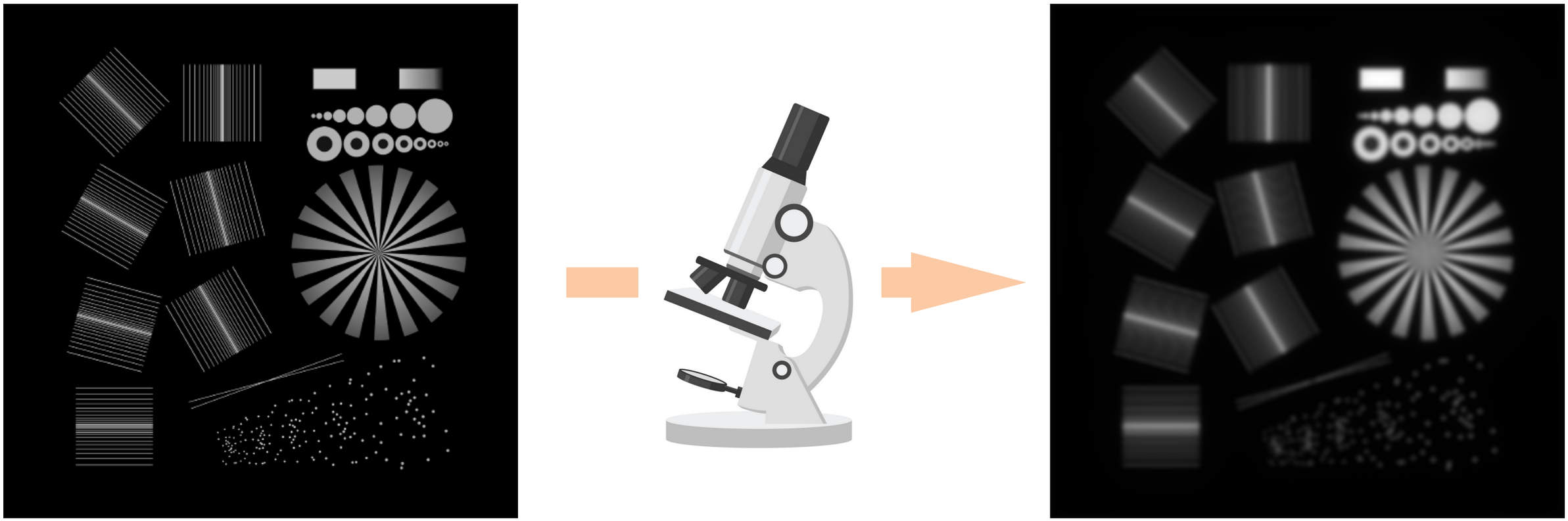
Resolution Limit in Microscopy

Diffraction Phenomenon

- Limited physical aperture of optical components
- Restrict the spatial extent of the wavefront

The system response to a source point is a blurry spot





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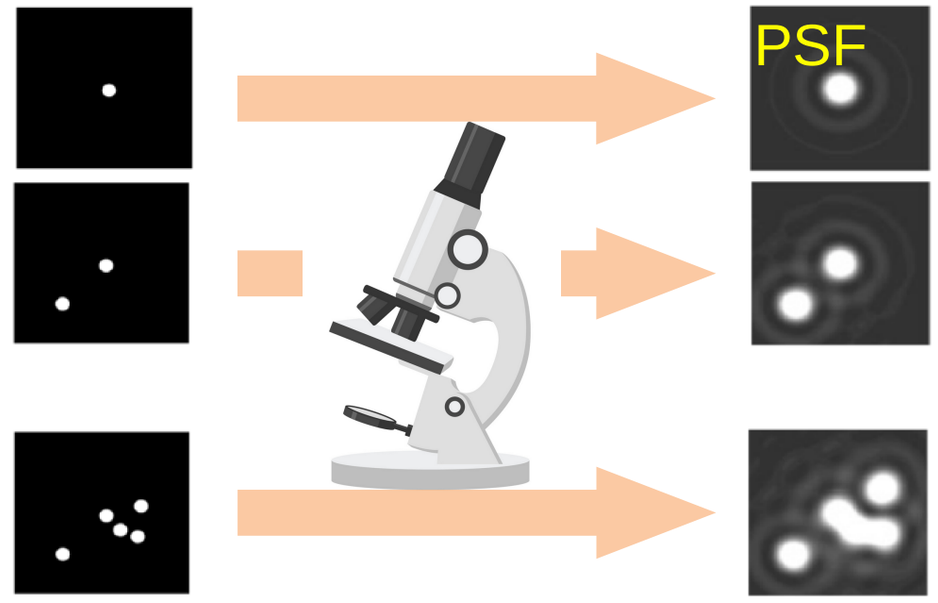
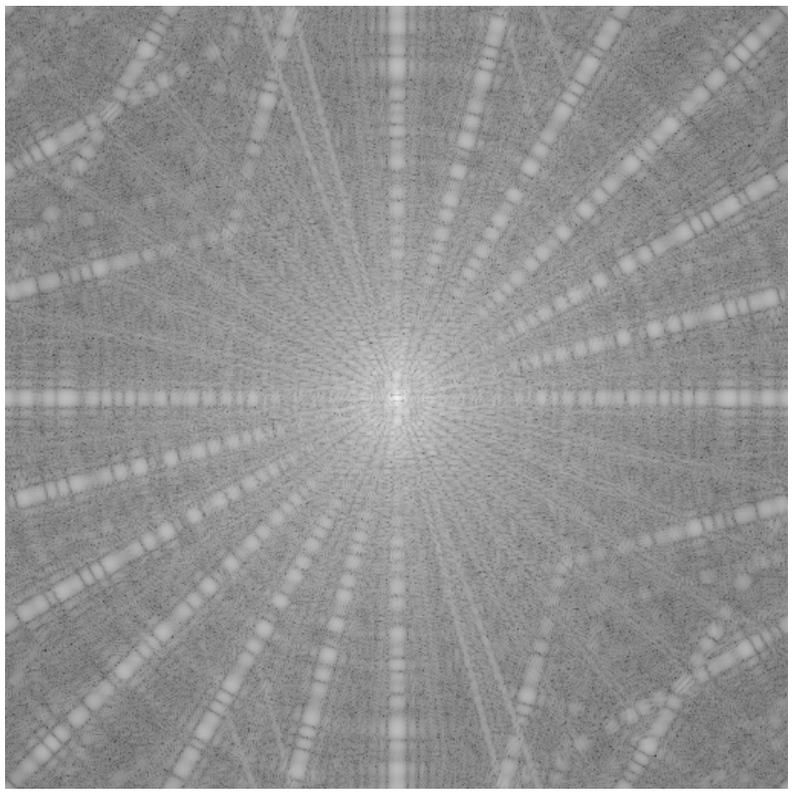
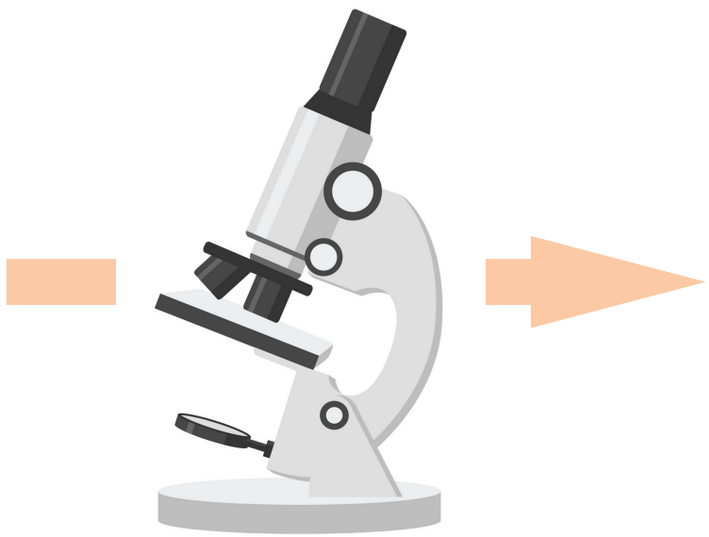
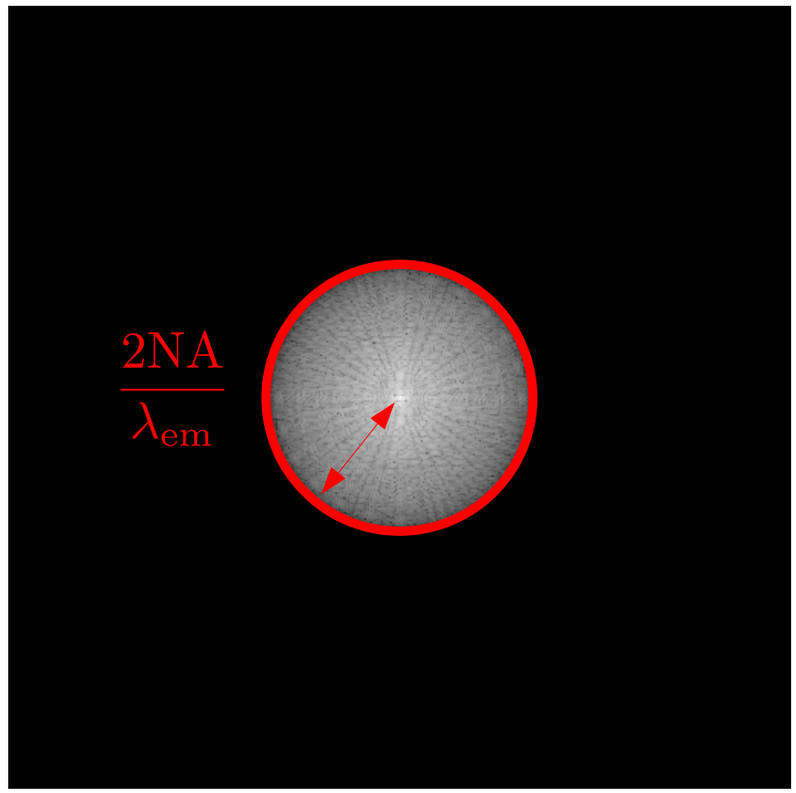




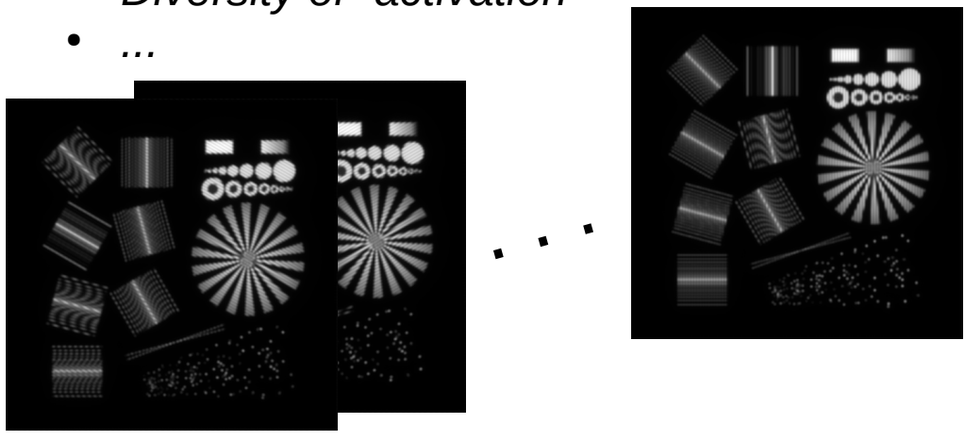




Resolution Limit in Microscopy

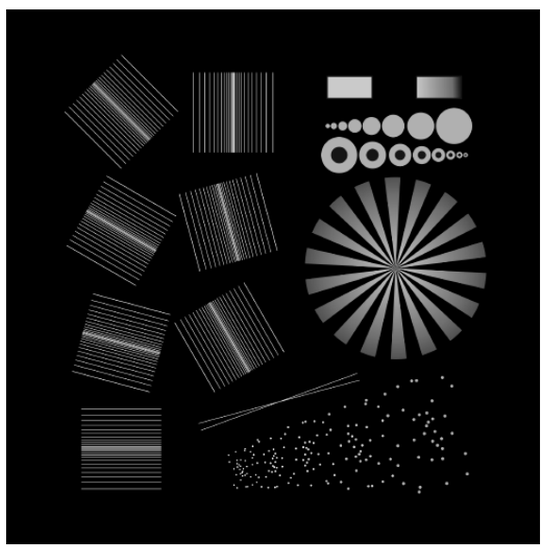
1) Acquire a set of diffraction limited images

- *Diversity of illuminations*
- *Diversity of orientations*
- *Diversity of “activation”*
- *...*



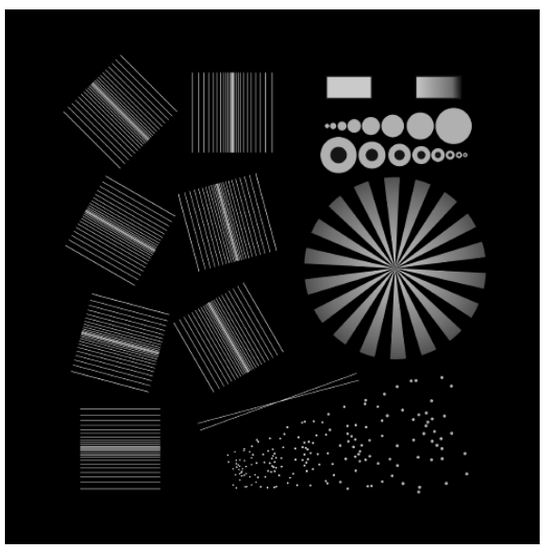
2) Numerical reconstruction

Sample



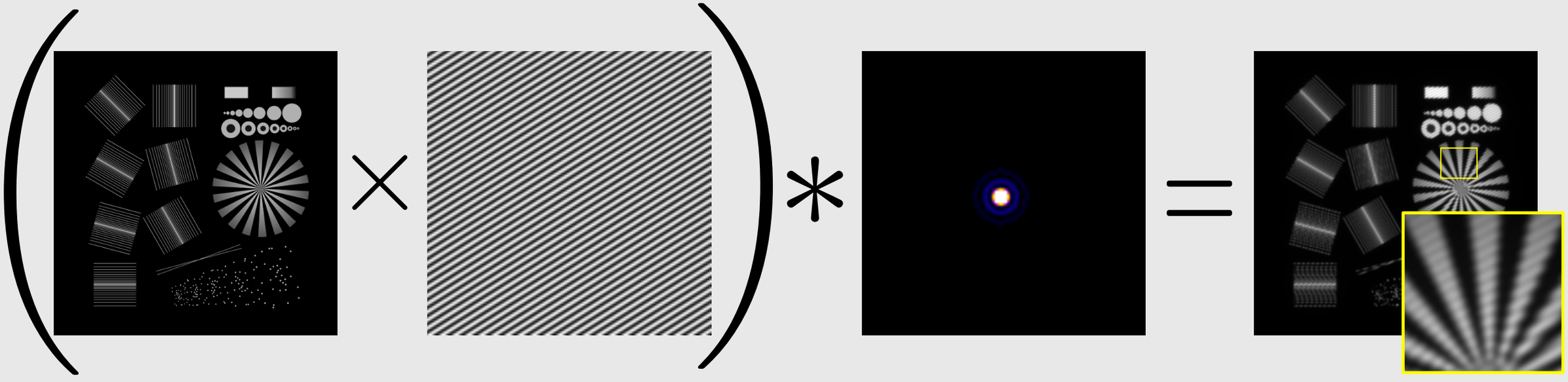
!! Diffraction !!

“Ideal” image

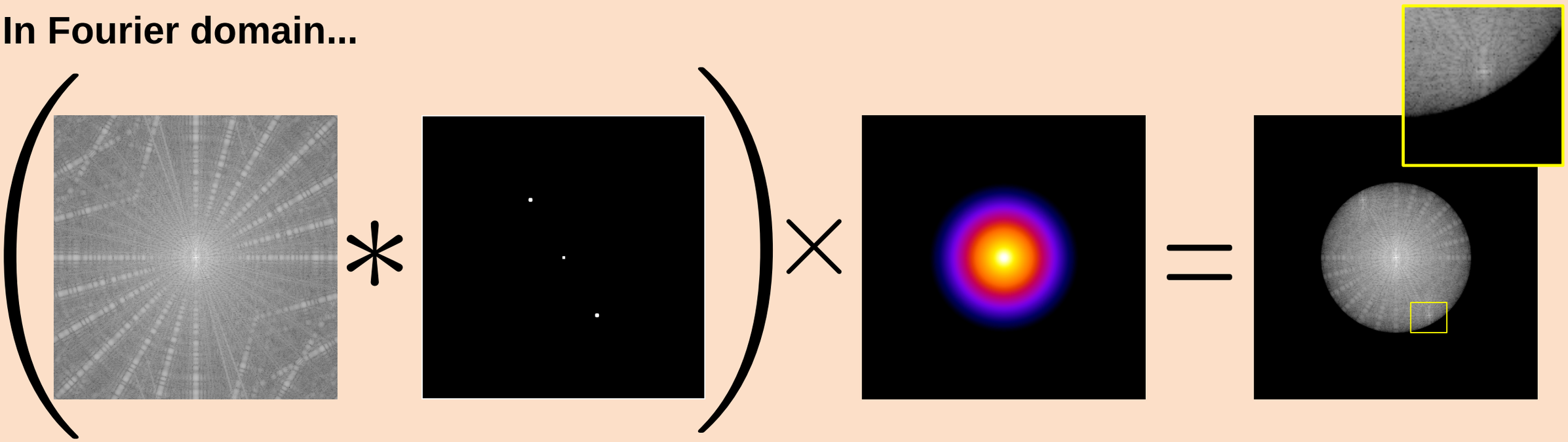


Structured Illumination Microscopy – Image Formation

Modification of the illumination (sinusoidal pattern)

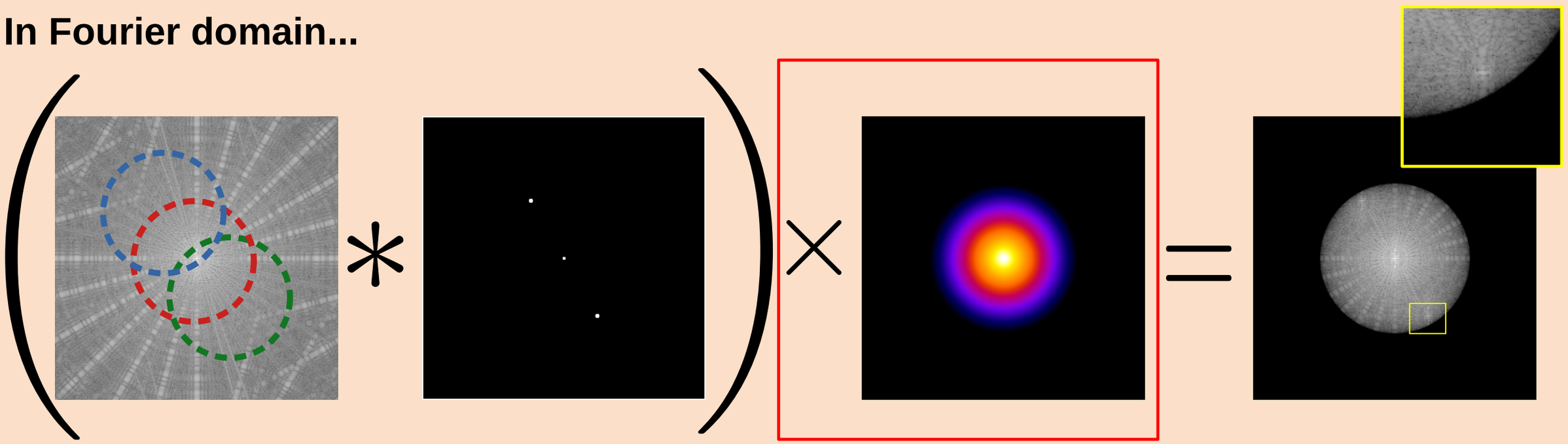


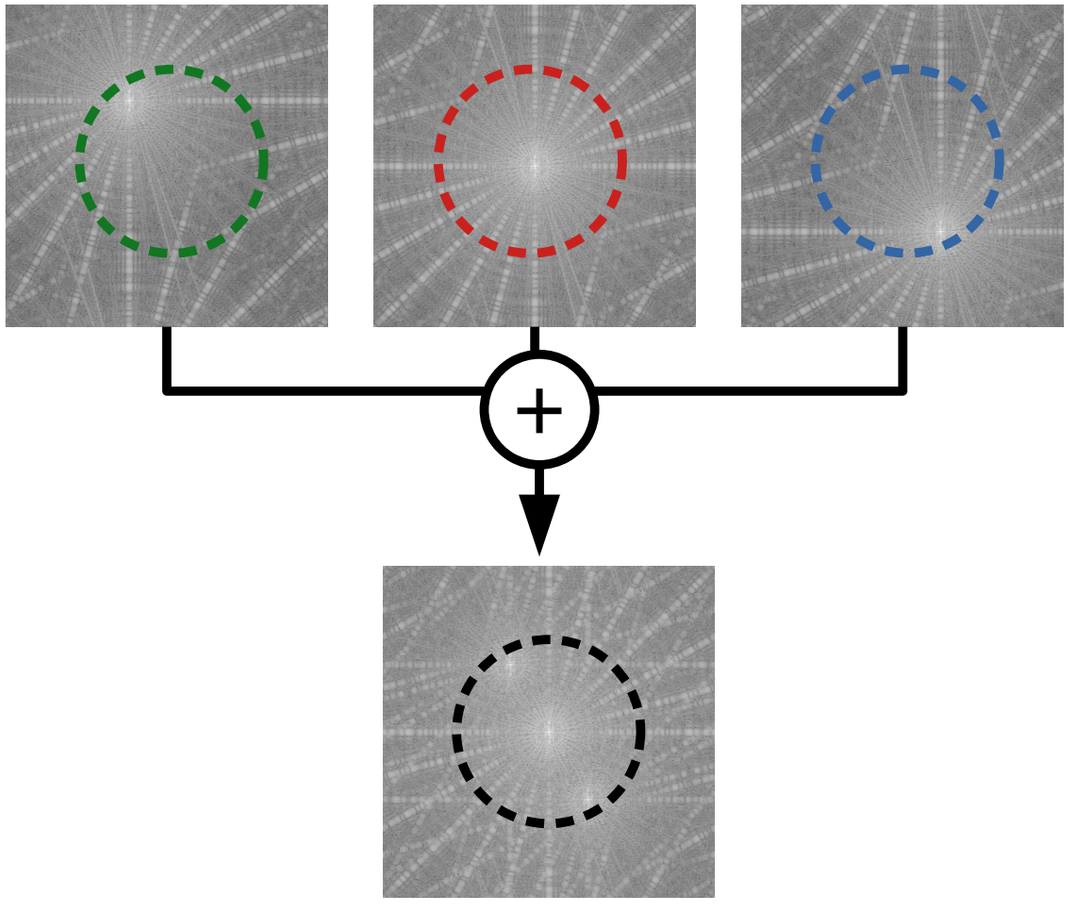
In Fourier domain...



Structured Illumination Microscopy – Image Formation

In Fourier domain...





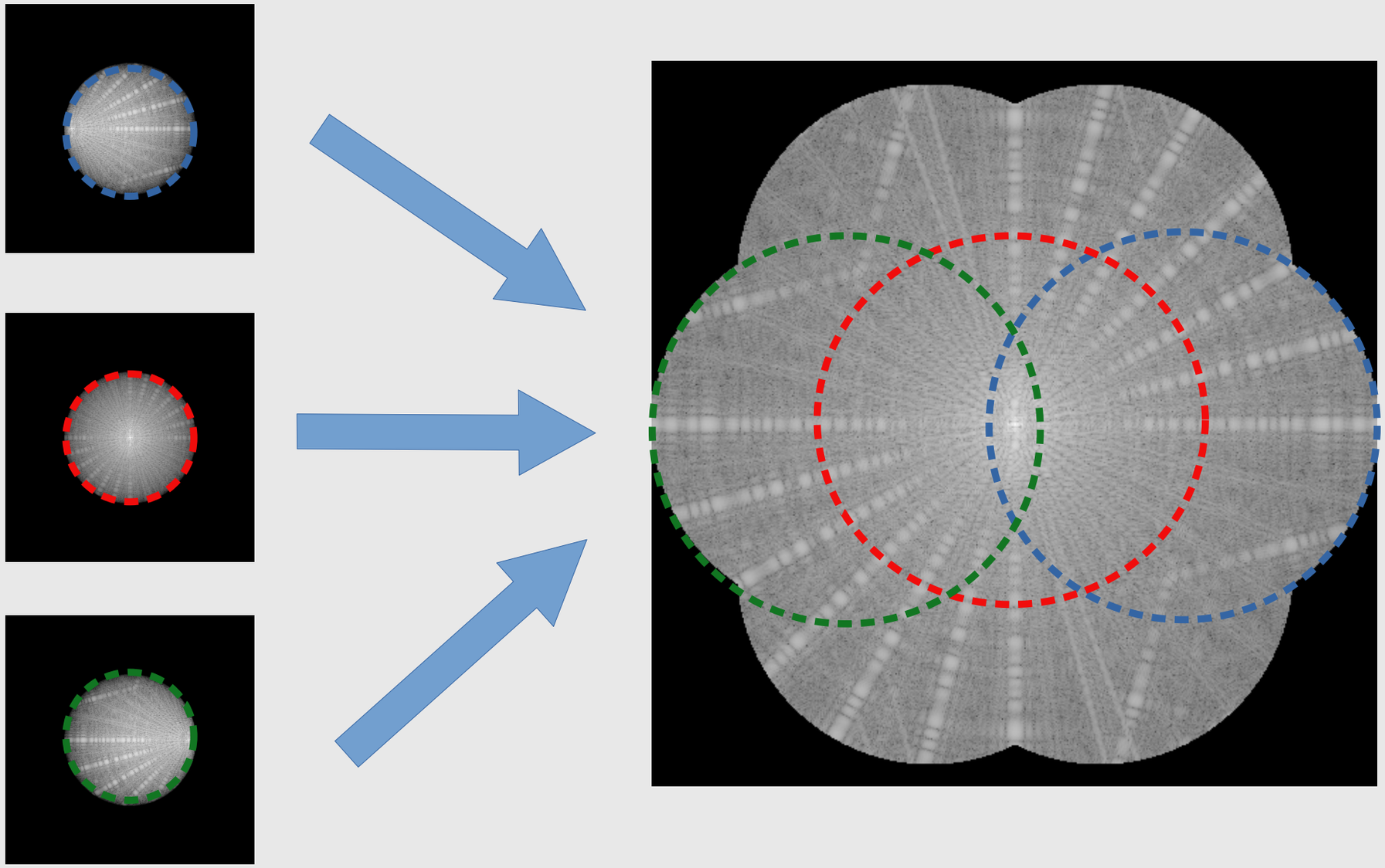
Using structured illuminations, high-frequency components of the sample are shifted into the OTF bandwidth.

Reconstruction is needed to “unmix” the three frequency components

… and shift them back to their correct location in the Fourier plane.

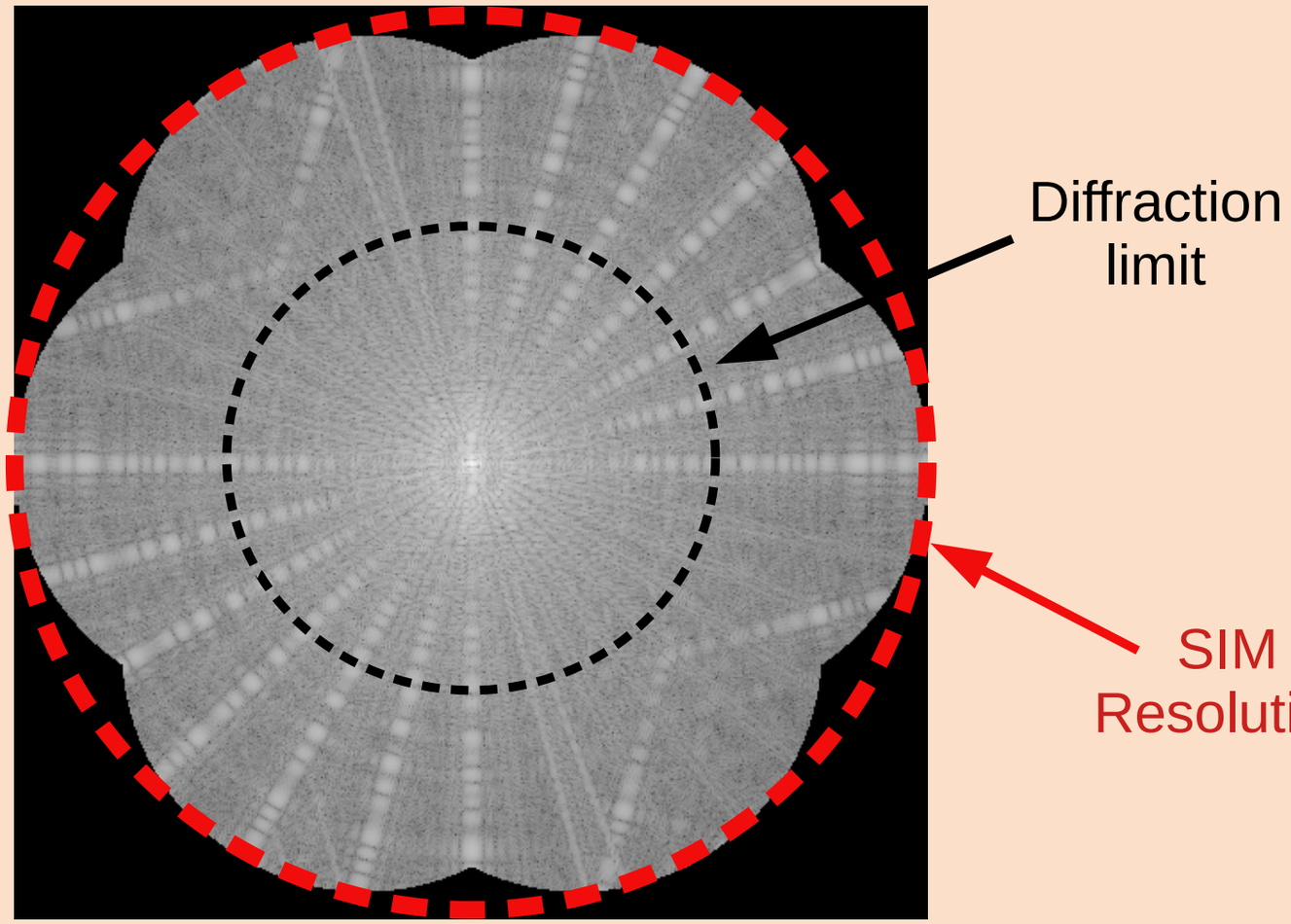
Structured Illumination Microscopy – Reconstruction

Recombination of Fourier components – Taking multiple orientations

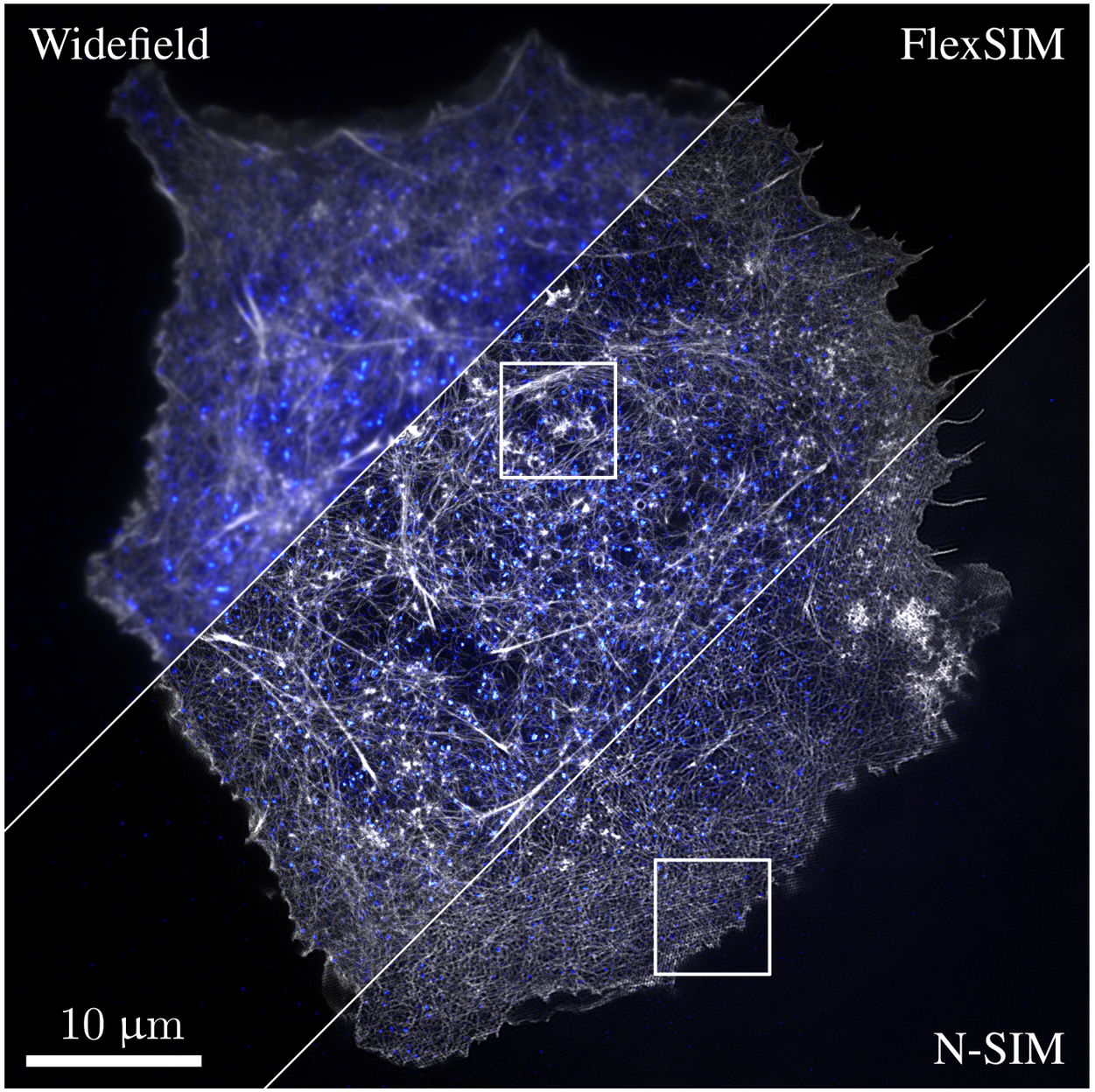


Structured Illumination Microscopy – Reconstruction

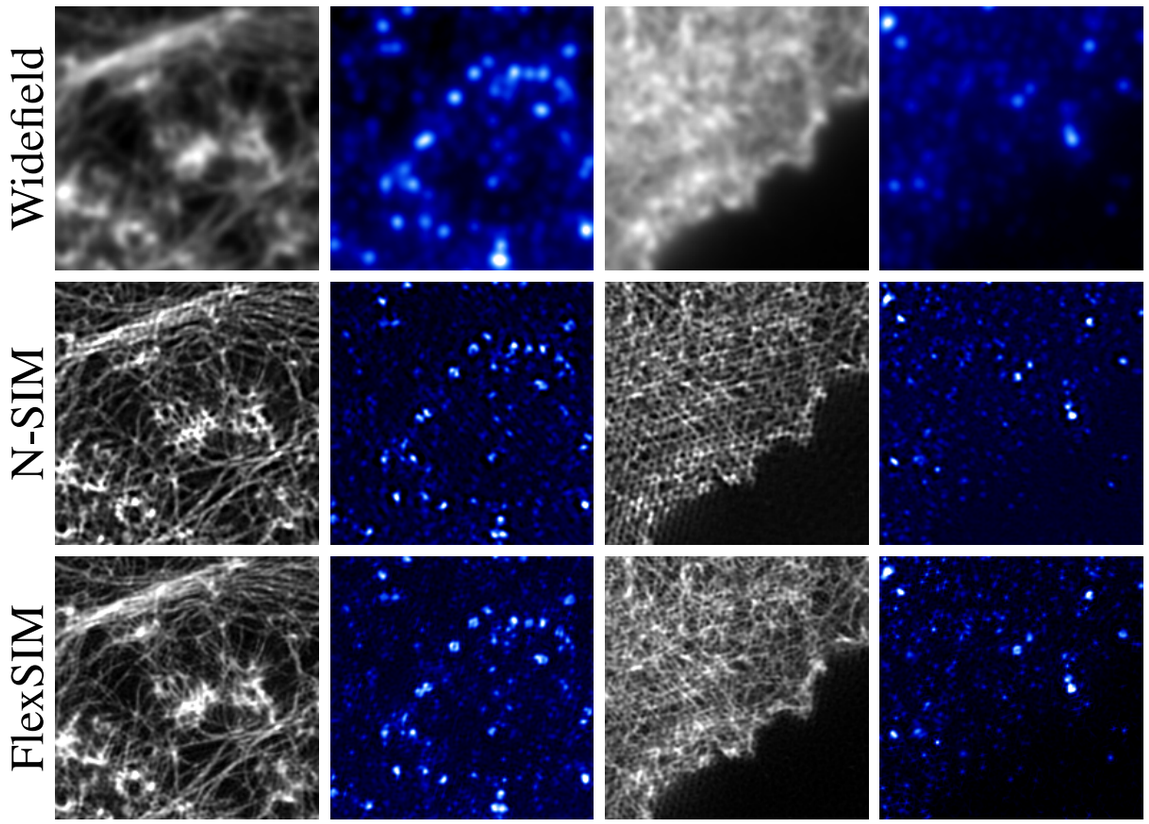
SIM resolution gain : Up to a factor of two



Reconstruction Matters



COS-7 cells imags, Nikon N-SIM-S in TIRF-SIM mode
Actin network in gray - Cathrin in blue
C. Leterrier, NeuroCytoLab



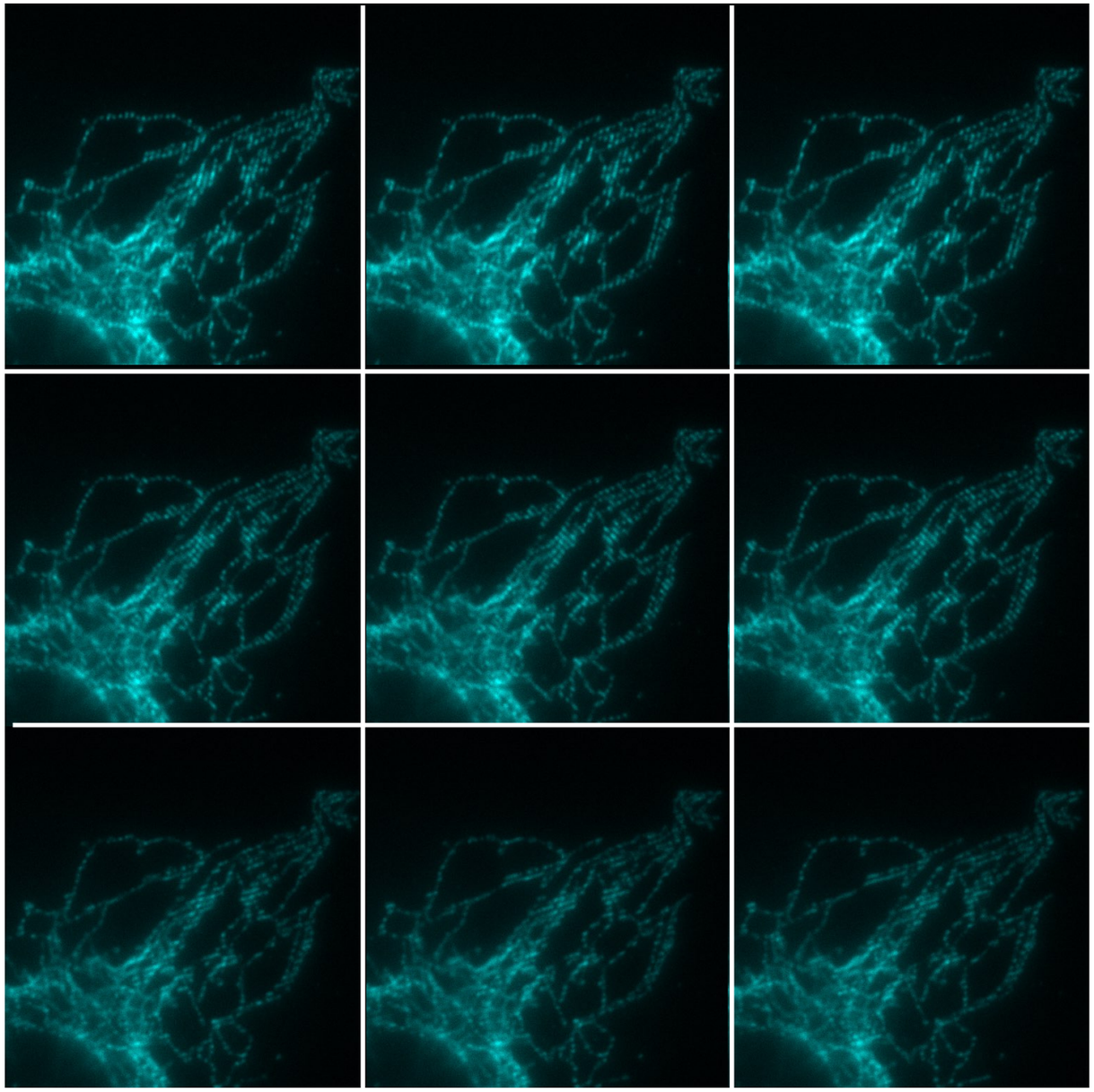
N-SIM Software of the microscopy

FlexSIM Matlab software package

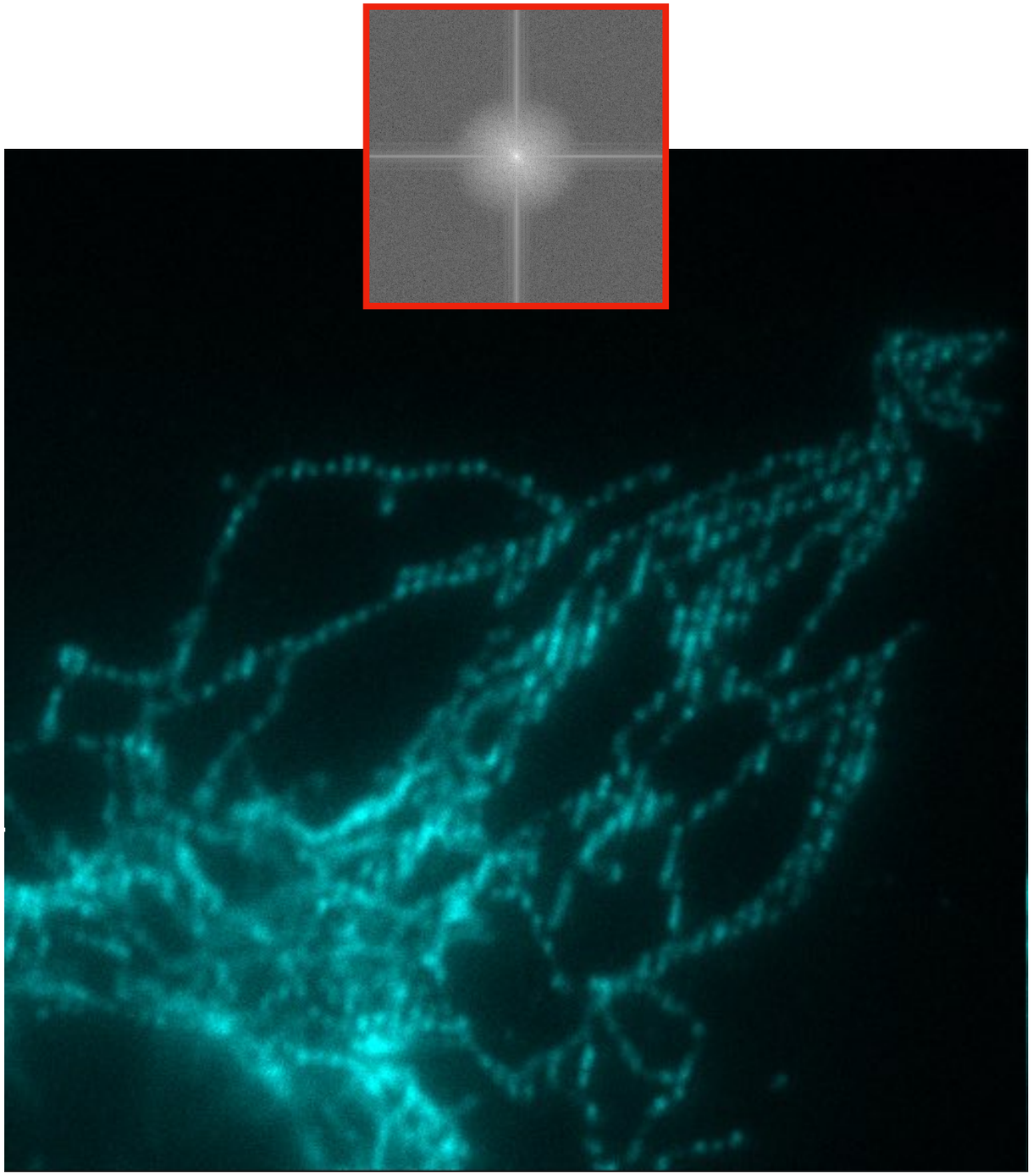
- Estimation of the patterns
- Remove the background

E. Soubies, Surpassing Challenges in SIM, biorxiv, 2023

SIM Acquisition



Thierry Laroche
BIOP EPFL



Reconstruction SIM Nikon

SIM Reconstruction