Structured Illumination Microscopy (SIM)

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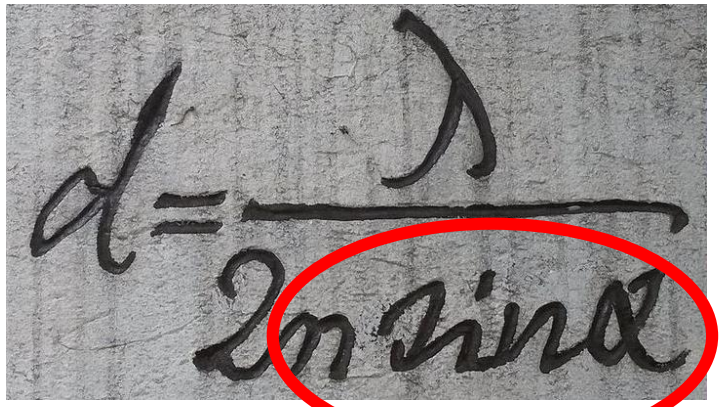
Light Microscopy Core Facility, IMG CAS

Motivation

- Standard microscopy

  <u>Abbé law</u> about diffraction barrier is valid:

  - Maximum lateral (**x**,**y**) resolution **(d)** in classical light microscopy: ~ **<u>200 nm</u>**;
  - Maximum axial (**z**) resolution: ~ **<u>500 nanometers</u>;**
  - Depending upon
    - **the average wavelength of illumination (λ),**
    - **the objective numerical aperture (NA) (=describing the quality of the objective).**



= NA

Motivation

- Structured illumination microscopy (SIM)

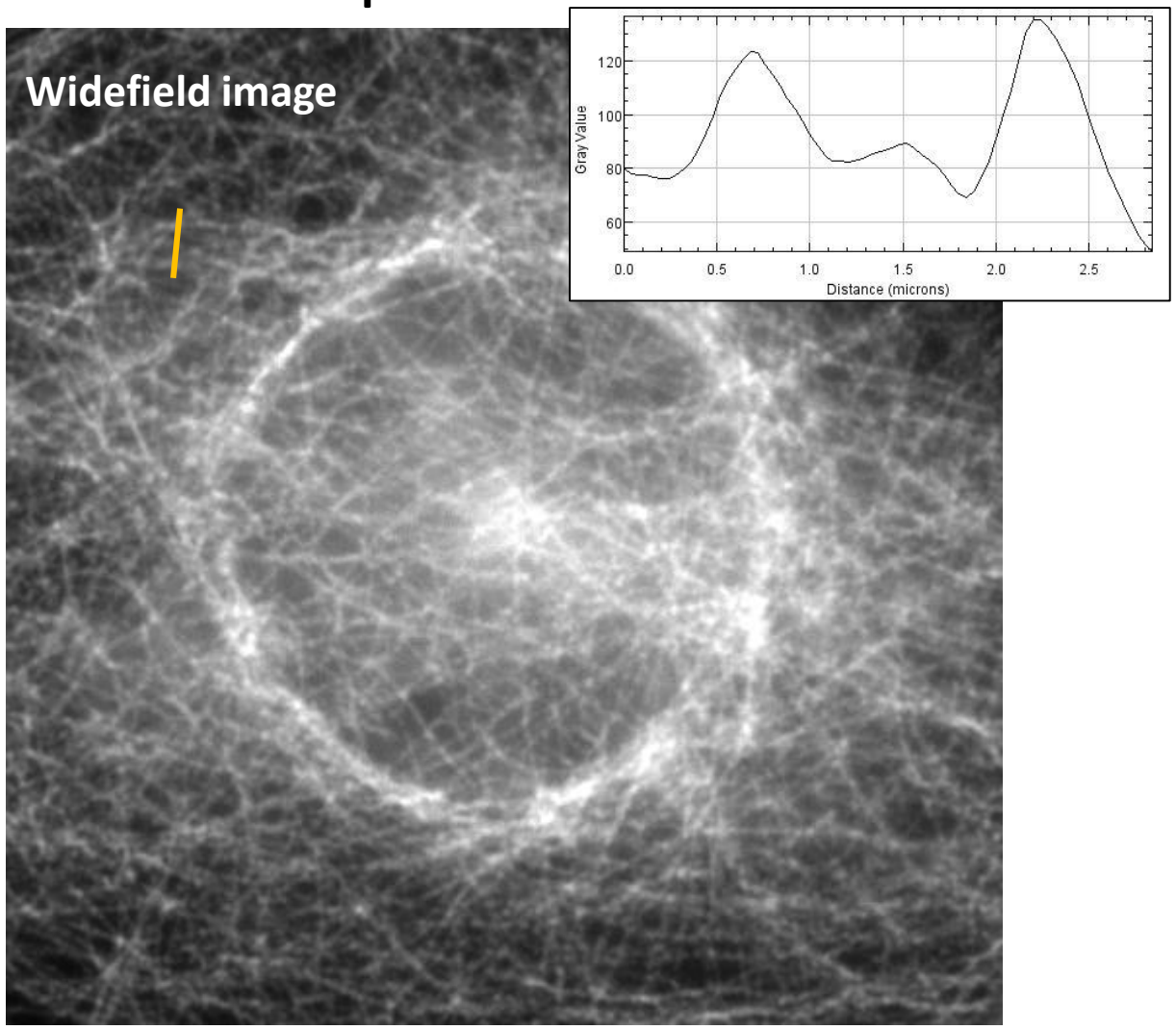
  → **Abbé law about diffraction barrier was broken!**

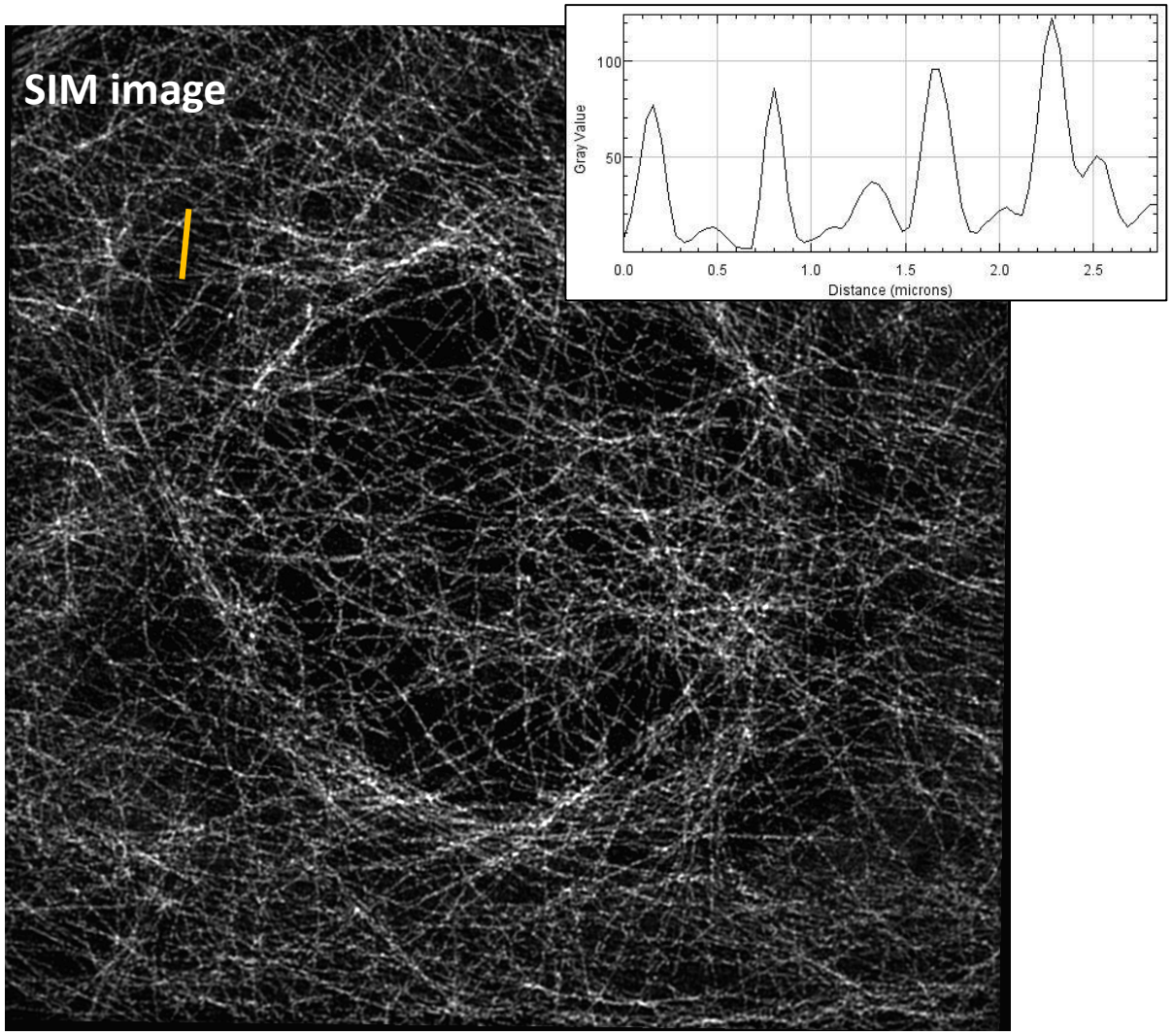
  - The lateral resolution: ~ **100 nanometers**,
  - the axial resolution: ~ **300 nanometers**,

  - i.e., **doubling** the resolution of standard microscopes.

  → Now it is possible to visualize the spatial and temporal relationships between subcellular structures (speckles, nuclear pores, histones, Cajal bodies, etc.)

Example data from WF and SIM





Tubulin in He-La cells; exc. 555nm, em. 609nm; OMX SR-SIM Deltavision

Pros & Cons of SIM

- Relatively technically easy solution – built upon **a standard fluorescence microscope**.
- The widespread availability of **dyes and fluorescent proteins** for labelling specimens.
- Easy conducting **multicolour imaging**.
- **3D sectioning**.

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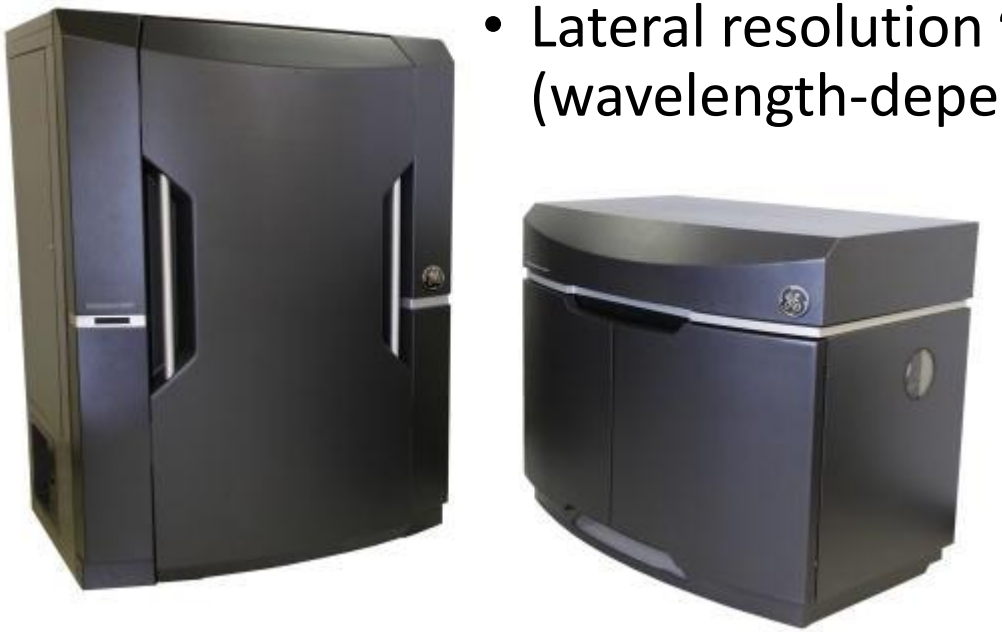
- **3D sectioning**.

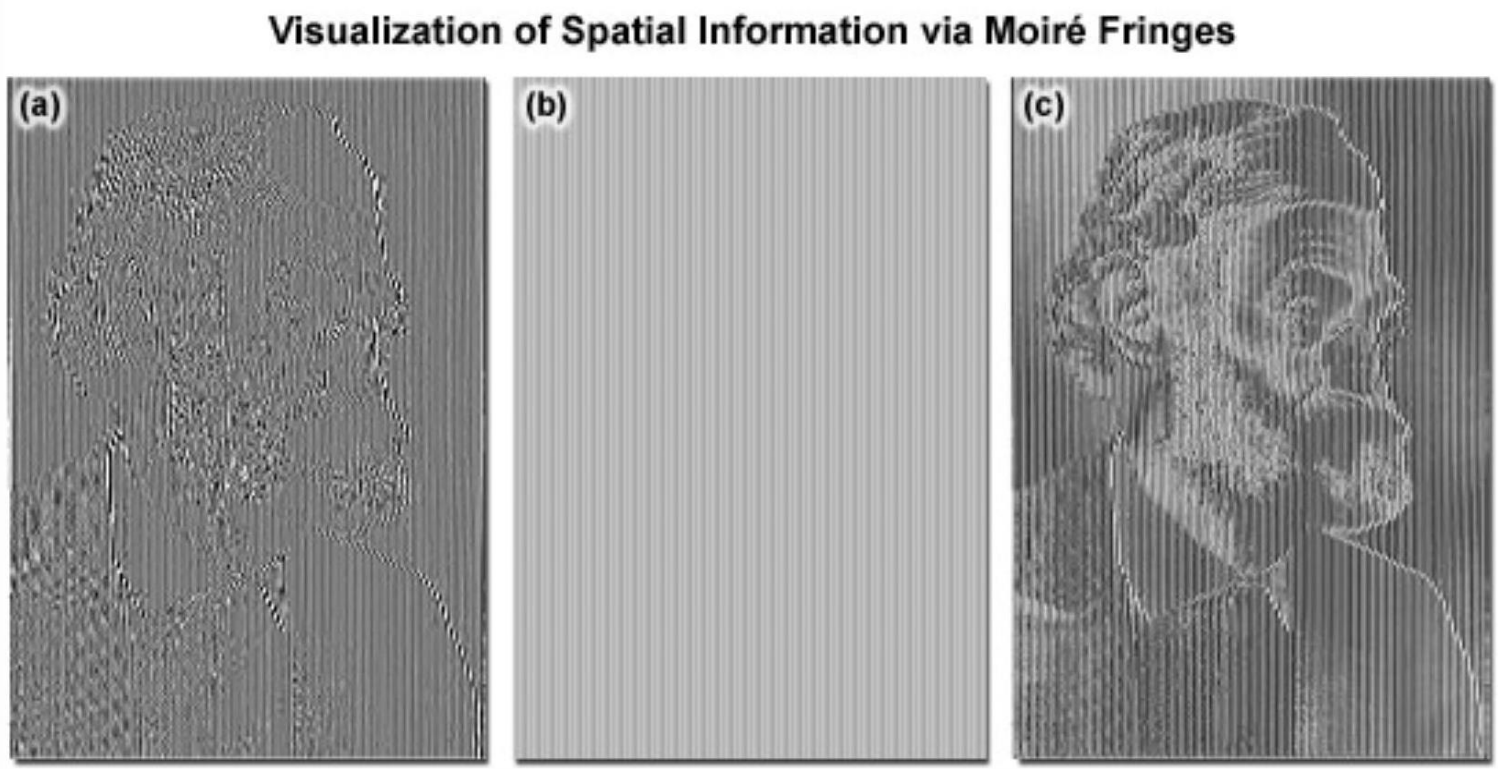
- **Acquisition time** (capturing 15 images per one optical section is required).
- Increased **photobleaching**.
- Refractive **index mismatch** and/or **poor sample quality** can induce artifacts.

DeltaVision OMX

- The fastest commercial SIM machine on the market:
  - Installed at IMG CAS in December 2015.
  - Acquiring 15 images per an optical section takes down to **tens of miliseconds** → **In vivo** super-resolution imaging possible!
  - Lateral resolution **~120 nm**, axial resolution **~340 nm** (wavelength-dependent).

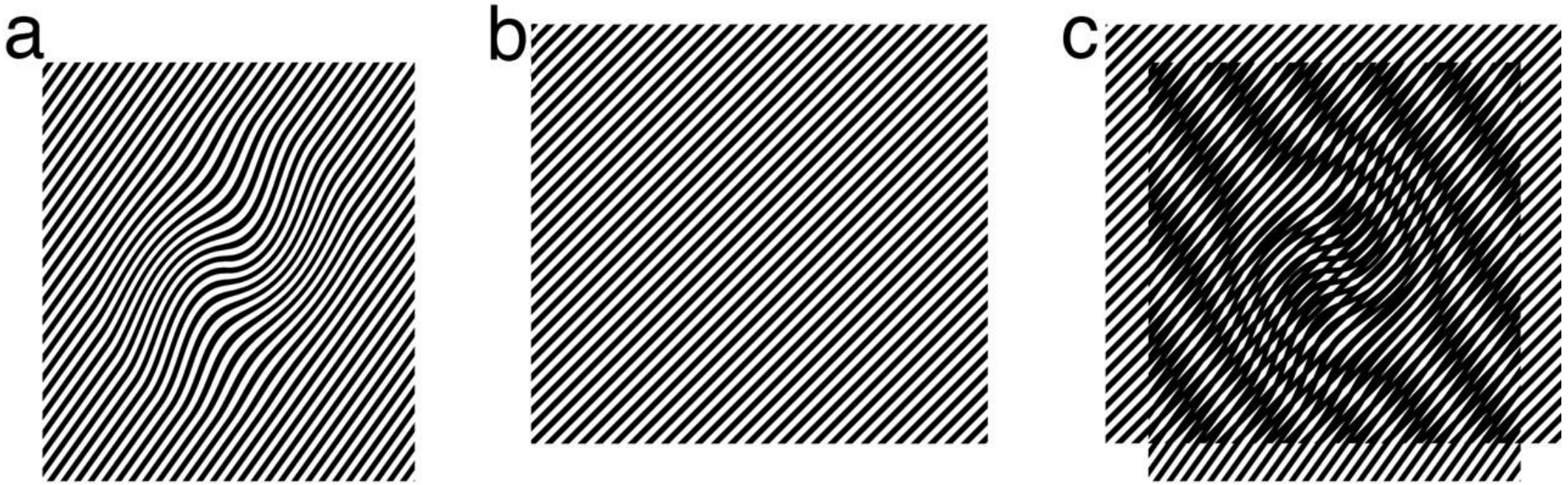


Basic principle of SIM



a) spatial details of a portrait of **Ernst Abbe**
b) a grid with a linear structure
c) mixing (a) with (b) results in **lower frequency moiré fringes** that make the portrait much easier to recognize

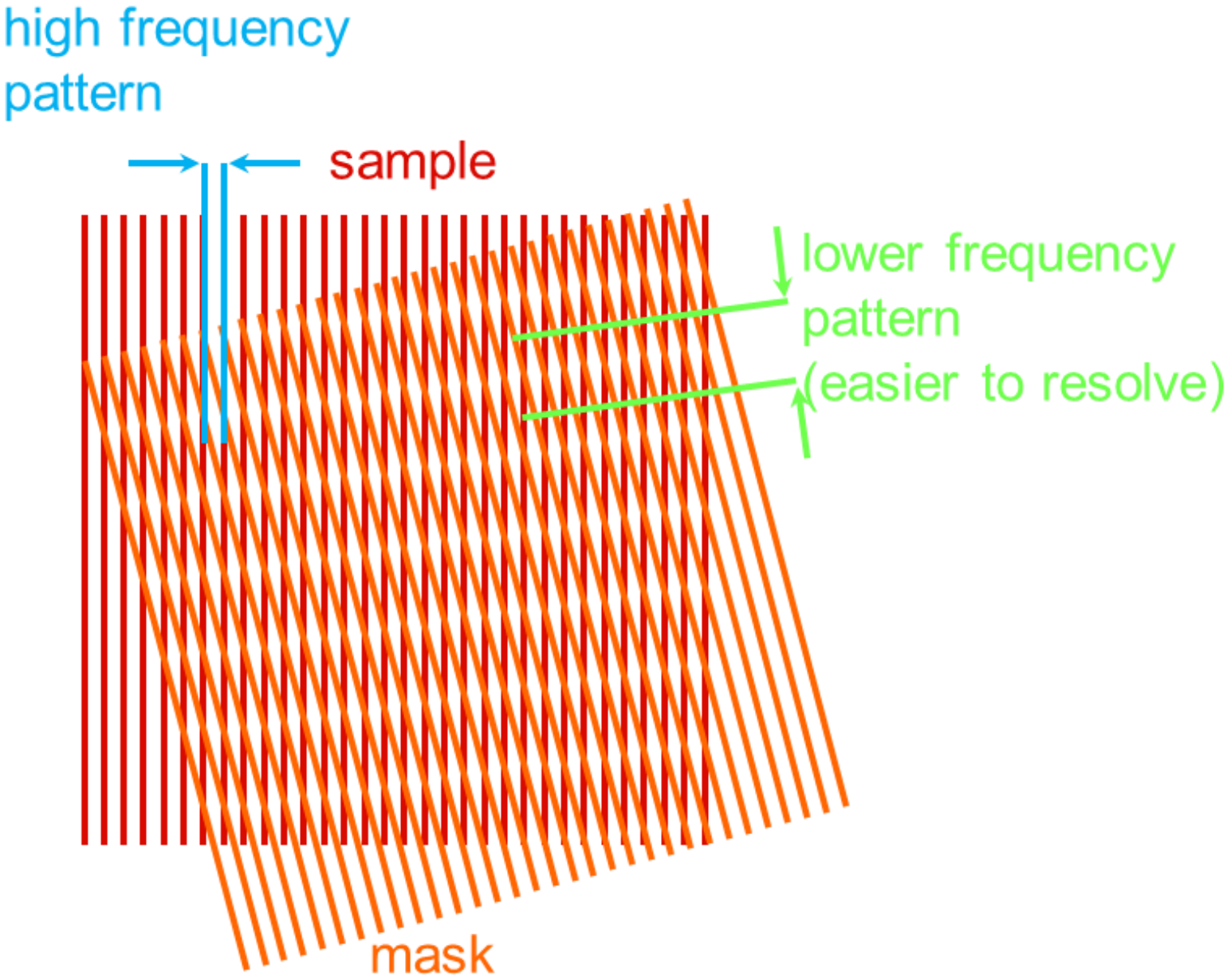
Moiré



- Resolution extension through the moiré effect.
- If an unknown sample structure (*a*) is multiplied by a known regular illumination pattern (*b*), a beat pattern (moiré fringes) will appear (*c*).

source: pnas.org/content/102/37/13081/F1.expansion.html

Moiré



source: physik.uni-bielefeld.de/biopho/index.php/en/research/superresolution/sim

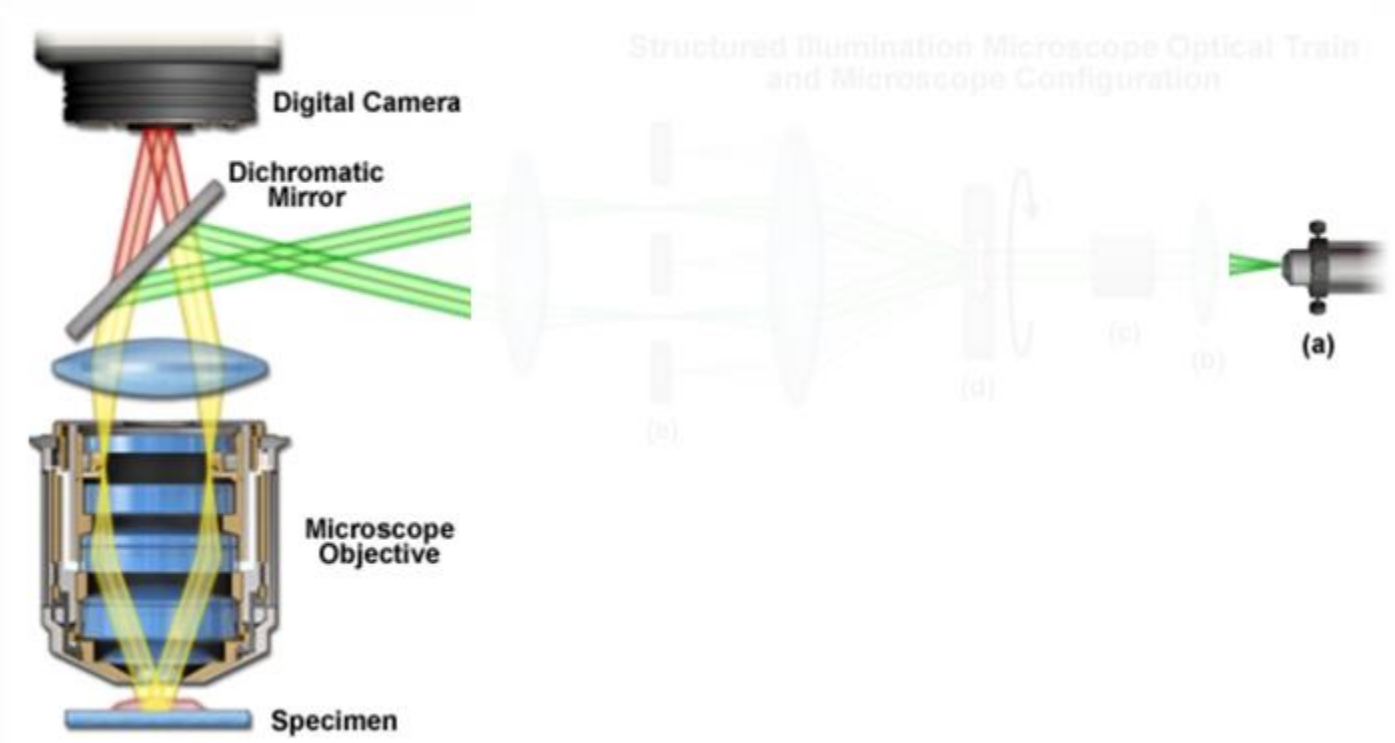
Basic principle of SIM

→Transforming **high frequencies (details)** in specimens

by **grid illumination** into **low frequencies (coarser structures)**

that can be acquired by a standard fluorescence microscope.

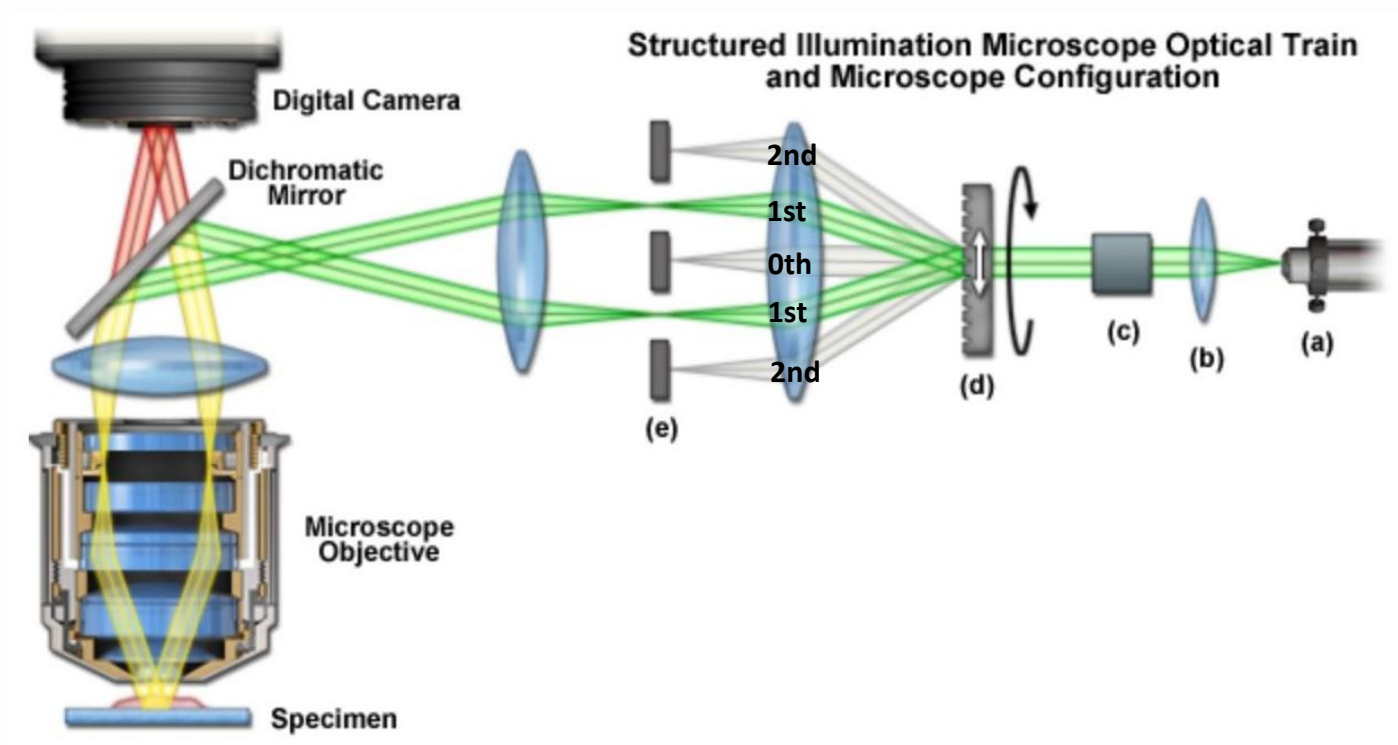
Fluorescence microscope – a schematic



a) laser – a source of excitation

source: zeiss-campus.magnet.fsu.edu/articles/superresolution/supersim.html

SIM – a schematic (**lateral resolution** increased)



a) laser – a source of excitation; b) collimator; c) polarizer;

d) **diffraction grating**; e) beam block

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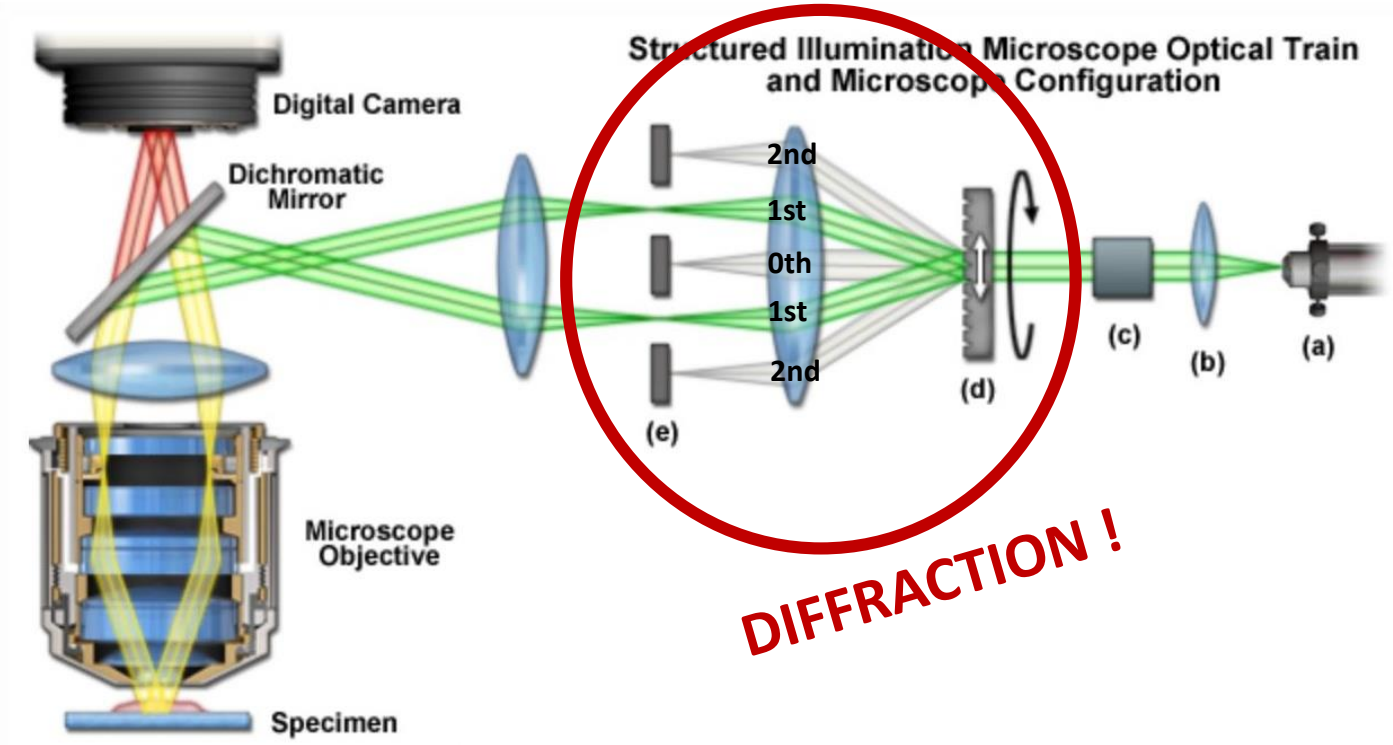

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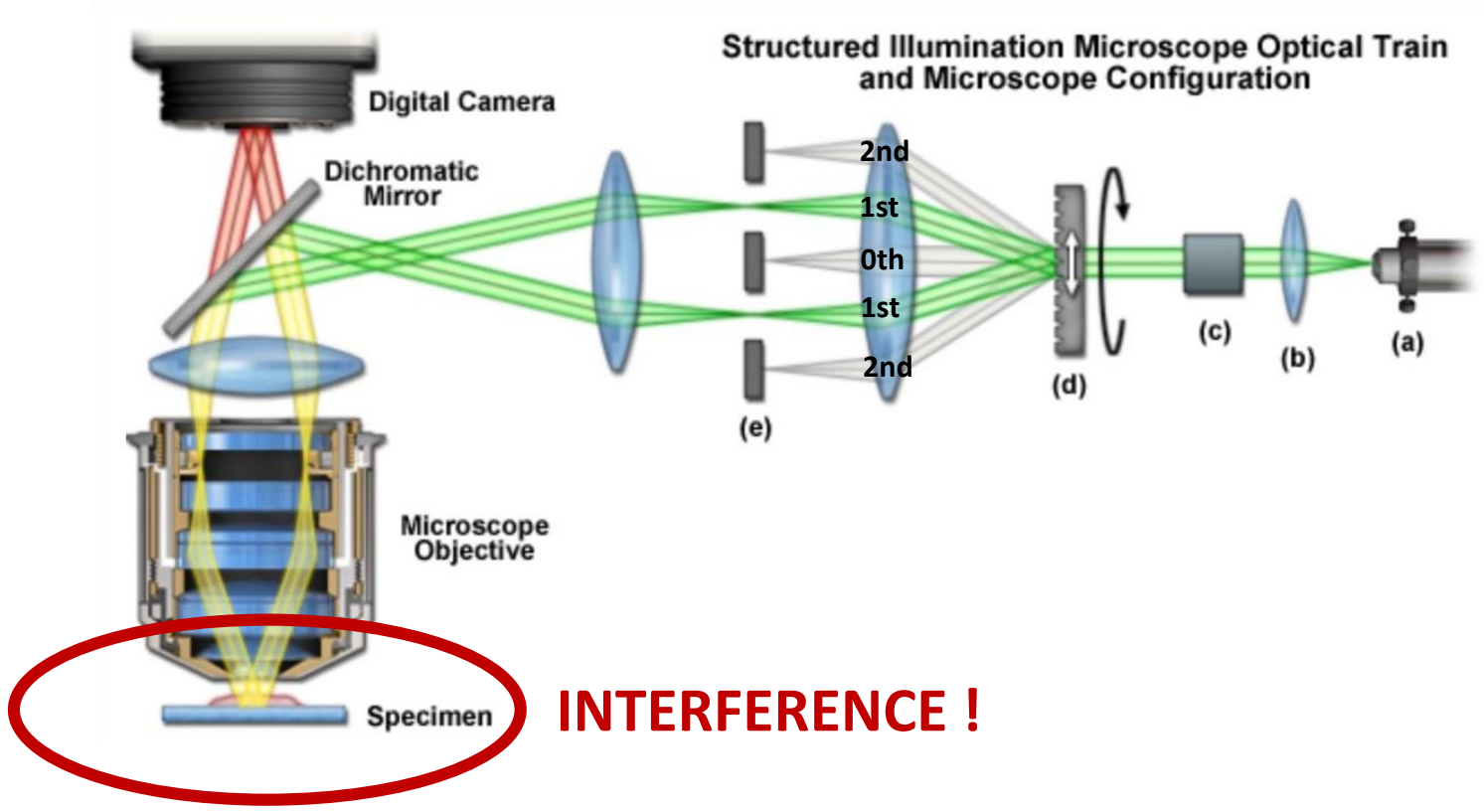

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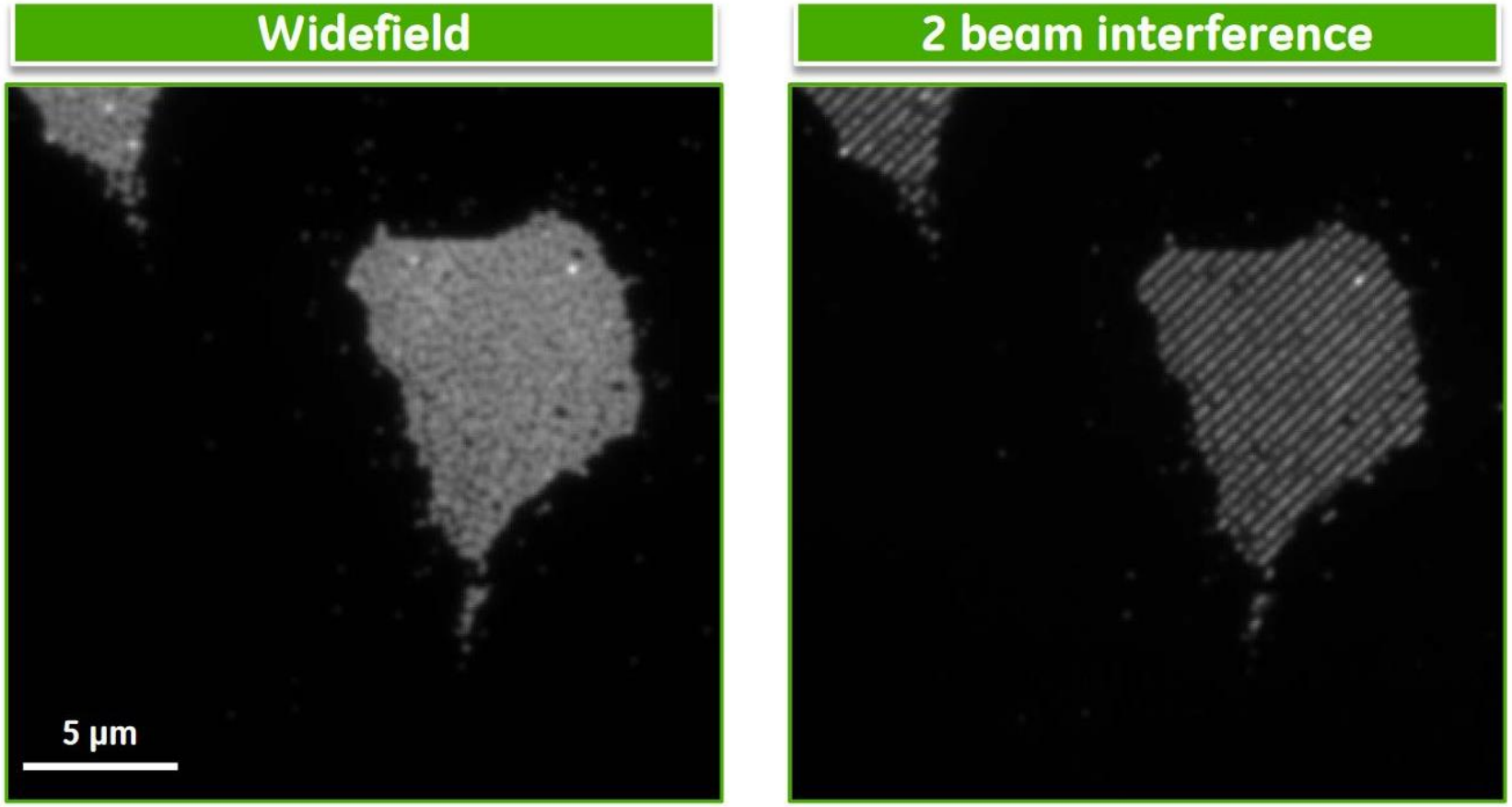
2-Beam Interference Grid Pattern illuminates the specimen

Introduces stripes into image



source: gelifesciences.com

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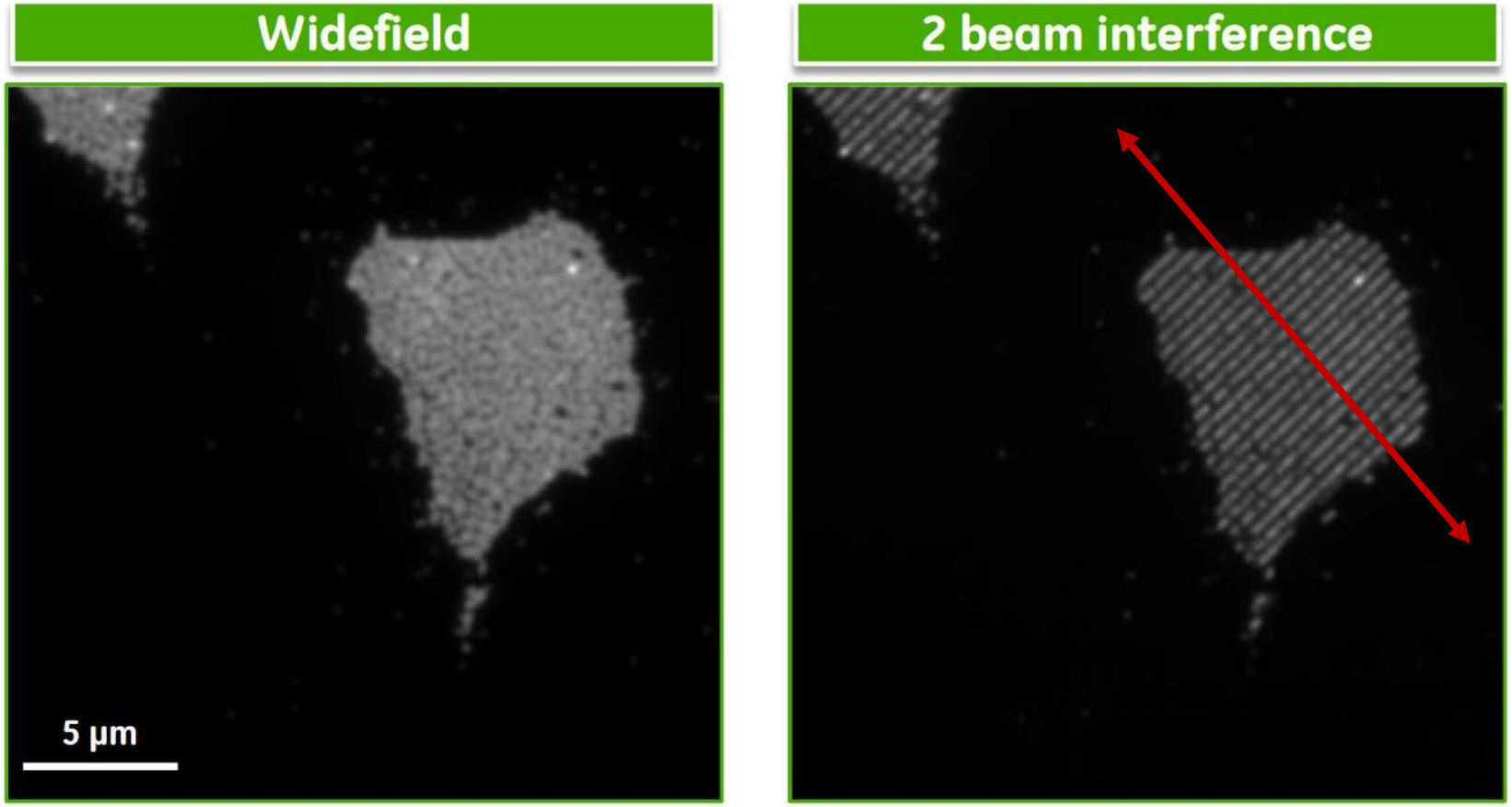
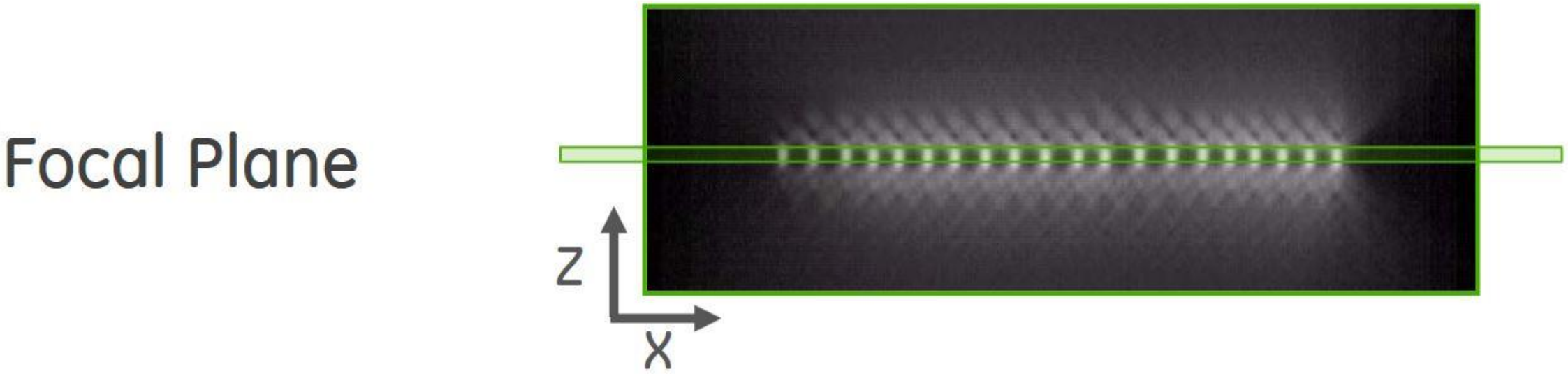


Resolution increased only in the depicted direction!

source: gelifesciences.com

2-Beam Interference Grid Pattern

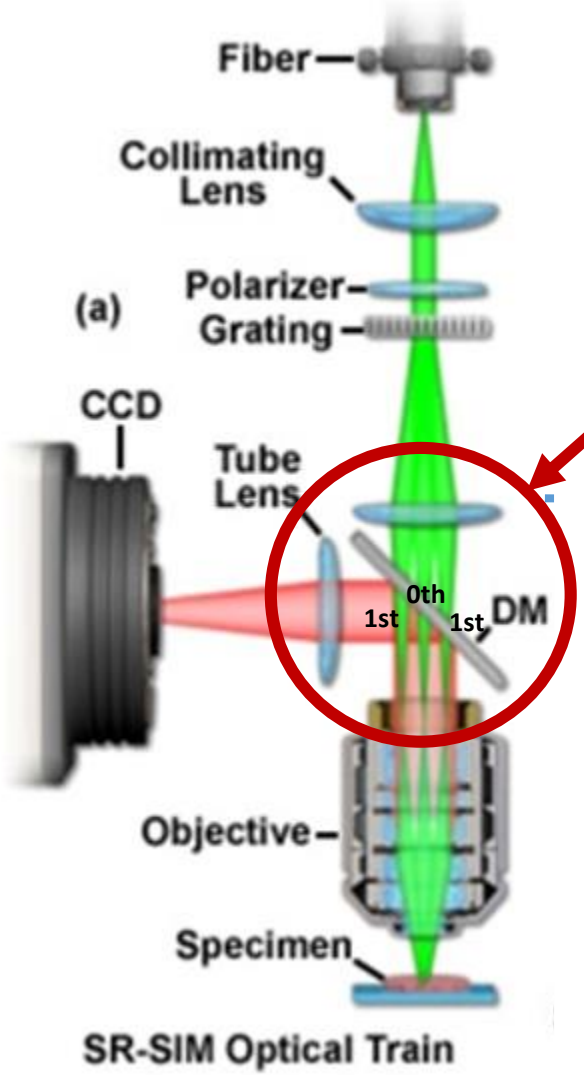
- No increase in contrast or resolution in Z!



source: gelifesciences.com

3-Beam SIM setup

3 BEAMS!



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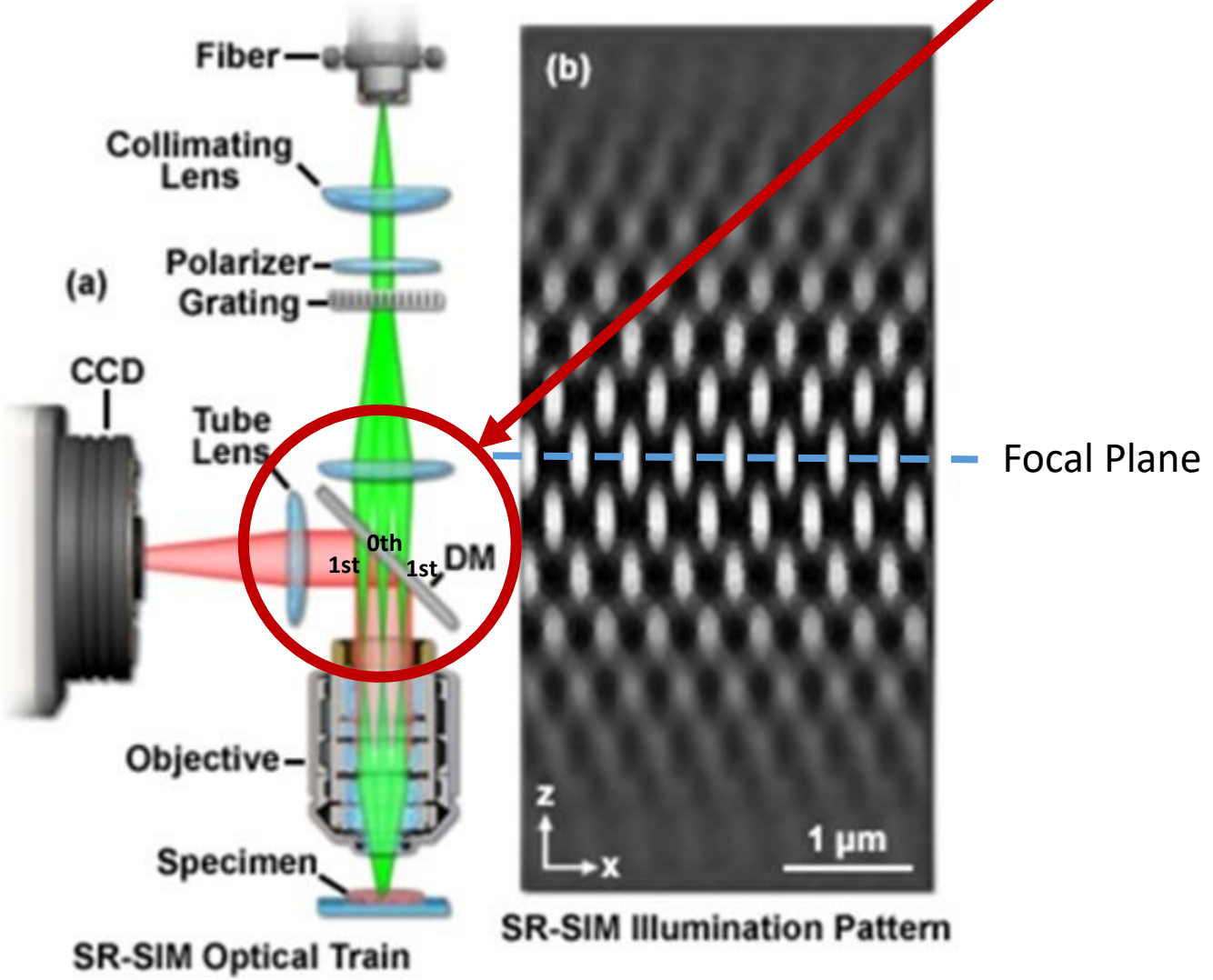

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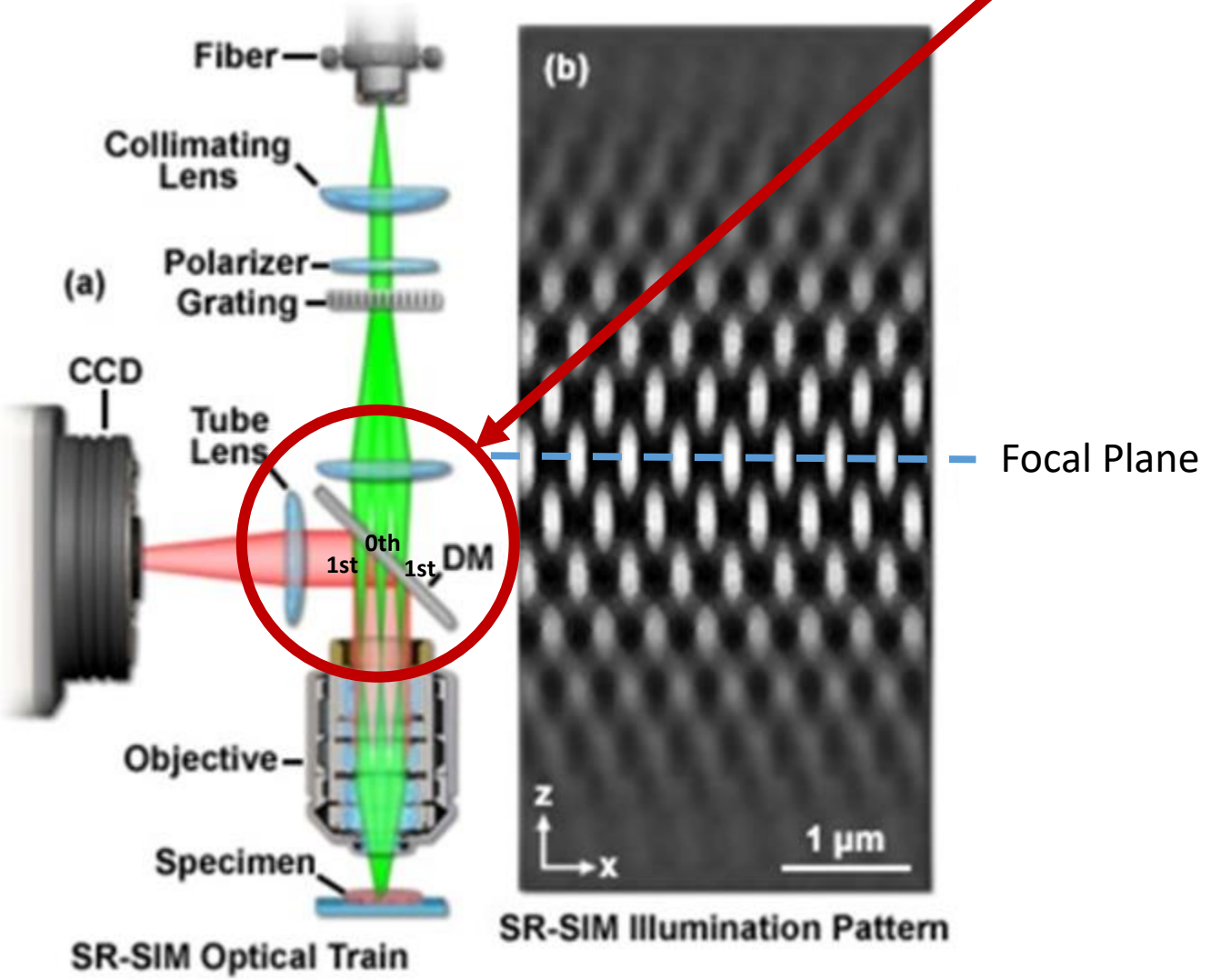


3D SIM

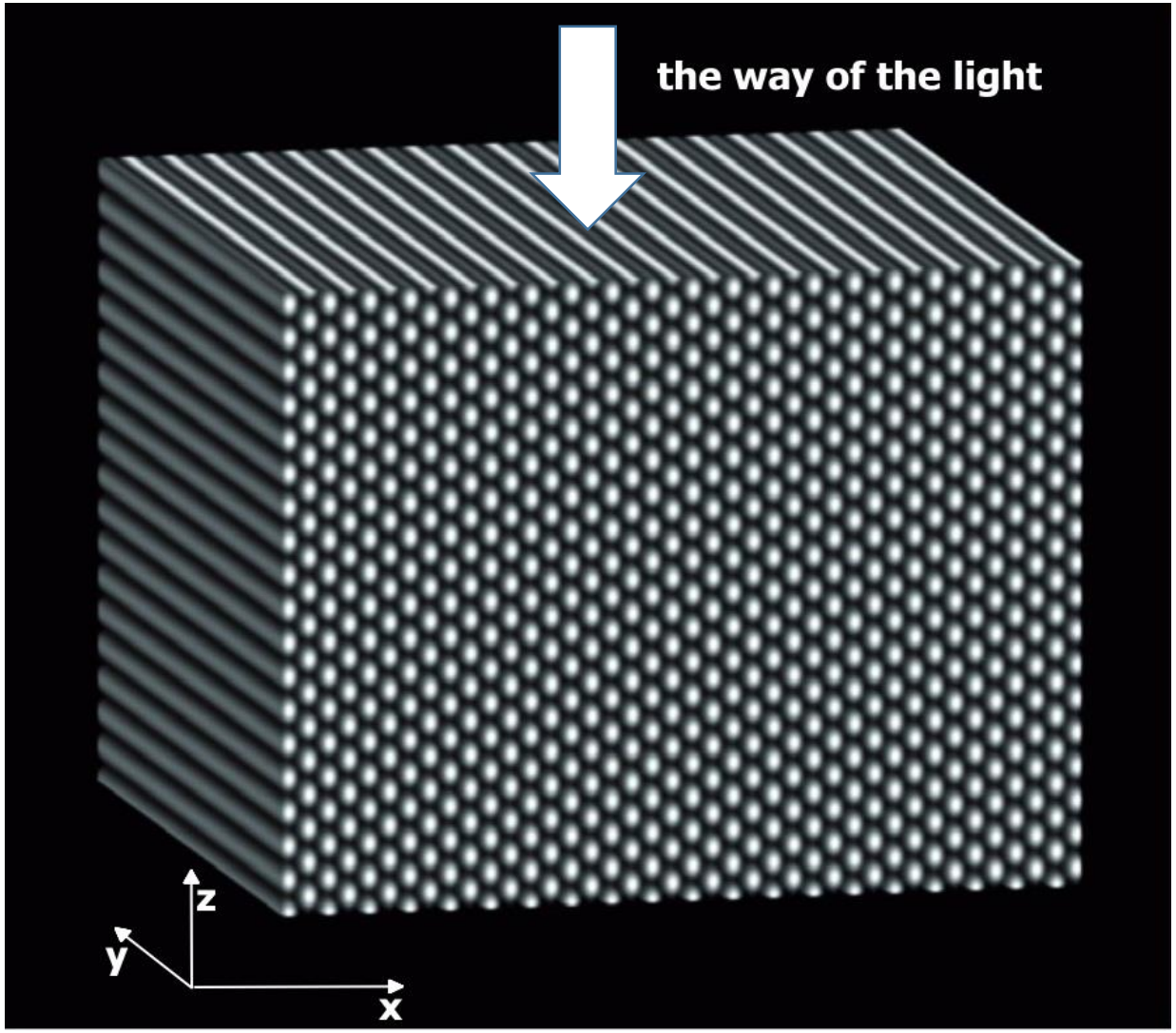
→ 3D pattern gives

2x increase

in axial resolution

source: zeiss-campus.magnet.fsu.edu/articles/superresolution/supersim.html

3D Light Grid – simulation



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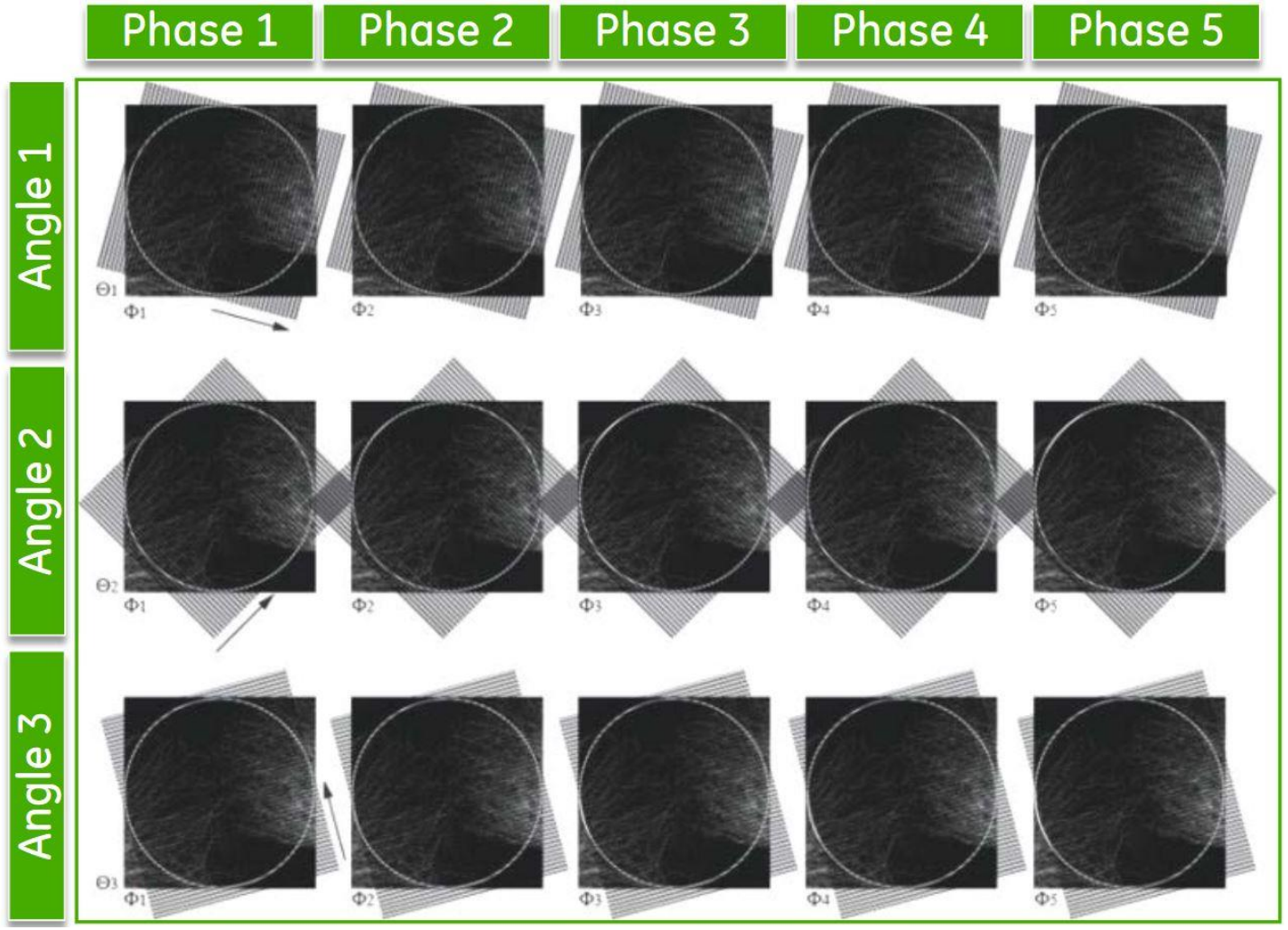
3D-SIM Imaging

Image with striped pattern at **3 angles** & **5 phases**



15 stripped widefield images per one optical layer

source: gelifesciences.com

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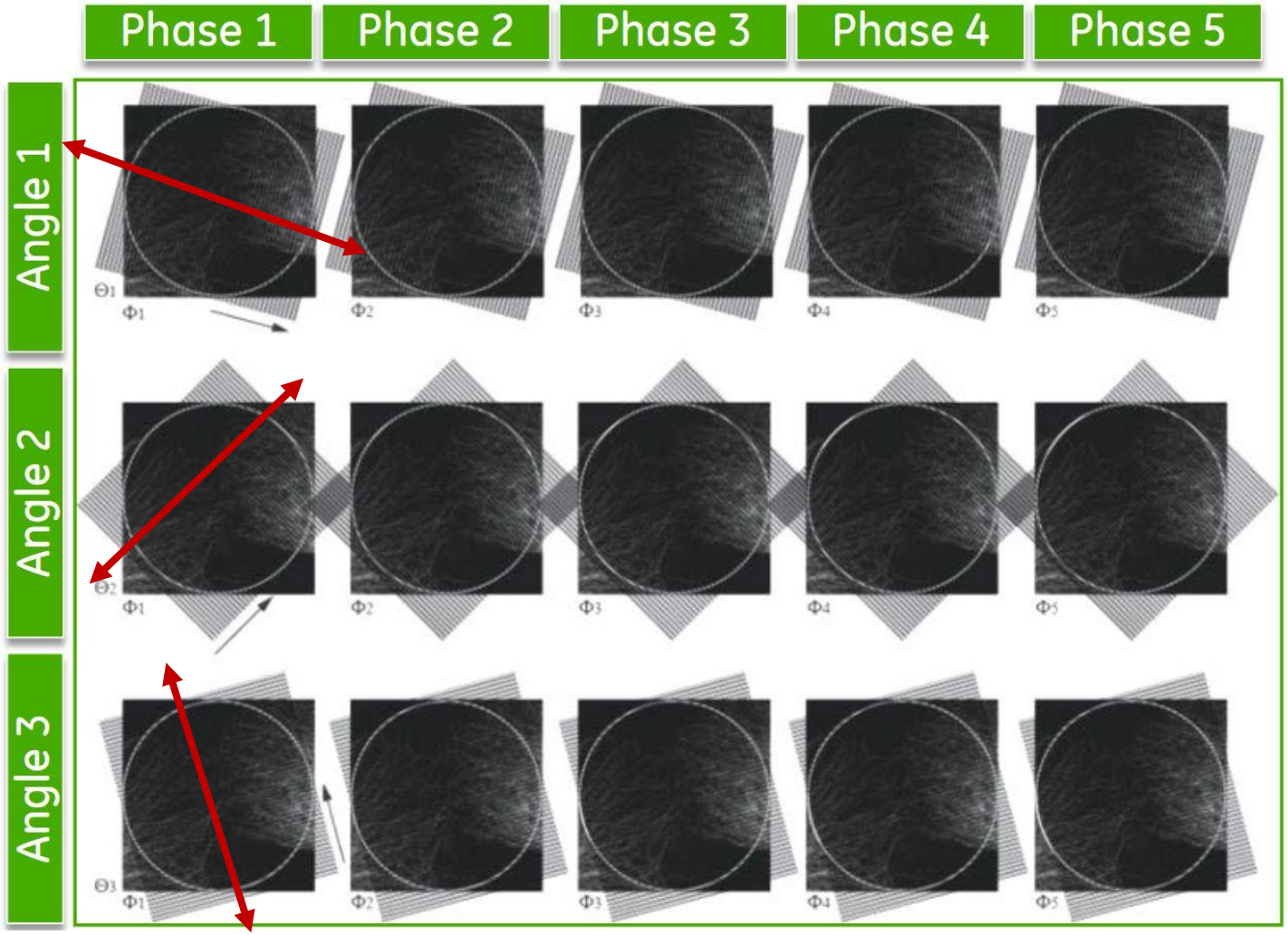

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SIM – reconstruction – **summary**

- Transformation of acquired pictures into Fourier space (Fourier transform)
- Separation of components
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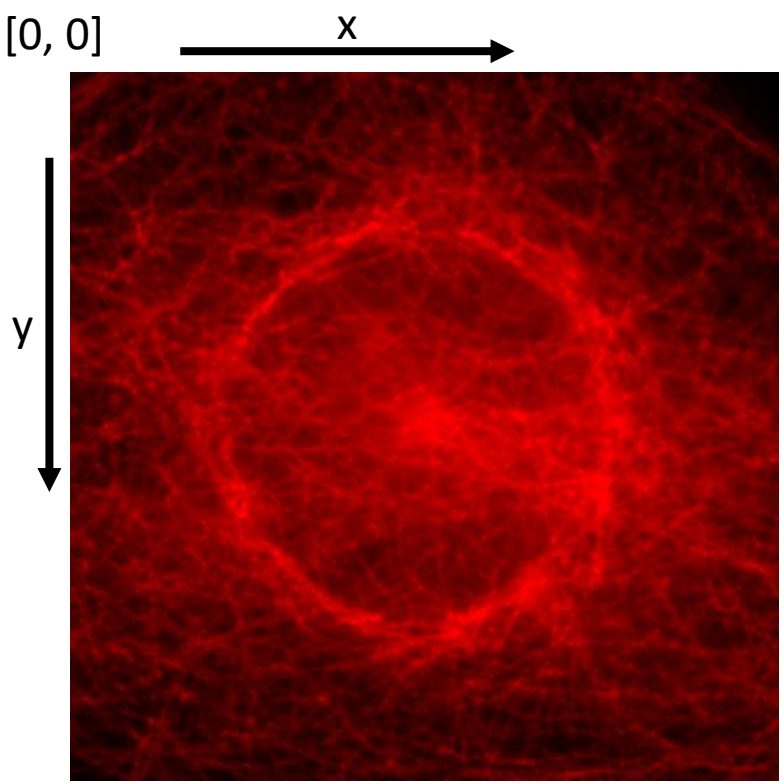
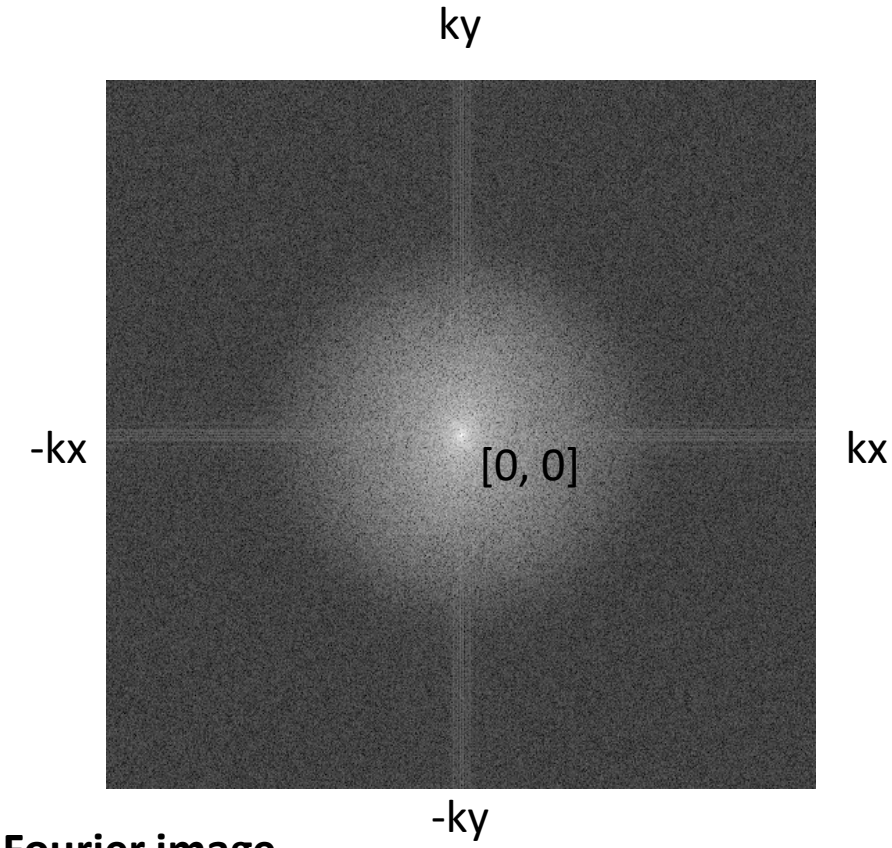
SIM – reconstruction – Fourier transform

Transformation of a **spatial image** into a **Fourier image!**



Fourier transform



Spatial image

x, **y** – spatial coordinates

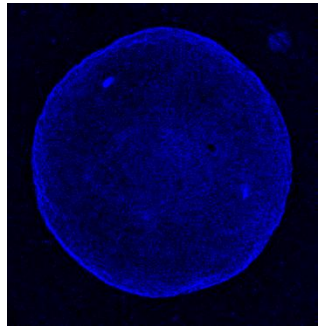
intensity – amount of fluorescence emission at [x, y]

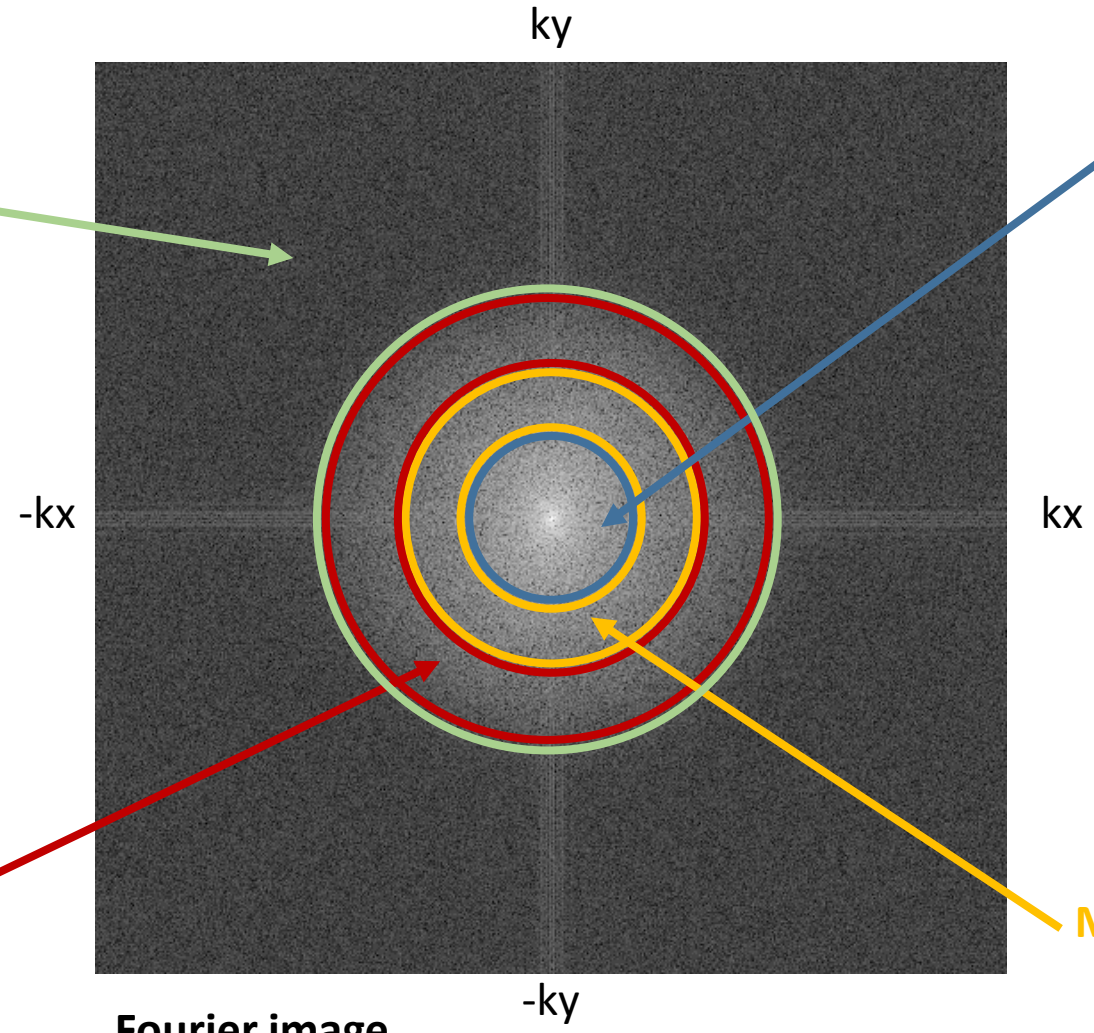
Fourier image

kx, **ky** – spatial frequences

intensity – occurrence of spatial frequence at [kx, ky]

SIM – reconstruction – Fourier transform





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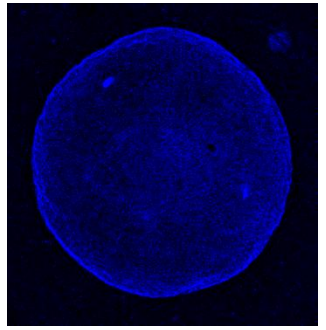

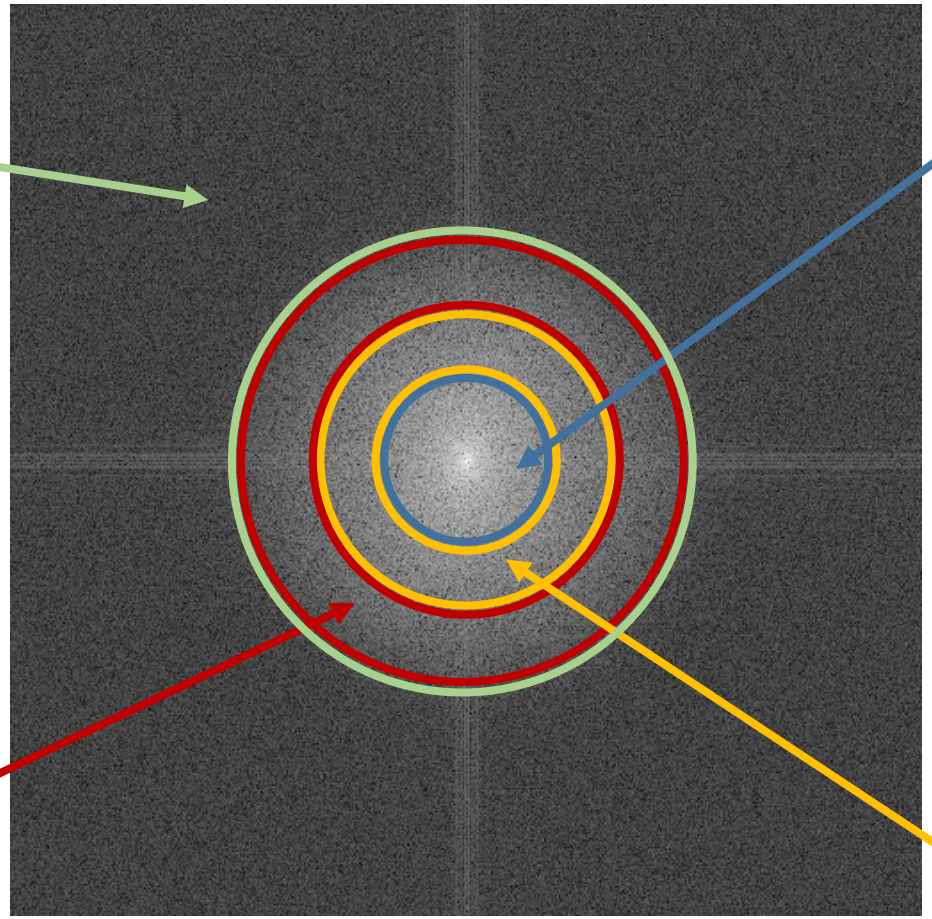

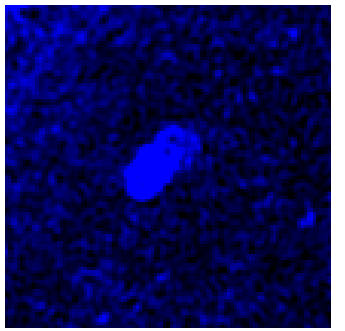

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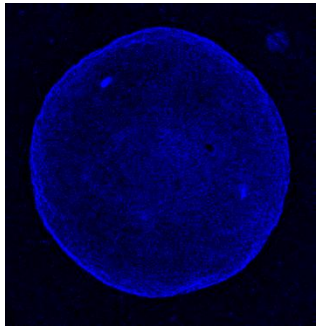


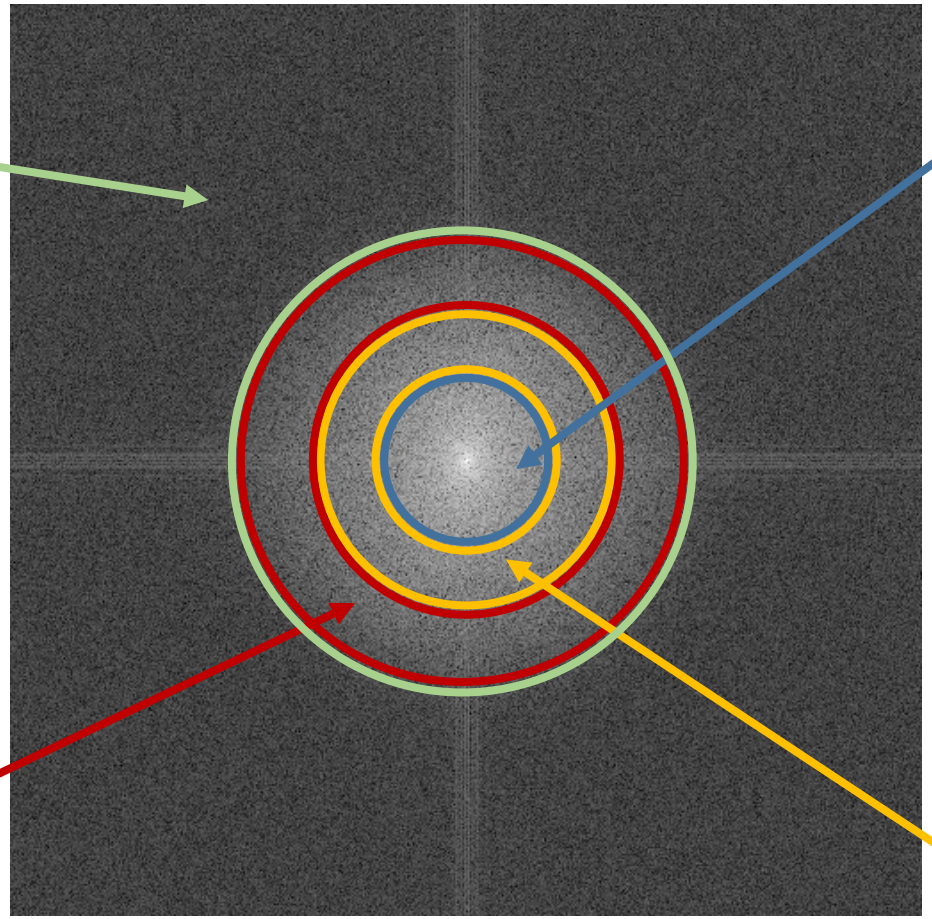
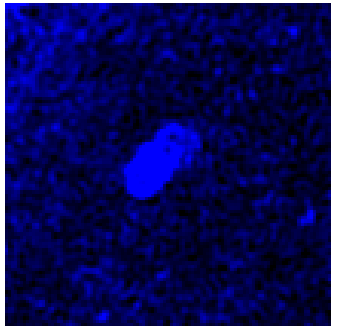
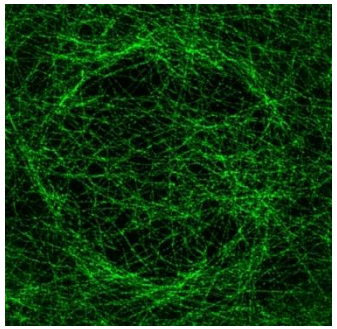


Image noise

Coarse structures





Subtle structures - details

Middle-sized structures

Fourier image

kx, **ky** – spatial frequences

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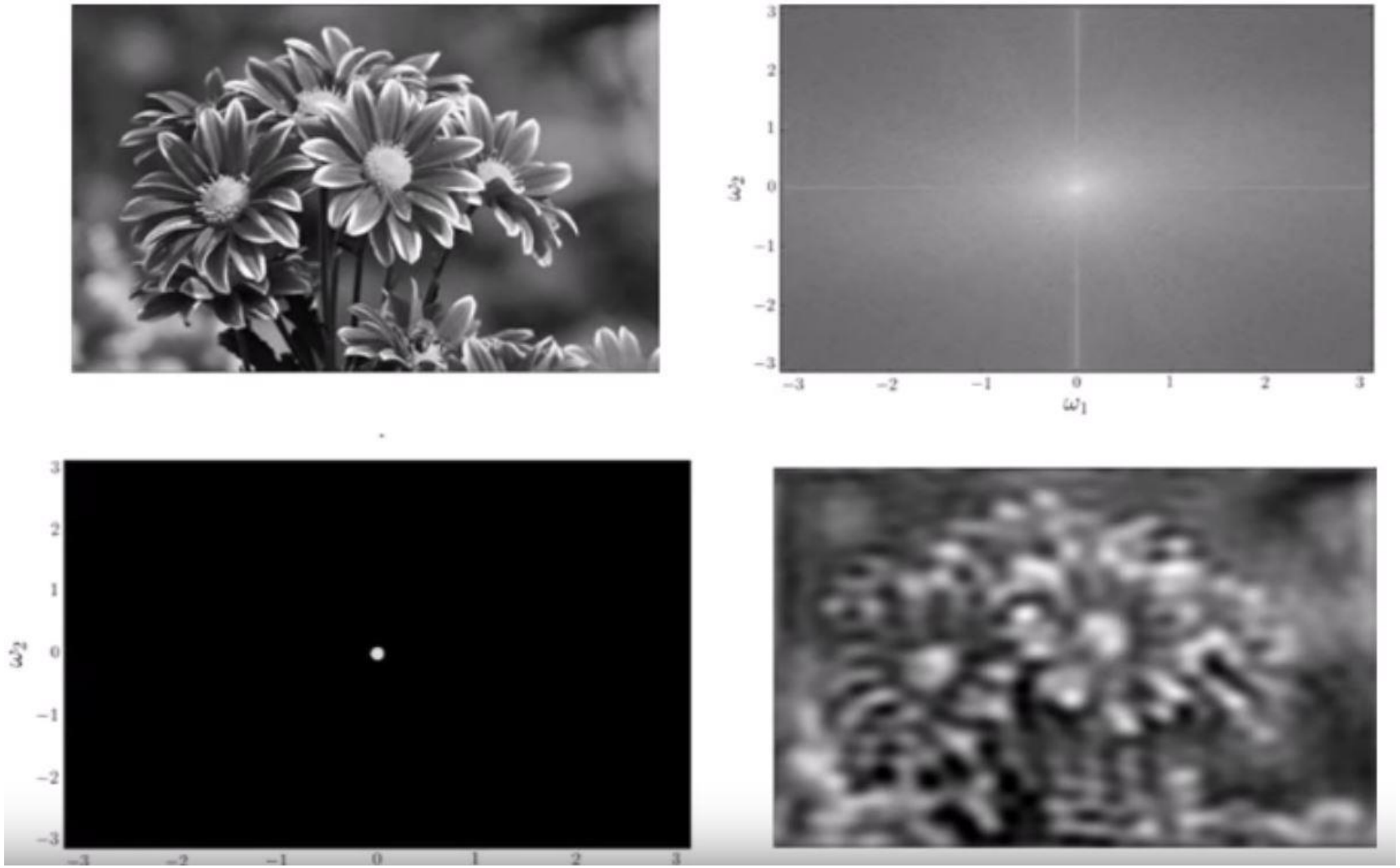
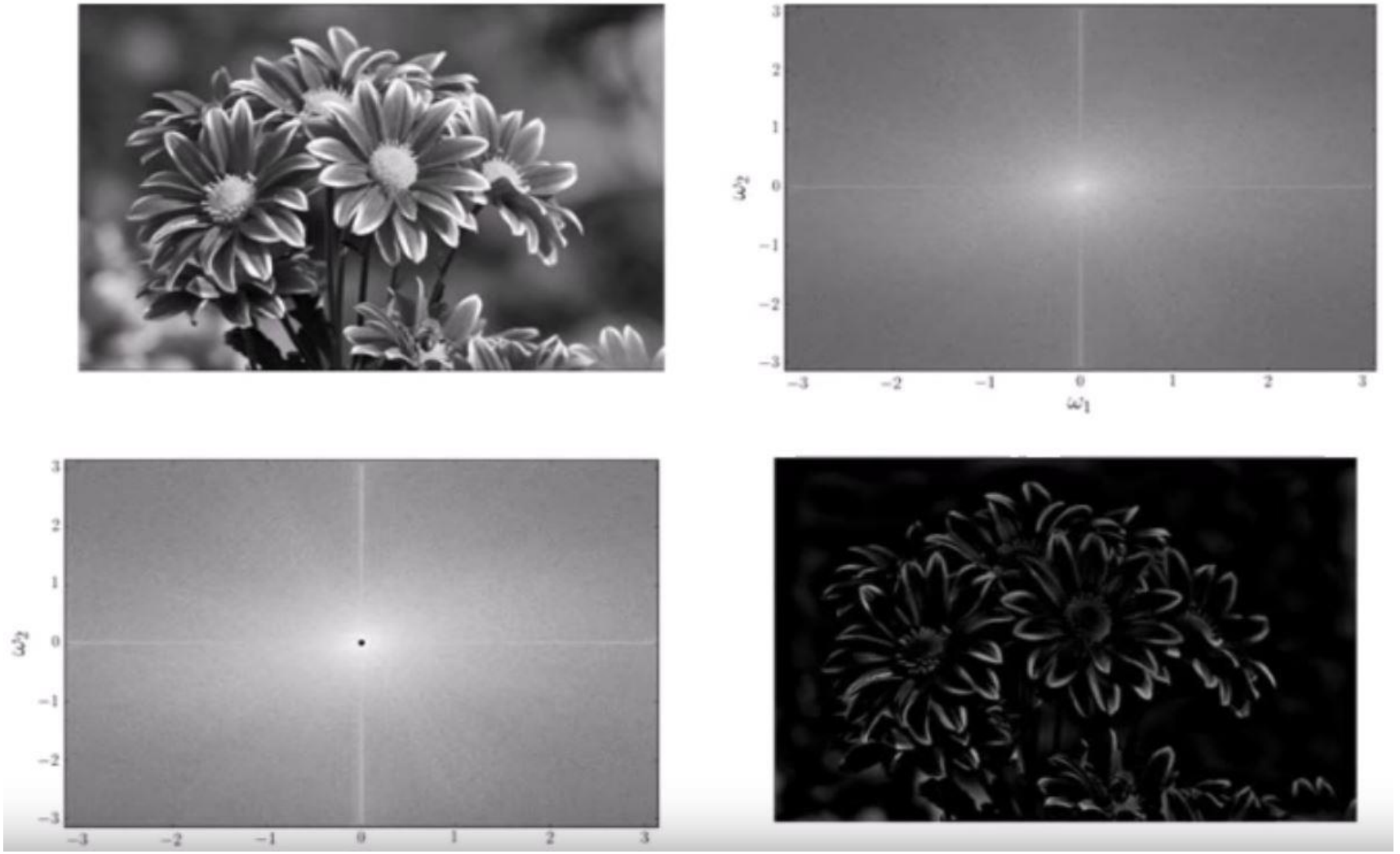
SIM – reconstruction – Fourier transform



source: https://www.youtube.com/watch?v=oACegp4iGi0

SIM – reconstruction – Fourier transform



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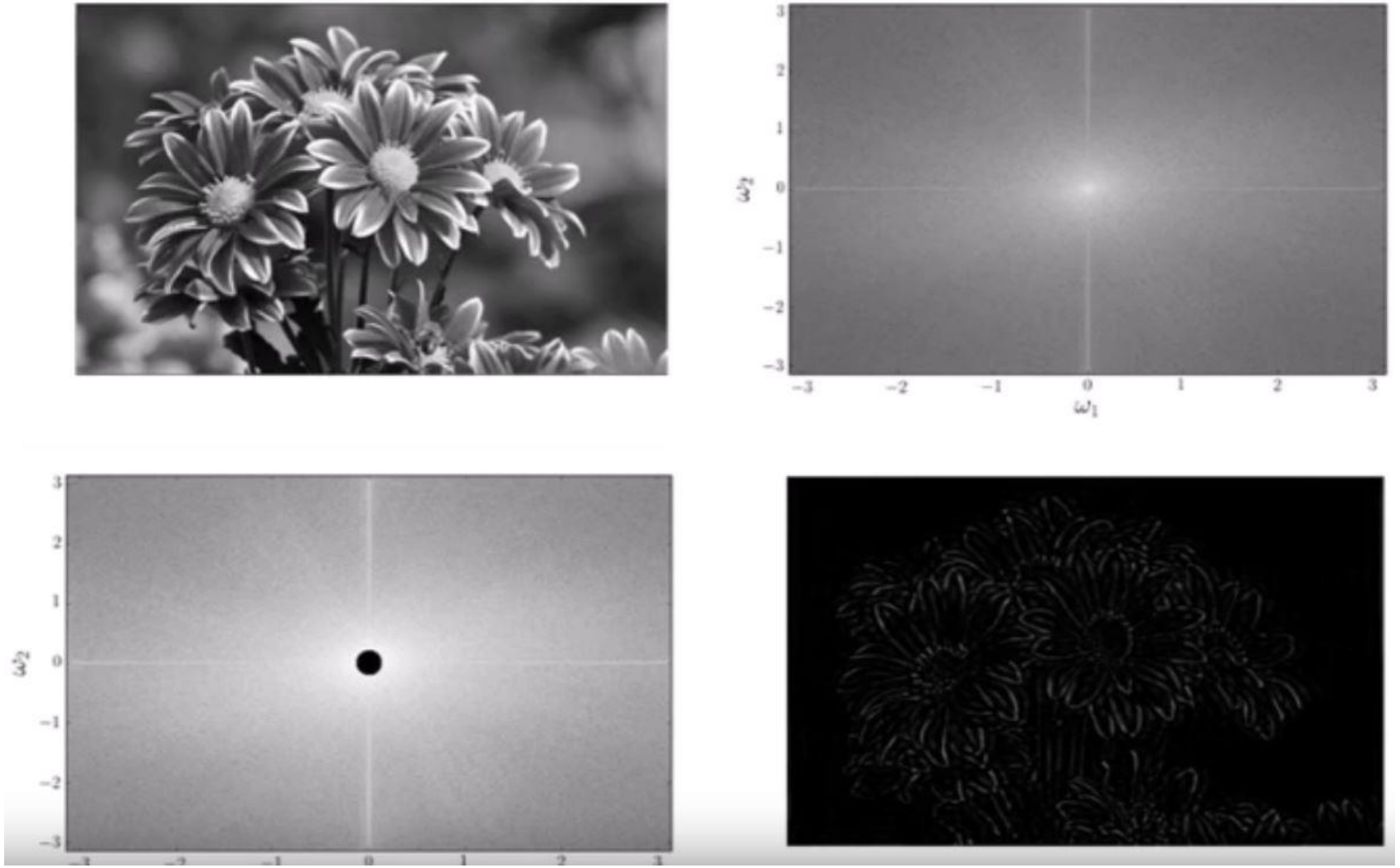

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SIM – reconstruction – **summary**

- **Transformation of acquired pictures into Fourier space** (Fourier transform)
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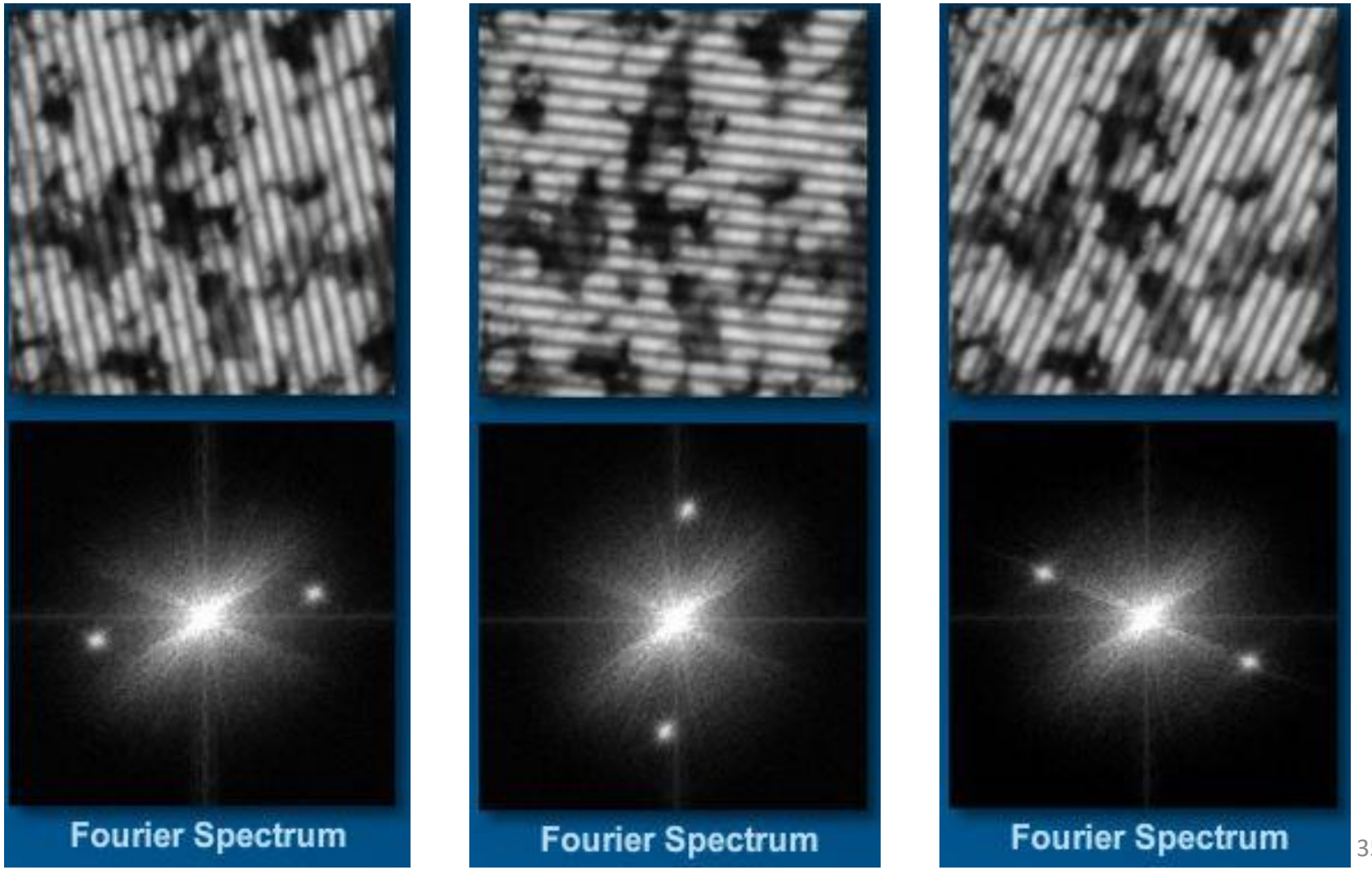
SIM – reconstruction – **Transformation into Fourier space** (grids rotated by 60°)



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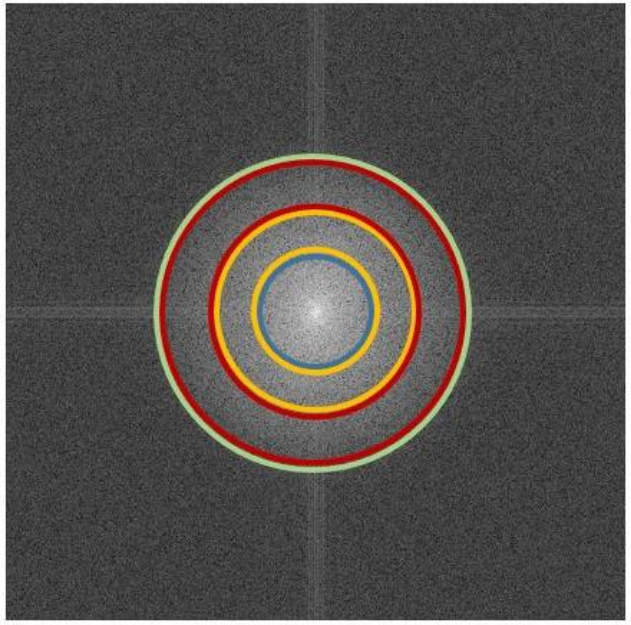

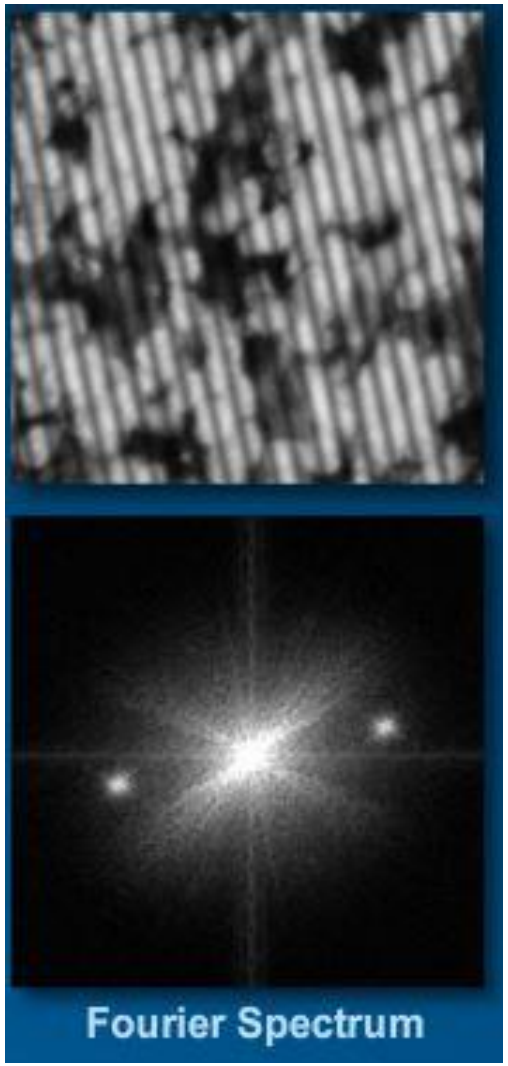

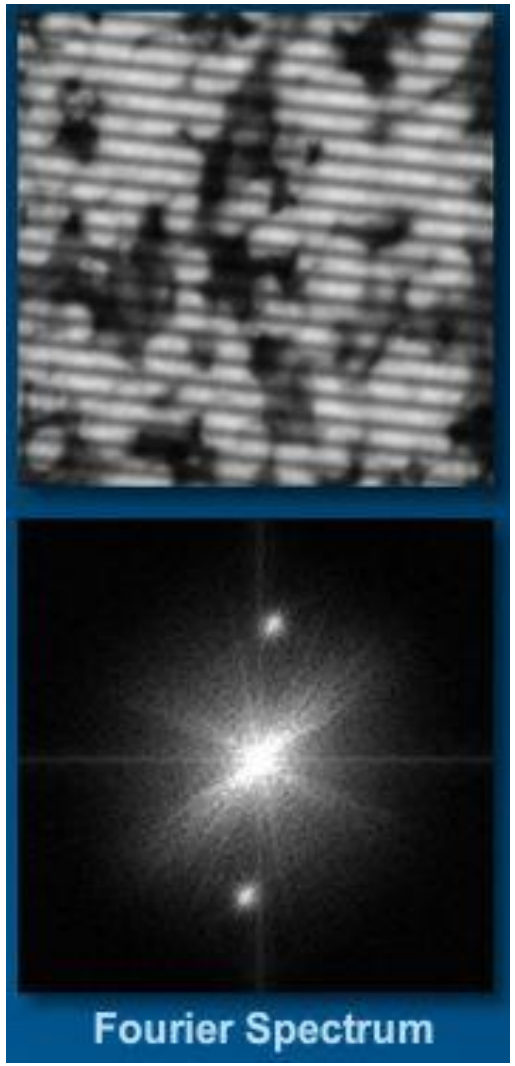

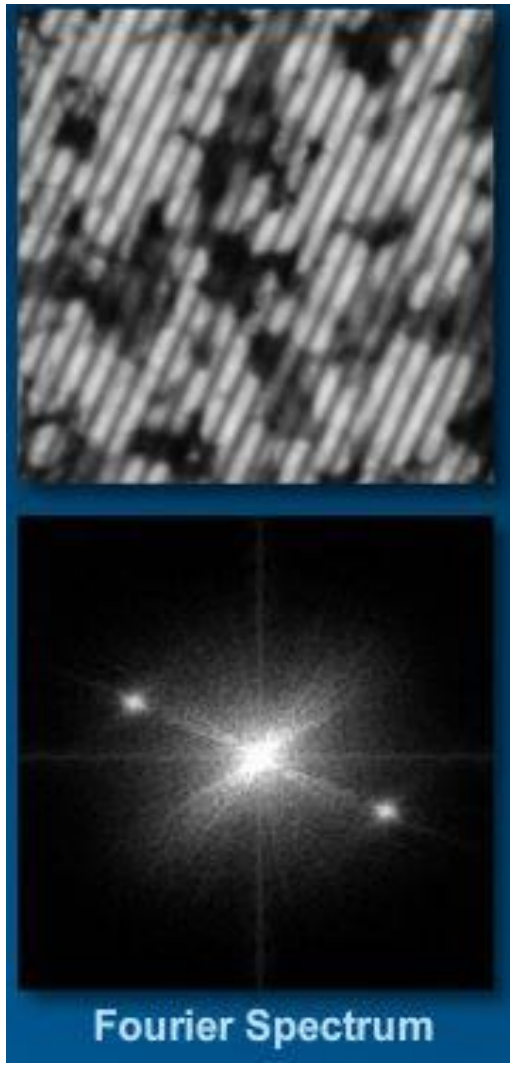

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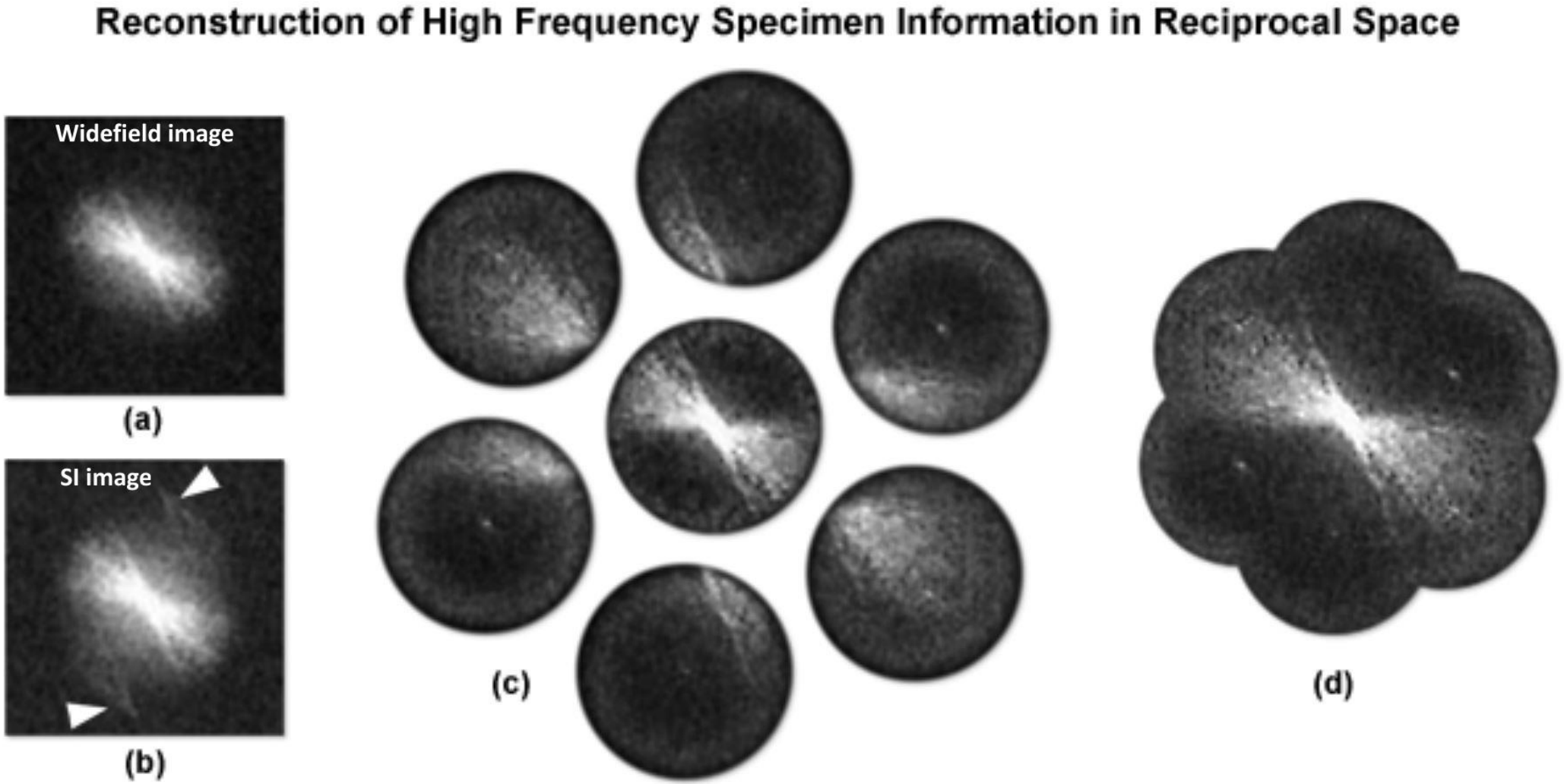

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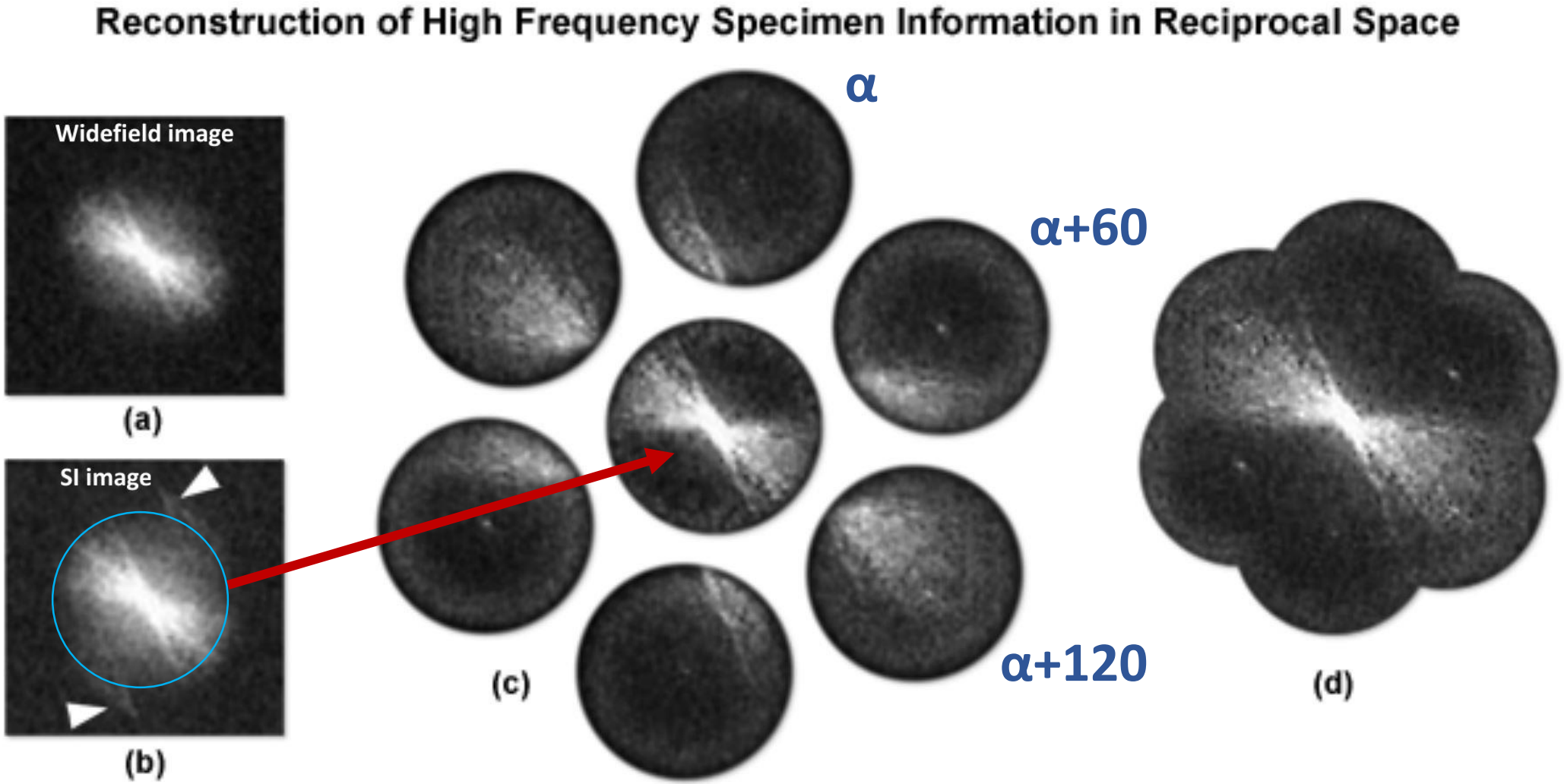

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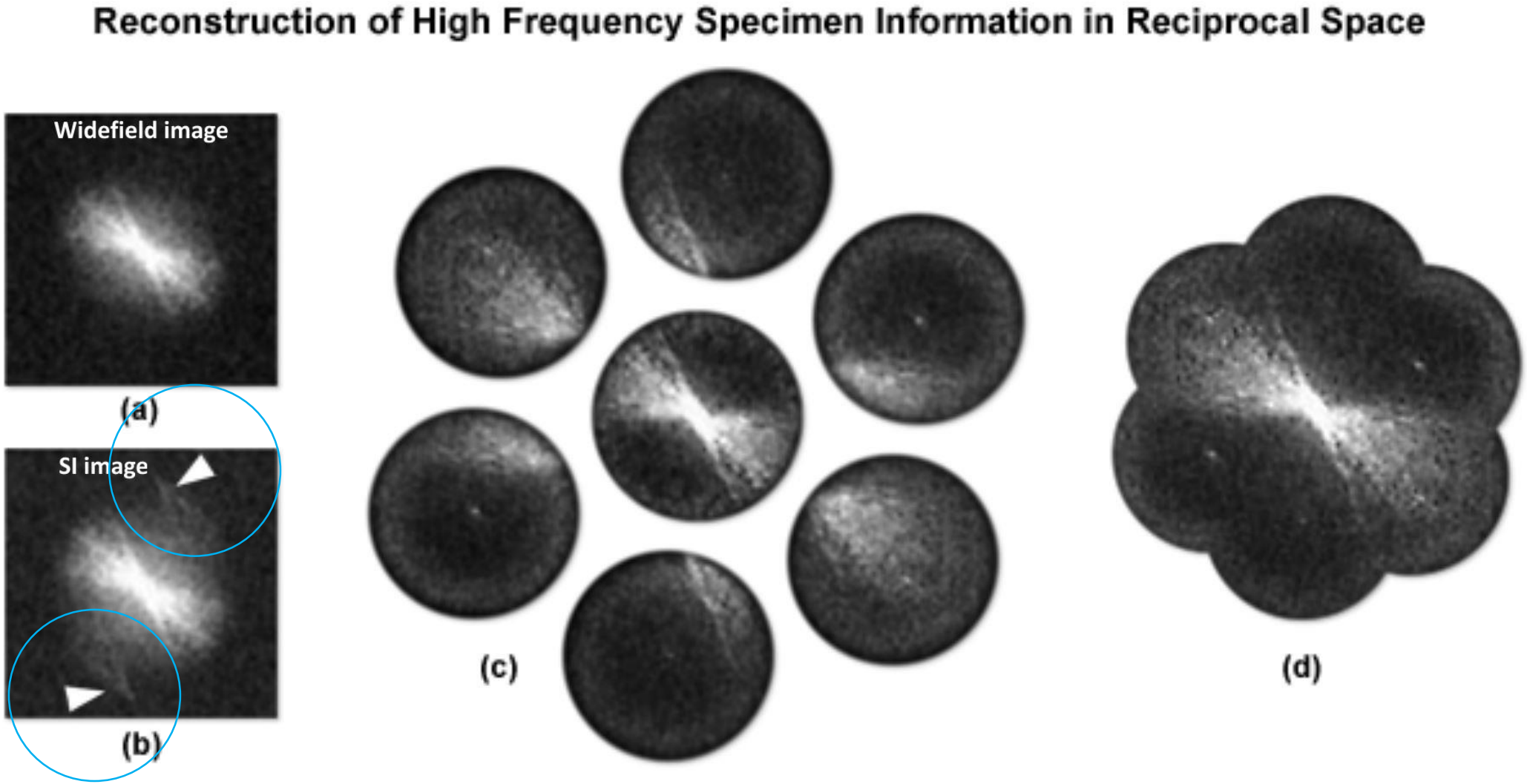
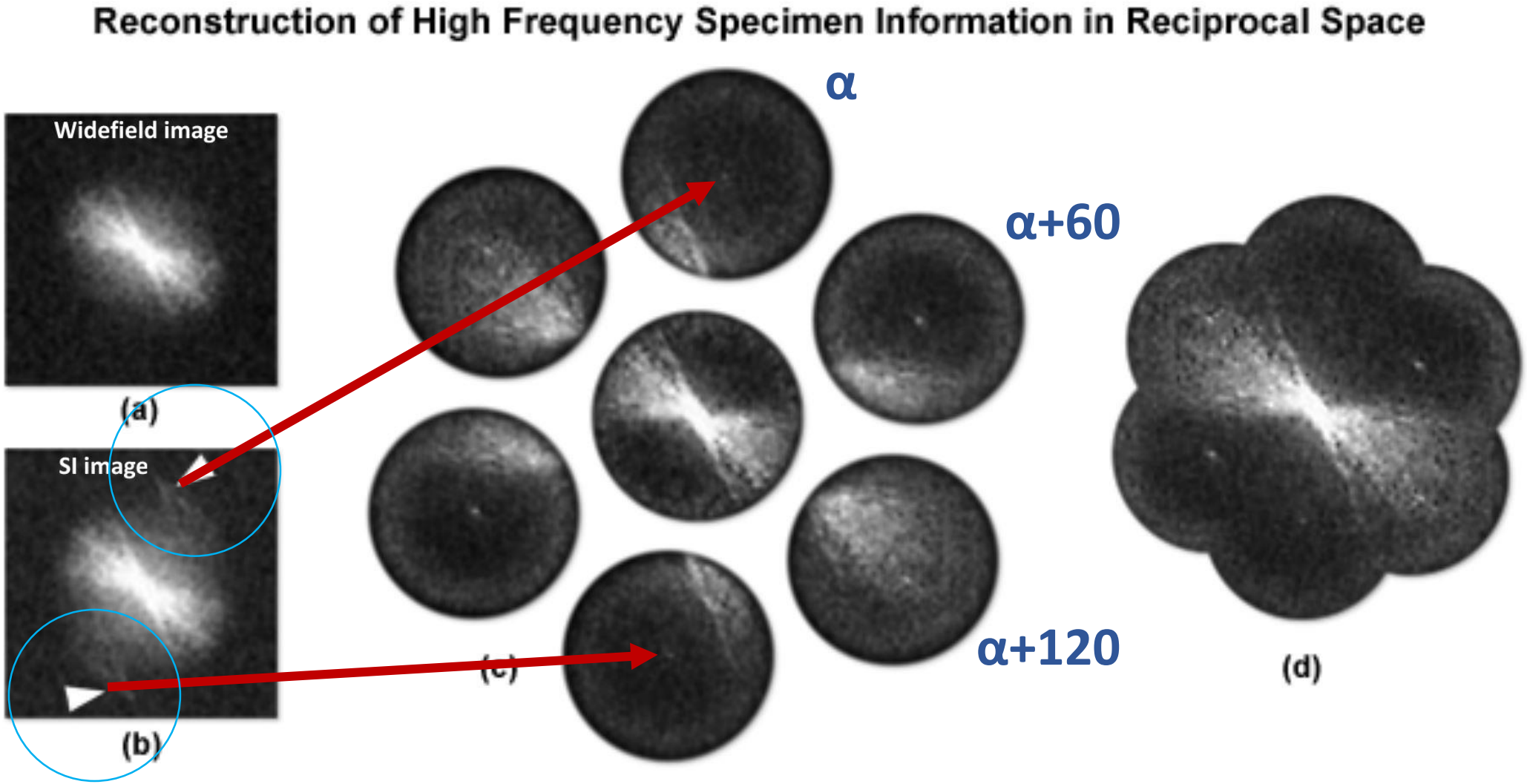


source: zeiss-campus.magnet.fsu.edu

SIM – reconstruction – Separation of components

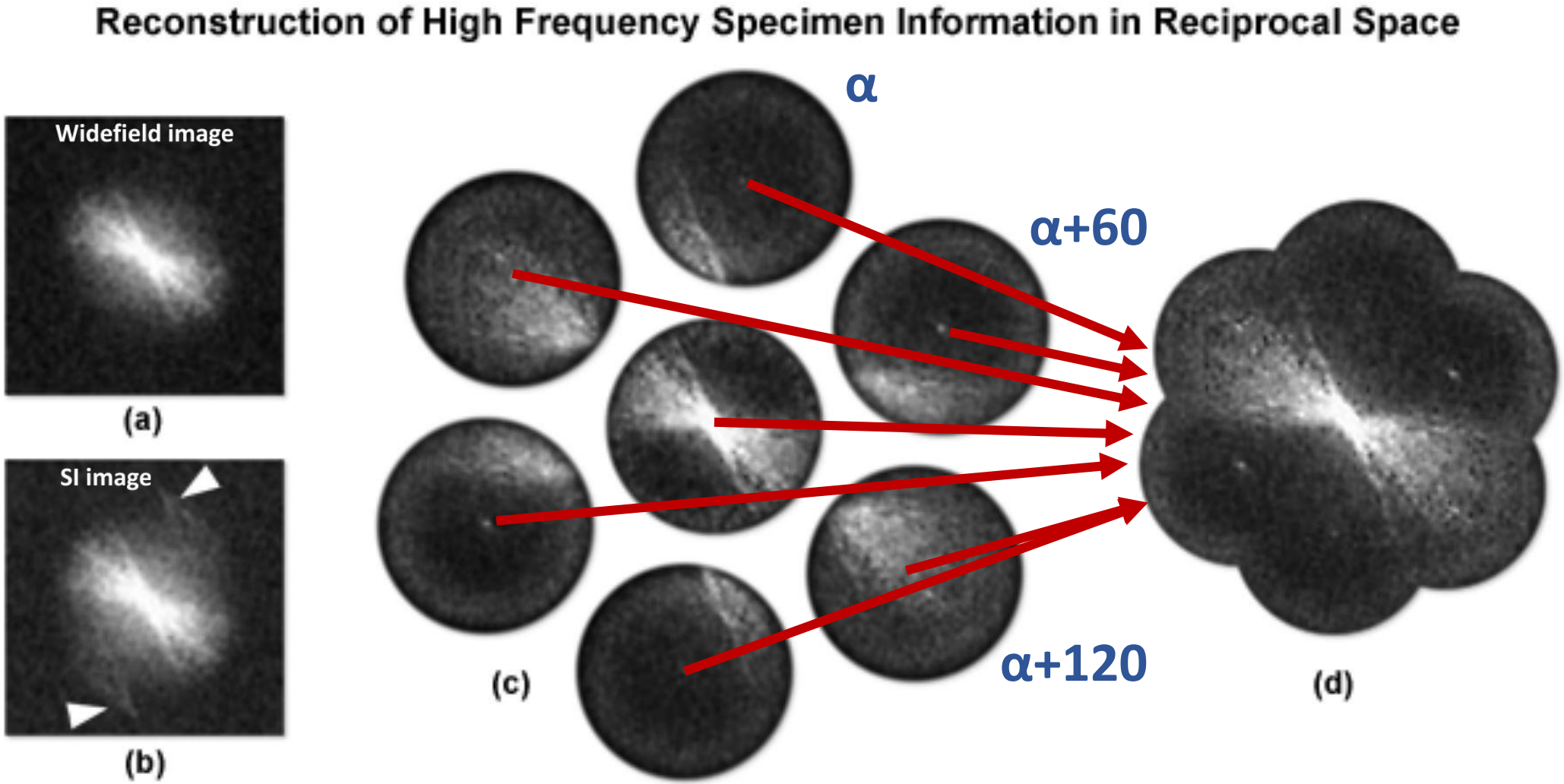


source: zeiss-campus.magnet.fsu.edu

SIM – reconstruction – **summary**

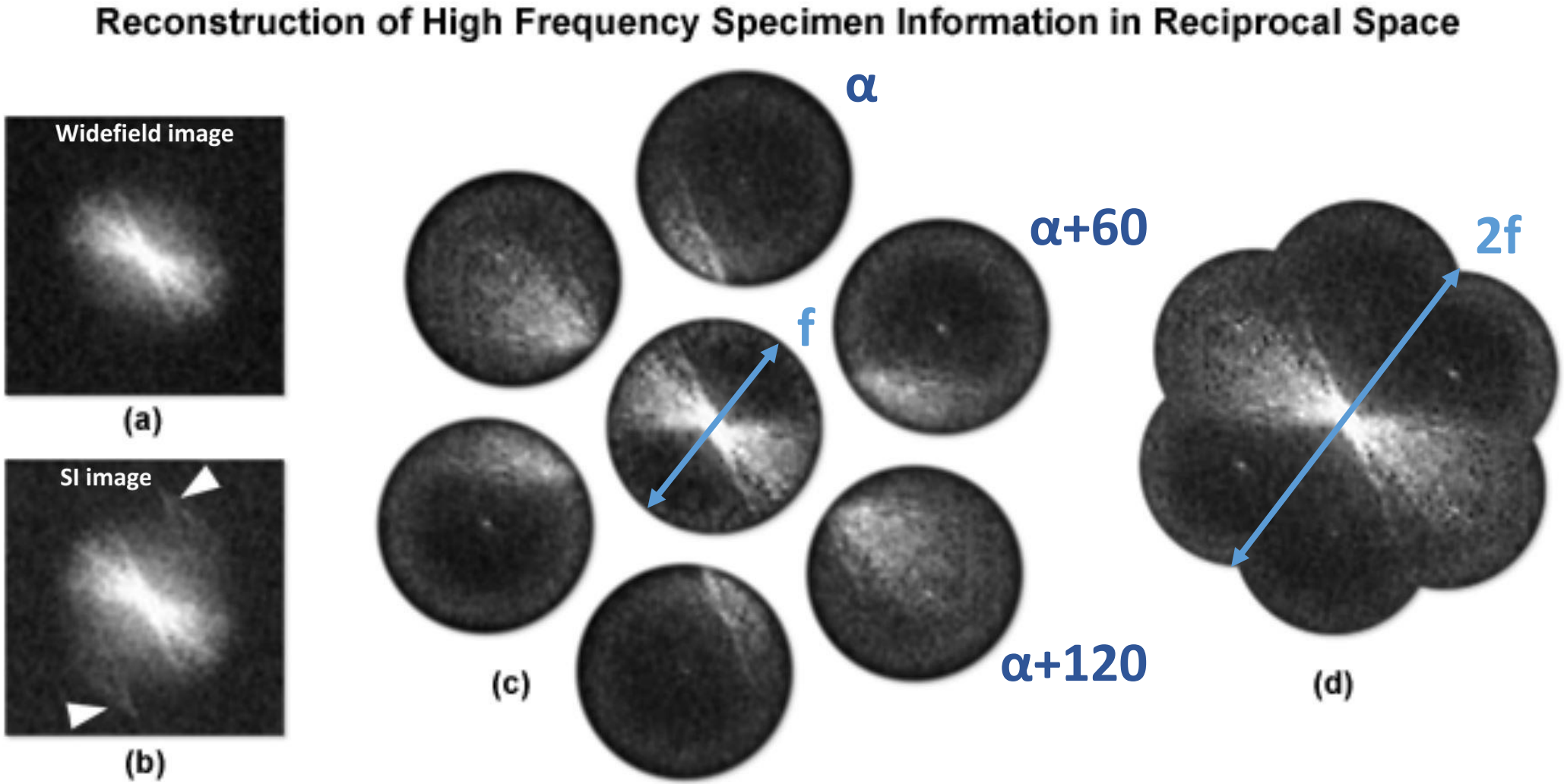
- Transformation of acquired pictures into Fourier space (Fourier transform)
- Separation of components
- **Alignment of separated components**
- Inverse Fourier transform of the reconstructed Fourier image

SIM – reconstruction – Alignment of separated components



source: zeiss-campus.magnet.fsu.edu

SIM – reconstruction – Alignment of separated components



source: zeiss-campus.magnet.fsu.edu

SIM – reconstruction – **summary**

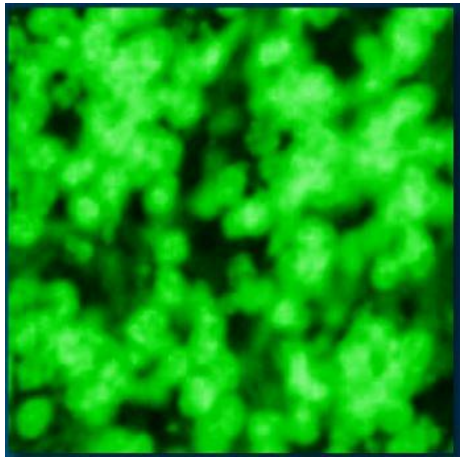
- Transformation of acquired pictures into Fourier space (Fourier transform)
- Separation of components
- Alignment of separated components
- **Inverse Fourier transform of the reconstructed image**

SIM – reconstruction – Inverse Fourier transform of the reconstructed image

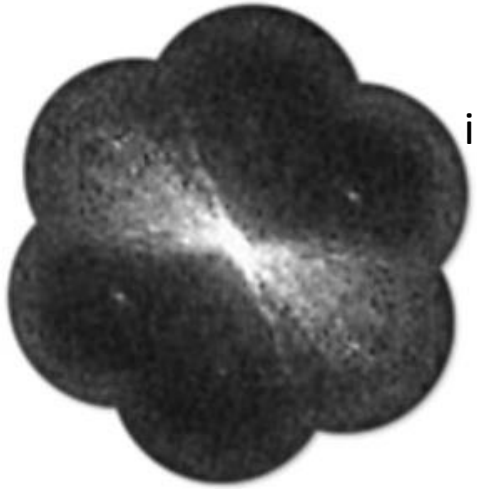
Fourier images

spatial images

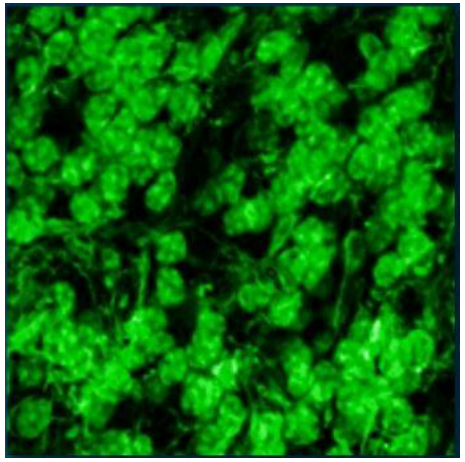
inverse Fourier transform



Widefield image



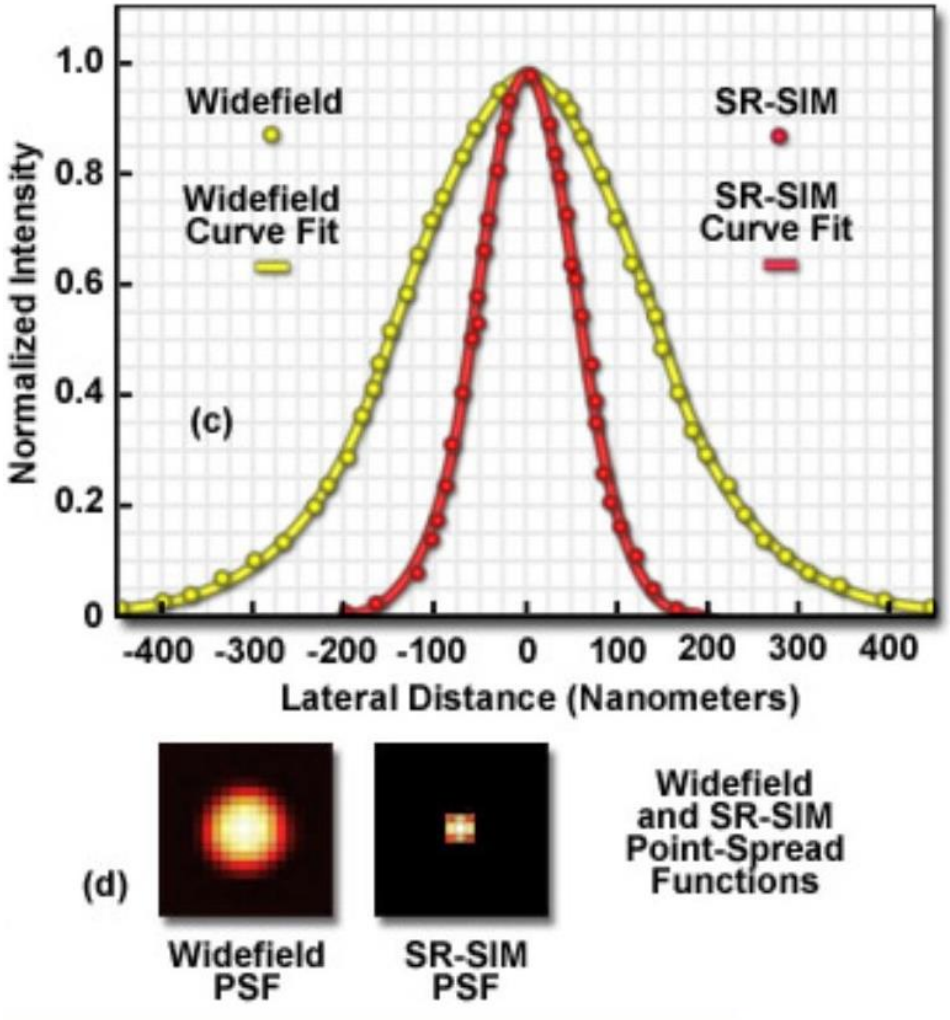
inverse Fourier transform



Super-resolution SIM image

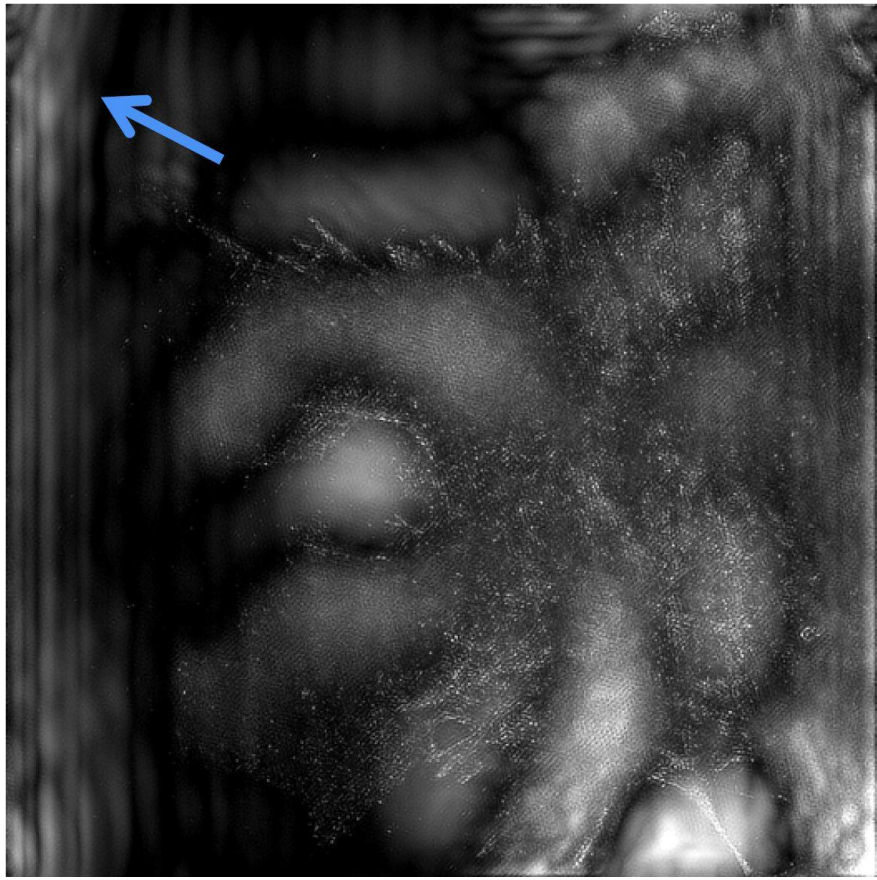
source: zeiss-campus.magnet.fsu.edu

SIM – point spread function (PSF)



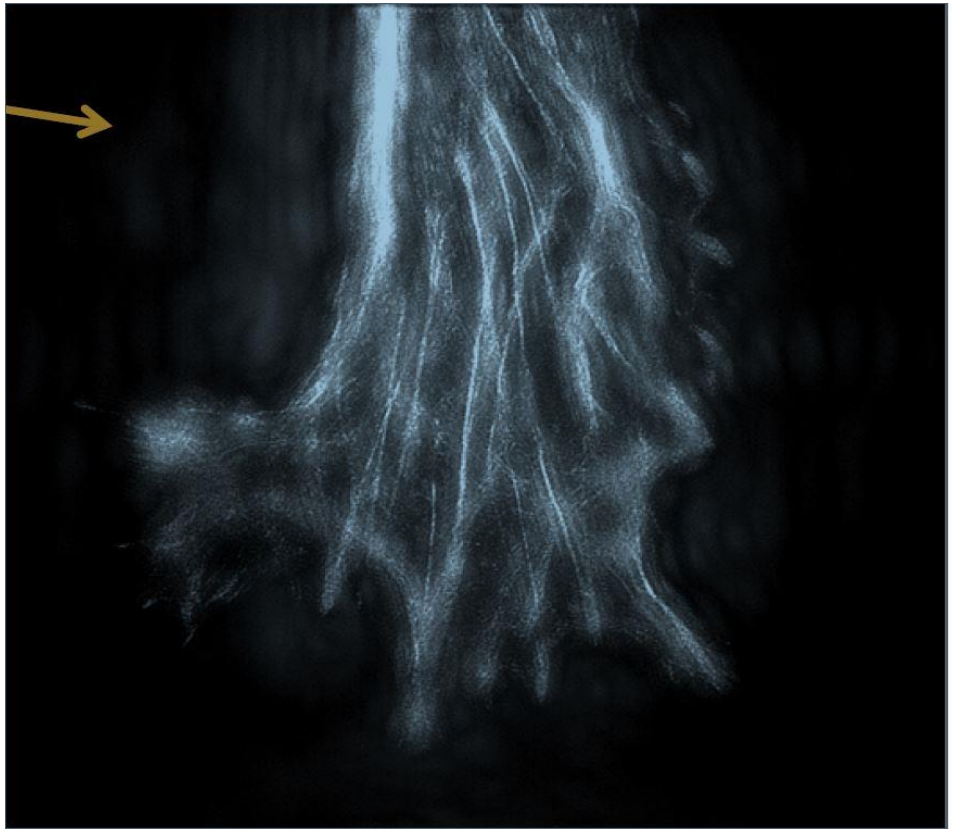
Reconstructing SIM data - artifacts

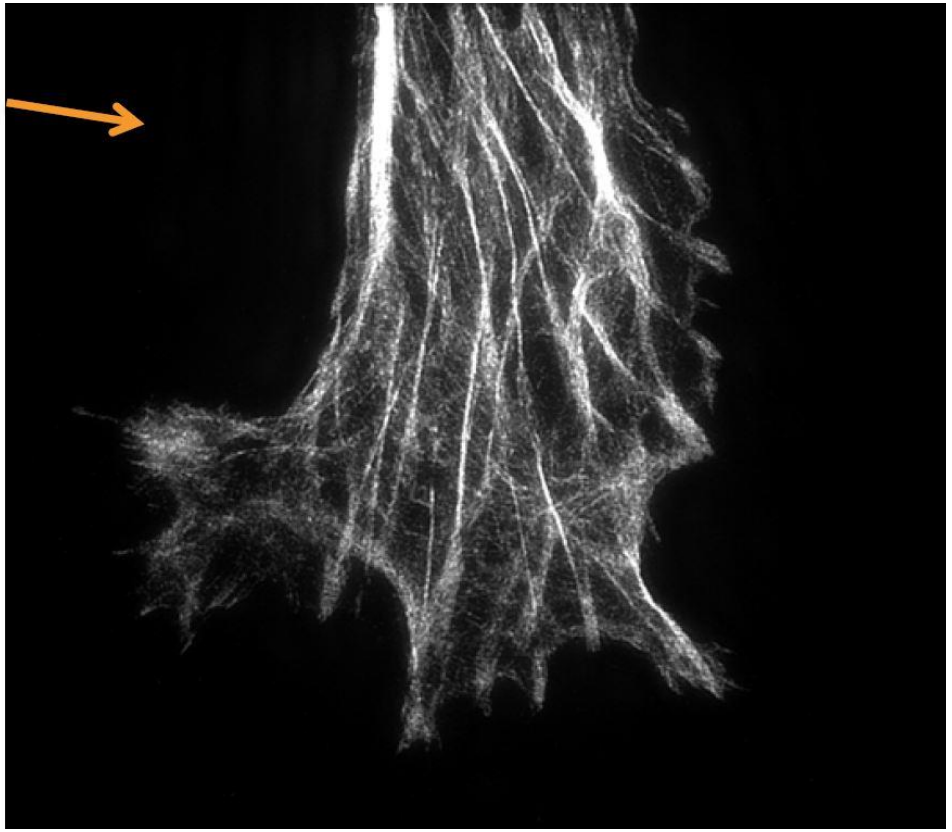
- **Photobleaching** causes „**haloing**“ or „**doubling**“



source: www.gelifesciences.com/gehcls_images/GELS/Pdf%20documents/2015-05-06-Pellett-Bitesizebio-Seminar.pdf

„Haloing“ or „doubling“





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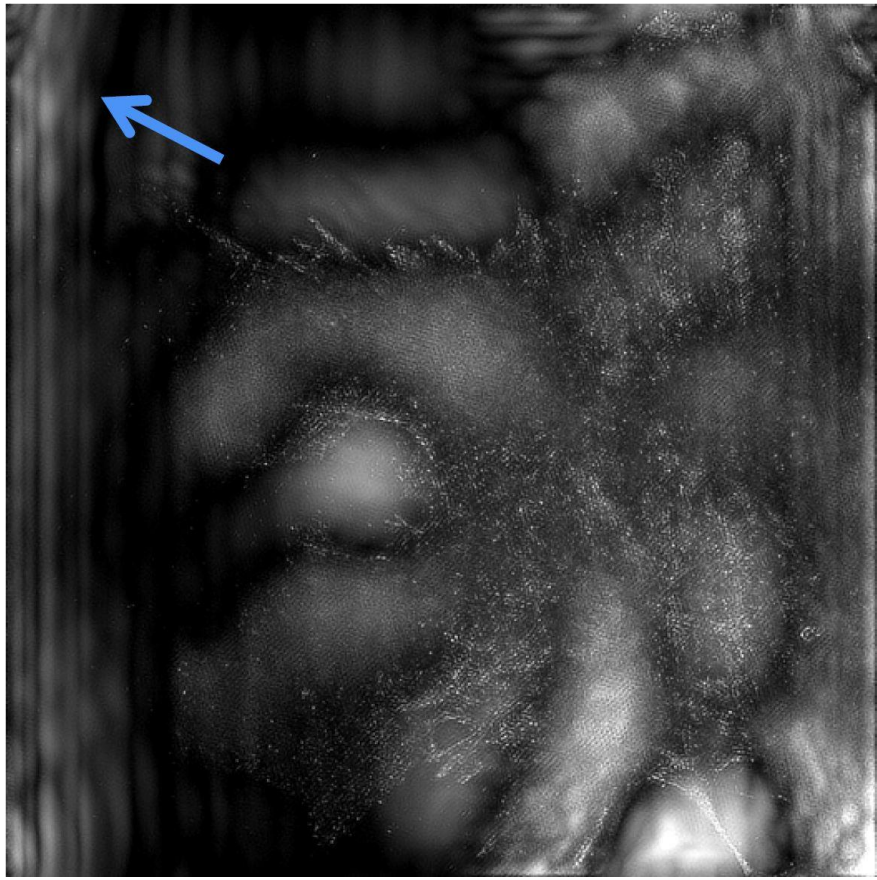
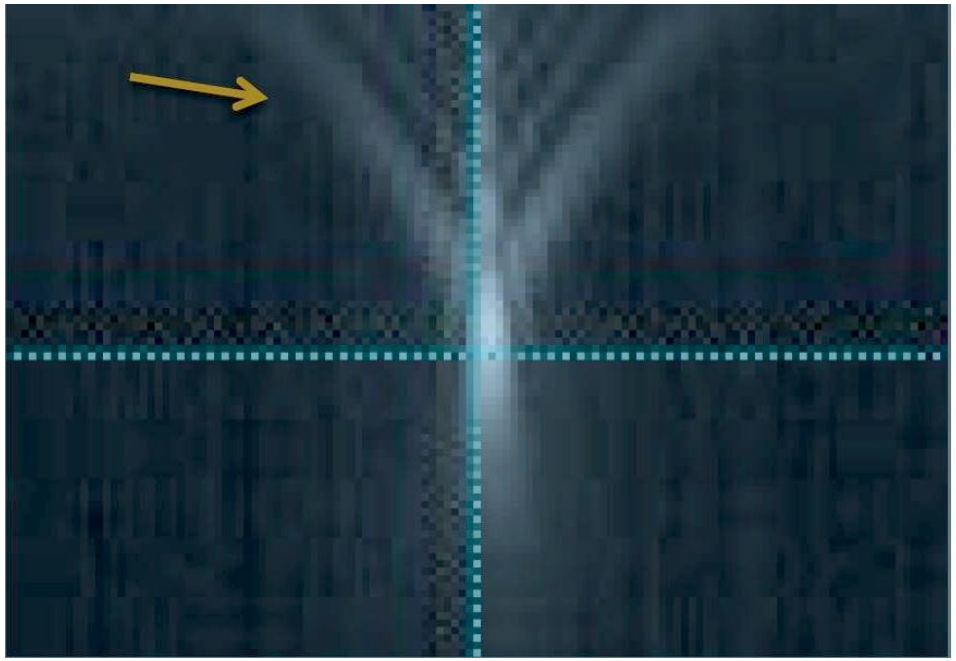
Reconstructing SIM data - artifacts

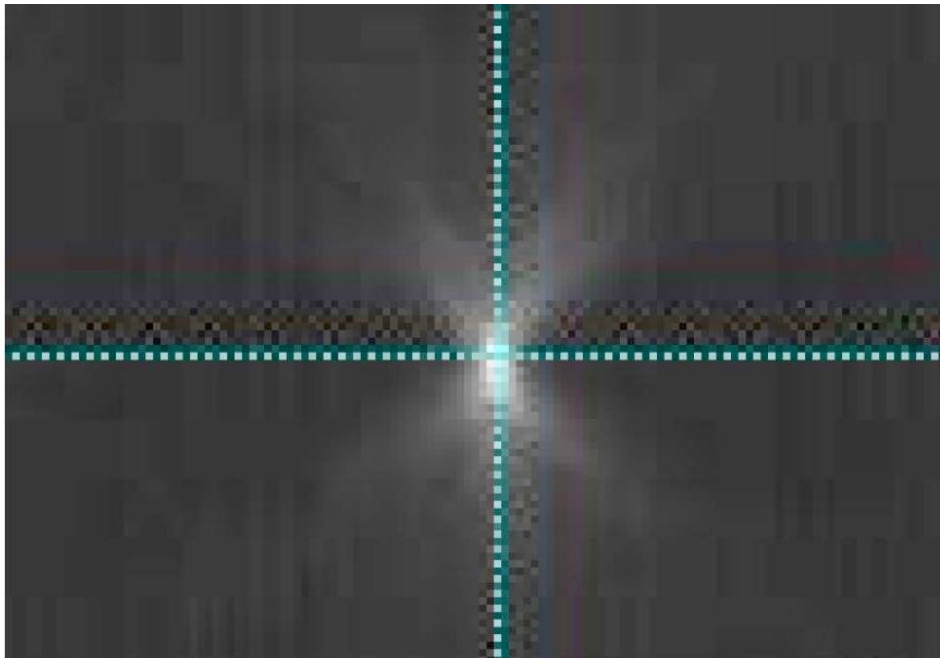
- **Photobleaching** causes „**haloing**“ or „**doubling**“
  - Due to an asymmetric PSF.



source: www.gelifesciences.com/gehcls_images/GELS/Pdf%20documents/2015-05-06-Pellett-Bitesizebio-Seminar.pdf

Asymmetric PSF





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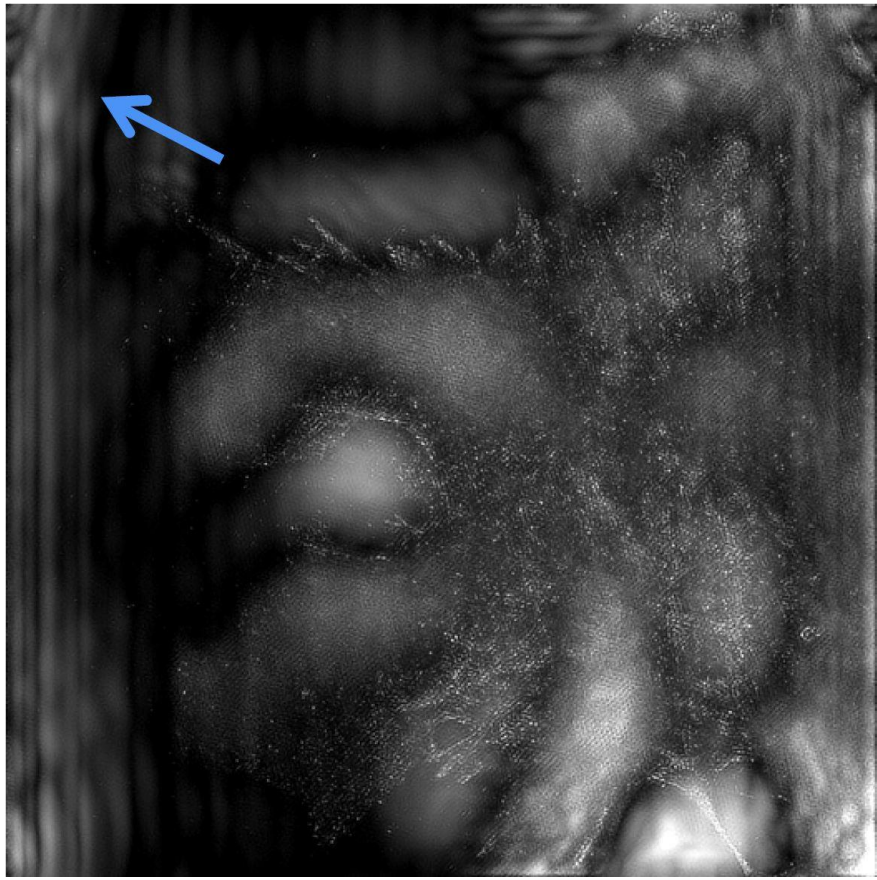
Reconstructing SIM data - artifacts

- **Photobleaching** causes „**haloing**“ or „**doubling**“
  - Due to an asymmetric PSF.
  - Do not bleach more than ~30% across the stack
    - Watch maximal image intensities during the experiment



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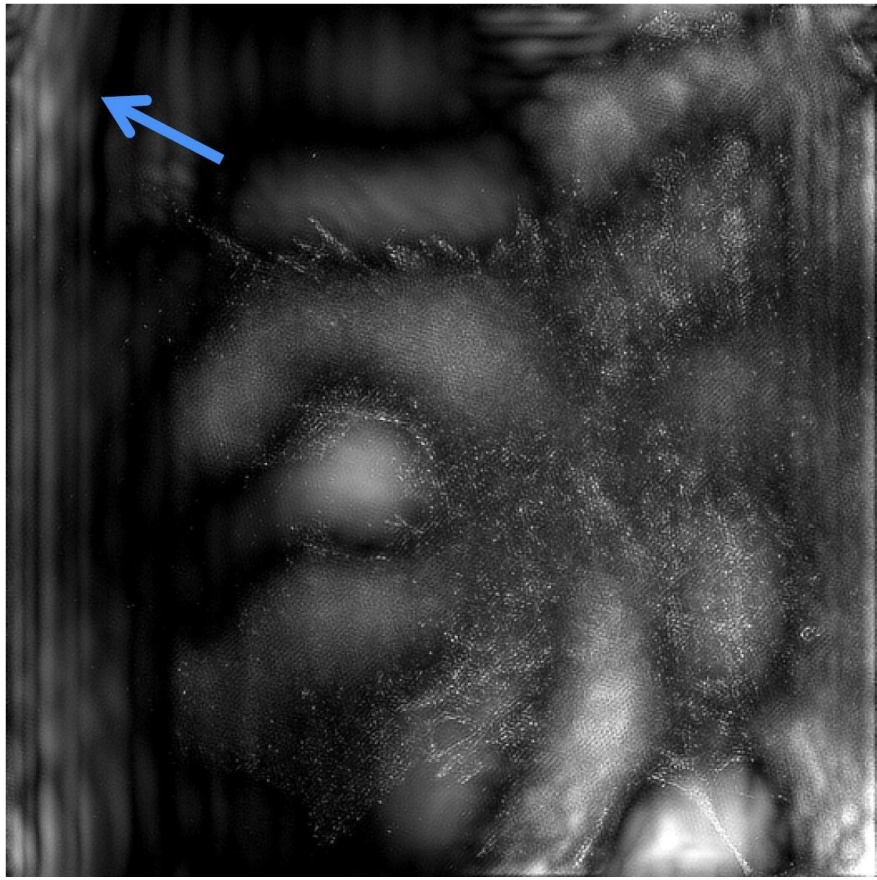
Reconstructing SIM data - artifacts

- **Photobleaching** causes „**haloing**“ or „**doubling**“
  - Due to an asymmetric PSF.
  - Do not bleach more than ~30% across the stack
    - Watch maximal image intensities during the experiment
  - If have „haloing“ in reconstruction, change imaging parameters to minimize photobleaching:
    - Lower %T, increase exposure
    - Increase %T, decrease exposure
    - Smaller z stack (fewer # of optical sections)
    - **Use antifade reagents (NPG, DABCO…)**



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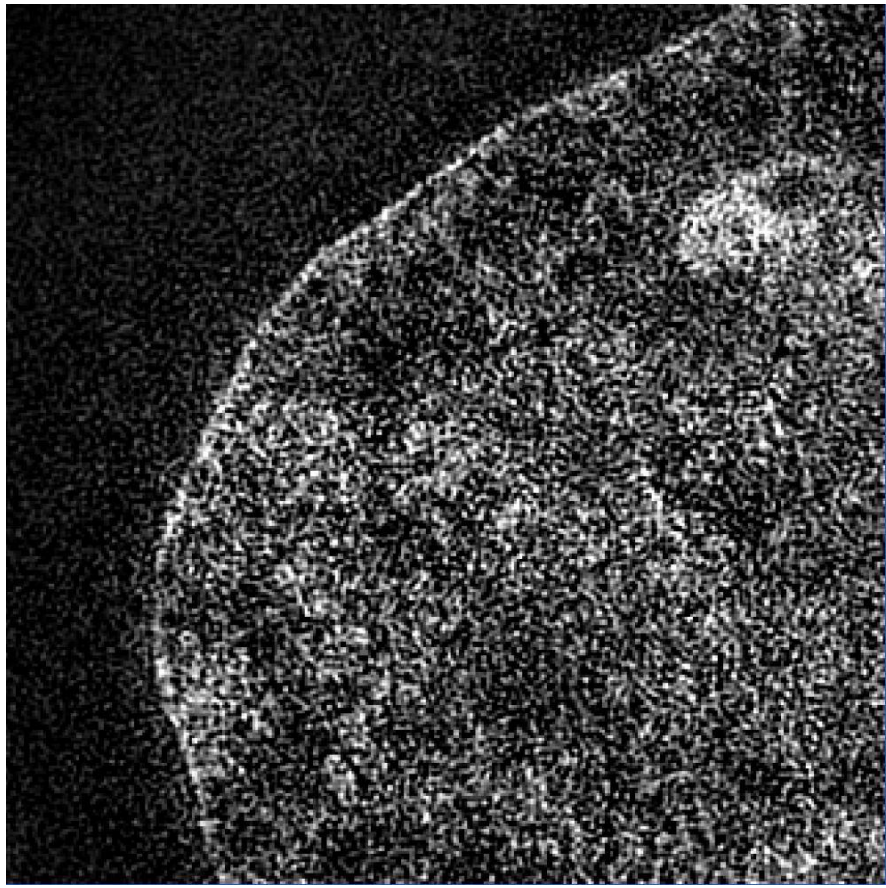
Reconstructing SIM data - artifacts

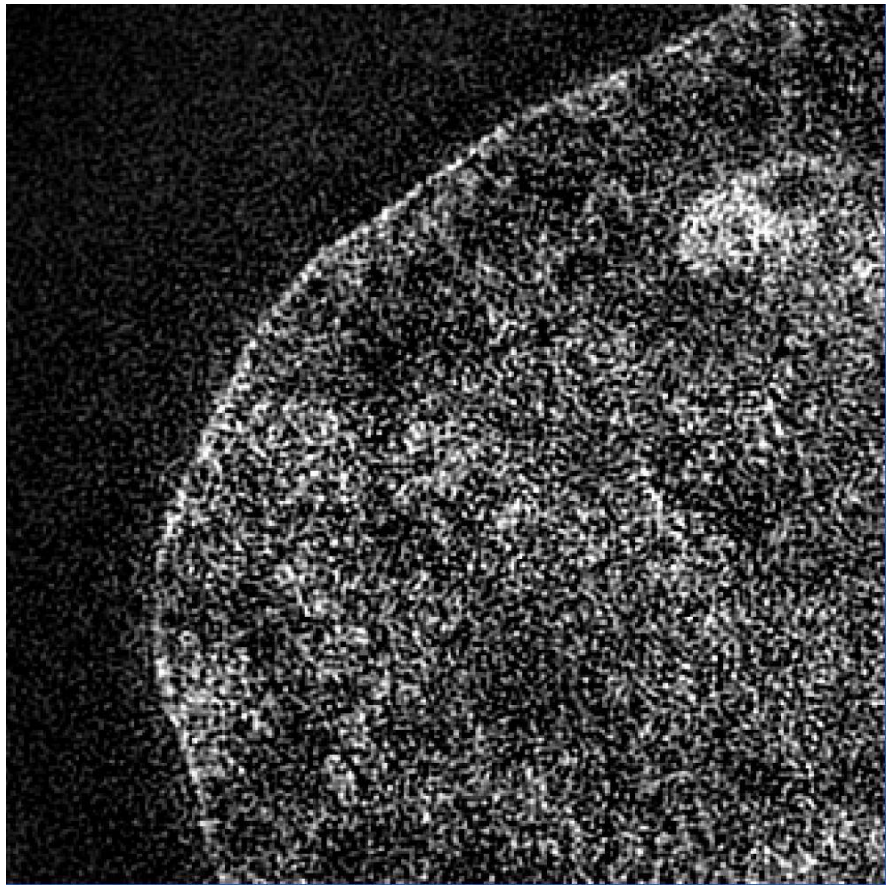
- **Diffuse labeling** & **bad S/N** gives „**honeycomb**“
  = Regular/hexagonal pattern



source: www.gelifesciences.com/gehcls_images/GELS/Pdf%20documents/2015-05-06-Pellett-Bitesizebio-Seminar.pdf

Reconstructing SIM data - artifacts

- **Diffuse labeling** & **bad S/N** gives „**honeycomb**“
  = Regular/hexagonal pattern
- Not all samples are appropriate for SIM
  - SIM works best with samples that have discrete structures
  - Diffuse labeling and high background will give honeycomb



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Reconstructing SIM data - artifacts

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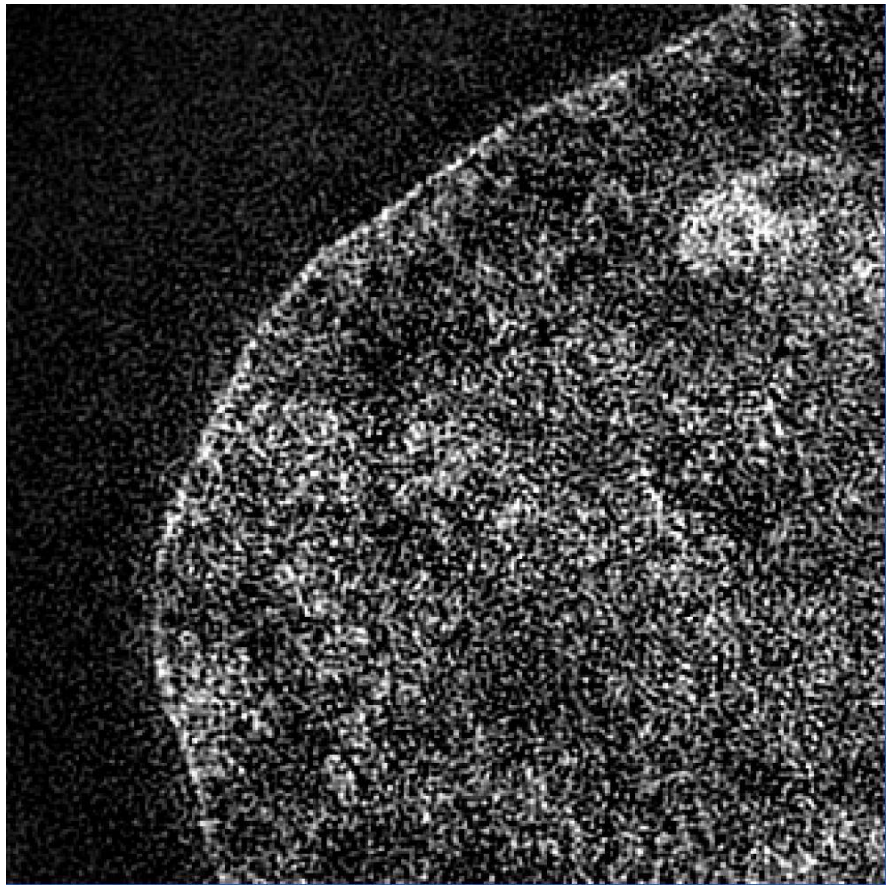
  - Diffuse labeling and high background will give honeycomb
- Increase S/N **without photobleaching**



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Reconstructing SIM data - artifacts

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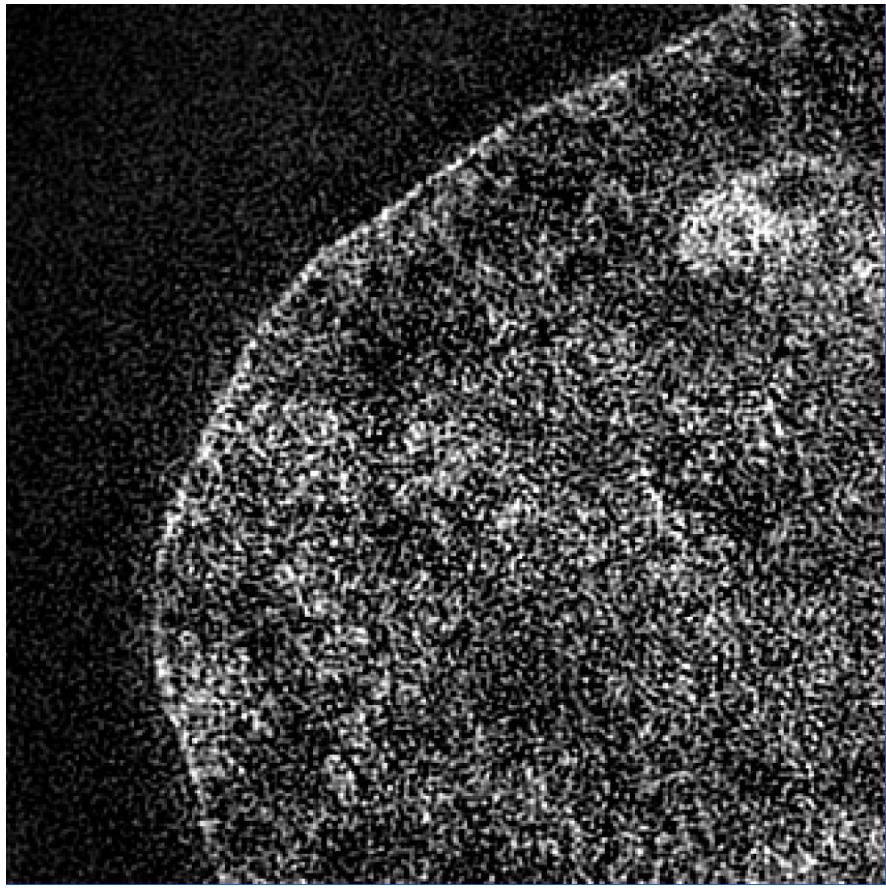
  = Regular/hexagonal pattern
- Not all samples are appropriate for SIM
  - SIM works best with samples that have discrete structures
  - Diffuse labeling and high background will give honeycomb
- Increase S/N **without photobleaching**
- Increase **Wiener filter constant**
  - This removes high frequency data
  - Decreases resolution!



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SIM – summary

- We learned:
  - Advantages and drawbacks
  - Principles and basic set-ups
  - Reconstruction algorithm of super-resolution images
  - Reconstruction artifacts

Thanks for your attention!