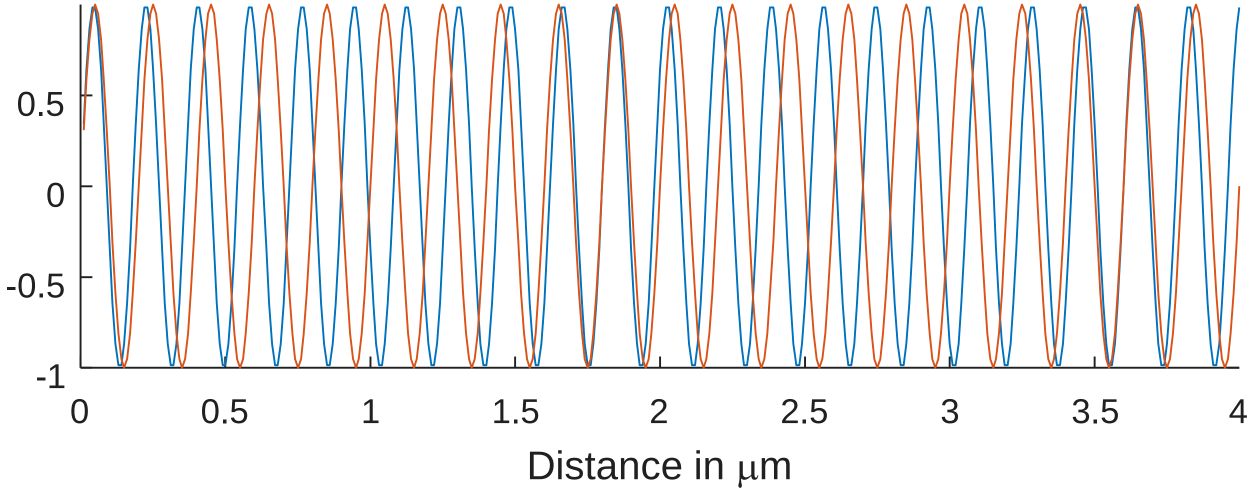
SIM, biosensors

Administrative

- HW 7 due 11/17
- Final 12/19 – 4:30 – 7:00 in this room
- Grad student presentations
  - Teams of 2 (one team of 3)
  - Read a modern (previous 2 years) paper on optics
  - Prepare 12 minute presentation
  - Paper can not be from your current or previous lab
  - Let me know teams by 11/29

- Last class
  - 2D Fourier transforms
  - Intro to SIM
- This class
  - More SIM
  - Biosensors

Beats move the invisible frequencies lower, so we can detect them with our scope

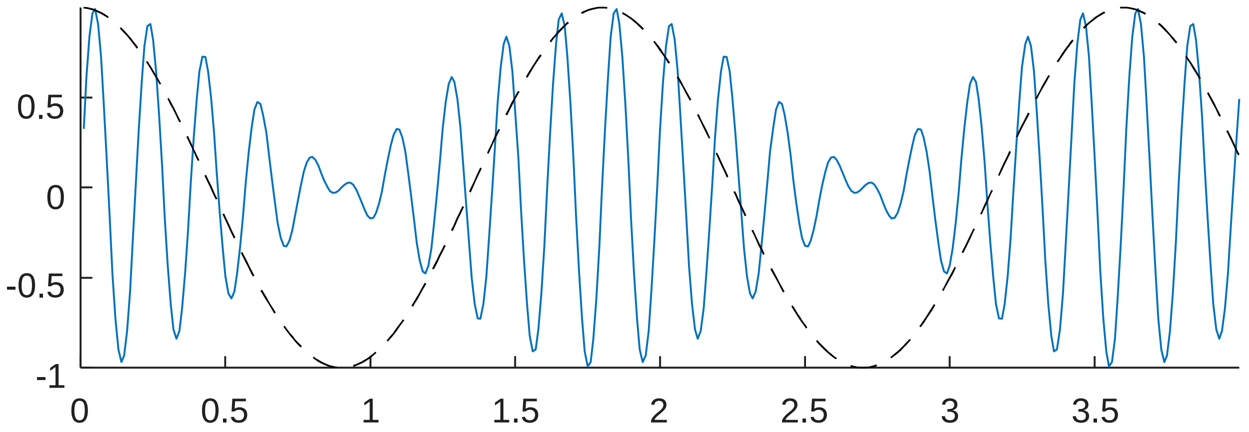


180 nm wave
(Invisible)

200 nm wave
(Resolution limit)

$$f_{beat} = |f_1 - f_2|$$

$$f = c/\lambda$$

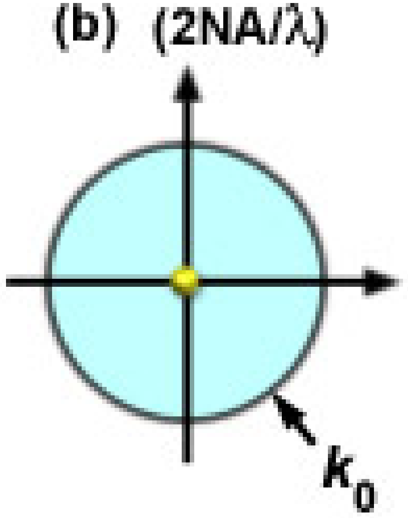


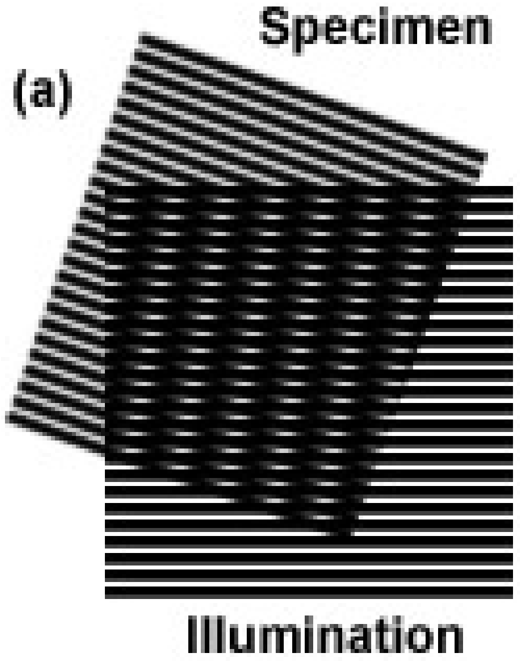
1200 nm wave
(Visible)

Spatial frequencies

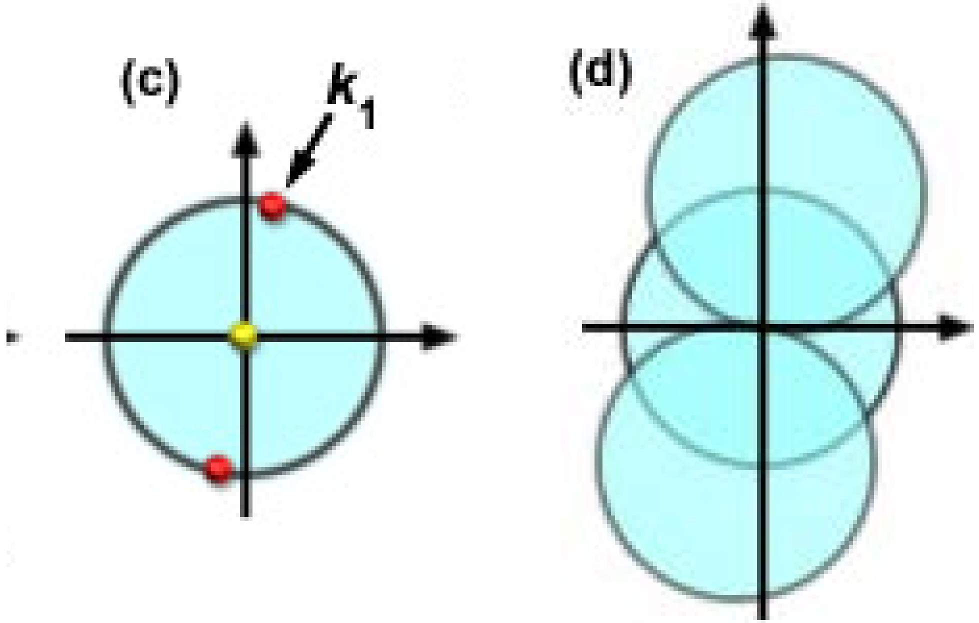
k_0 = 1/diffraction limit

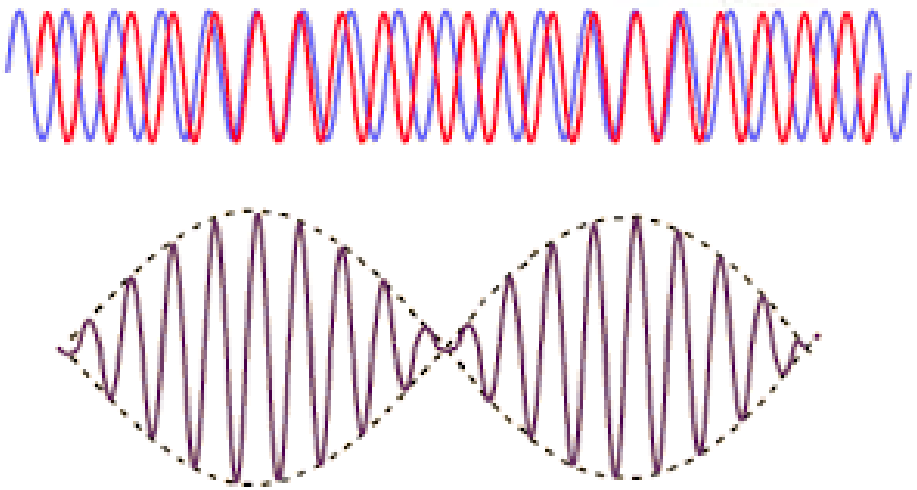
$k_0 = 2NA / \lambda_{em}$ - Maximum observable frequency





Moire fringes can take a two high frequencies that are offset, and make a lower freq appear

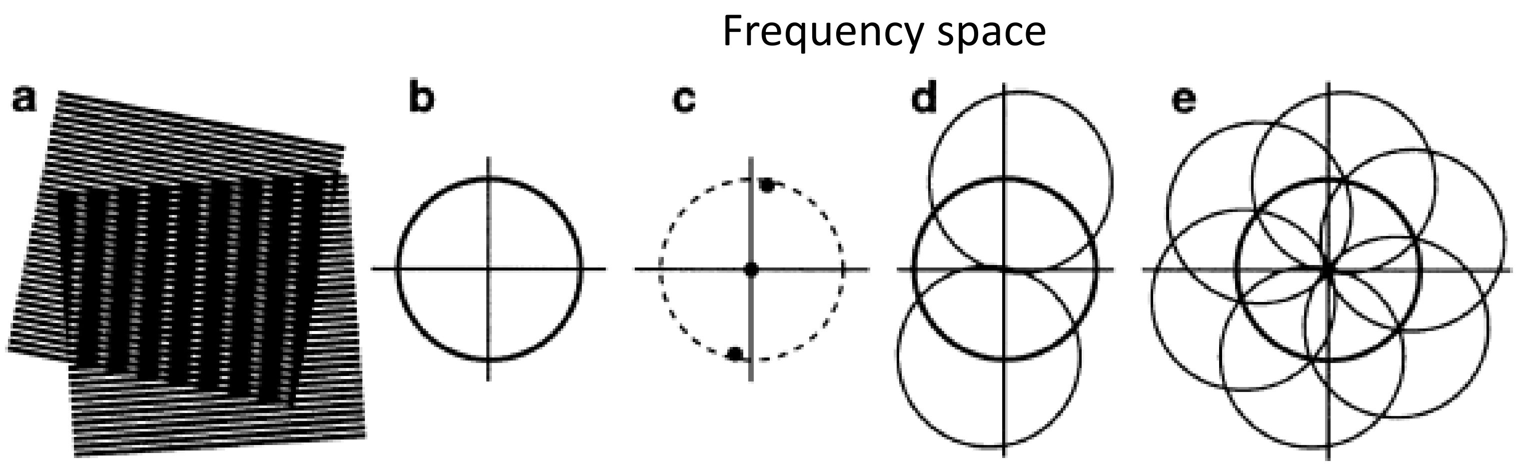


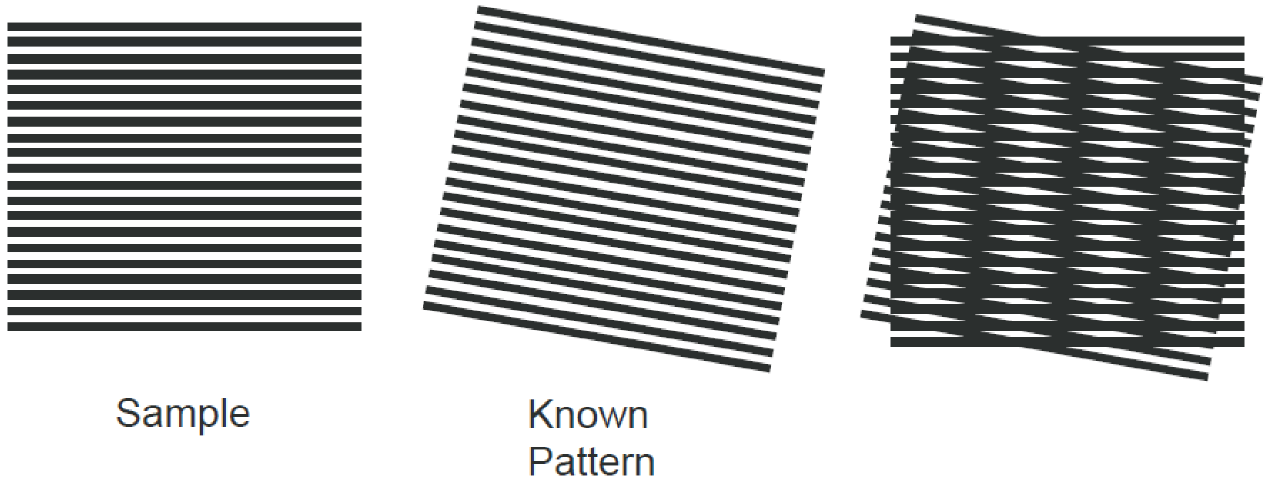


Similar to beat frequencies – two high frequency waves sum together for a low frequency output

We can pattern the illumination to convolve higher order frequencies

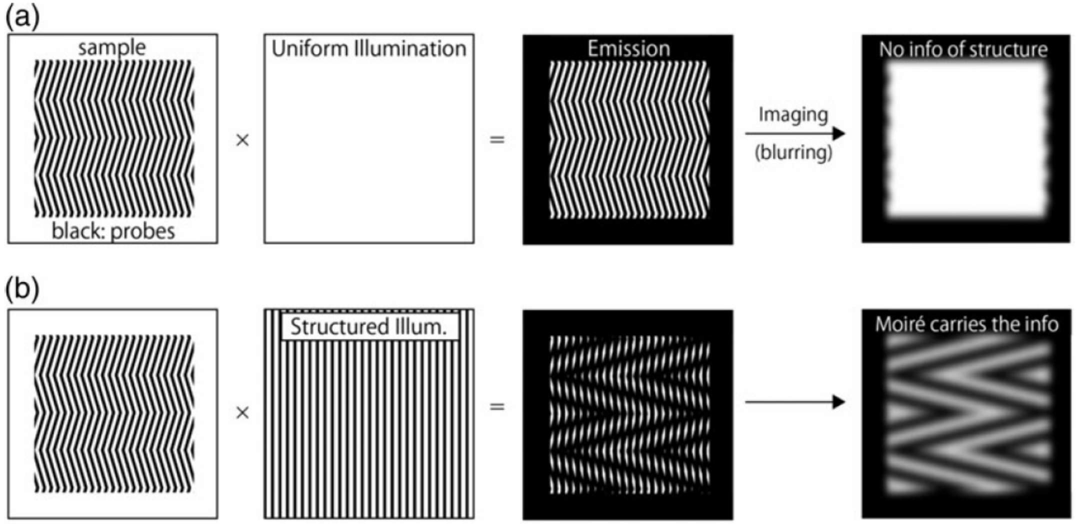
- Super imposing the same frequency at an angle results in Moire' fringes
- We can pattern our illumination to have a sinusoid on the sample
- The frequency of our patterned sinusoid will move the resulting information in frequency space to the edges
- We can add in higher frequency information contained in the sinusoidal illumination pattern
- Repeating this process at many angles enables full reconstruction with increased frequency content

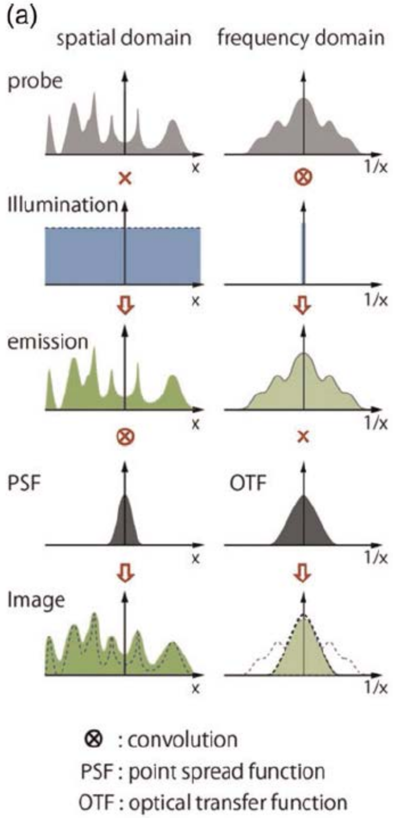




SIM Theory

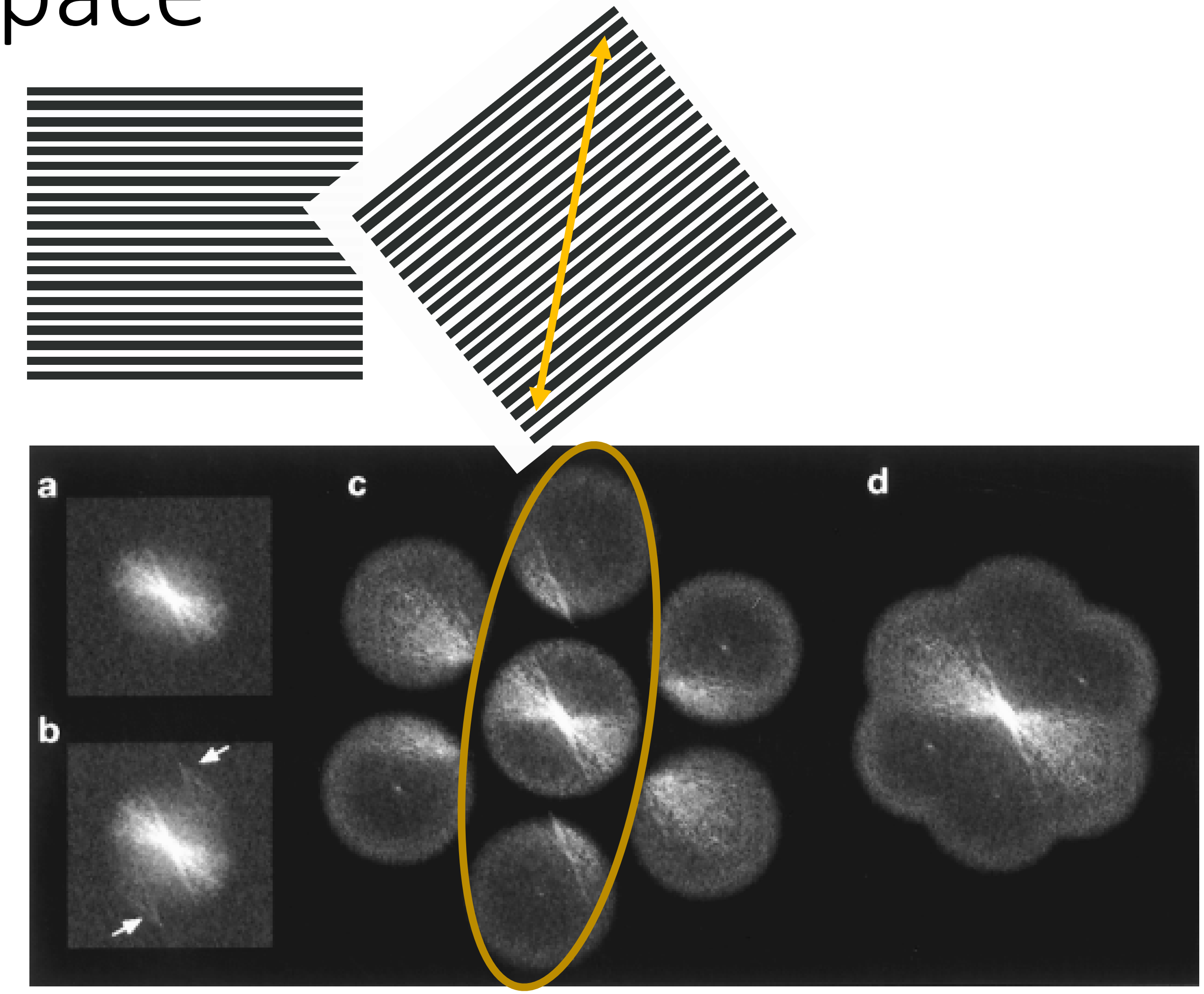
- Structured illumination adds higher order frequencies
- In the frequency domain, those higher orders are now transmitted through the NA
- All processing is carried out in frequency domain, then IFFT to convert back to the image

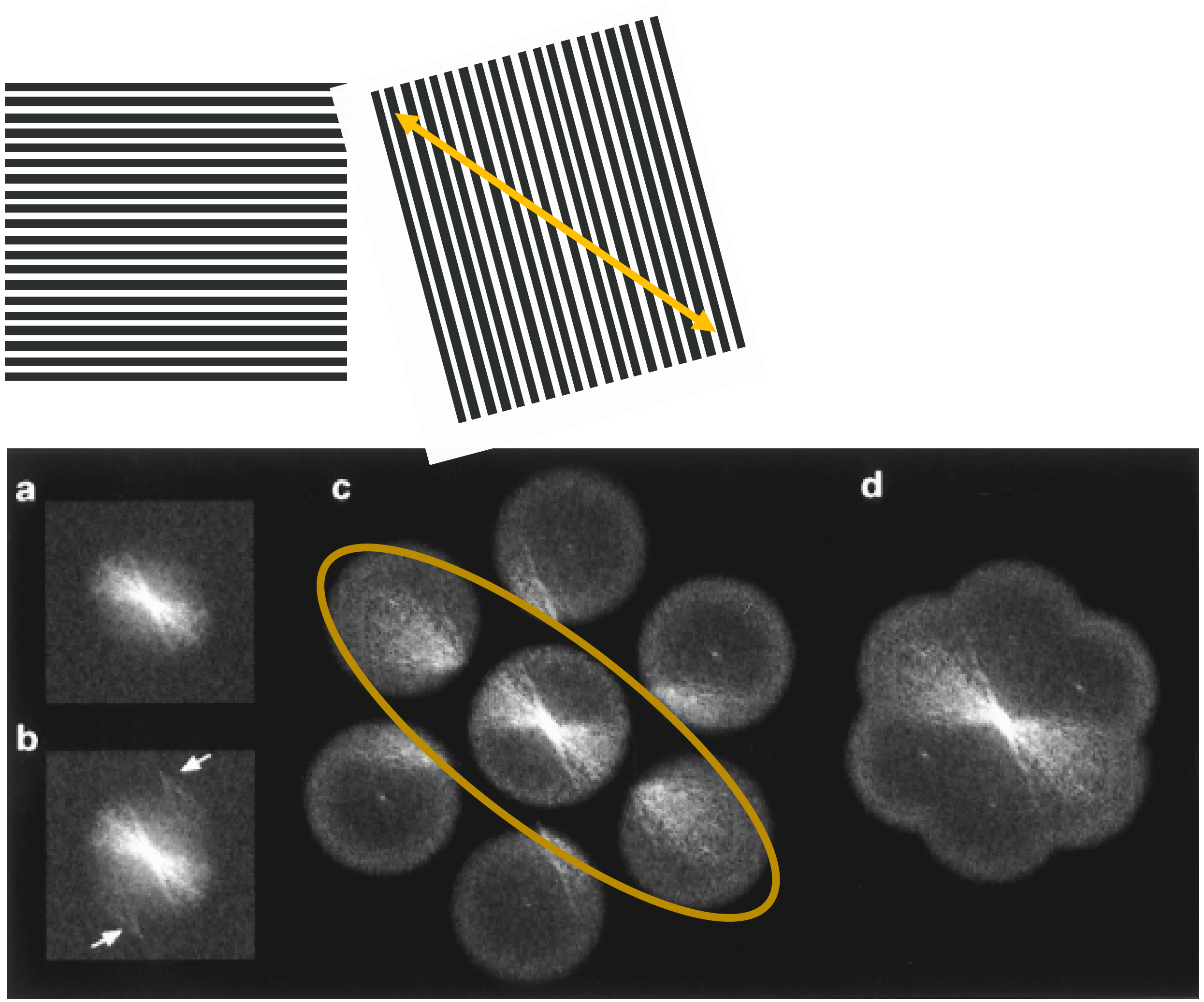


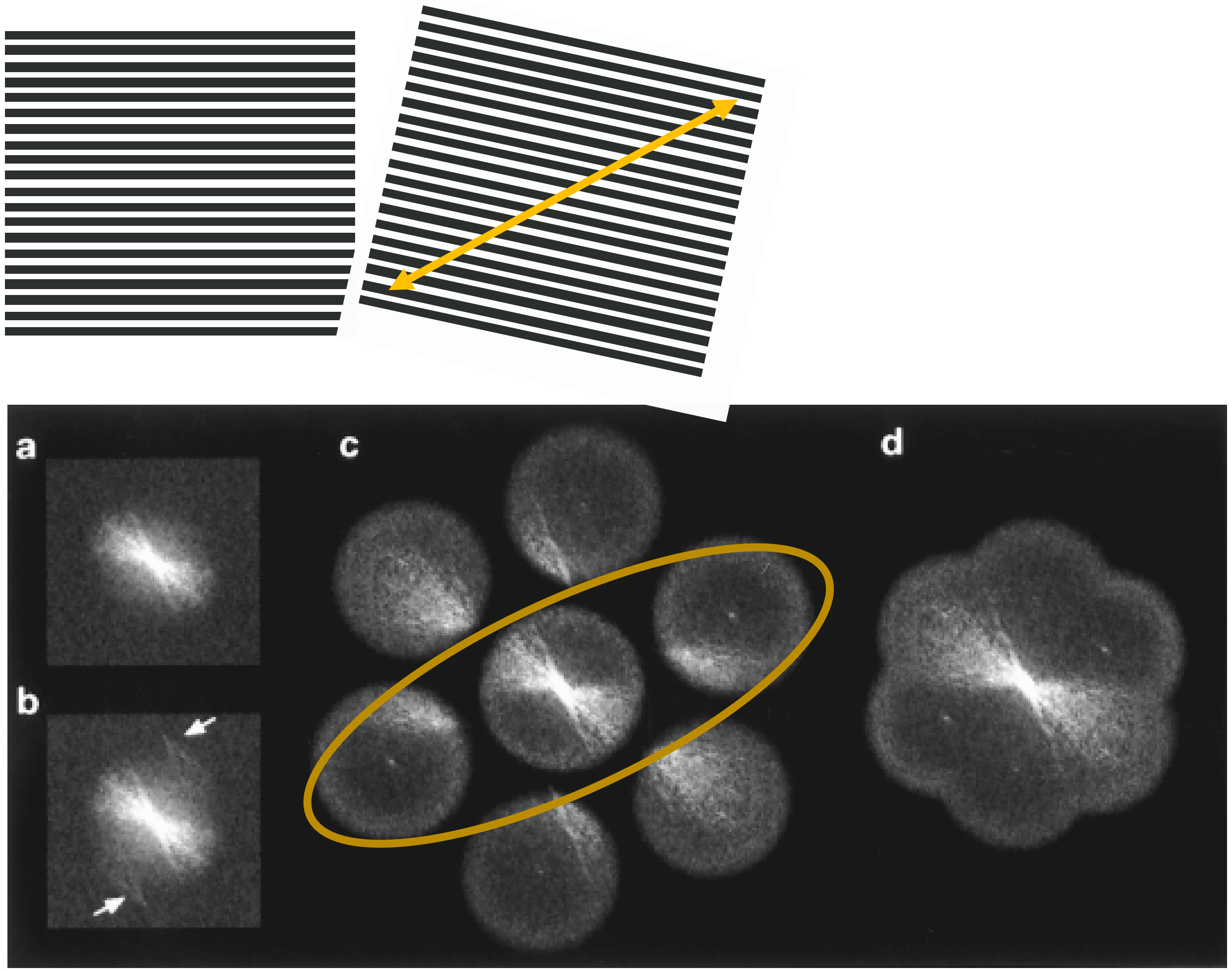


Repeat pattern many times to complete Fourier space

- To increase the resolution along one dimension, take the three sine wave patterns that will fill the entire image (3 translations of grating)
- To improve along another axis, rotate the grating, and take another 3 translations
- Total of at least 9 images to build 1 SIM frame

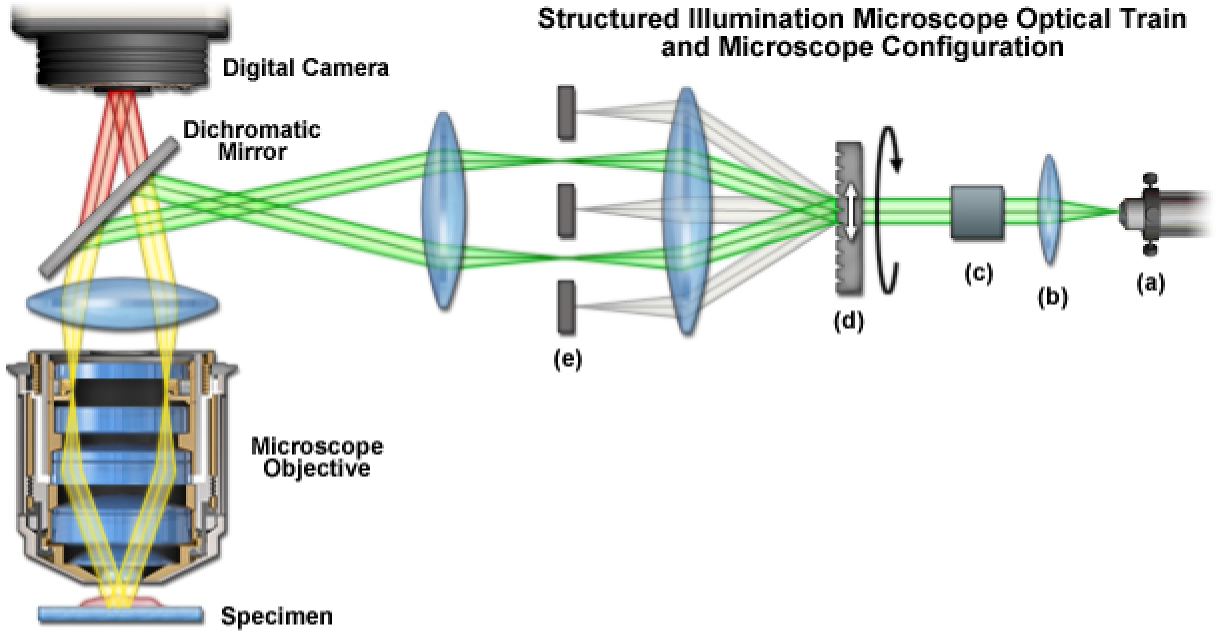


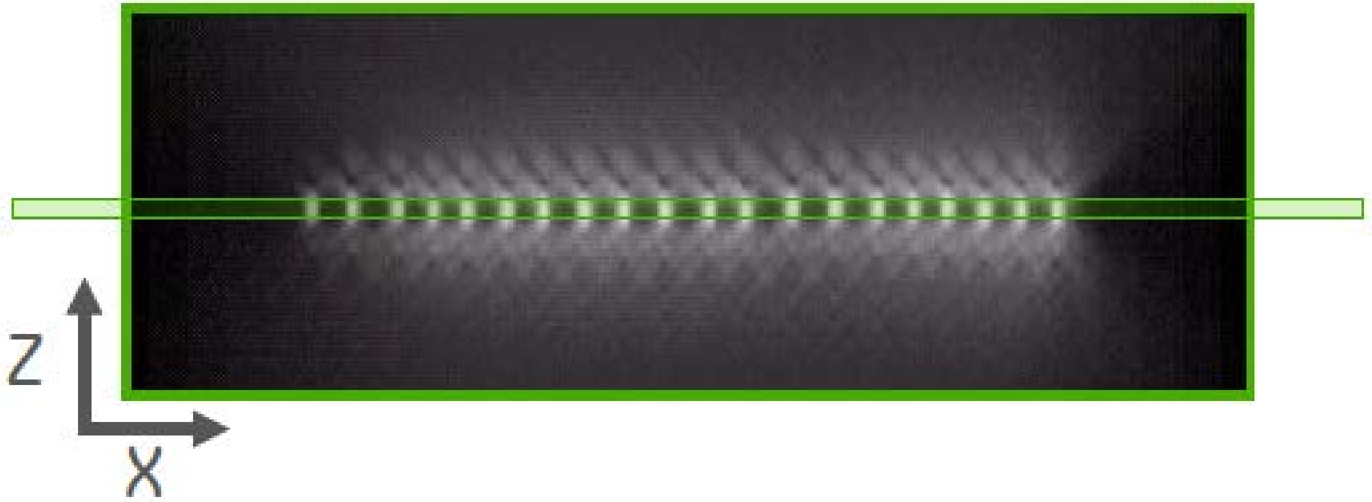

a
b
c
d


a
b
c
d

Generating SIM grids

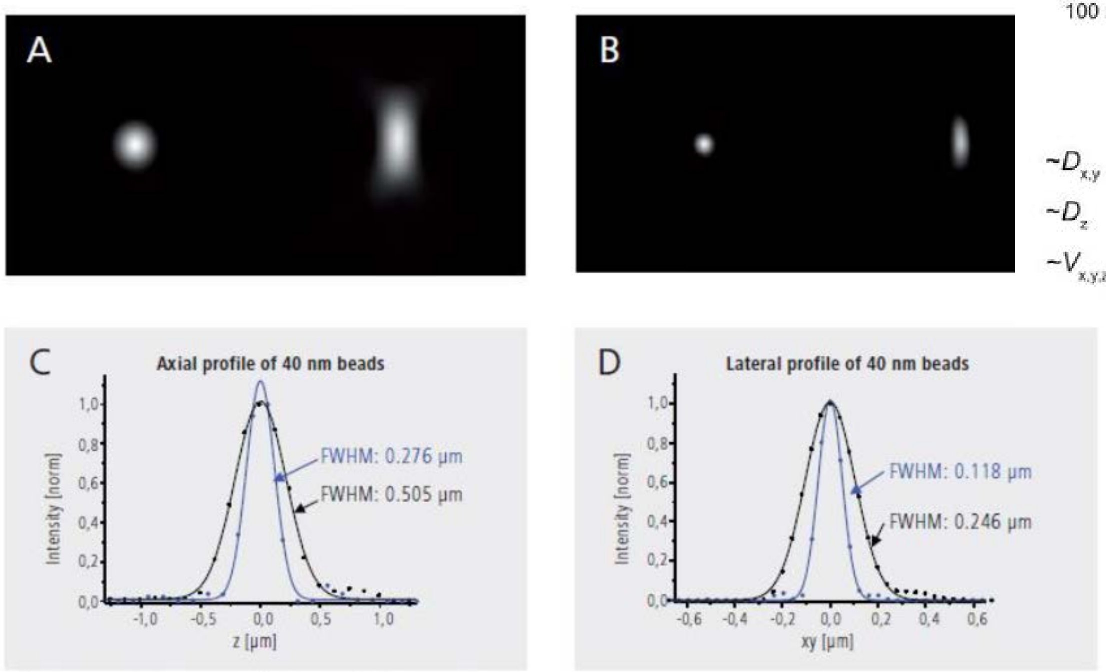
- Bring polarized light onto a diffraction grating
- Allow the 2 first order peaks through
- Focus on back aperture of objective
- Forms an image of a grating in the sample plane
- Rotate and translate diffraction grating

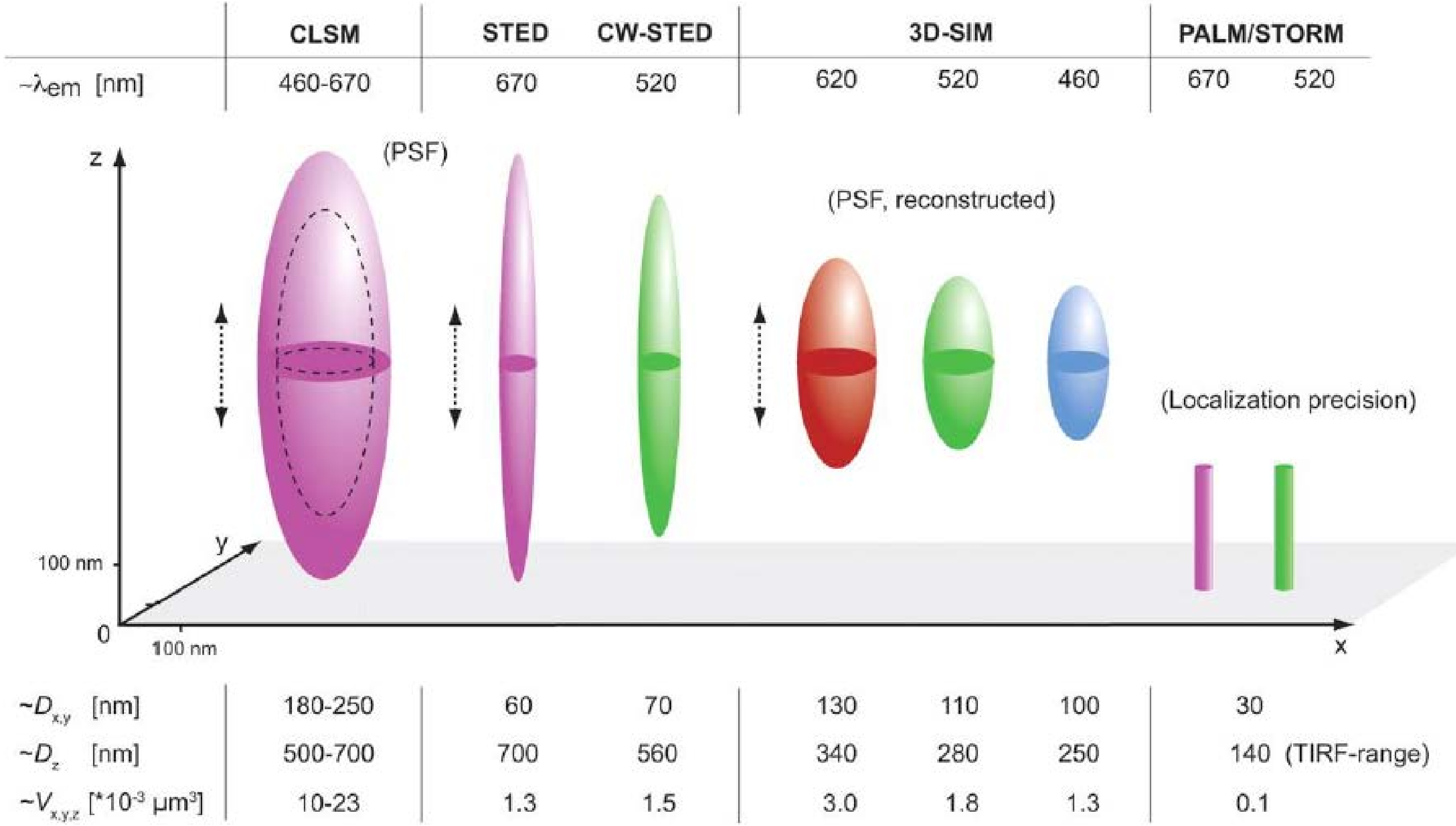




Resolution of SIM

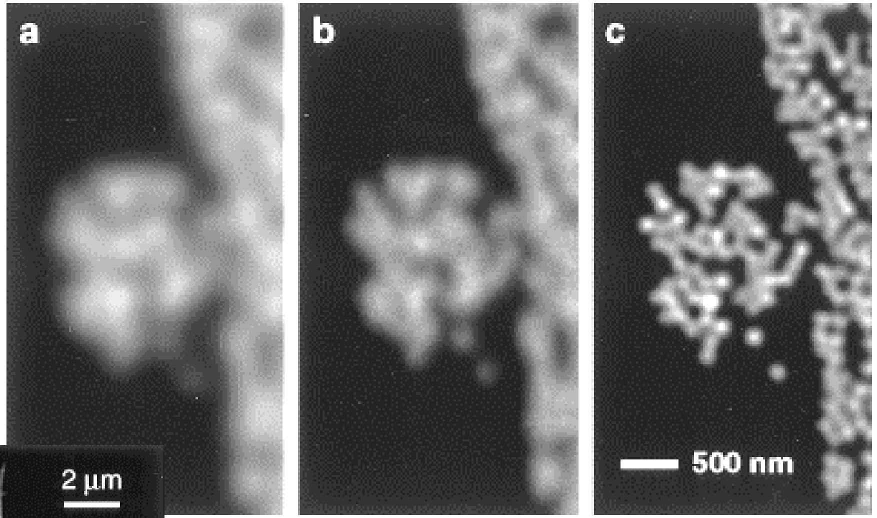
- Axial resolution enhanced by factor of 2

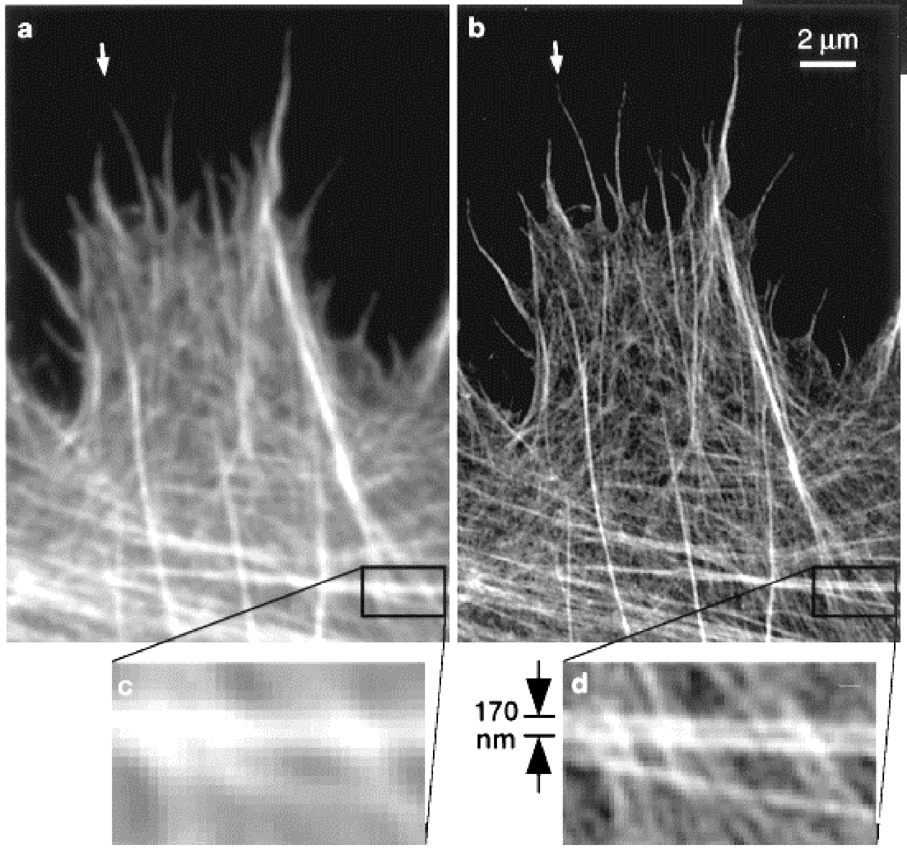




Structured illumination imaging

- Works with any dyes
- Requires repeated exposures with multiple gratings (9 times)
- Only need normal excitation levels (x9 for repeated exposures)
- Easy to grab multi channel data
- Improved axial resolution

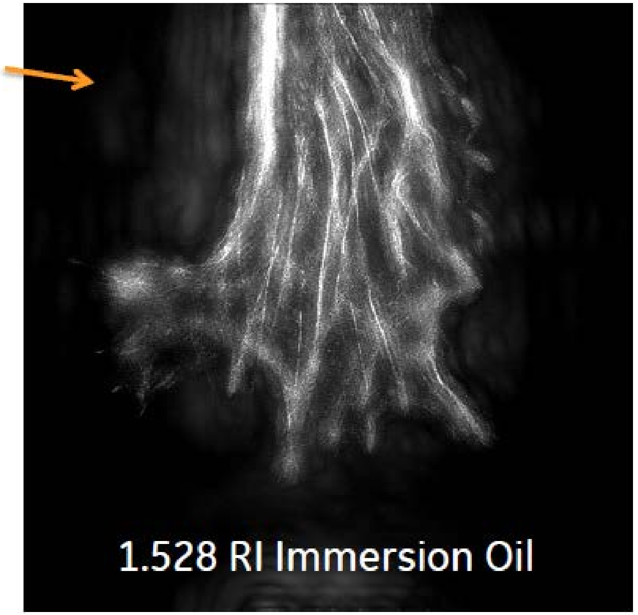




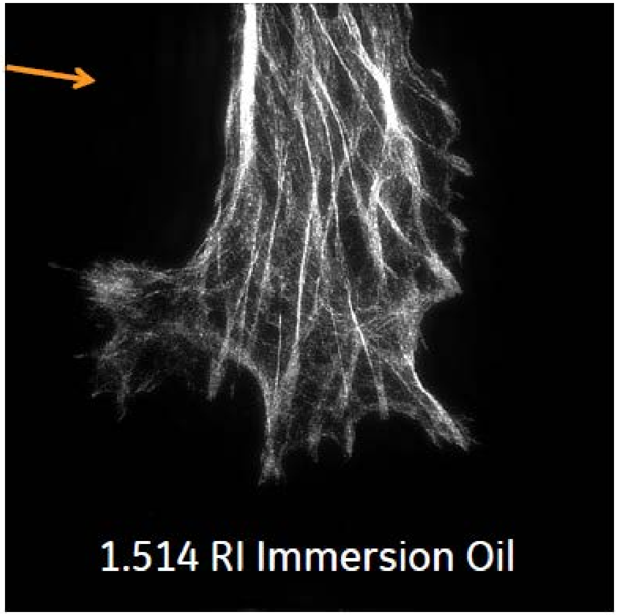
Cautions with SIM

- Thick samples are a no go, scattering ruins pattern
- Dyes require high SNR and low photobleaching (9 exposures per 1 image)
- If there is motion happening faster than the time of 1 SIM frame, it will contaminate image
- Need to ensure higher order diffraction peaks are blocked

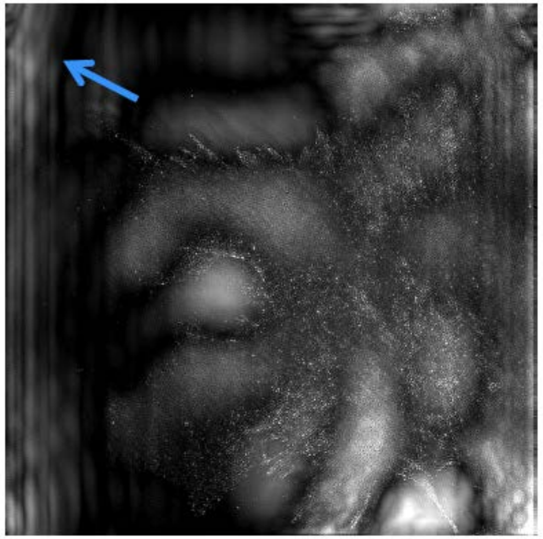
Mismatched oil



Matched oil



Max Projection



Photobleaching during SIM capture will result in “square edges”

2 Color SIM

- Gratings are optimal to 1 wavelength
- Can either change gratings, or live with sub-optimal reconstruction
- $PCC_{widefield} = .76$
- $PCC_{2ColorSIM} = .48$

Comparison of Widefield and SR-SIM Two-Color Imaging of Nuclear Pore Proteins

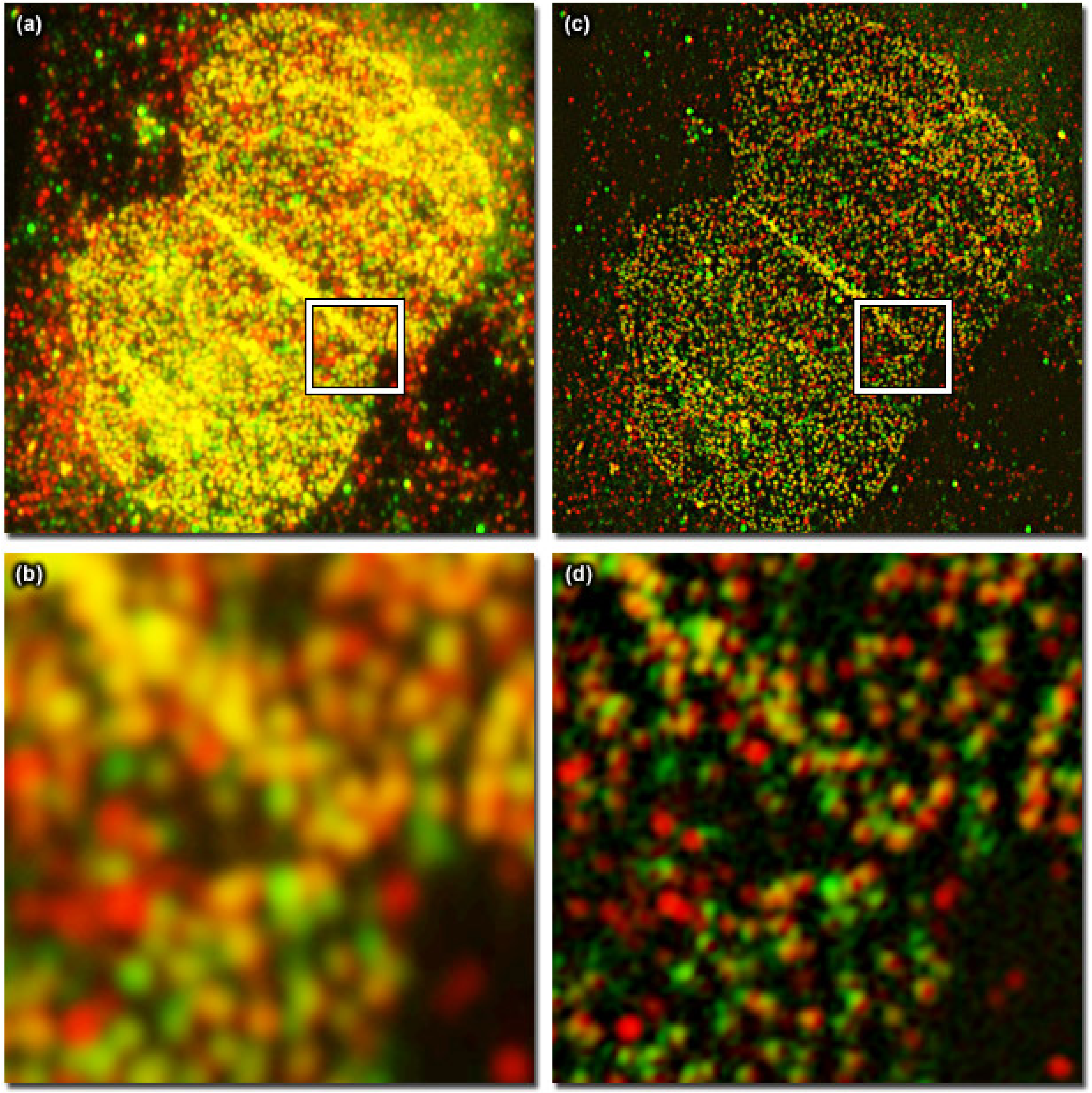


Figure 10

3D SIM

- Add in 0th order beam
- Sets up 3D lattice
- Need 15 images to from 3D SIM frame
- Improves axial resolution at the expense of acquisition time

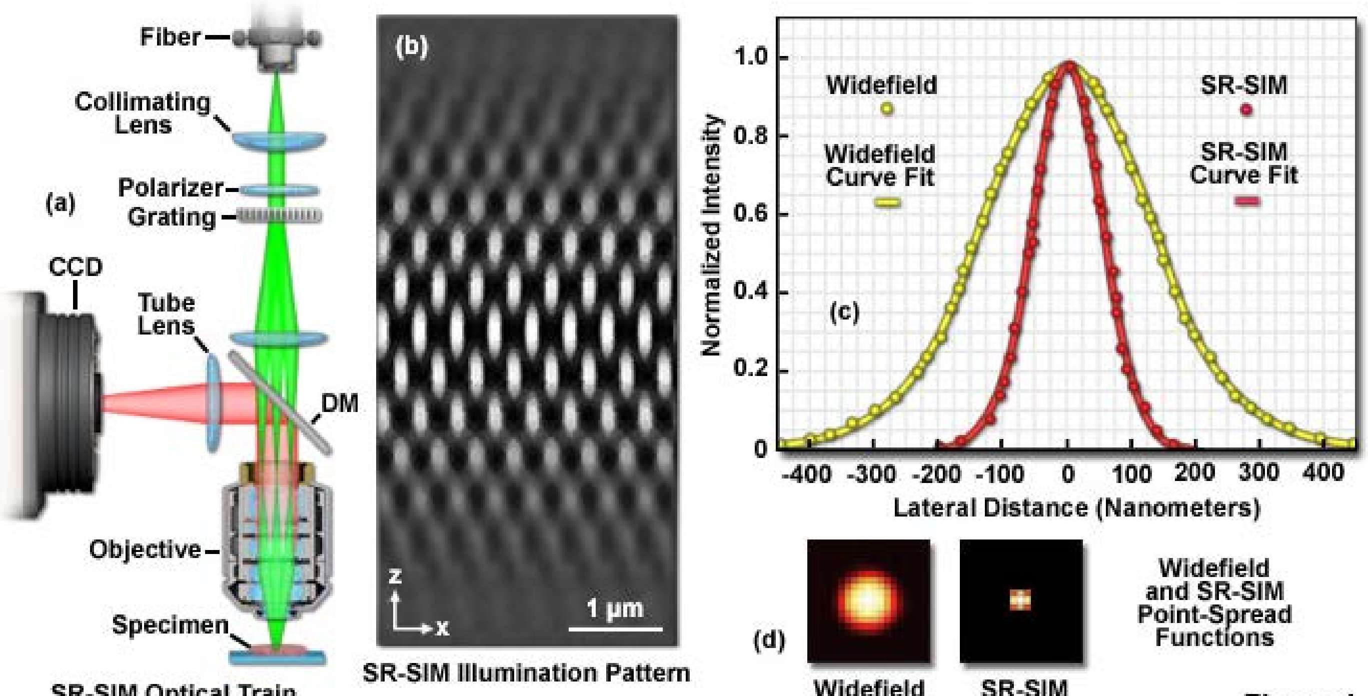


Figure 1

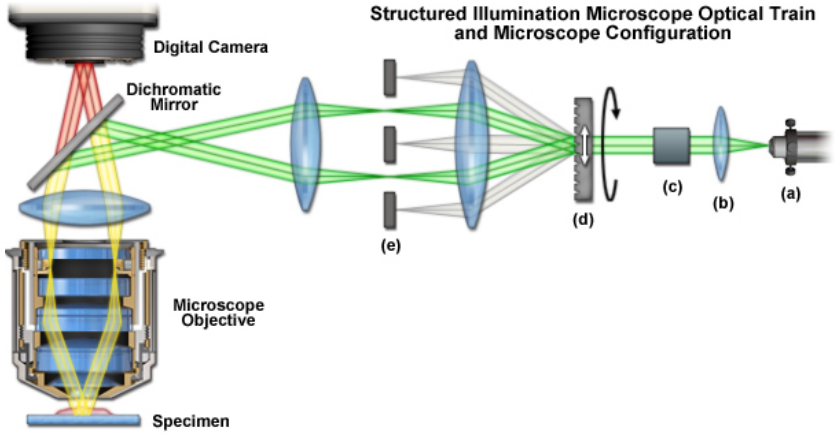
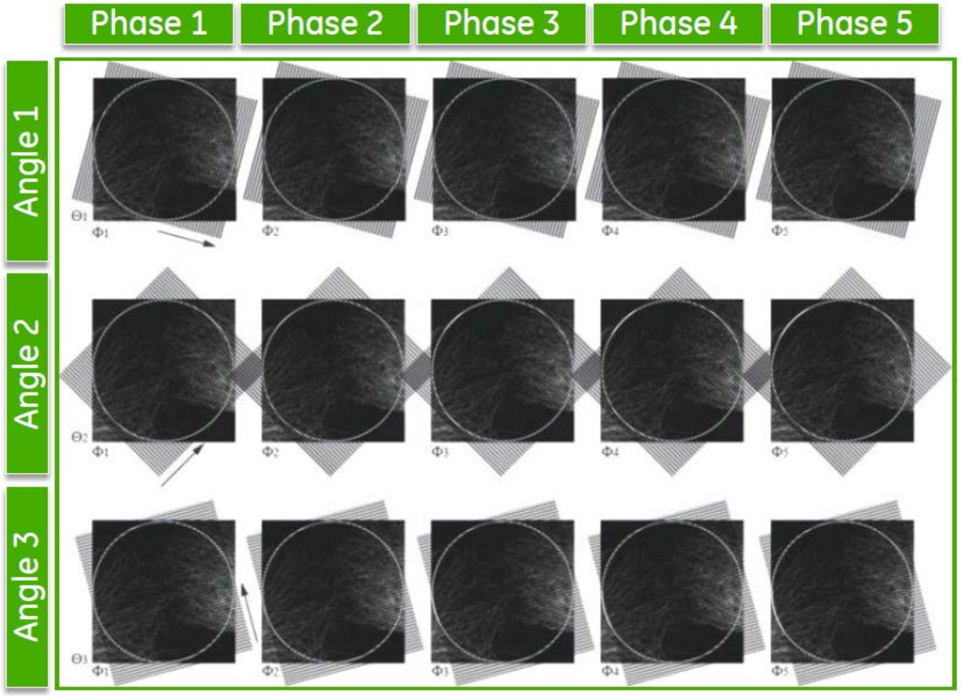
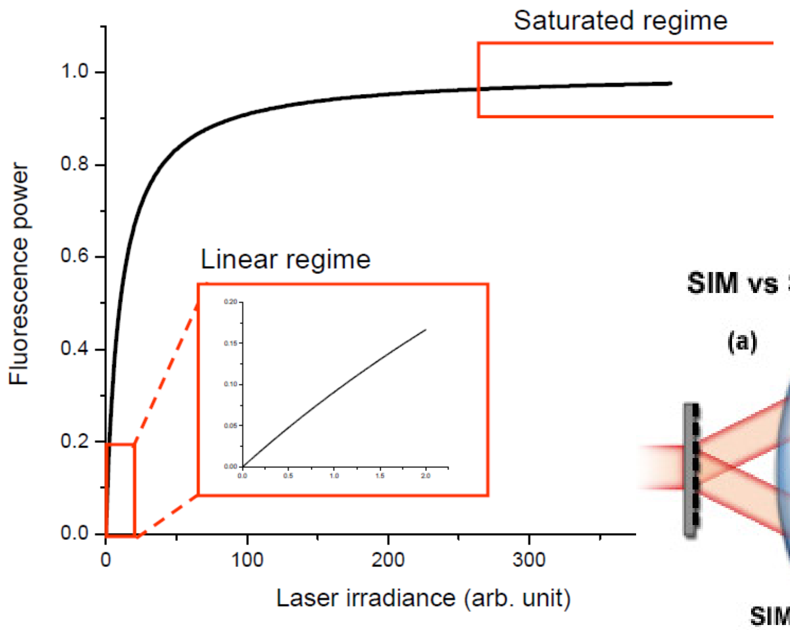


Figure 8



Saturated SIM – Super resolution

- Relies on non-linear fluorescence of samples
- Illumination pattern is no longer sinusoidal, also contains even higher frequencies
- Fluorescence increases with excitation intensity up to a point
- After saturation, it can not emit faster due to physical limitations



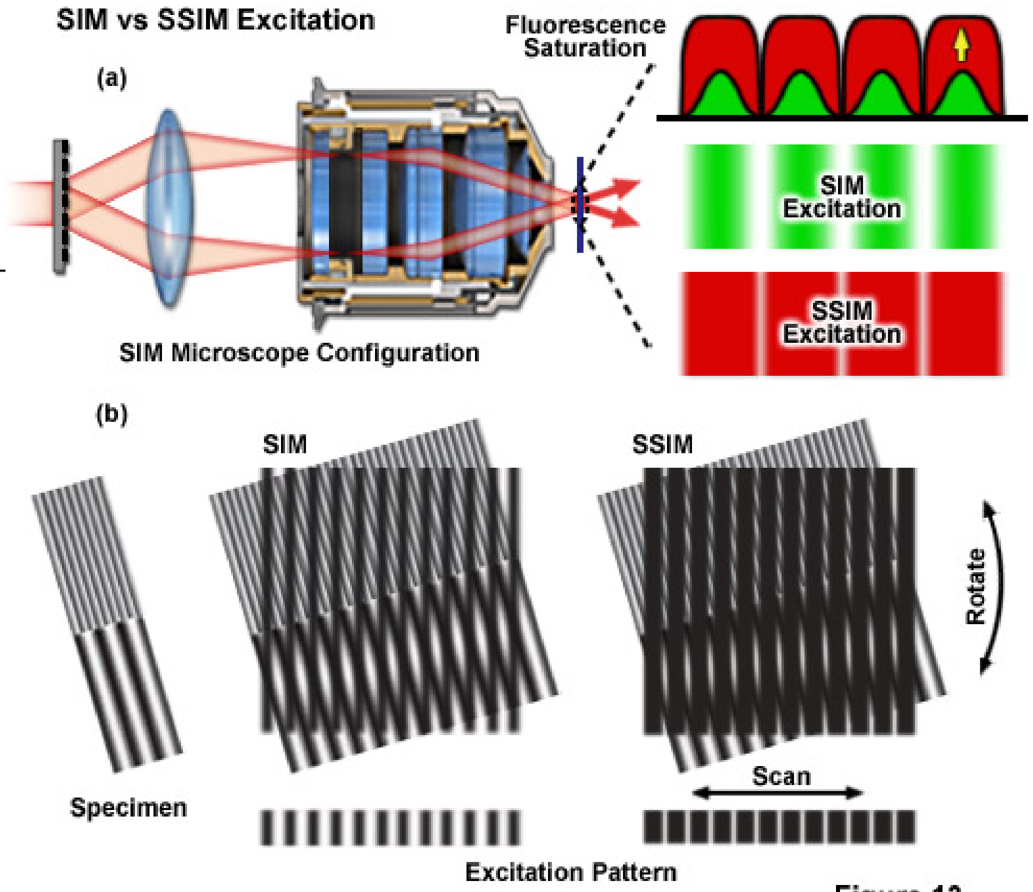


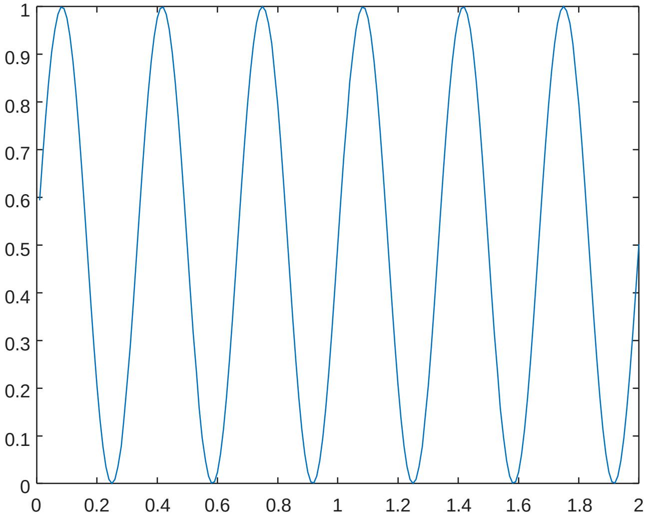
Figure 13

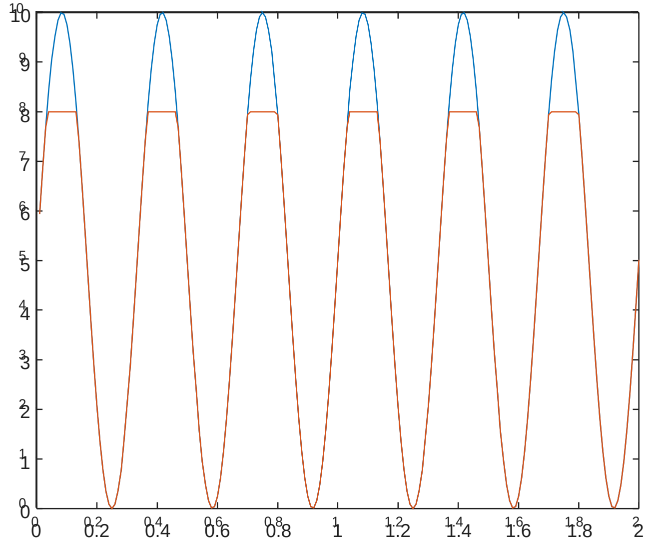
FFT of saturated patterns

Due to the saturation of fluorophores, if we apply enough intensity, we won’t get a pure sine wave

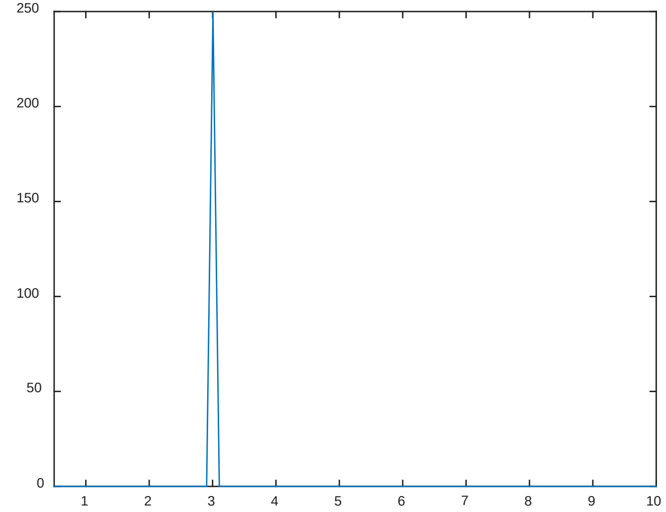
The clipped fluorescence will add additional frequencies in to the spectrum

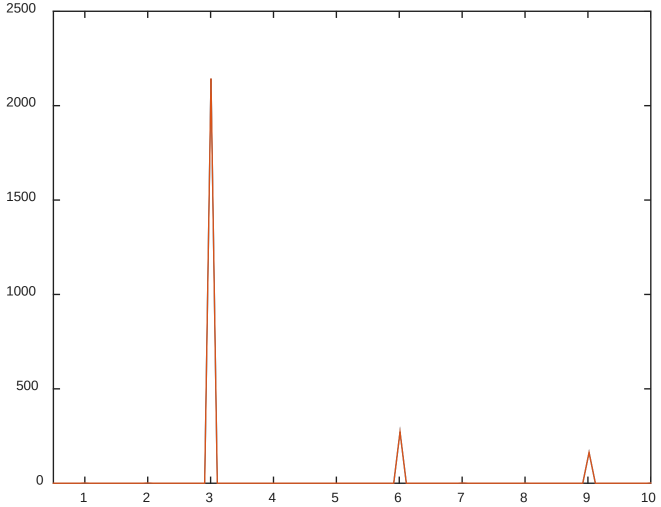
We can use these frequencies to go even higher in frequency space -> better resolution





FFT





SSIM

- Additional harmonics enable even higher frequency reconstruction
- Requires many individual exposures to generate 1 SSIM frame (36 frames to double resolution, 50 nm)
- Theoretically unlimited resolution if you can apply higher powers

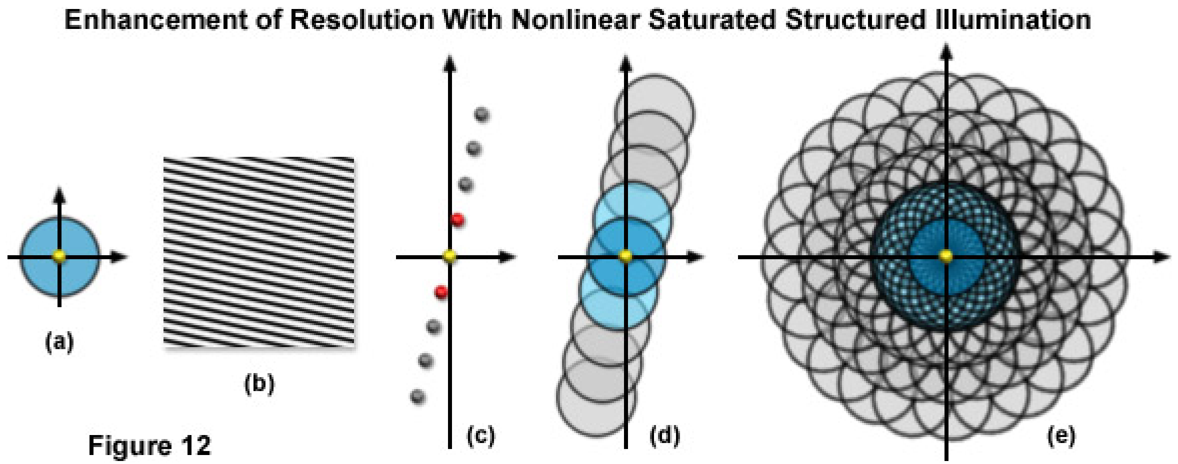
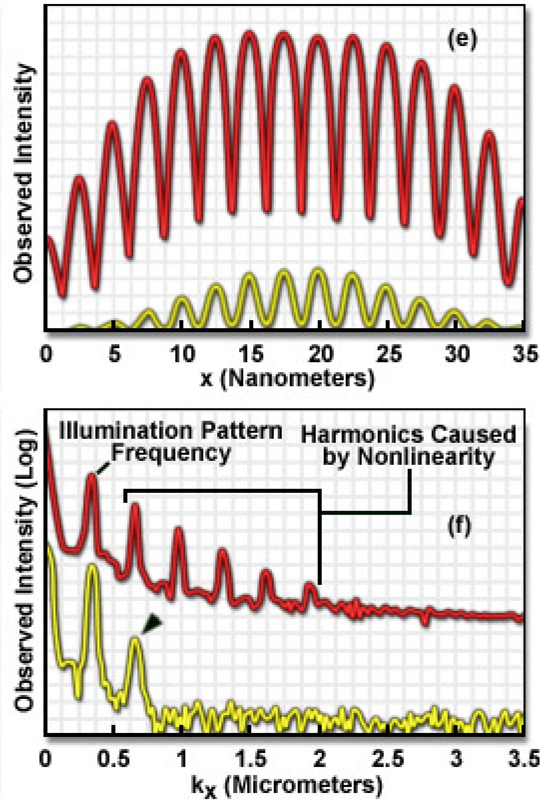
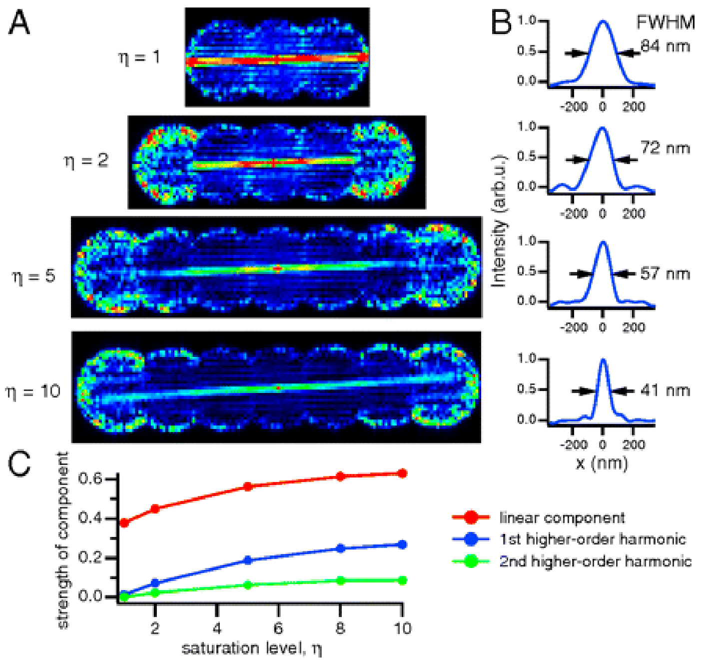


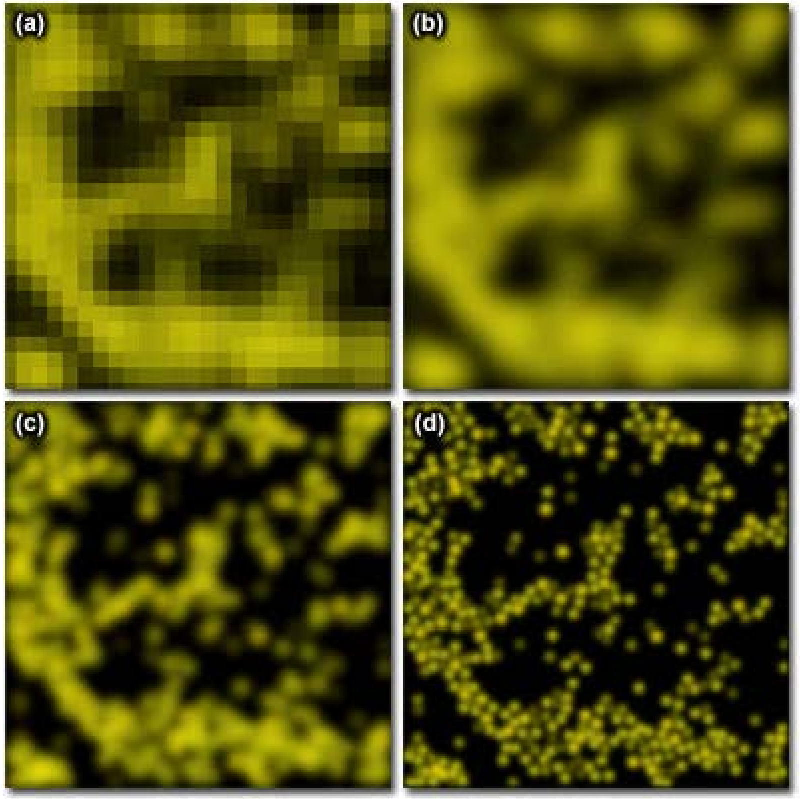
Figure 12





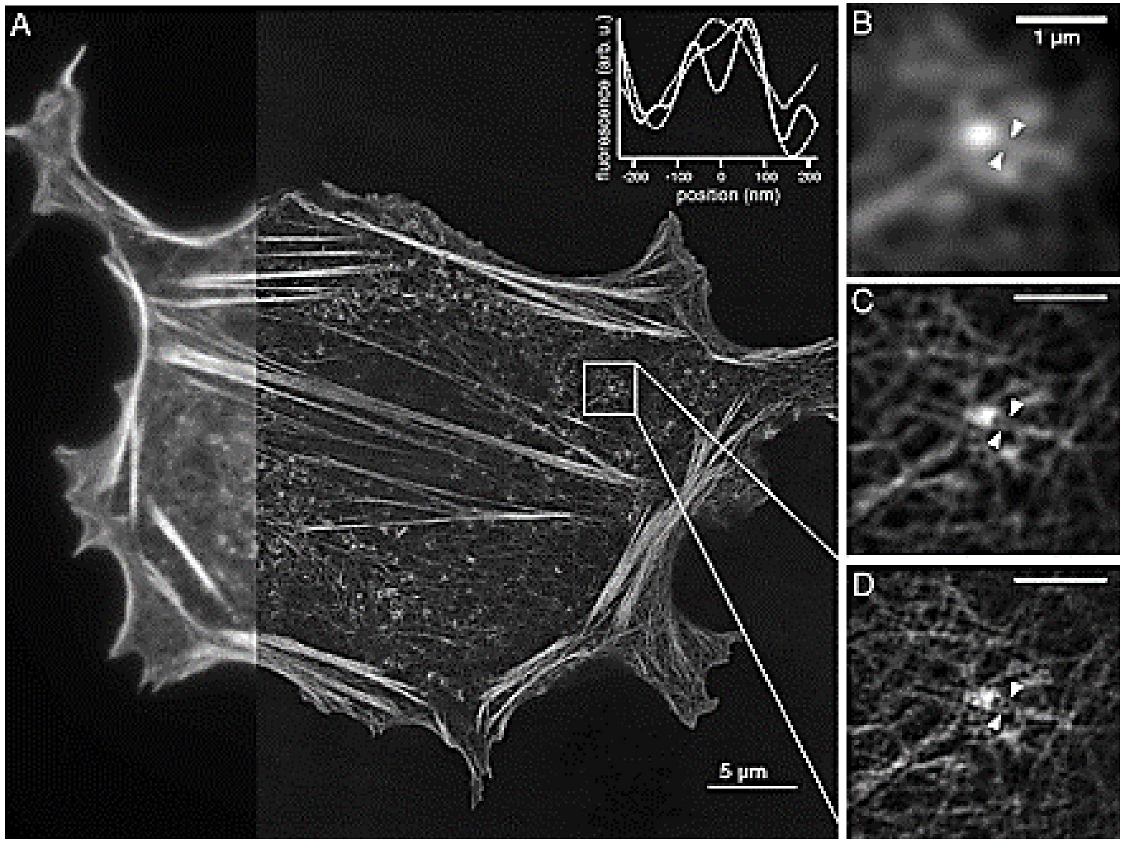
SSIM

Widefield WF-deconvolved



SIM SSIM Figure 11

CHO cell expressing Dronpa-Lifeact



On to Matlab…