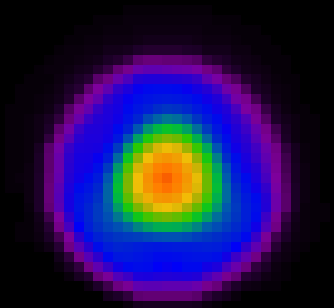
Your images are wrong!
So restore them…
…and, how
3D-SIM beats confocal

July 2016

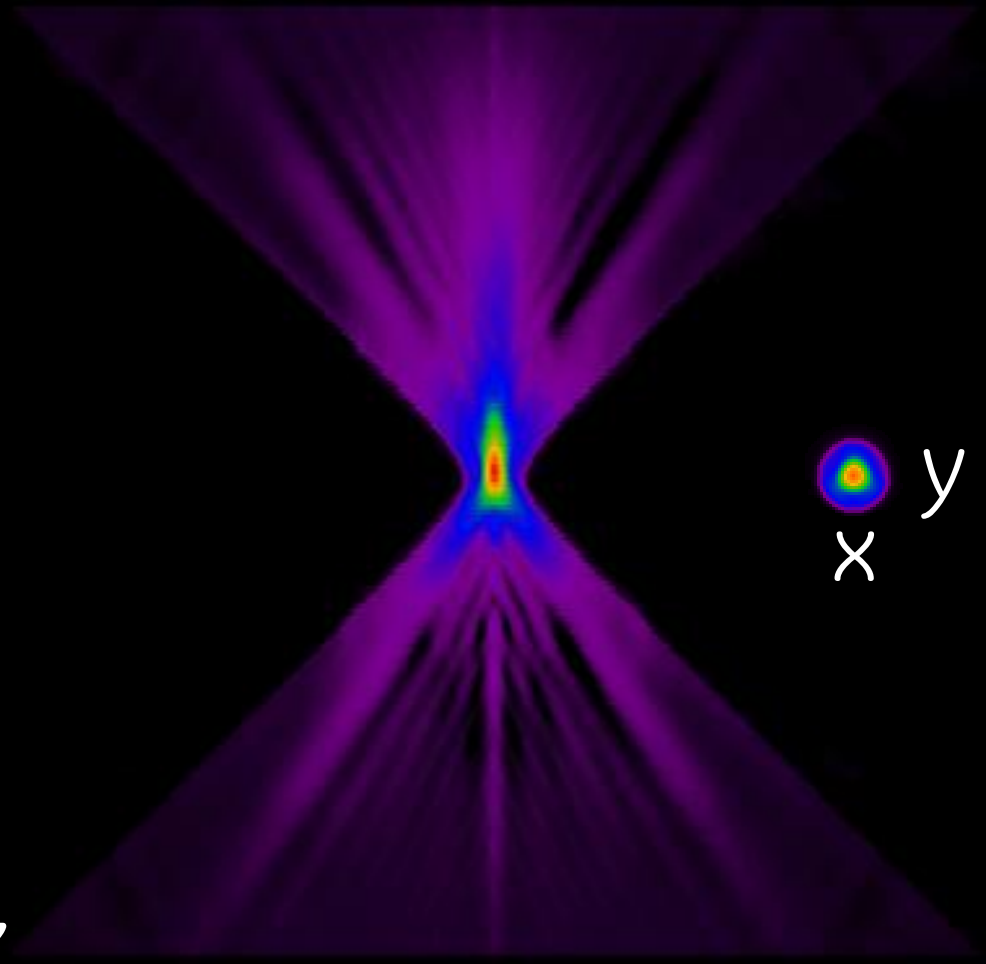
Daniel J. White Ph.D.

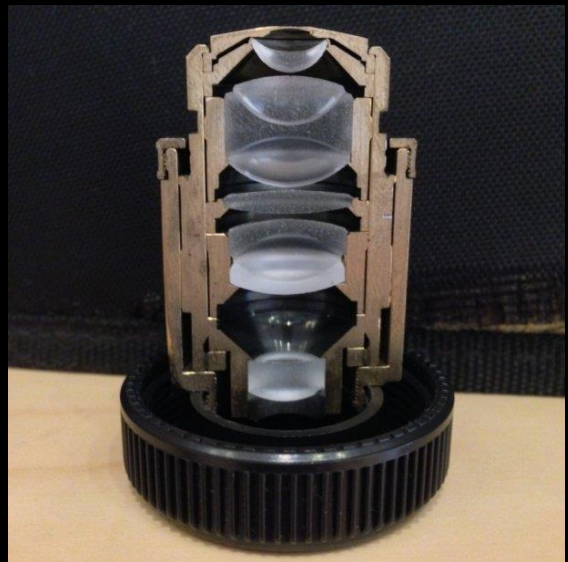
Microscopy Applications Scientist

image of a point
is NOT a point!



systematic error
Point Spread Function



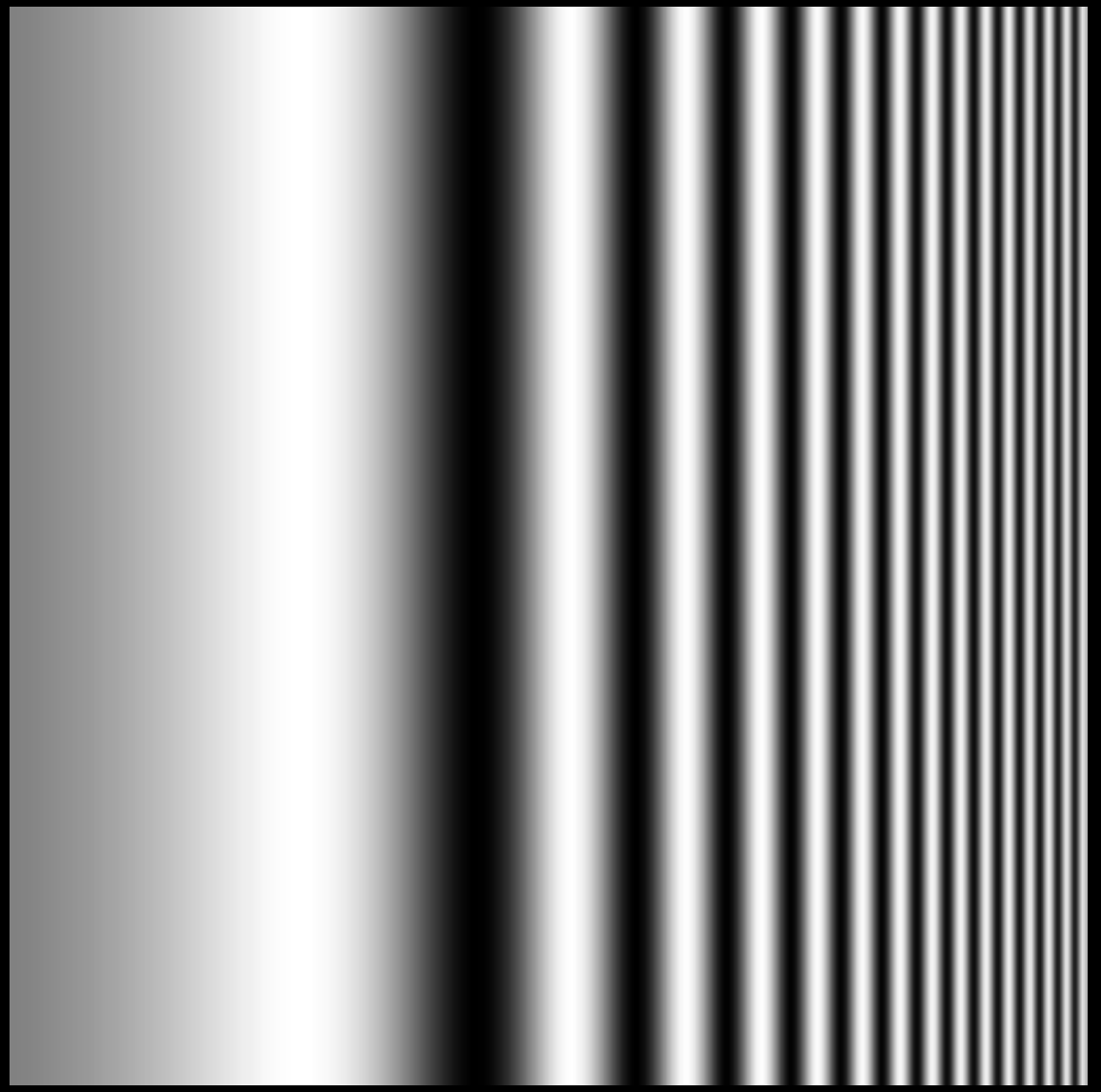


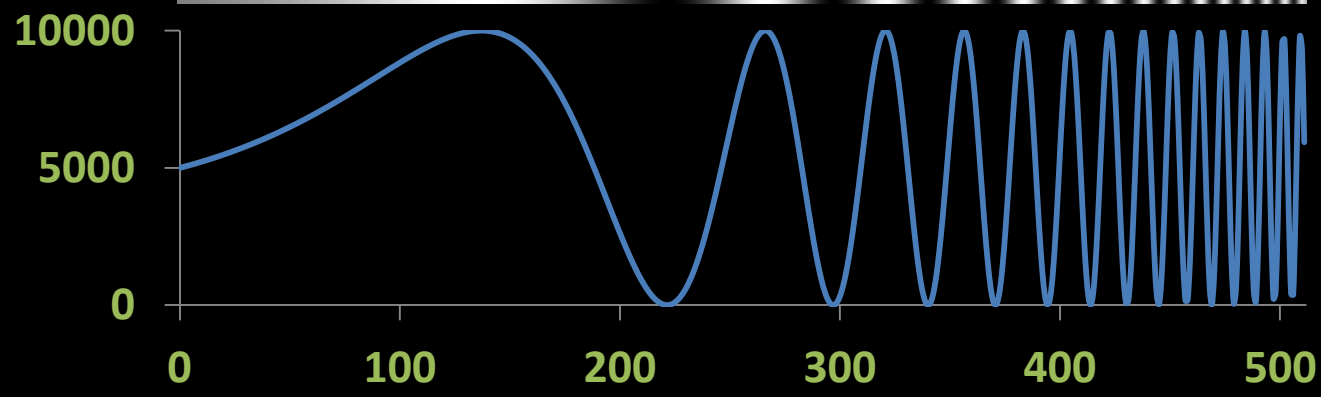
1.4 NA Lens PSF, Jesse Lipp, Dan White MPI-CBG

why take an image?
know how much stuff is where

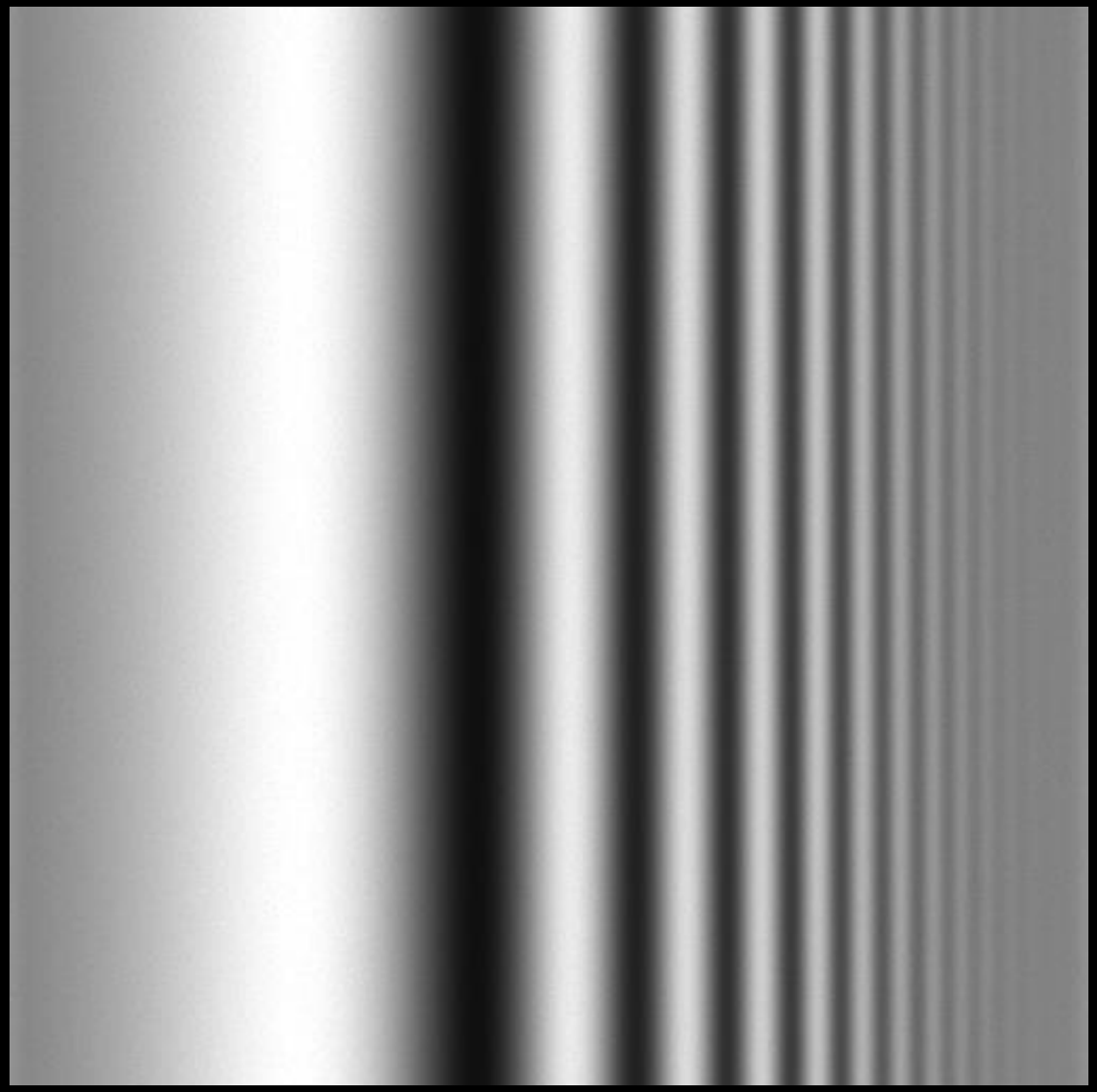
ground truth

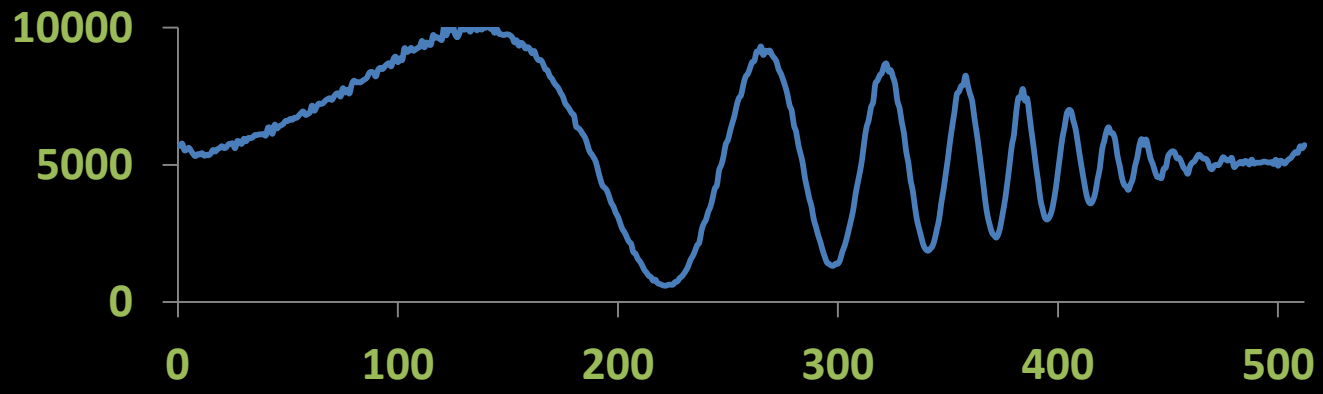
0 – 10,000 photons





lens kills contrast

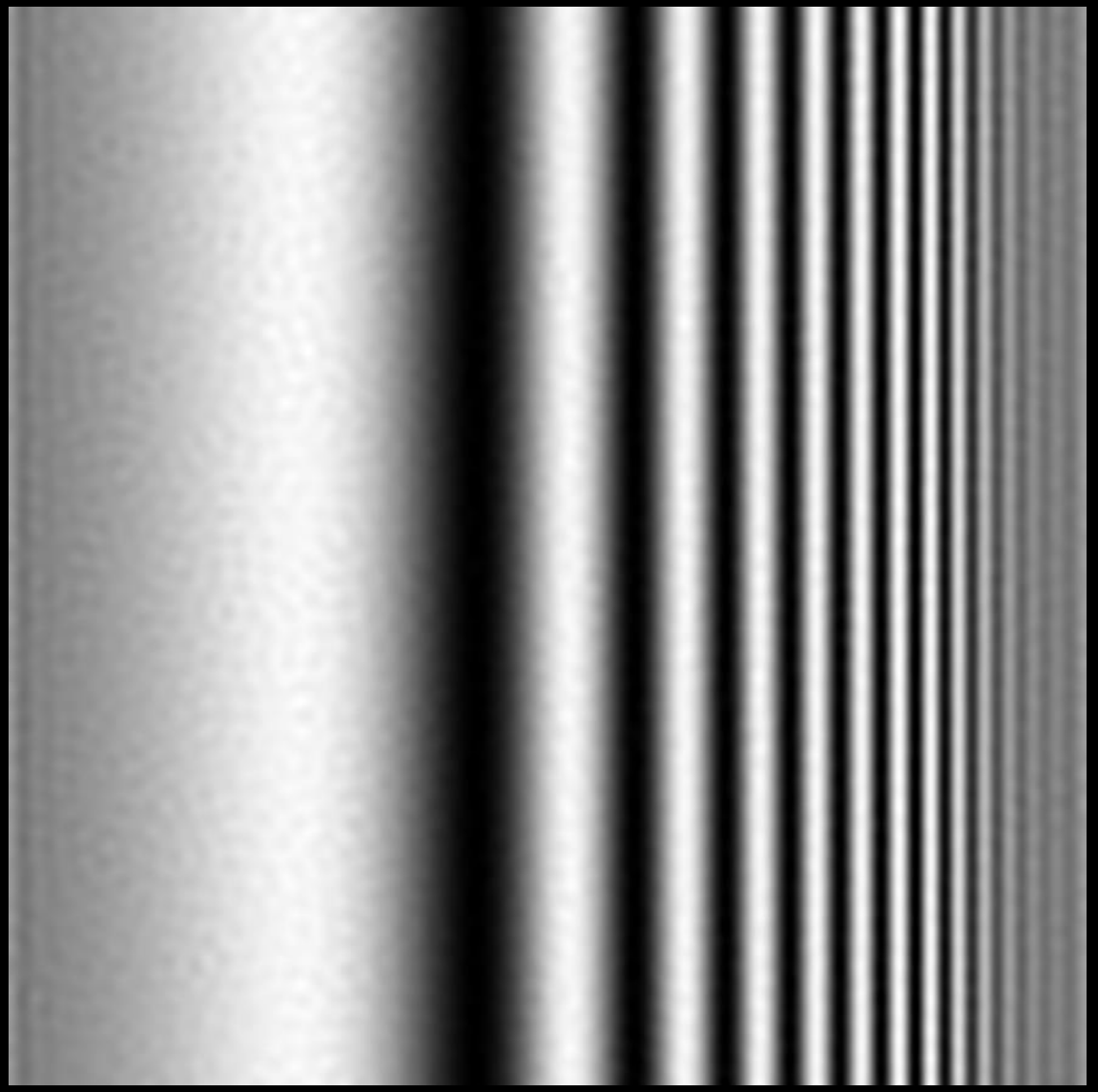


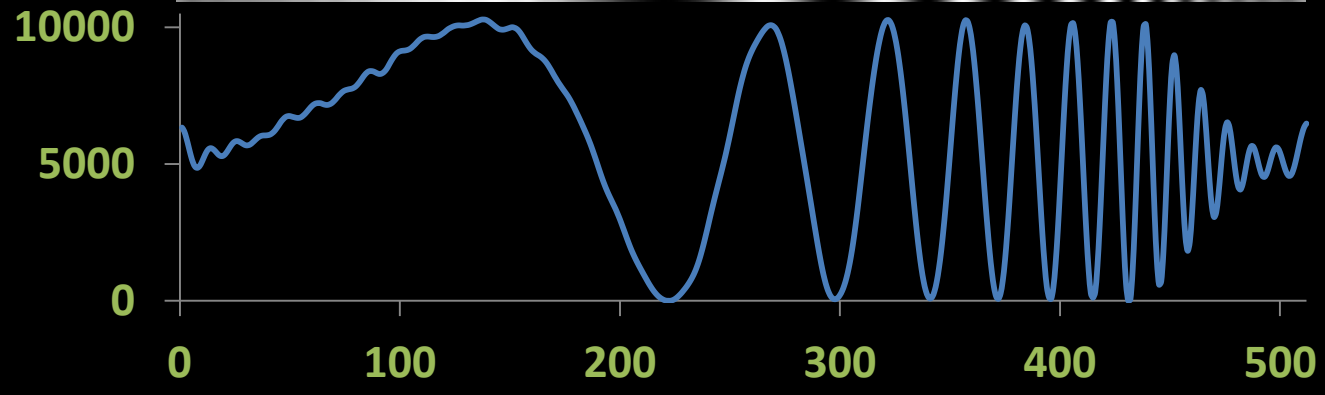


wrong answer!

contrast restoration

better answer!

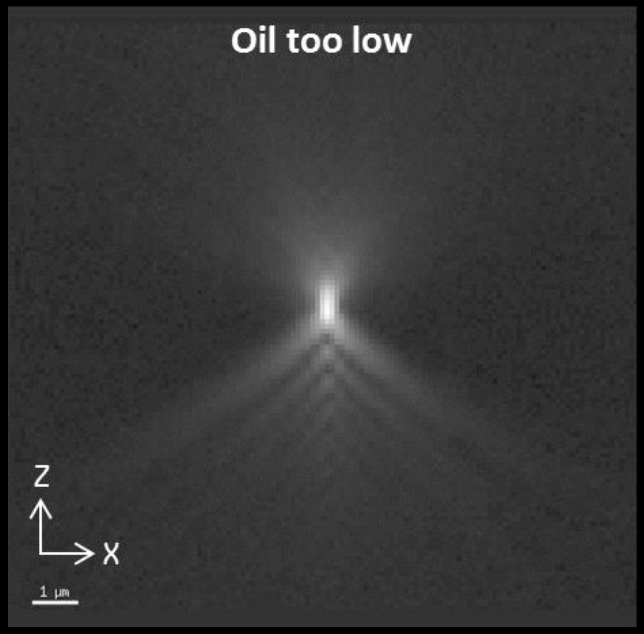


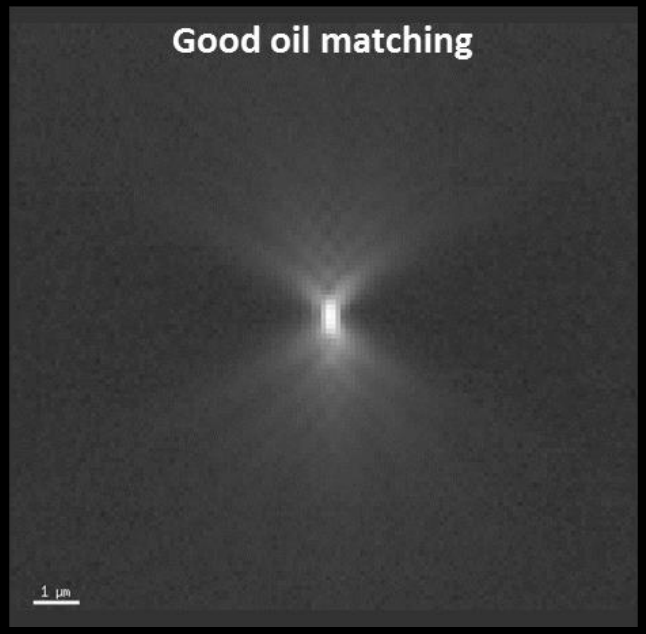


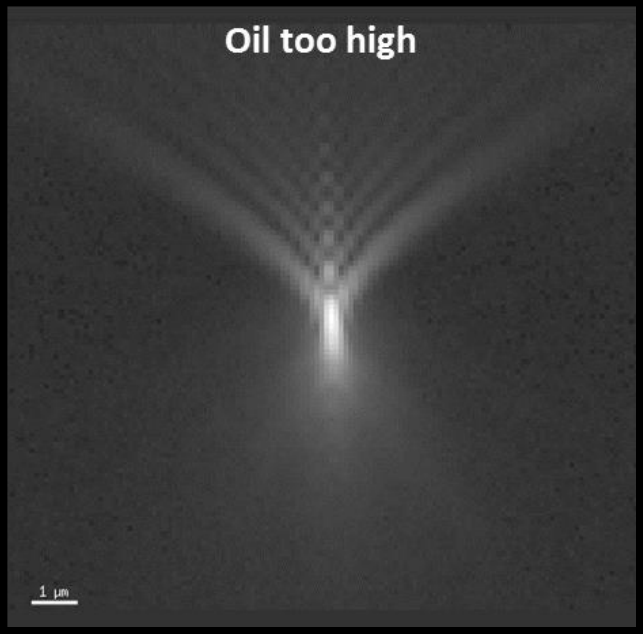
garbage in
garbage out

the DeltaVision difference
attention to detail

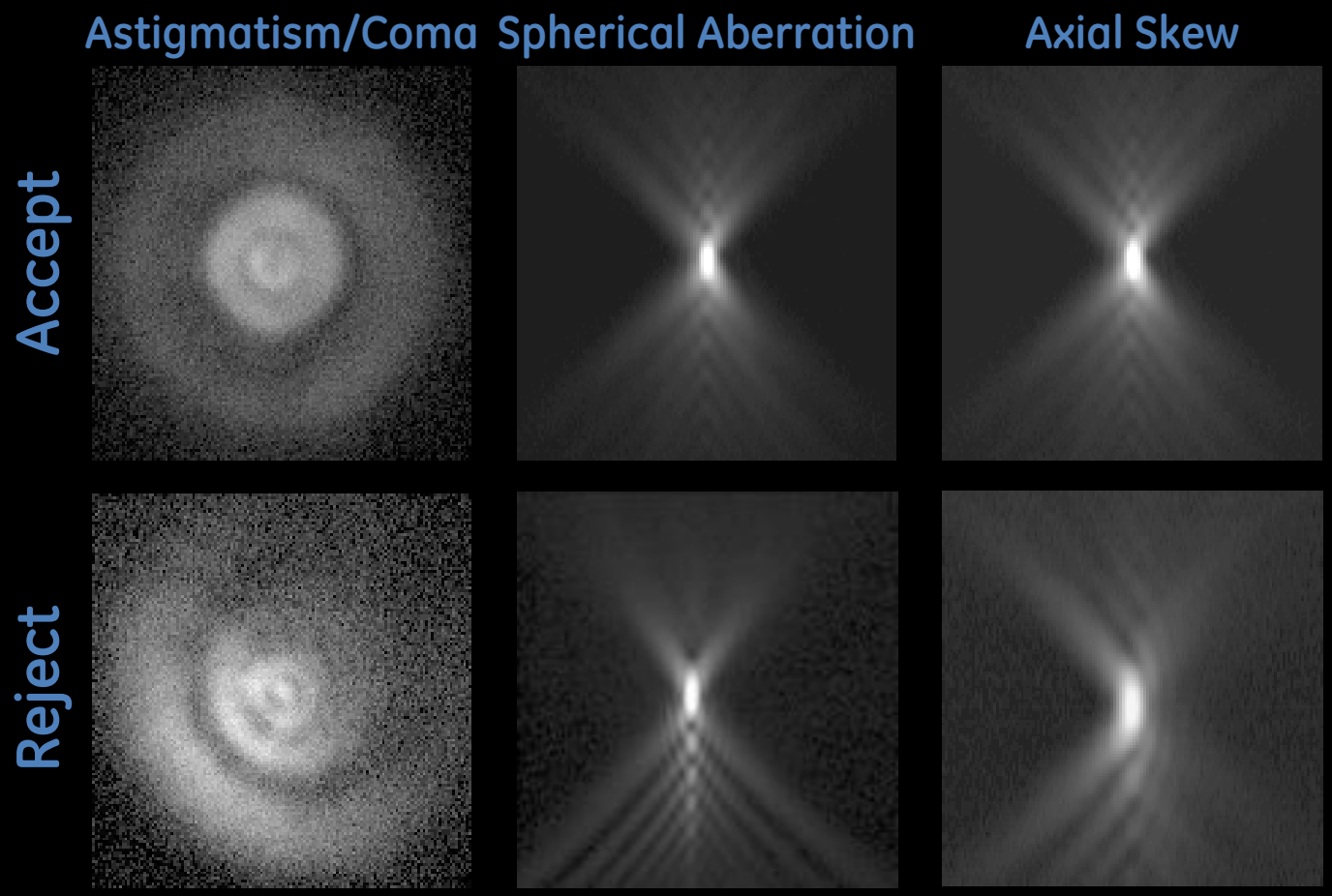
spherical aberration
fix with immersion oil





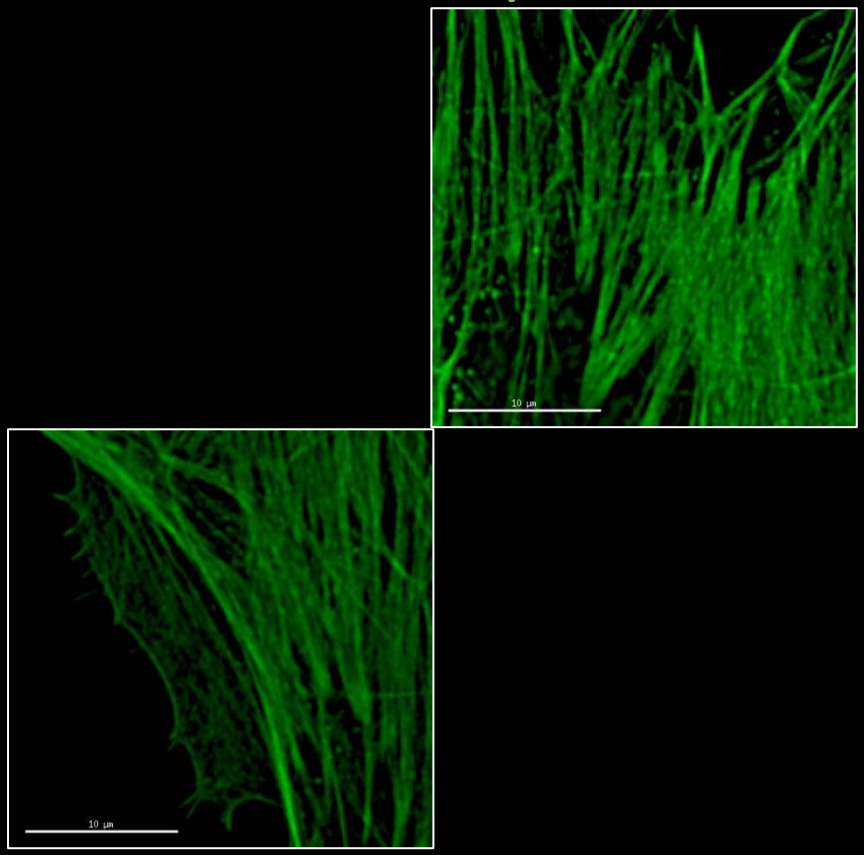


lenses are not perfect
we choose the best lenses

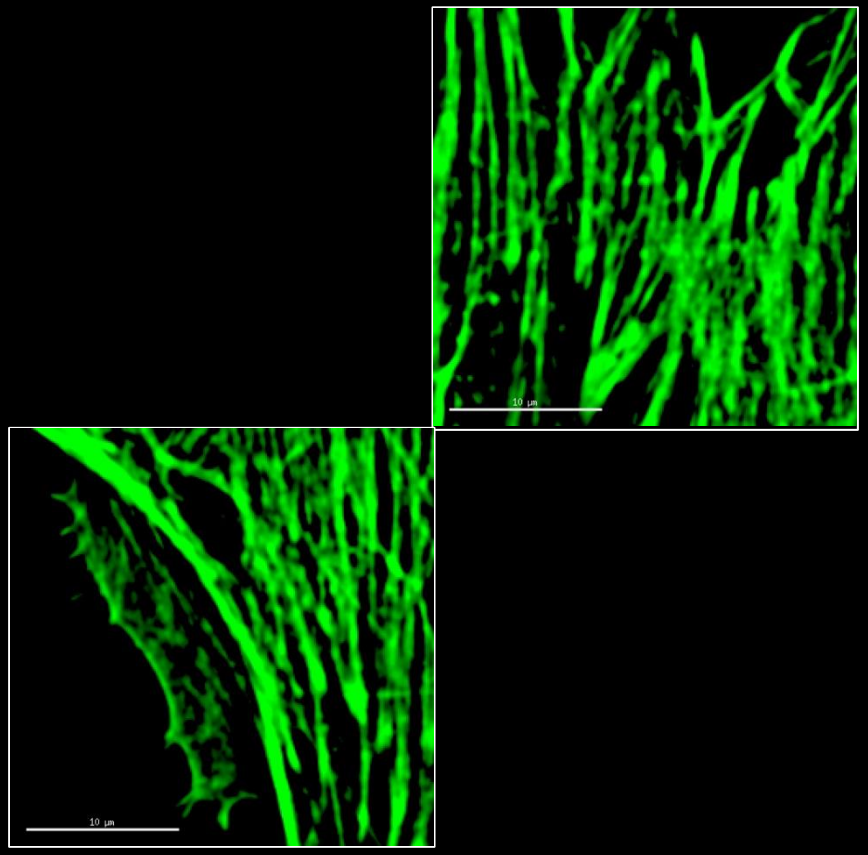


the theory is wrong!
we measure PSF for you

Restored – Empirical PSF



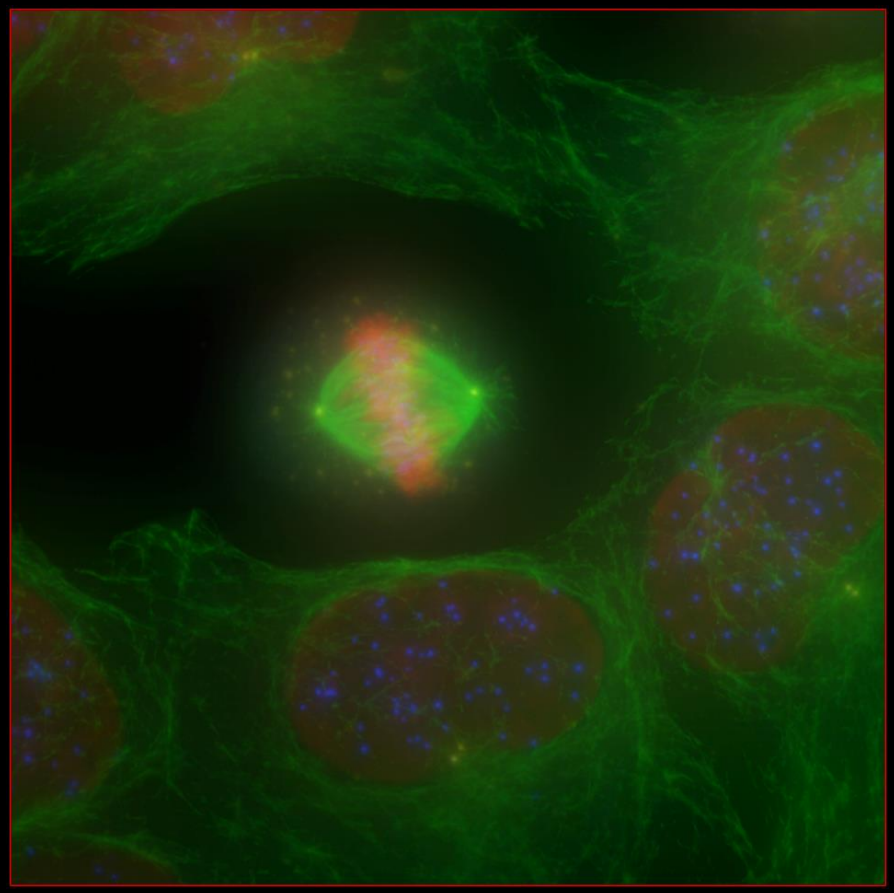
Restored – Theoretical PSF



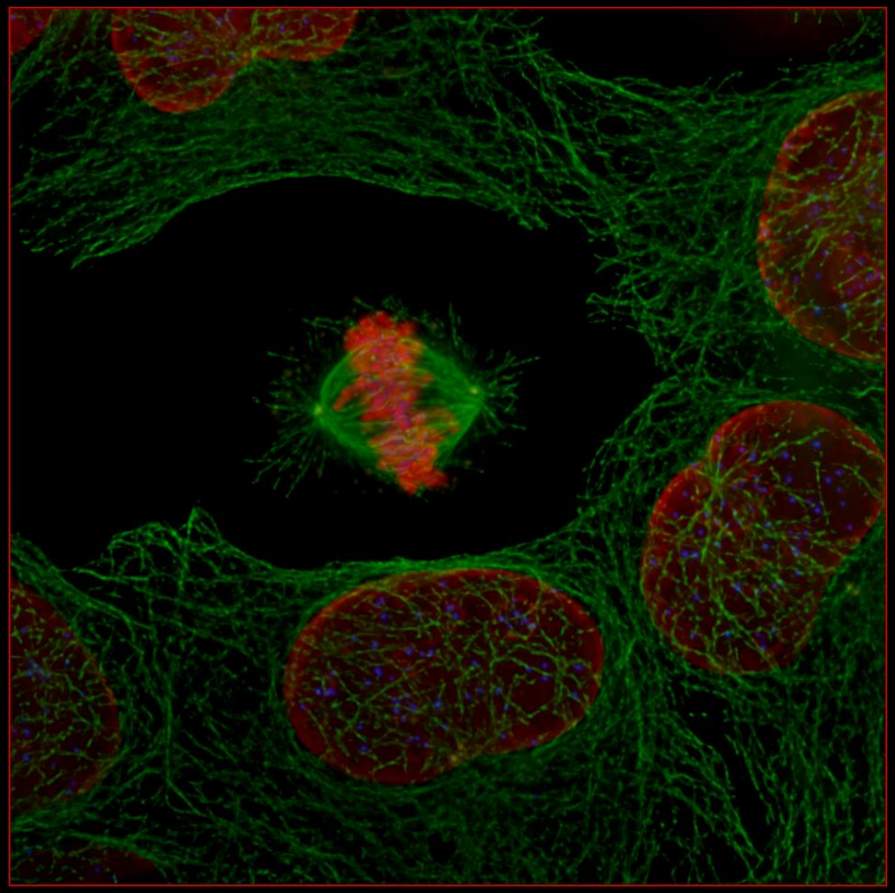
the DeltaVison difference

nothing escapes optimization!

DeltaVison contrast restoration
z-stack mono layer cultured cells on glass



raw image
max Z projection



deconvolved image
max Z projection

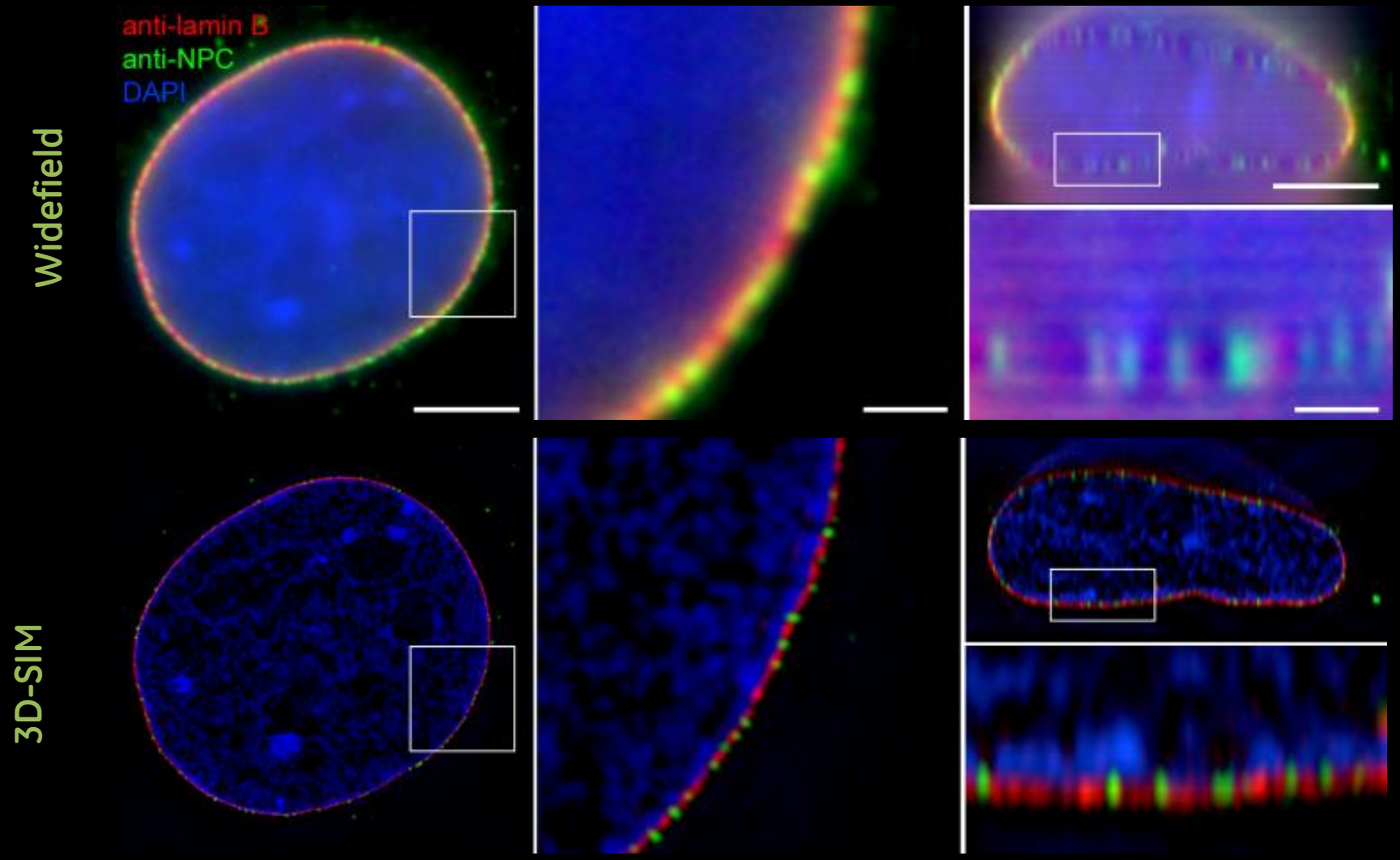
Images: Alex Bird / Dan White MPI-CBG

super resolution?

what?

why?

widefield vs. 3D-SIM



confocal vs. 3D SIM

CLSM

3D SIM

OMX SR

3D-SIM
structured illumination

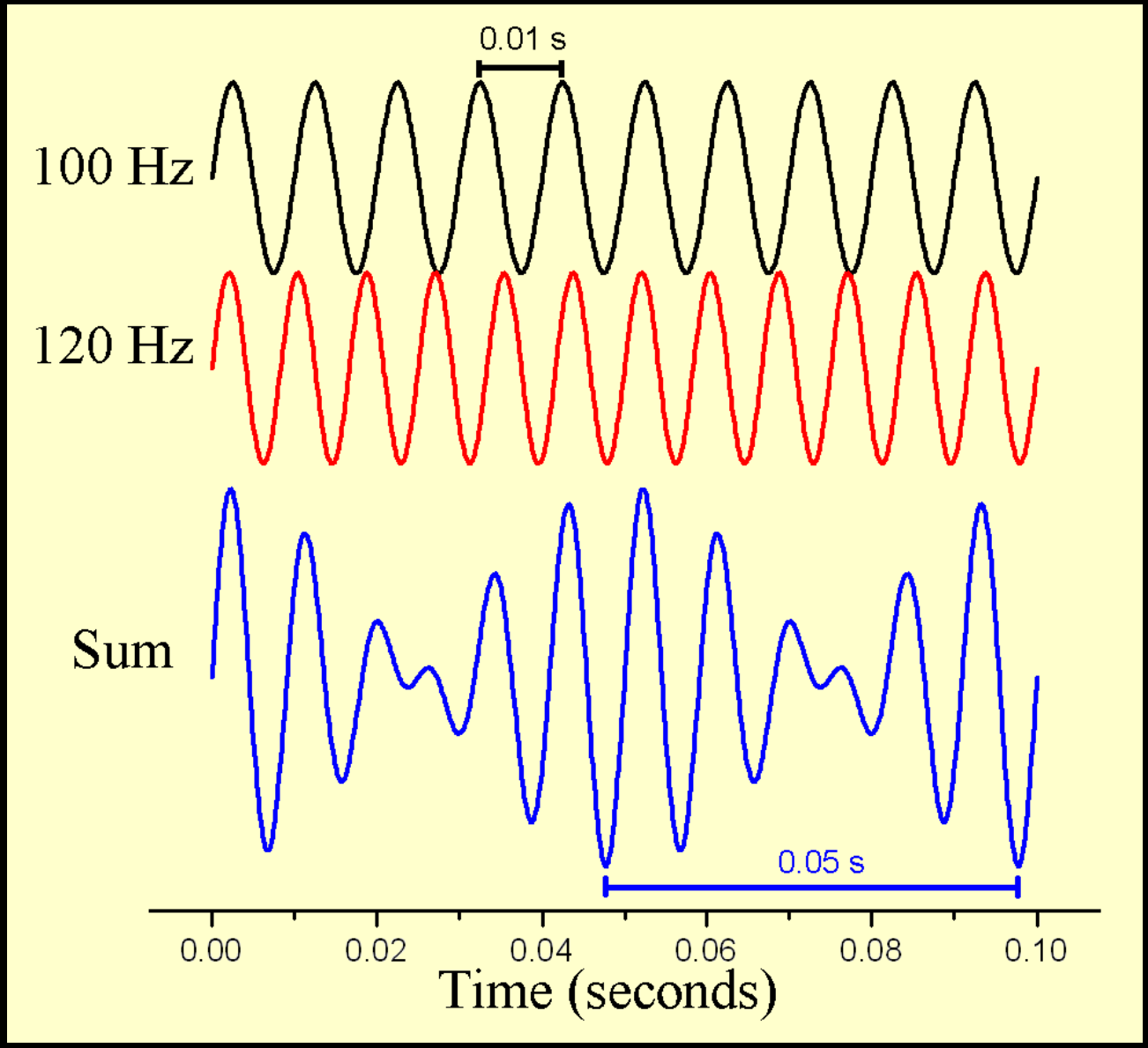
2x better resolution
much more contrast
low noise

Moiré Effect

Moiré Effect

Blurred

Moiré Effect

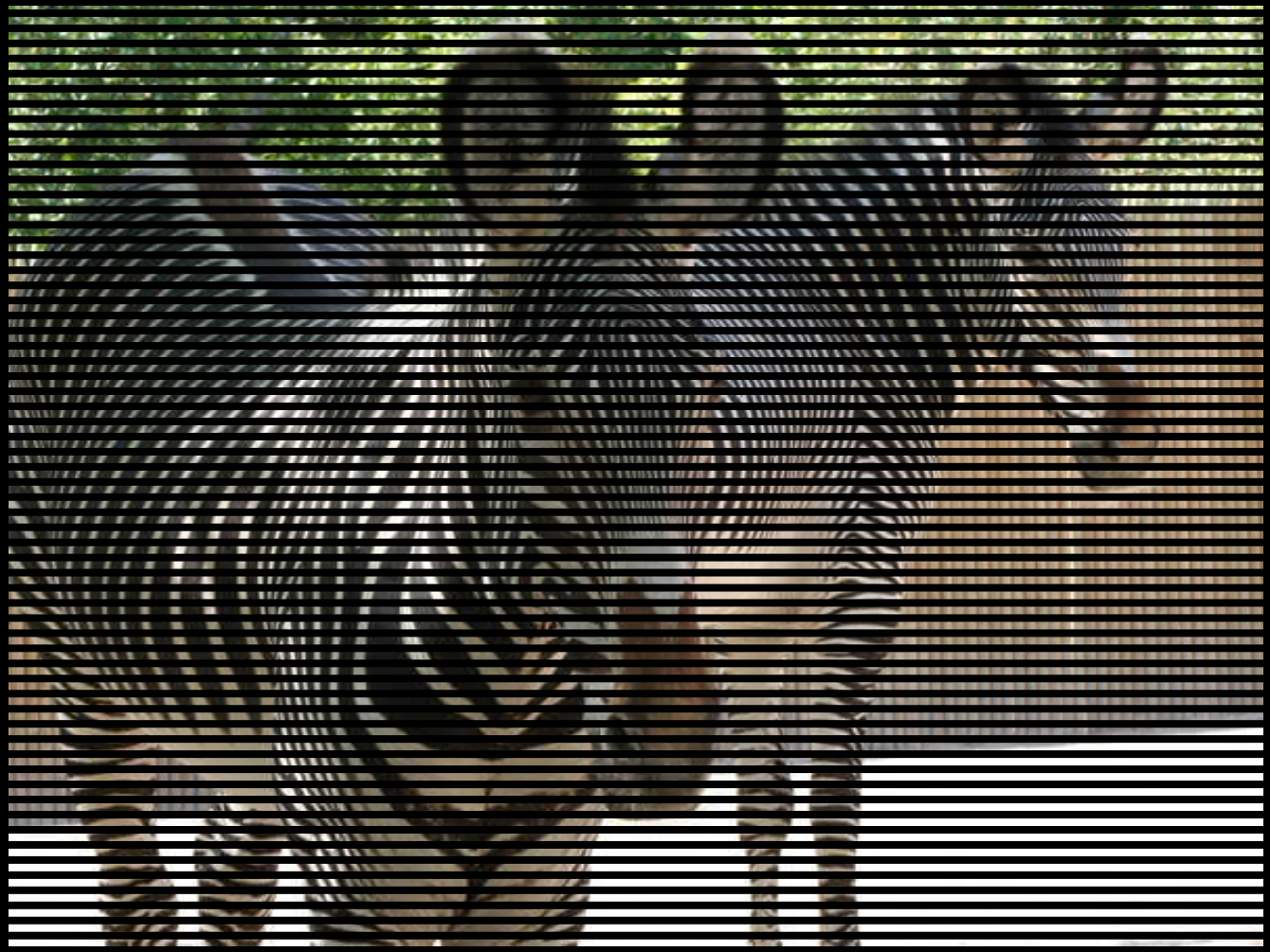


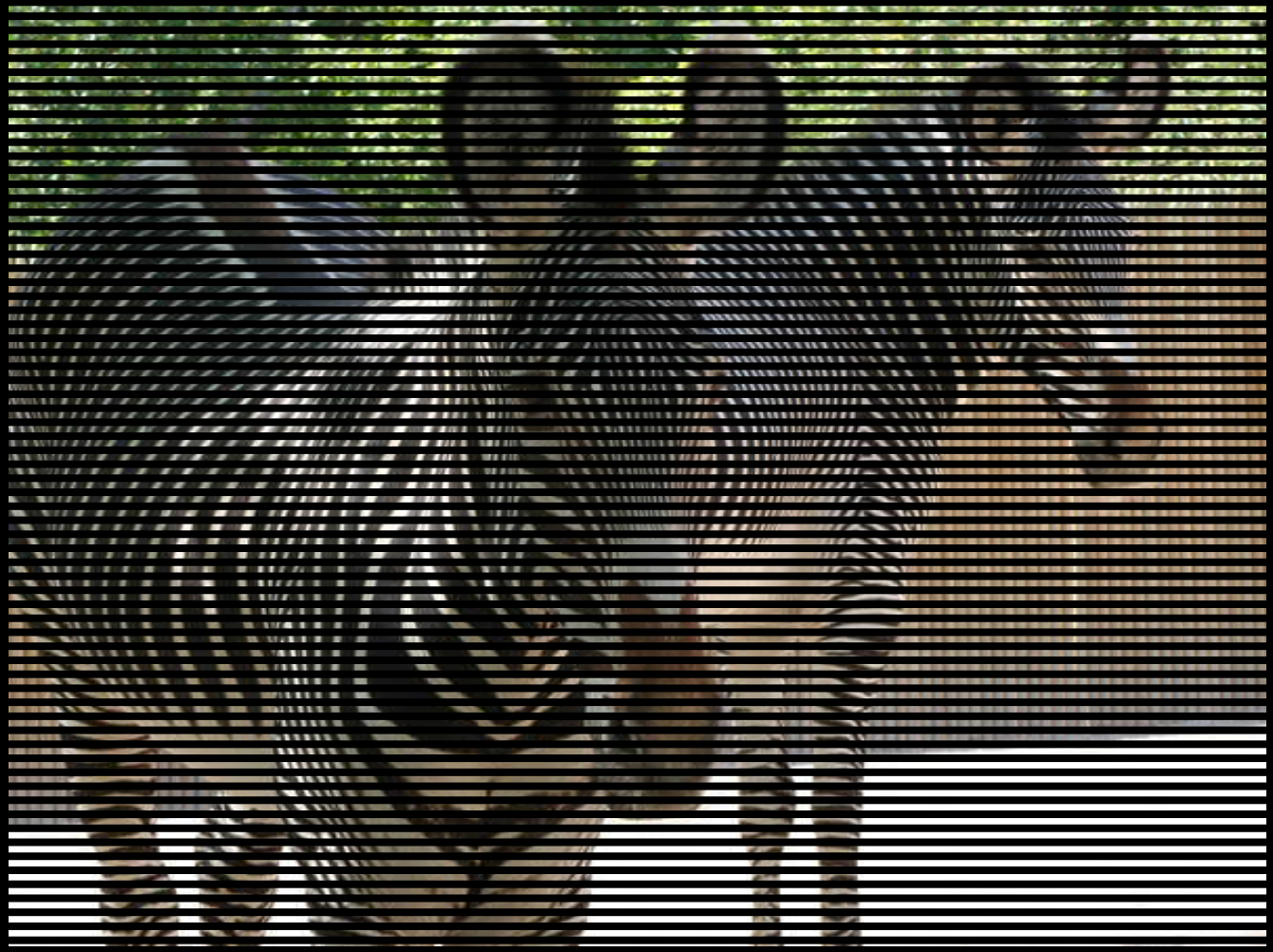
www.phy.mtu.edu/~suits/beats.html

Horses?

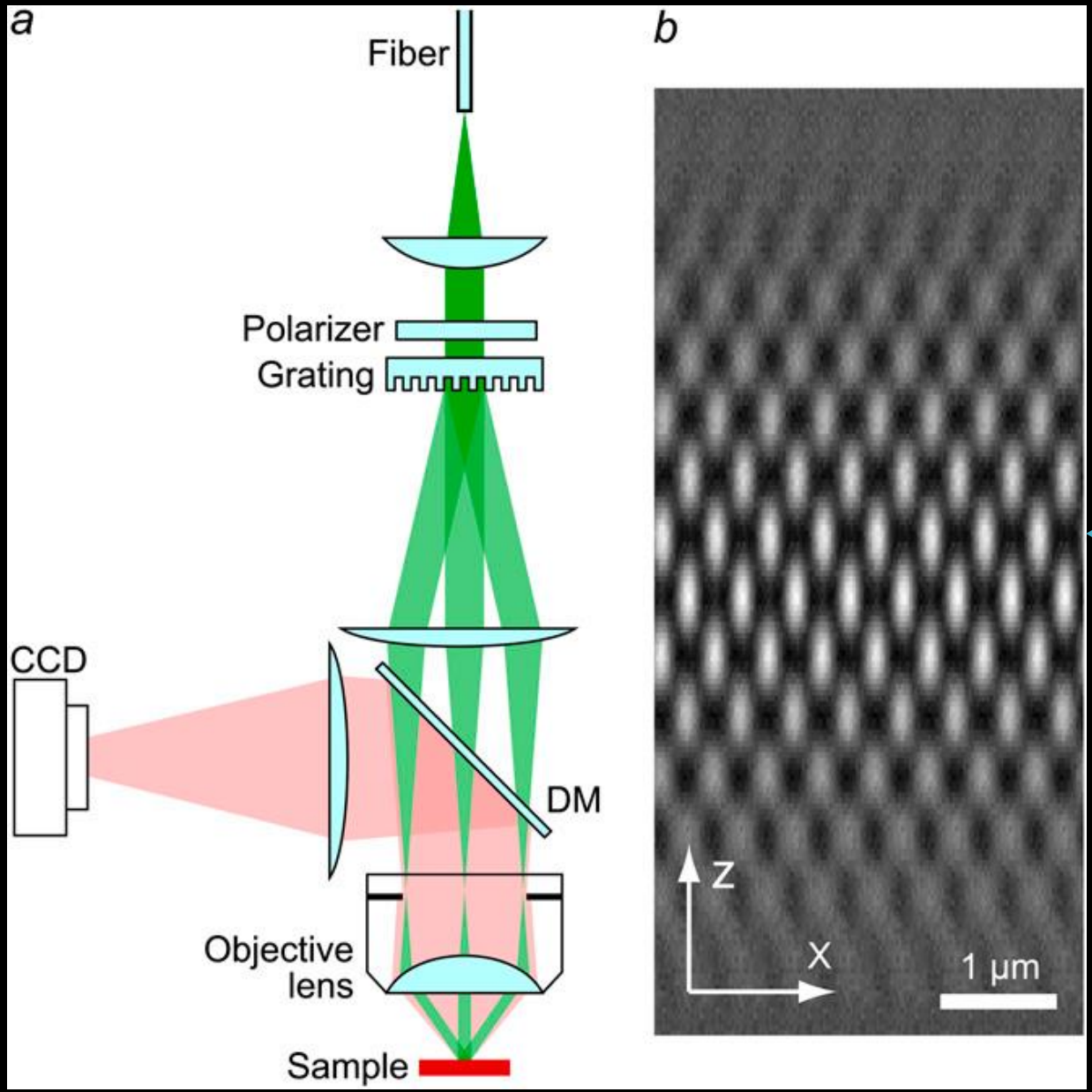
Courtesy Stephen Cody Ph.D, Monash Micro Imaging

Zebras





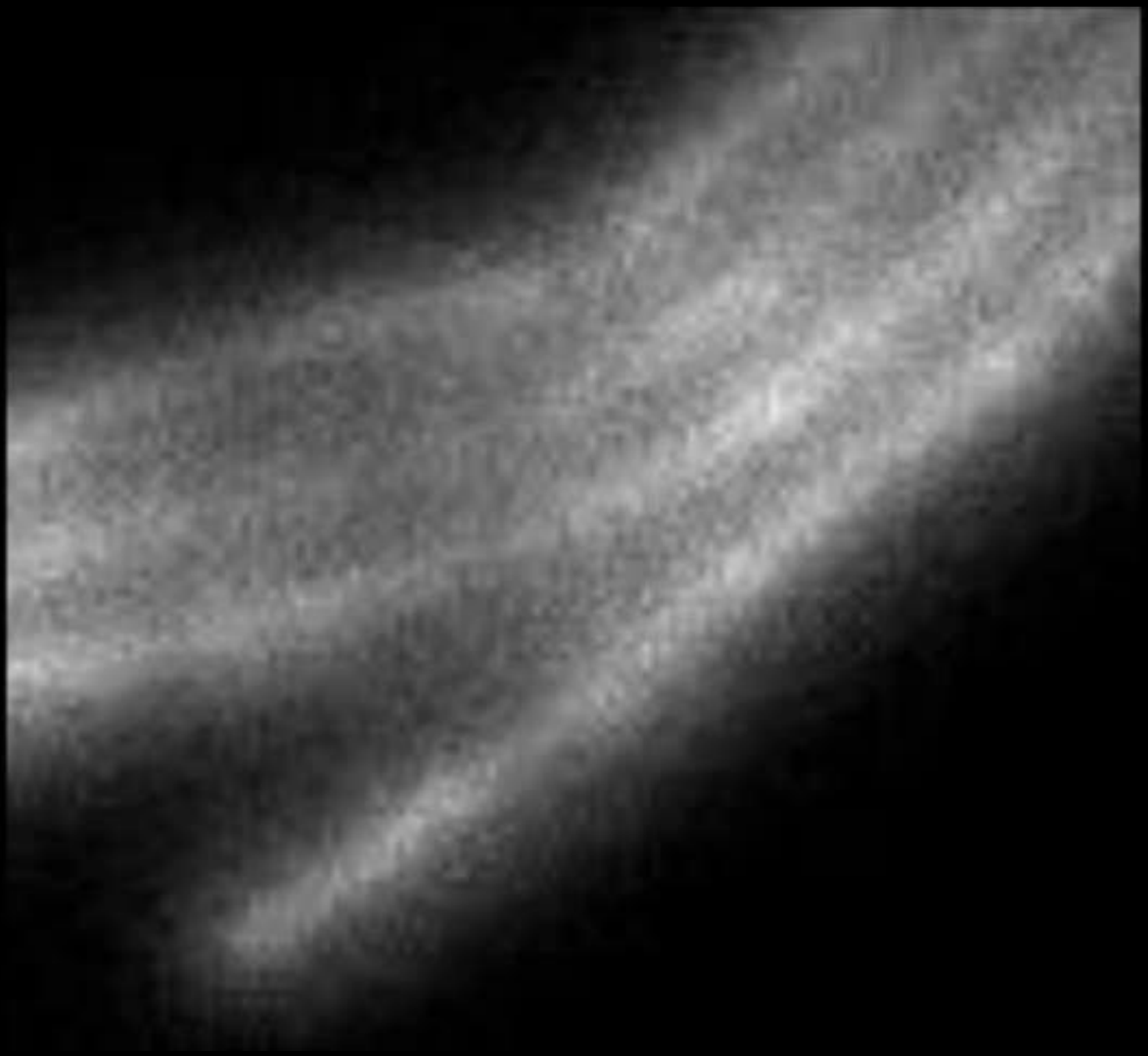
Structured Illumination Pattern Generator



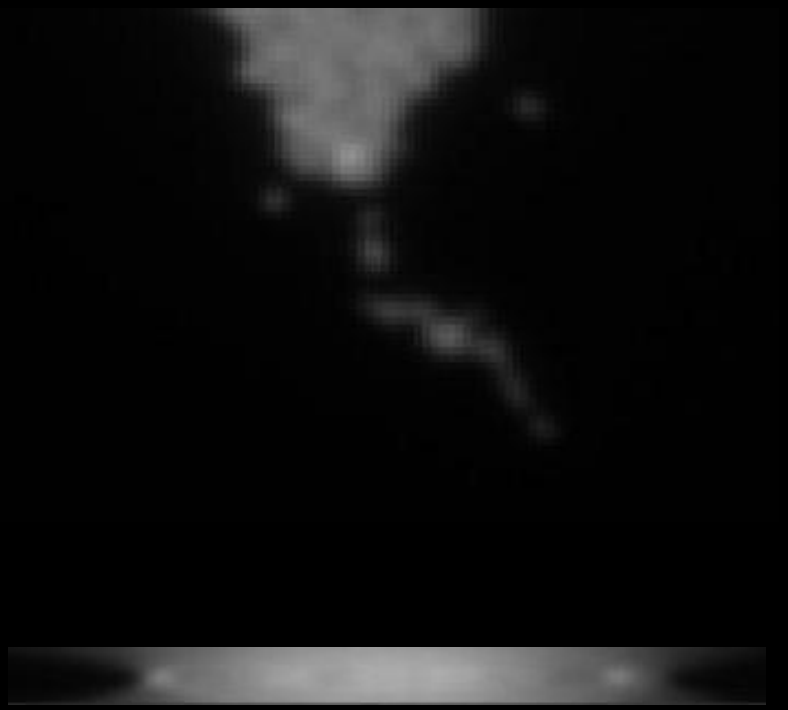
← focus

Structured Illumination Pattern Generator

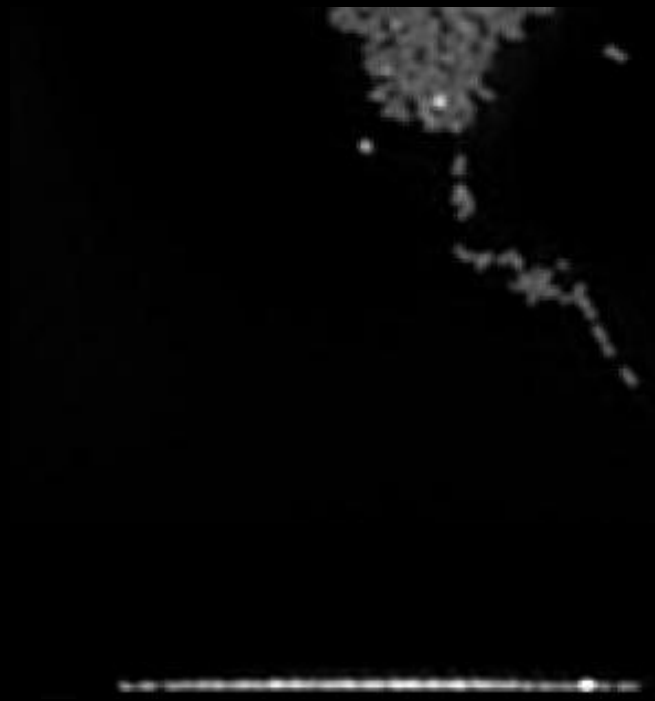
SIM pattern in action



Z axis
contrast improvement



Widefield



3D-SIM

resolution
contrast

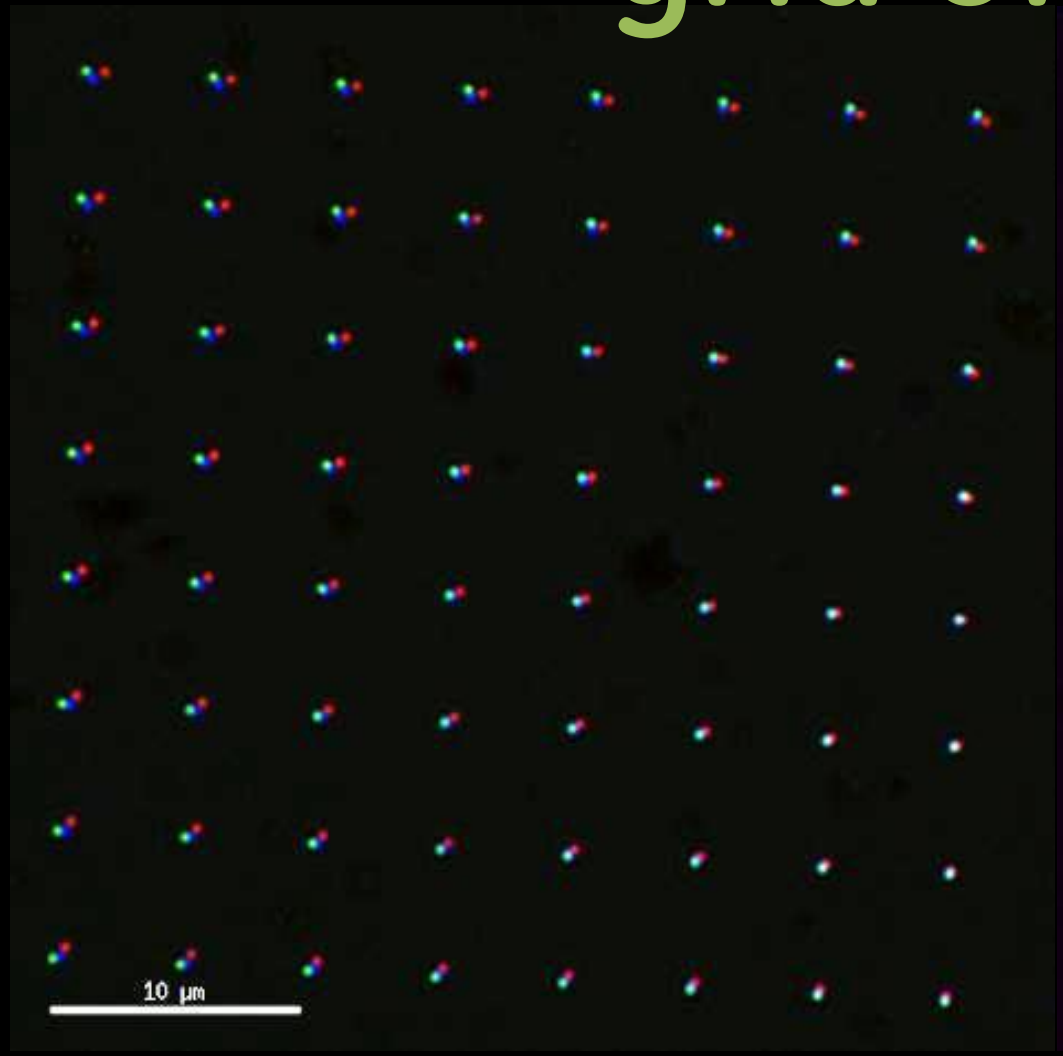
	widefield restored	3D-SIM
Lateral, xy		
Resolution*	225 nm*	130* nm
Contrast	145:1	3000:1
Axial, z		
Resolution*	450 nm*	330* nm
Contrast	3:1	3000:1

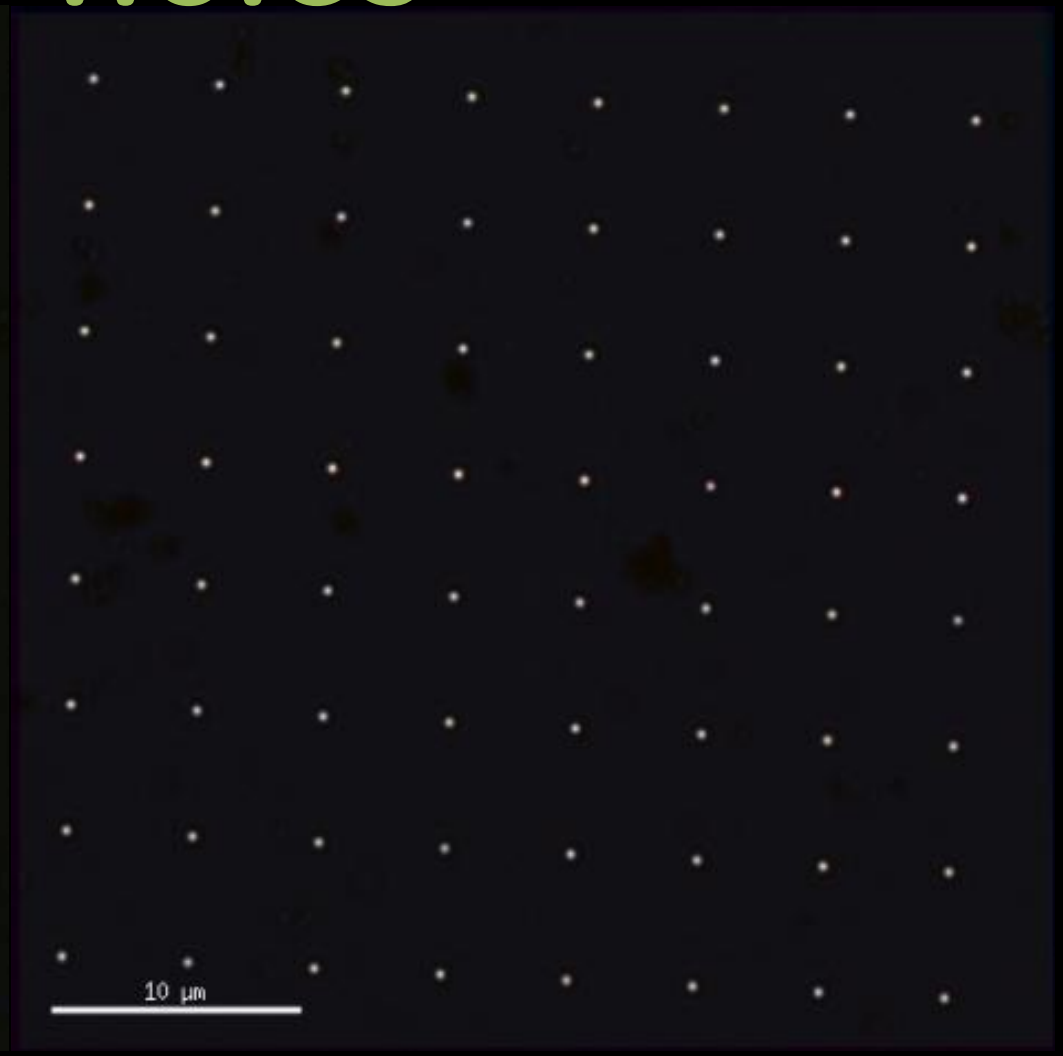
* wavelength dependent, measured at ~540 nm emission

the killer application

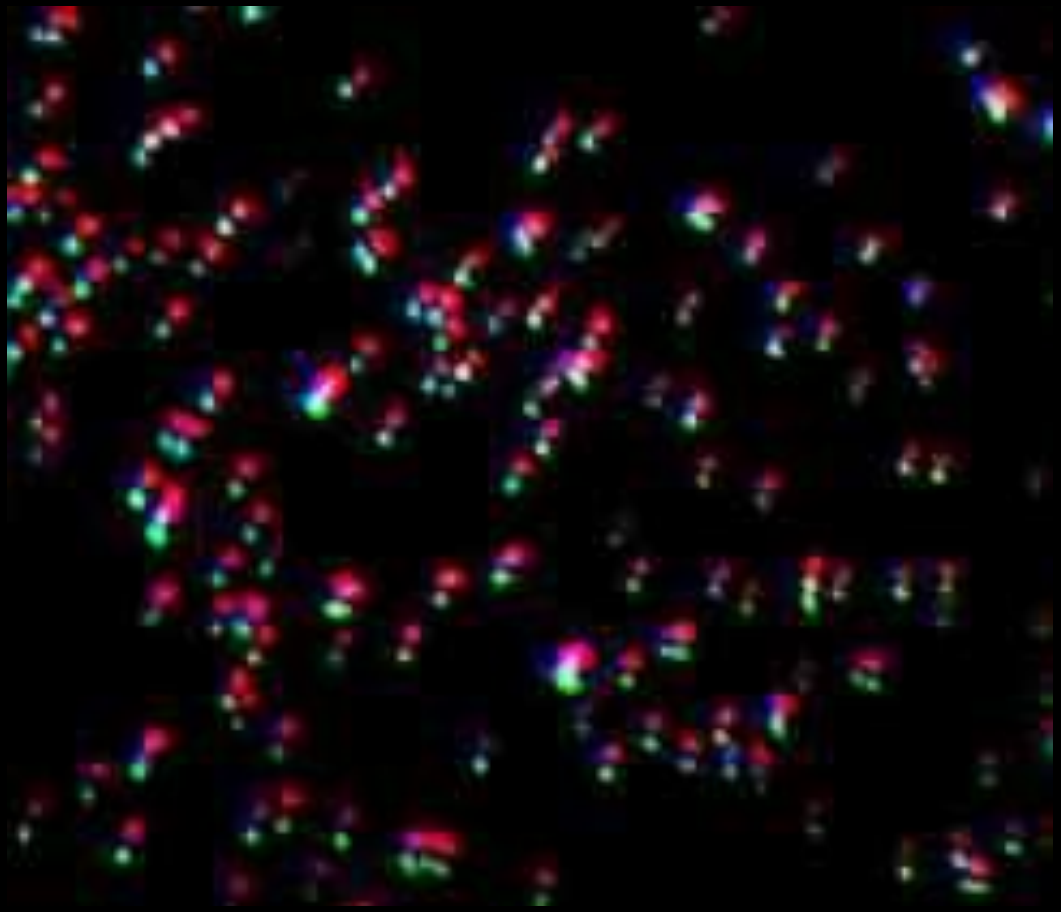
colocalization

color shift calibration
grid of holes

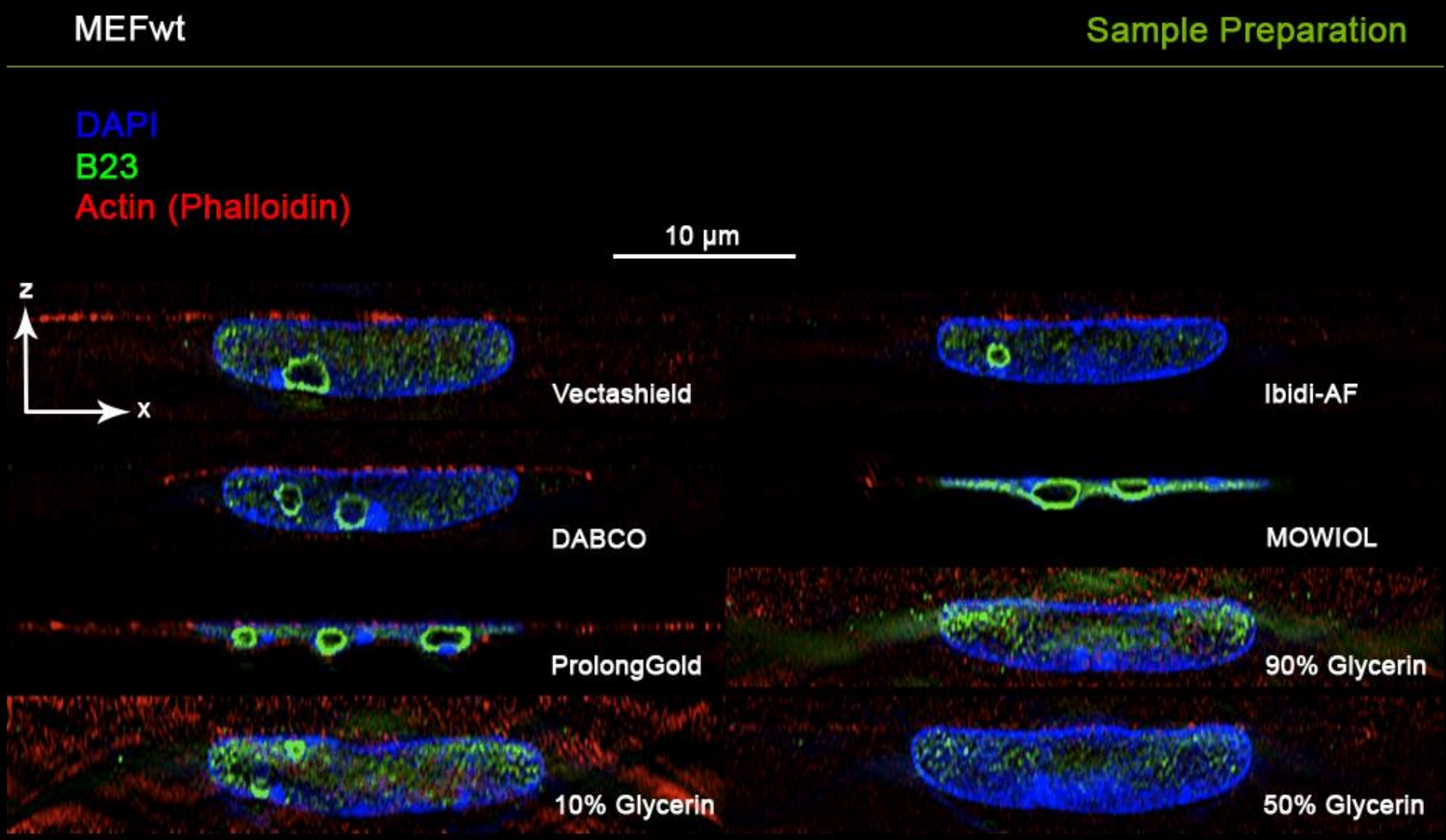




colocalization fixed!



One Last Thing…



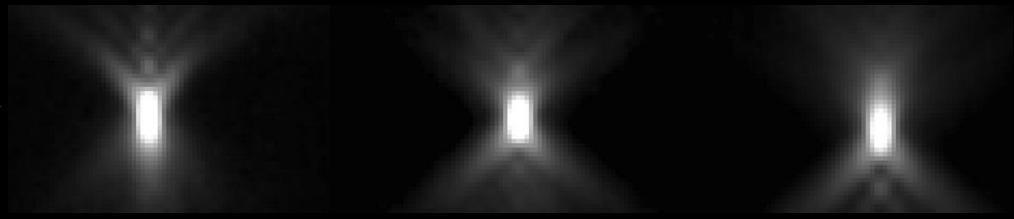
Images (3D-SIM): Andreas Maiser (LMU München)

Links and Further Info:

OMX Websites:

GE lifesceinces - OMX SR www.gelifesciences.com/dvomxsr

OMX@Harvard
omx.hms.harvard.edu



www.ibiology.org YouTube videos:
Microscopy: Point Spread Function (Jeff Lichtman)
youtube.com/watch?v=JQy94K94nL0
Microscopy: Deconvolution Microscopy (David Agard)
youtube.com/watch?v=MJ2FOFqr7hw
Microscopy: Structured Illumination (David Agard)
youtube.com/watch?v=i73HhpLJrqs

Abbe's Diffraction Experiments (Peter Evennett, RMS) vimeo.com/2150123

QED 2/4: Fits of Reflection and Transmission (Richard Feynman)
youtube.com/watch?v=kMSgE62S6oo How light *really* works.