

Saturated SIM (SSIM)

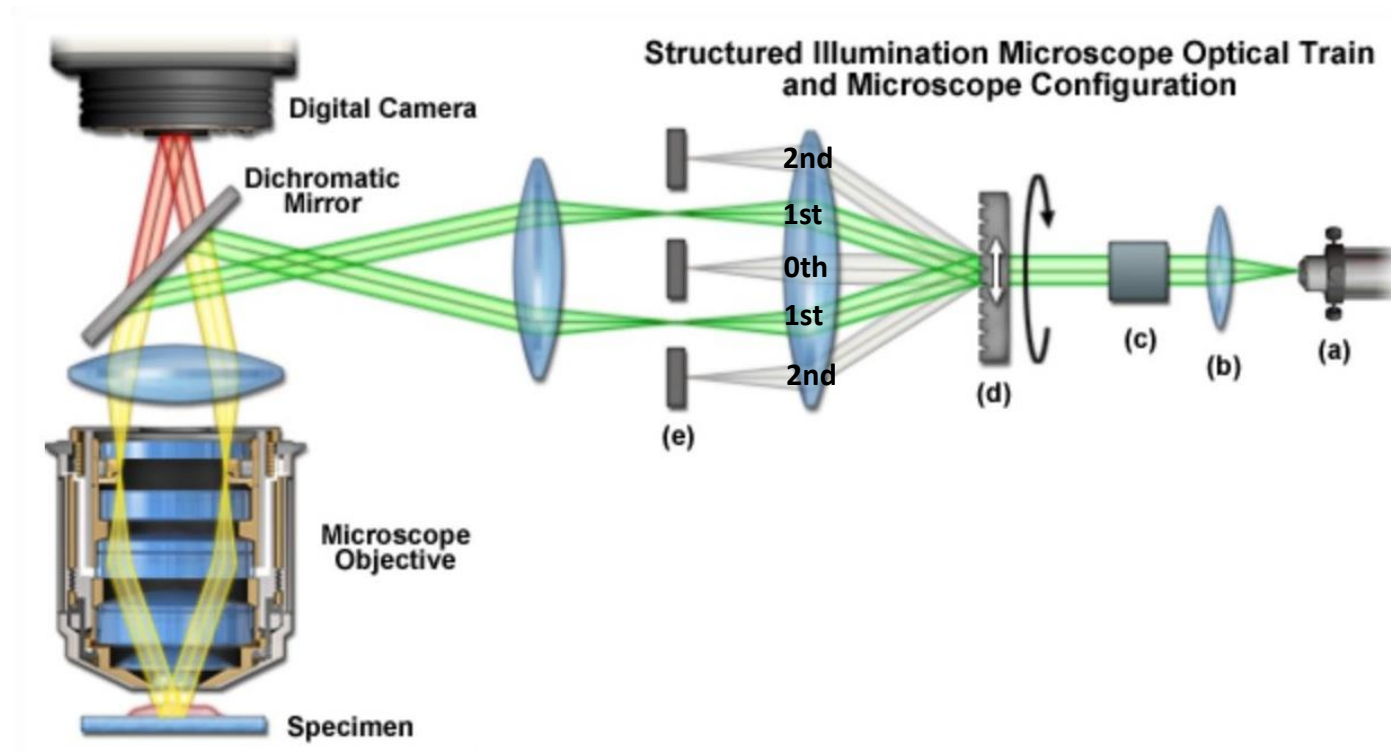
Ing. Martin Čapek, Ph.D.

Light Microscopy Core Facility, IMG CAS

Motivation

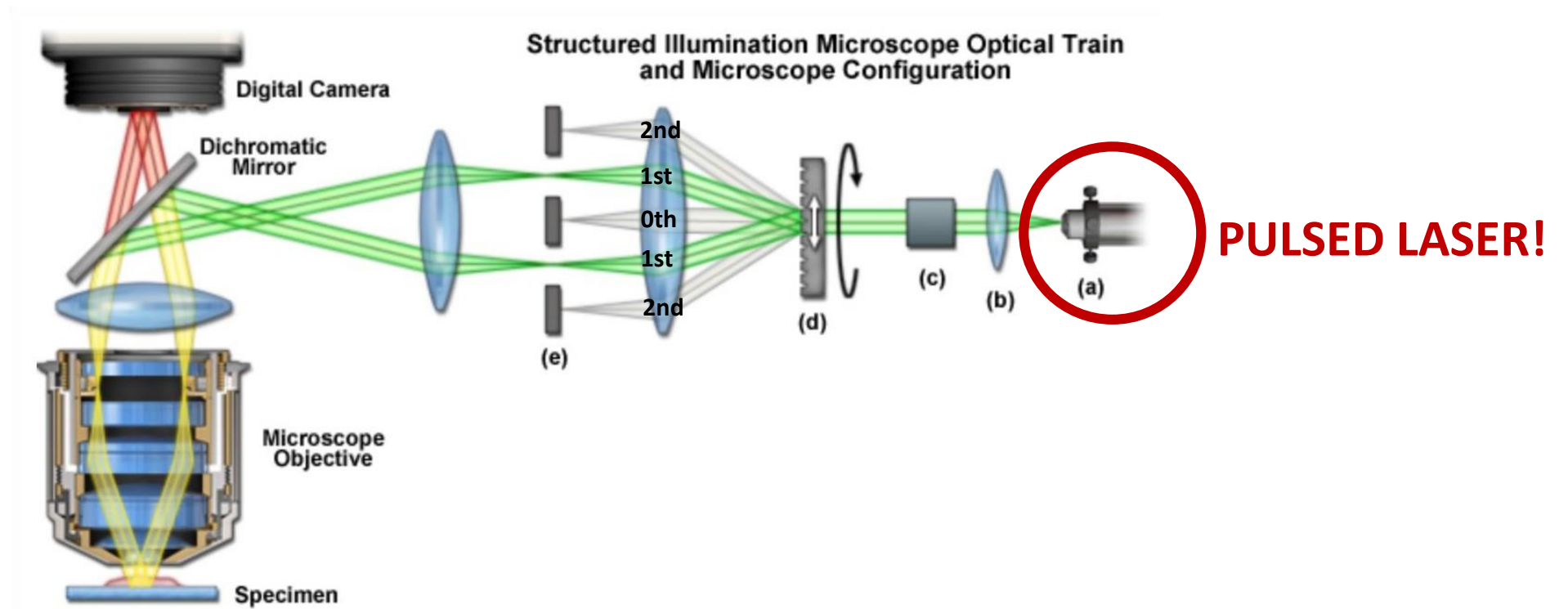
... to **increase resolution** of the microscope even more
to see **subtler details**.

2D SIM – a schematic (lateral resolution increased)



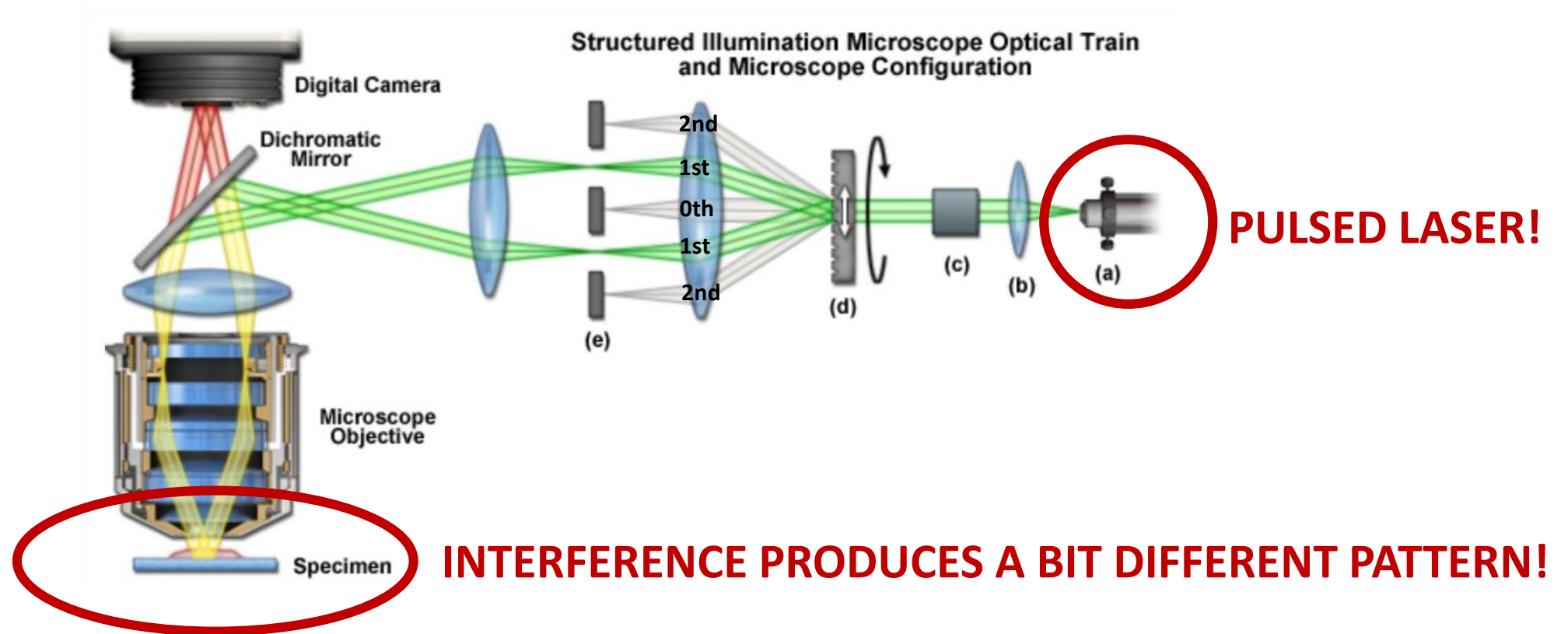
- a) laser – a source of excitation; b) collimator; c) polarizer;
d) **diffraction grating**; e) beam block

2D SIM – a schematic (lateral resolution increased)



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

2D SIM – a schematic (lateral resolution increased)

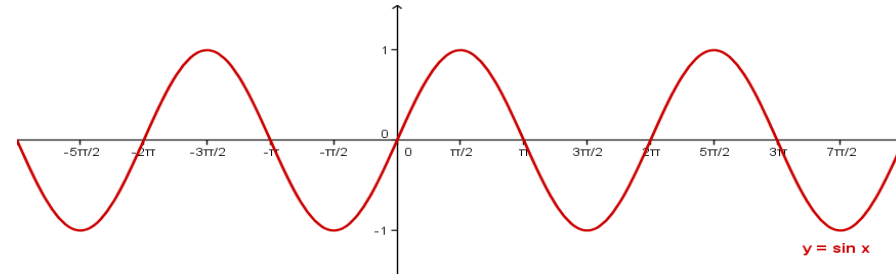
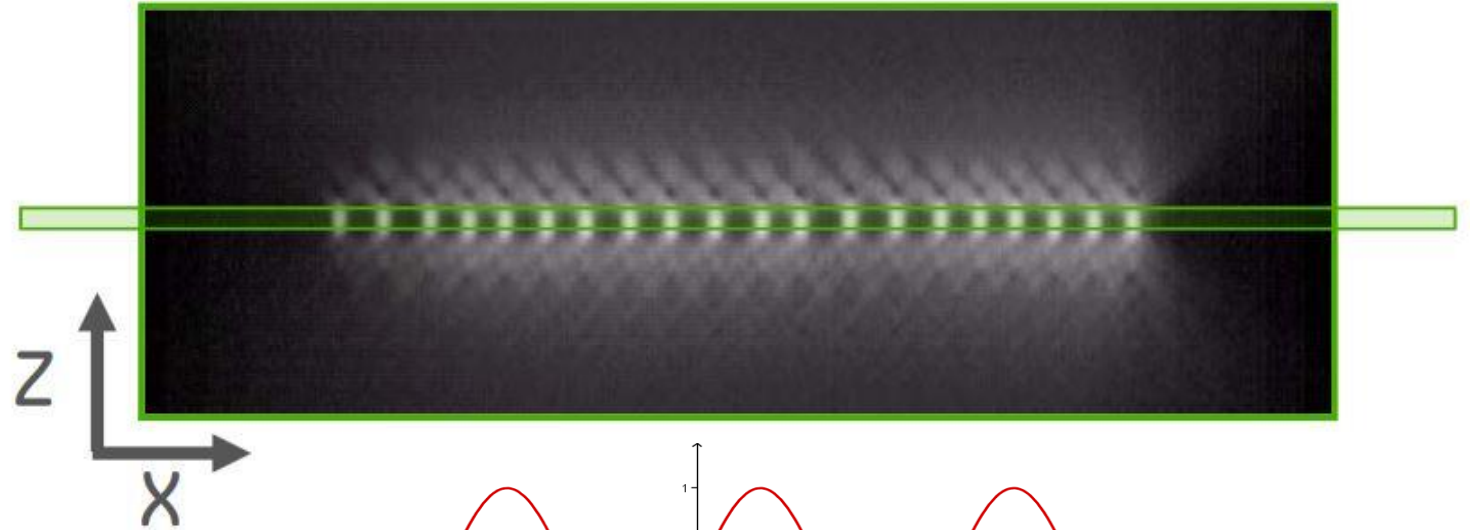


- a) laser – a source of excitation; b) collimator; c) polarizer;
d) **diffraction grating**; e) beam block

Standard 2D-SIM - Illumination

Focal Plane

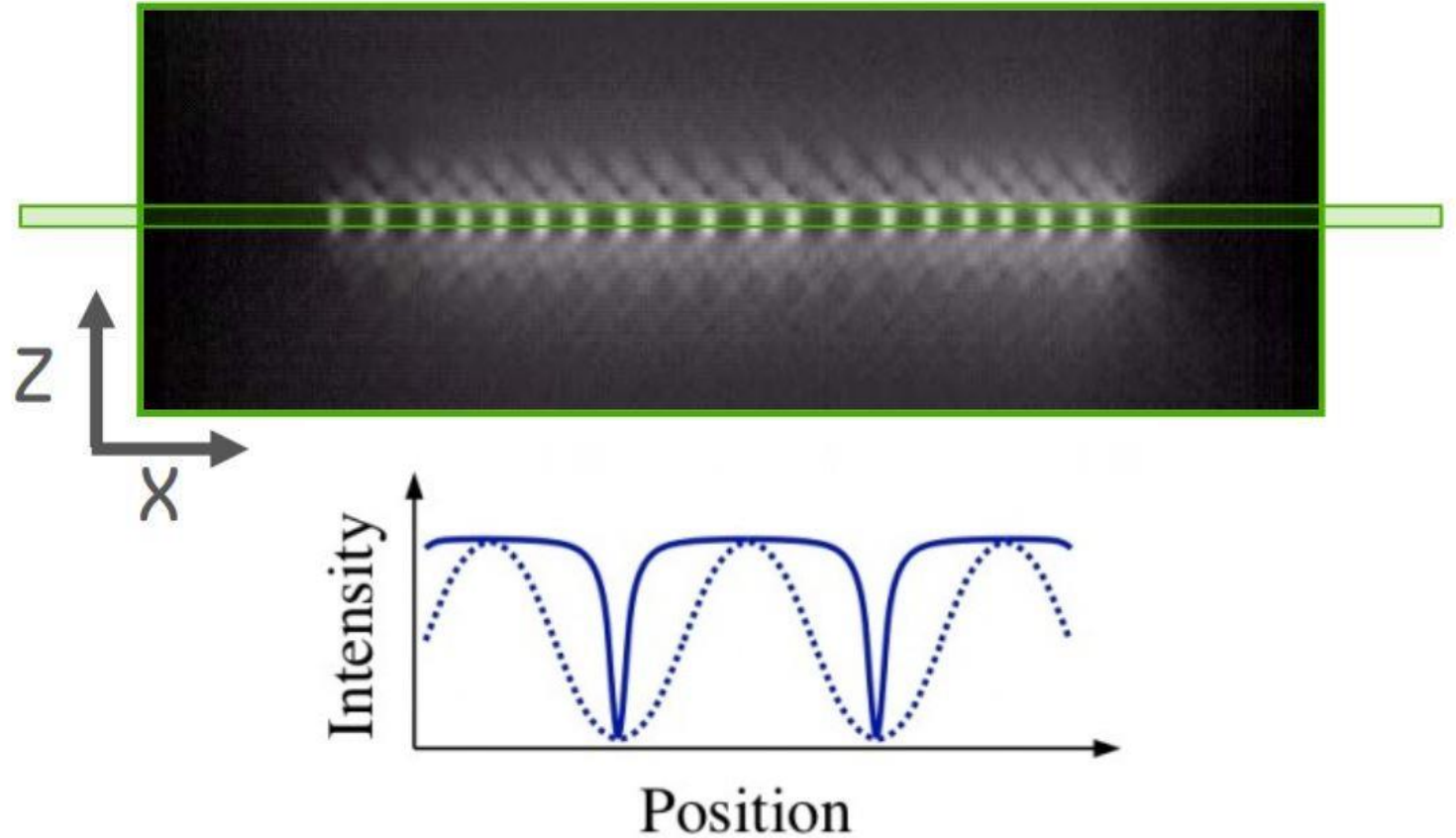
2 beam interference



Standard 2D-SIM + SSIM - Illumination

Focal Plane

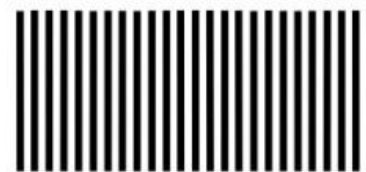
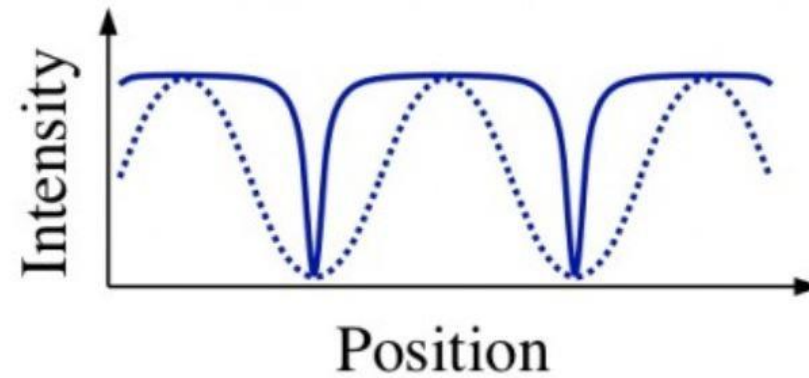
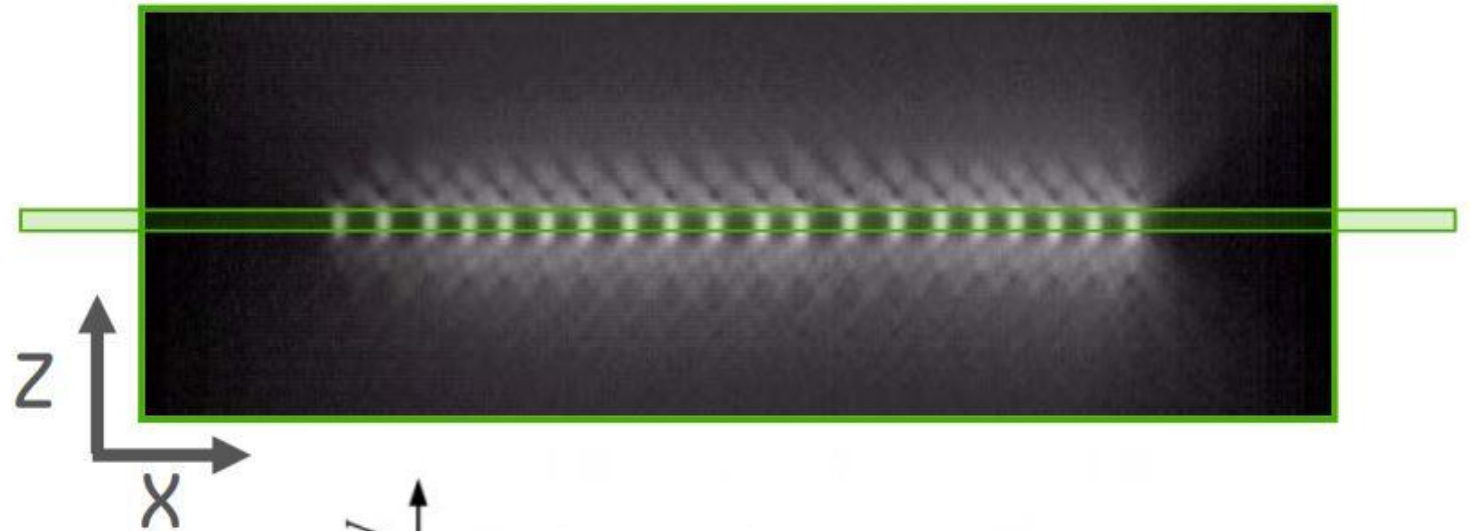
2 beam interference



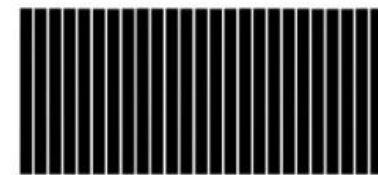
Standard 2D-SIM + SSIM - Illumination

Focal Plane

2 beam interference

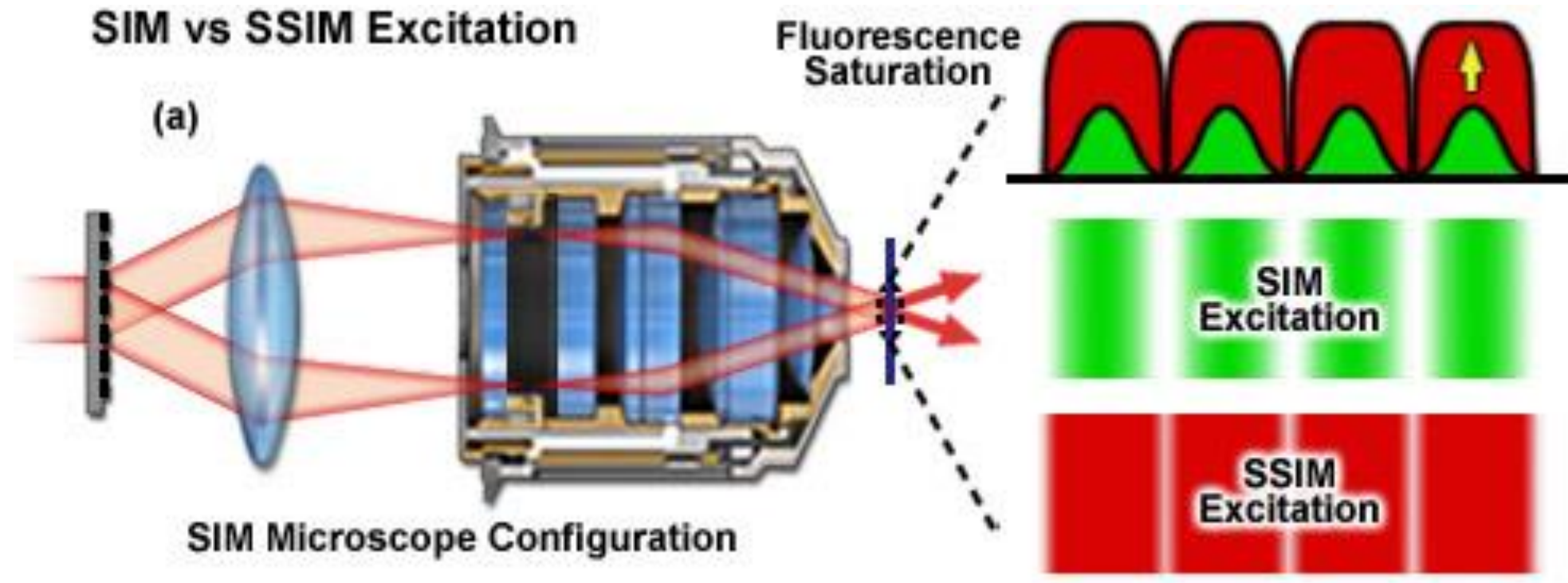


SIM

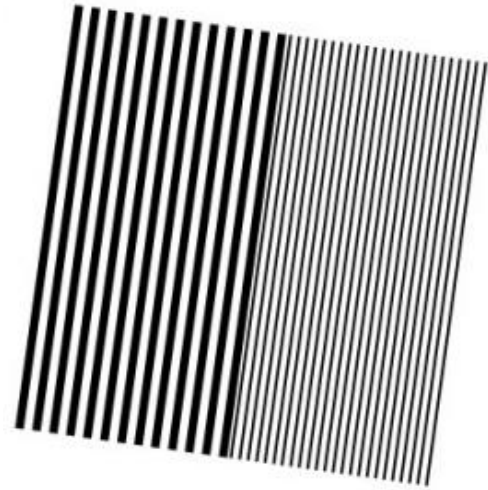


SSIM

Standard 2D-SIM + SSIM - Illumination



Effect of using high energy laser pulses...



Sample



SIM
excitation



SSIM
excitation

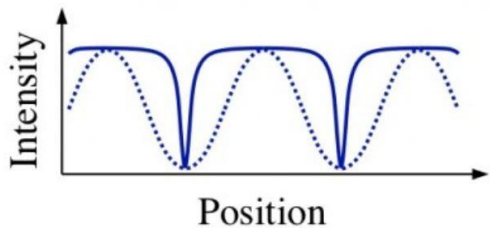
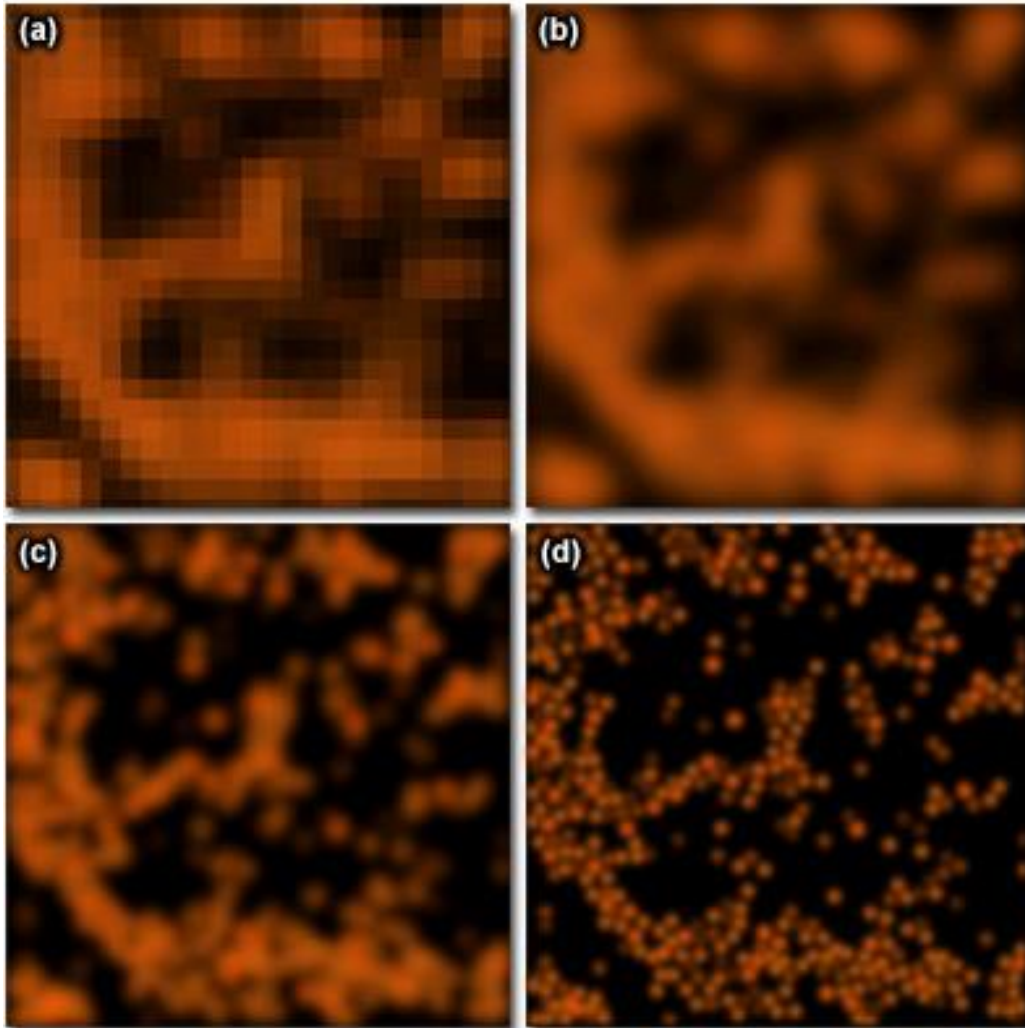
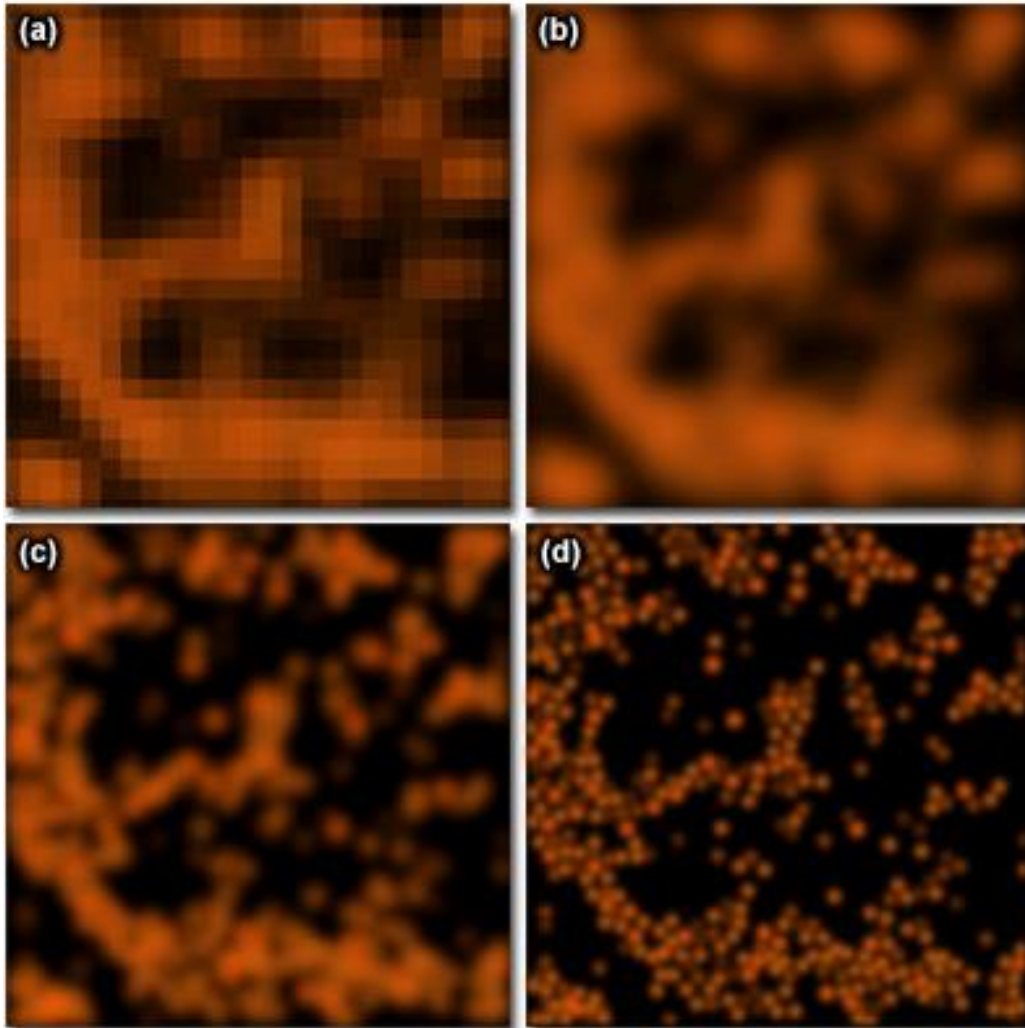


Image results - 50nm fluorescent beads



- (a) WF
- (b) WF + filtering
- (c) SIM
- (d) SSIM

Image results - 50nm fluorescent beads



(a) WF

(b) WF + filtering

(c) SIM

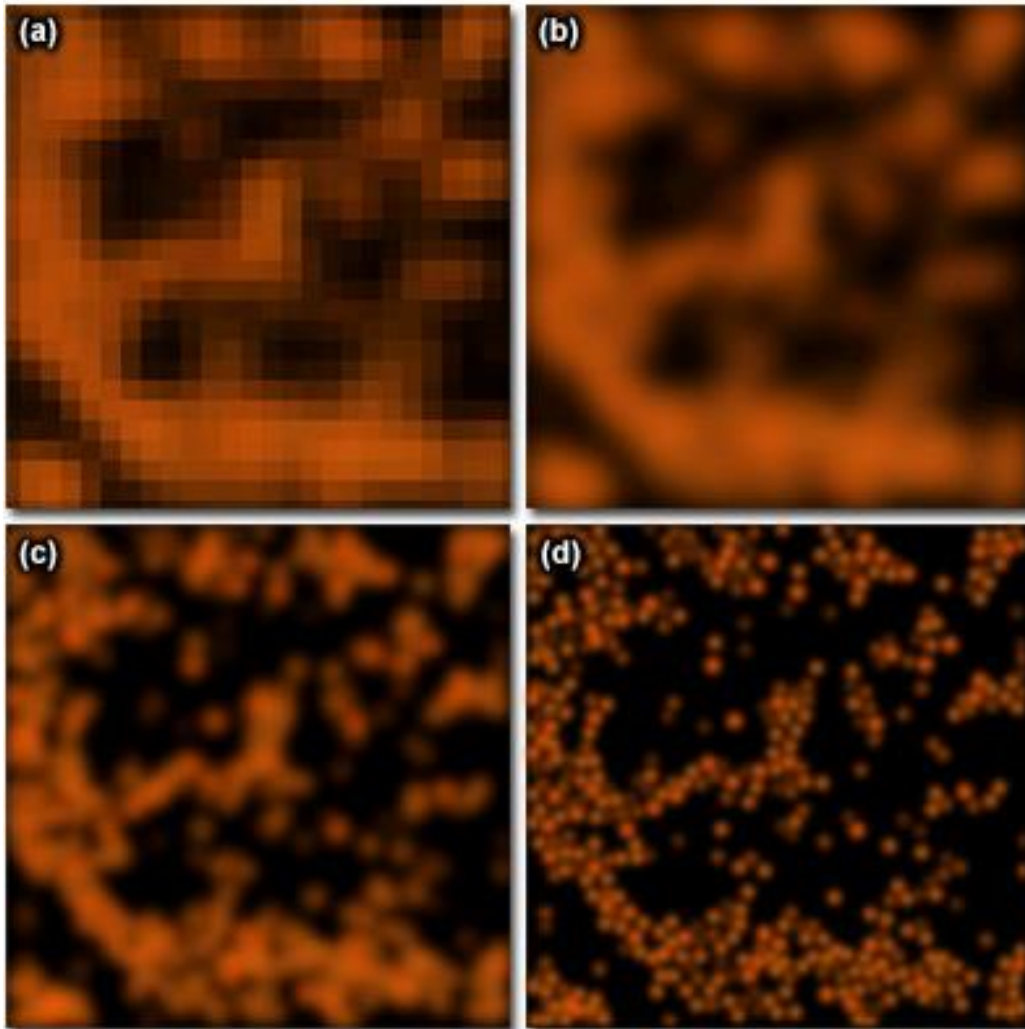
(d) SSIM



SIM – doubling the resolution

SSIM – quadruplying the resolution

Image results - 50nm fluorescent beads



- (a) WF
- (b) WF + filtering
- (c) SIM
- (d) SSIM



SIM – doubling the resolution
SSIM – quadruplying the resolution

**... increasing resolution is
theoretically unlimited, it depends on
light energy applied**

Problem

Highly **increased excitation light energy**

⇒ high **photobleaching**

Thanks for your attention!