OUTLINE

- **Presentation of the facility**
- **Equipment**
  what news?
- **Super-Resolution (SR) microscopy**
  an essential step
- **Open discussion**
  - Identify ways to optimize the facility's efficiency
  - Evaluate common needs
  - Discuss ways to fund new equipments & scientific contributions

The Bioimaging Core Facility

- Created in 2001

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- 15 acquisition systems
- 6 PC workstations
- 1 server 16 To
- Staff & Committee

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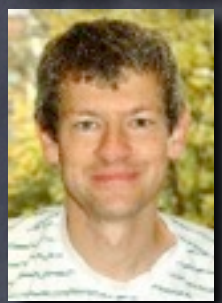
- 19'500 hours of use
- Biggest numbers of users
  (400 from 175 groups)
- A dozen of events
  (Demos, Talk, Courses, Meetings, ...)

(in 2013)

The Bioimaging Core Facility

The committee



Dr. Bernhard Wehrle-Haller (academic supervisor)

Pr. Alexandre Dayer (academic supervisor)

Dr. Sophie Leboube

Pr. Patrick Meraldi

The staff

François Prodon (coordinator)

Olivier Brun (technical assistance)

Sergei Startchik (Image Analysis Assistance)

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- Swiss bioimaging network
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(in 2013)

Groups' contribution

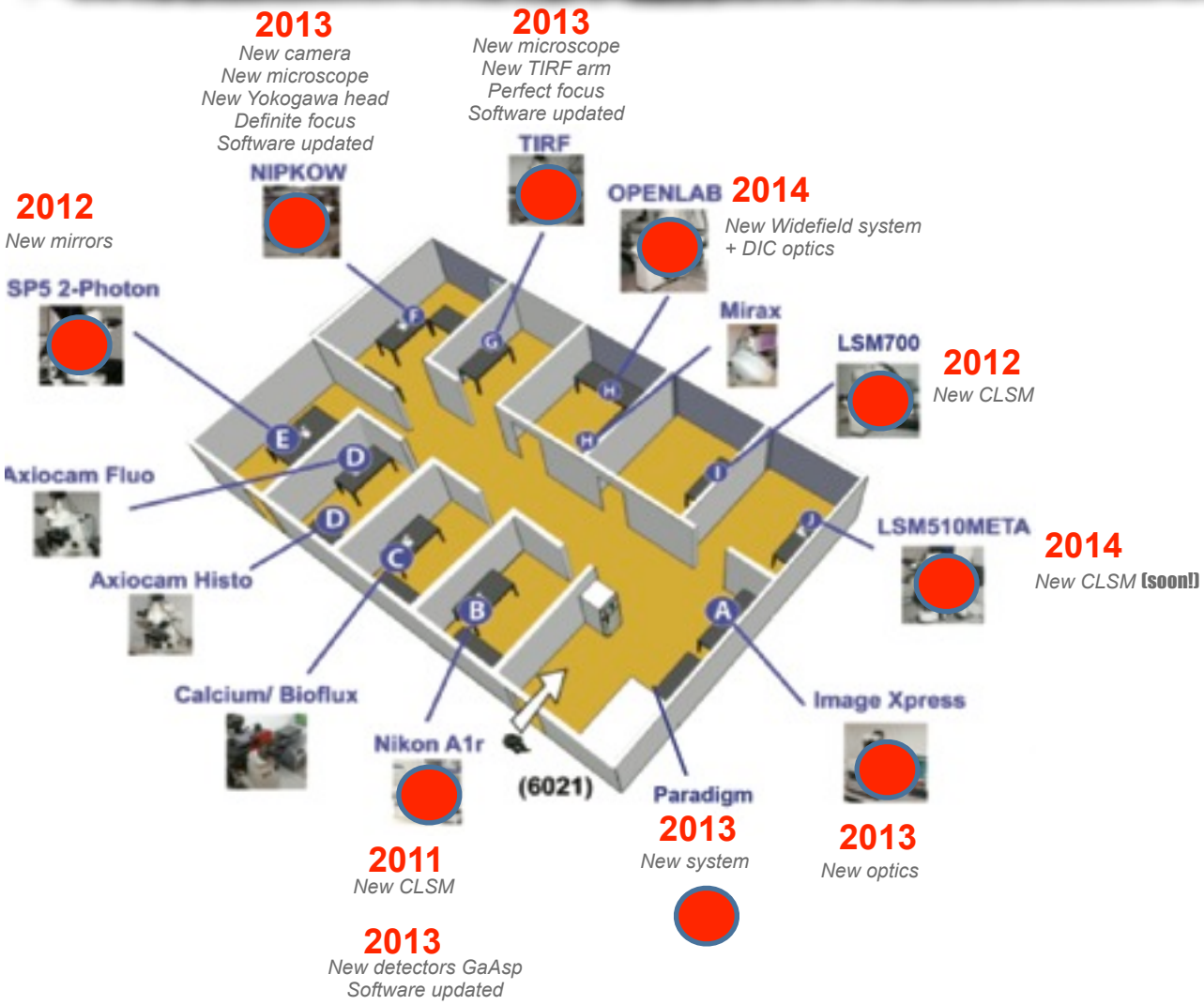
Half of the equipment was acquired with the help of a scientific contribution as shown in blue

+ Laser Capture LMD
+ Olympus VS120 (Slide reader)

Pre. Gotta
Pre. Wohlwend

Collaboration /partnership

Upgrades & new acquisitions



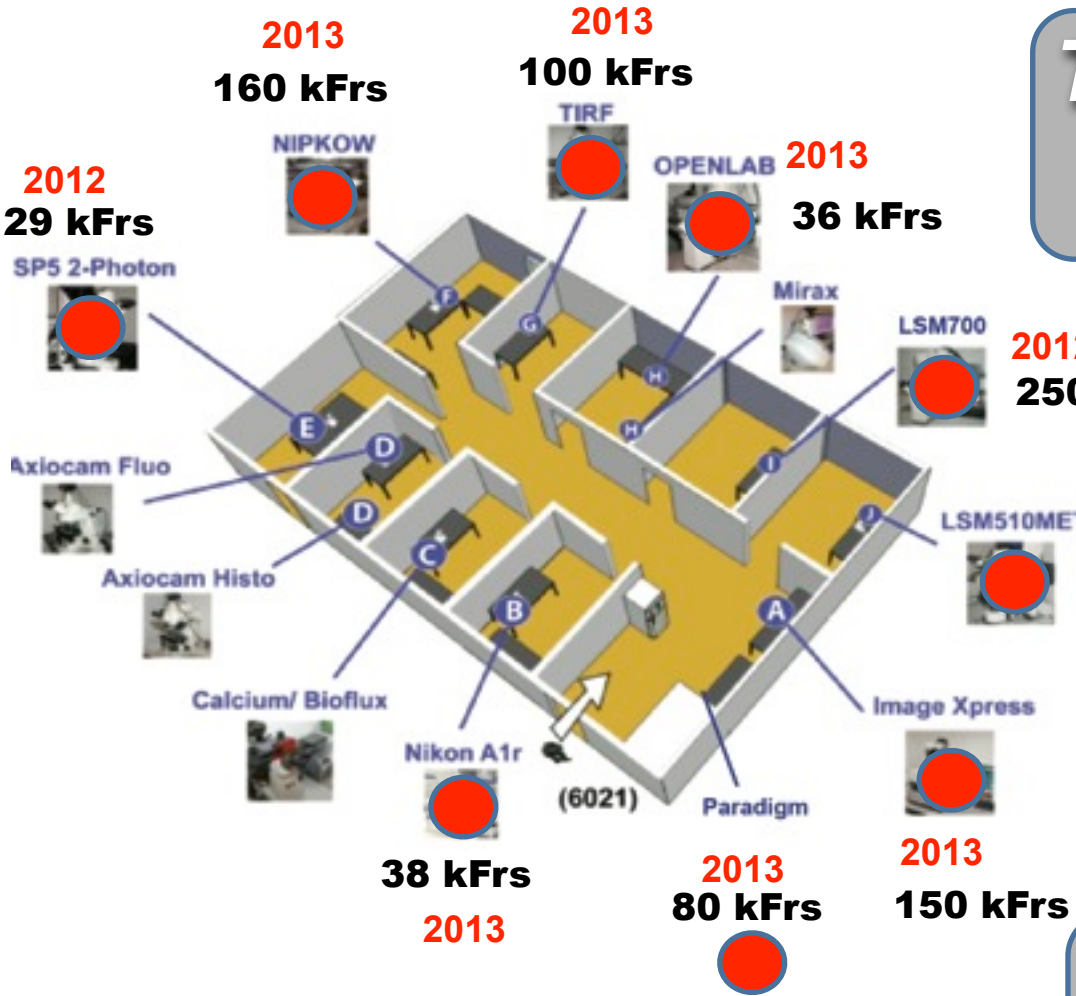
Two-thirds of the machine park was upgraded or renewed these last 2 years as shown in red

+ Laser Capture LMD
+ Olympus VS120 (Slide reader)

New camera, new module for automation (software) **2012**

New system **2013**

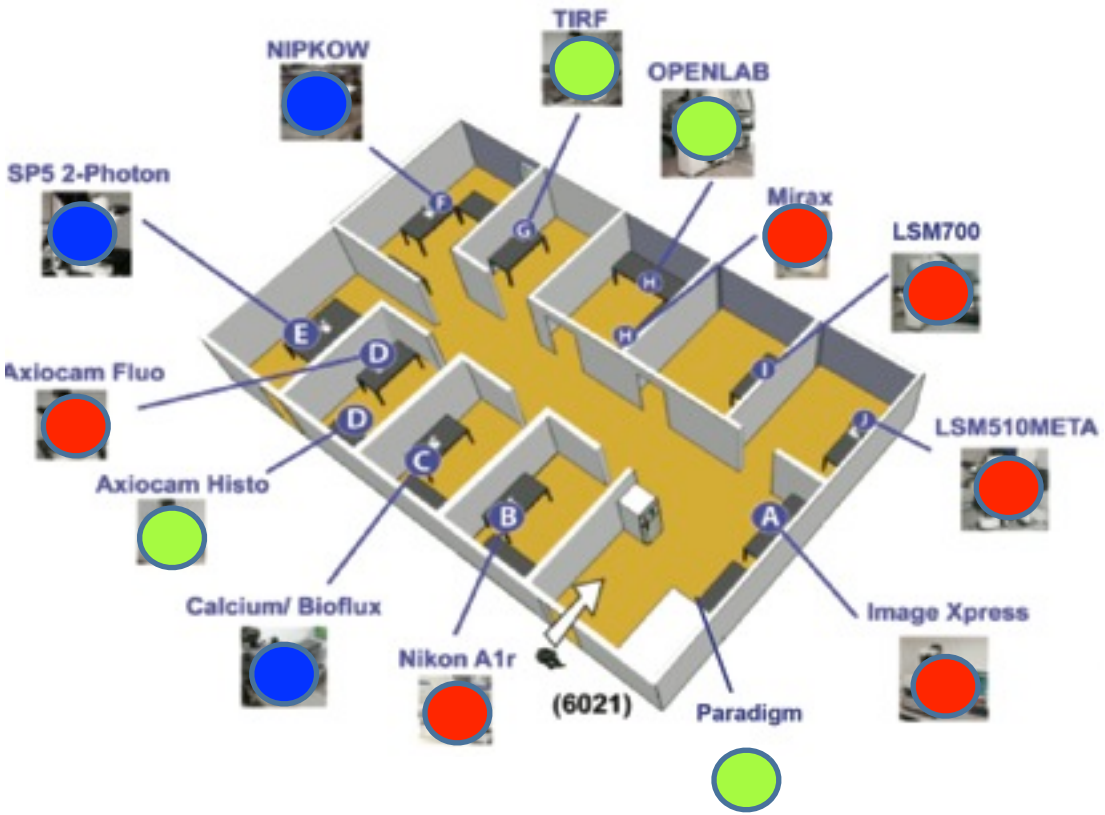
Upgrades & new acquisitions



Total (last 2 years): 1'493'000.-Frs

Running costs: 53'000.-Frs (2013) 95'000.-Frs (2012)

Different degrees of Occupancies



High
(>1500 hours)

Medium
(500–1500 hours)

Low
(<500 hours)

+ Laser Capture LMD
+ Olympus VS120 (Slide reader)

Number of Users & groups per machine

+ Laser Capture LMD 16/7
+ Olympus VS120 (Slide reader) 2/2

Number of Users & groups per machine

users/ groups (hours)

PC Mirax

14/10 (45 hours)

PC LSM510

PC MetaXpress

22/9 (206 hours)

PC MetaMorph RED

PC Imaris

41/27 (348 hours)

64/33 (1153 hours)

PC MetaMorph GREEN

58/28 (1052 hours)

Hours (total): 2804

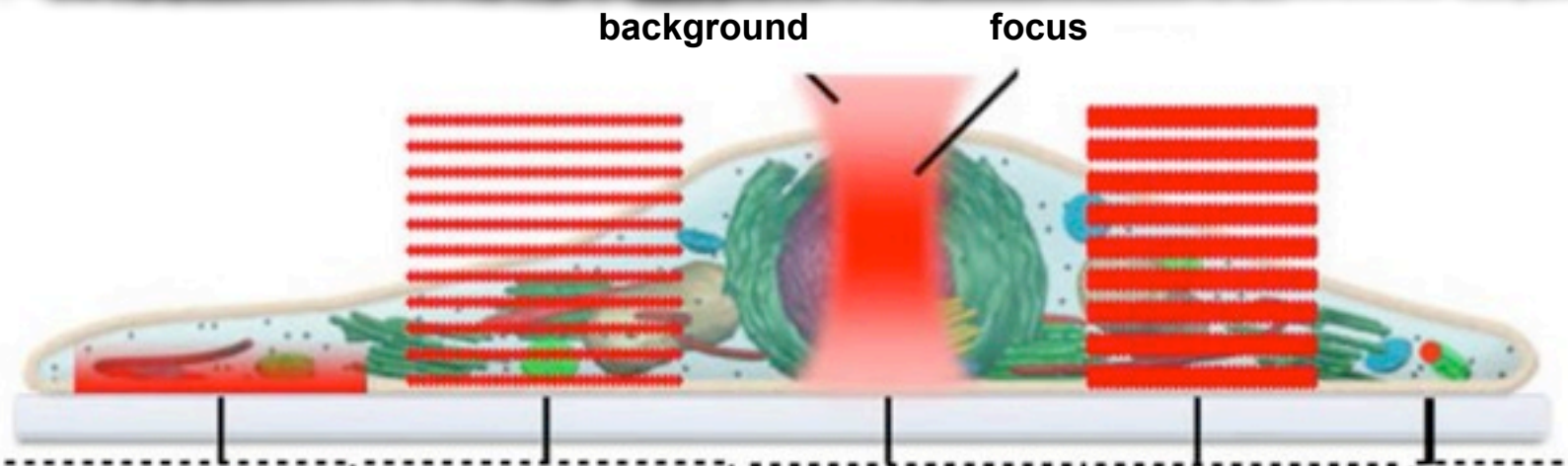
List of image processing softwares

- **ImageJ/ Fiji** (Open-source, freeware)
- **CellProfiler** (Open-source, freeware)
- **Metamorph** (3 fixed licences)
- **Definiens** (1 server licence)
- **Huygens** (1 licence + stabilizer + tracker options)
- **MatLab** (1 basic licence)
- **Imaris** (1 licence + filament tracer + colocalization...)
- **Leica/Zeiss/Nikon** (3 off-ine licences)

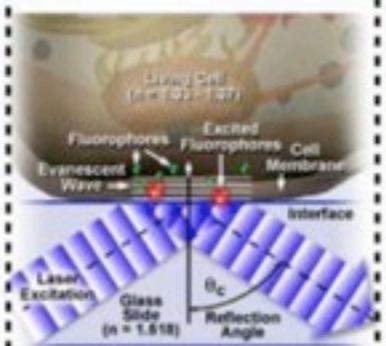
Total ≈80'000.-Frs (acquisition)

and then ≈ 8'000. -Frs /year (updates, 10 %)

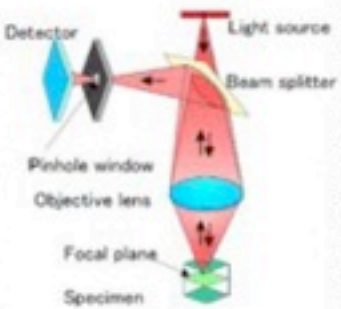
What type of technology is available?



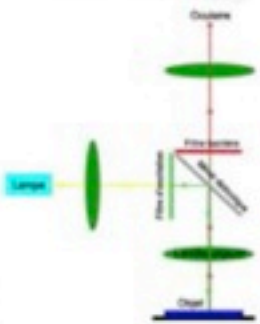
Evanescent Wave Microscopy (TIRFM*)



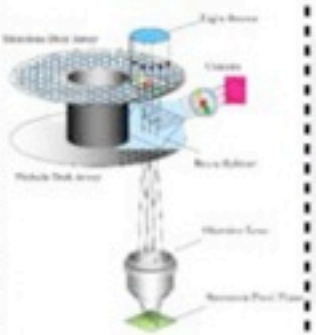
Confocal Laser Scanning Microscopy (CLSM)



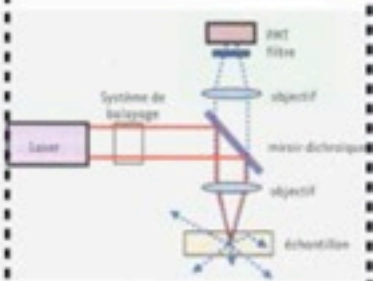
Fluorescence Widefield Microscopy



Nipkow Spinning Disk Confocal Microscopy



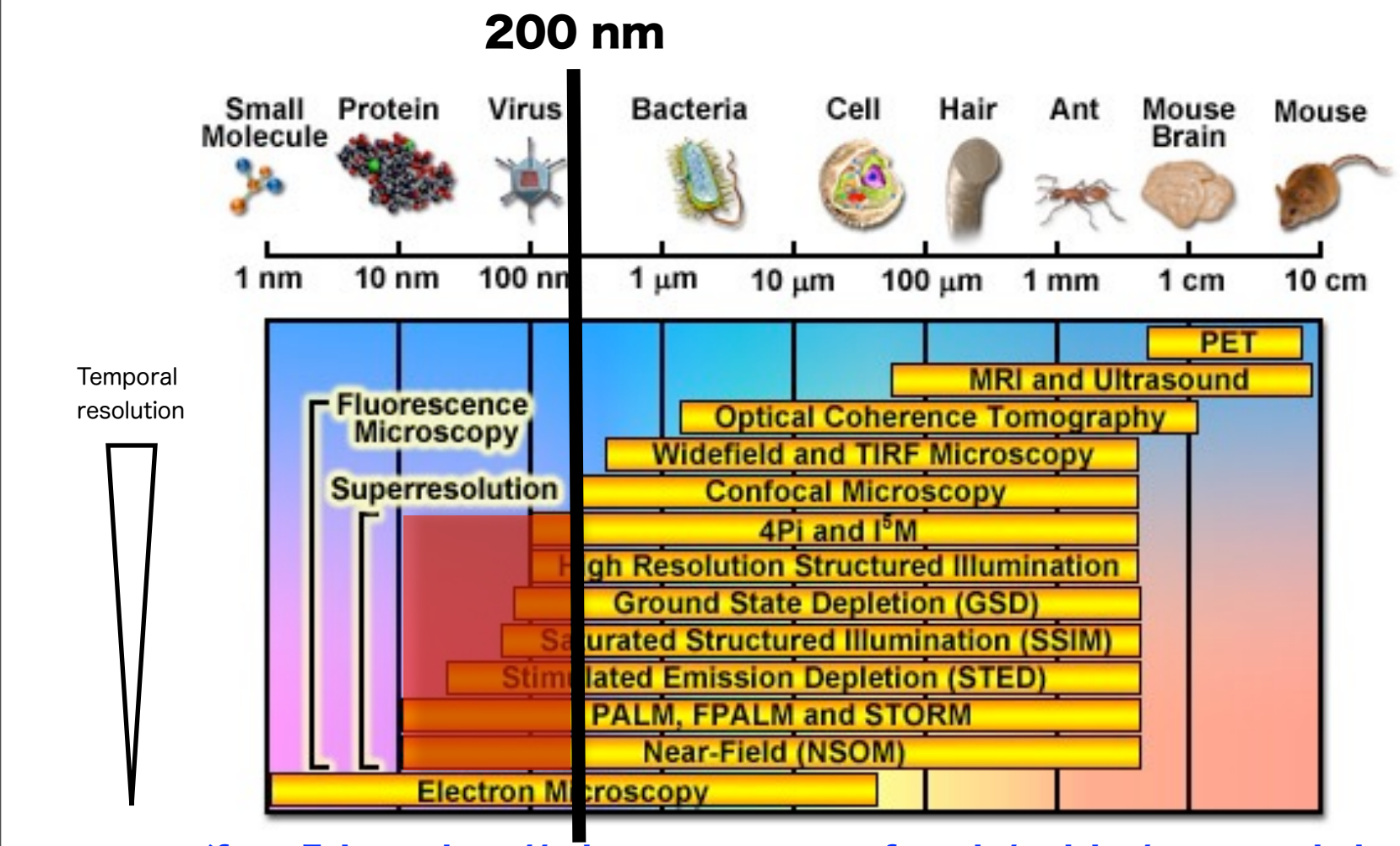
Two-Photon Microscopy



(*TIRFM :Total Internal Reflection Fluorescence Microscopy)

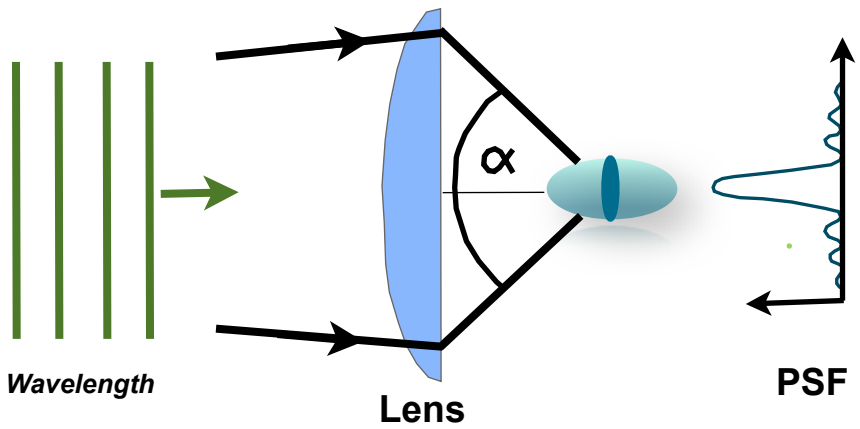
+ Laser Dissection Microscope
+ automated systems (Mirax/ slide scanner, ImageXpress/plate reader,….)

Spatial and temporal resolution of biological imaging techniques

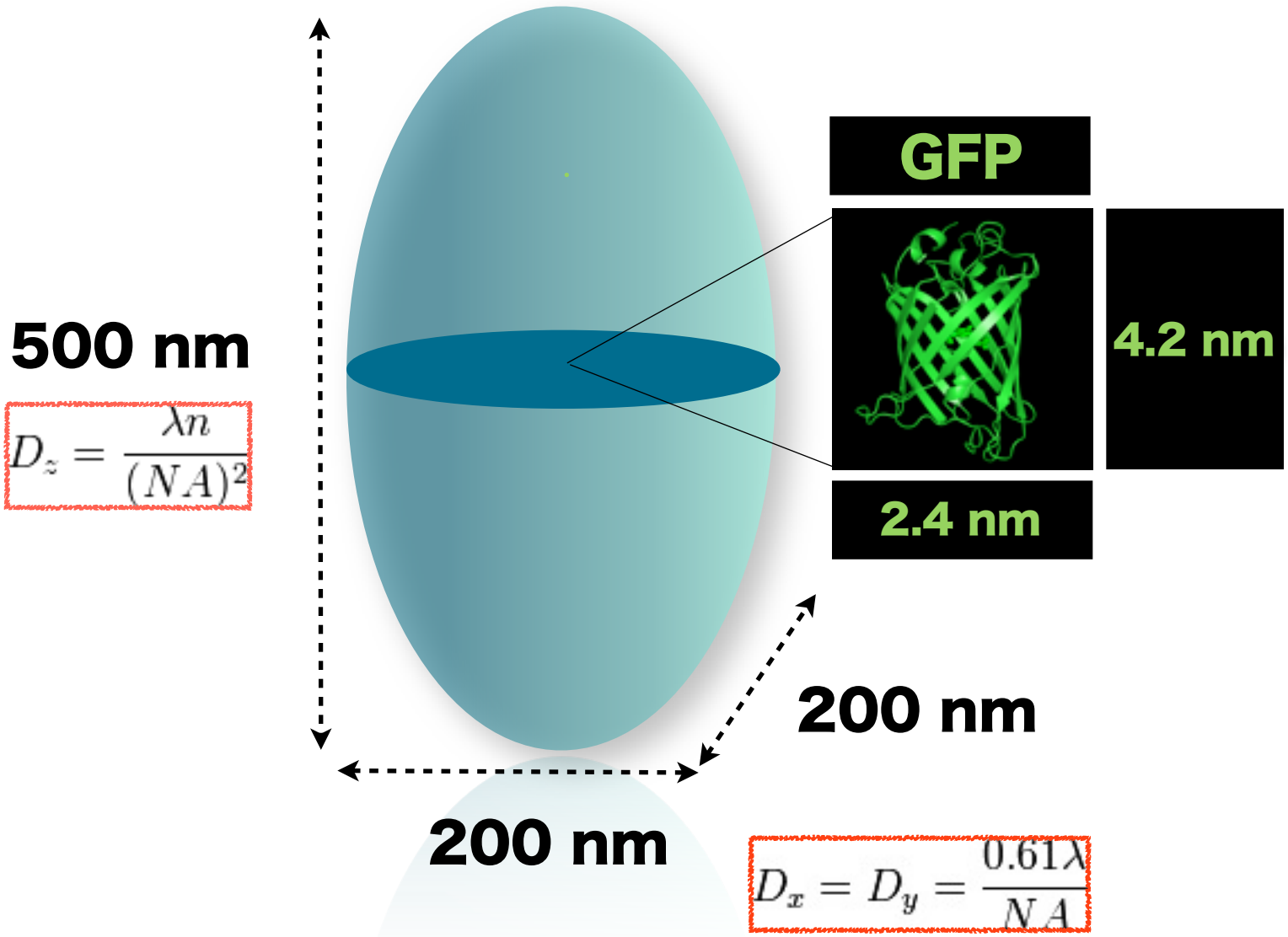


(from Zeiss at: http://zeiss-ampus.magnet.fsu.edu/articles/superresolution/introduction.html)

The microscope: an imperfect tool

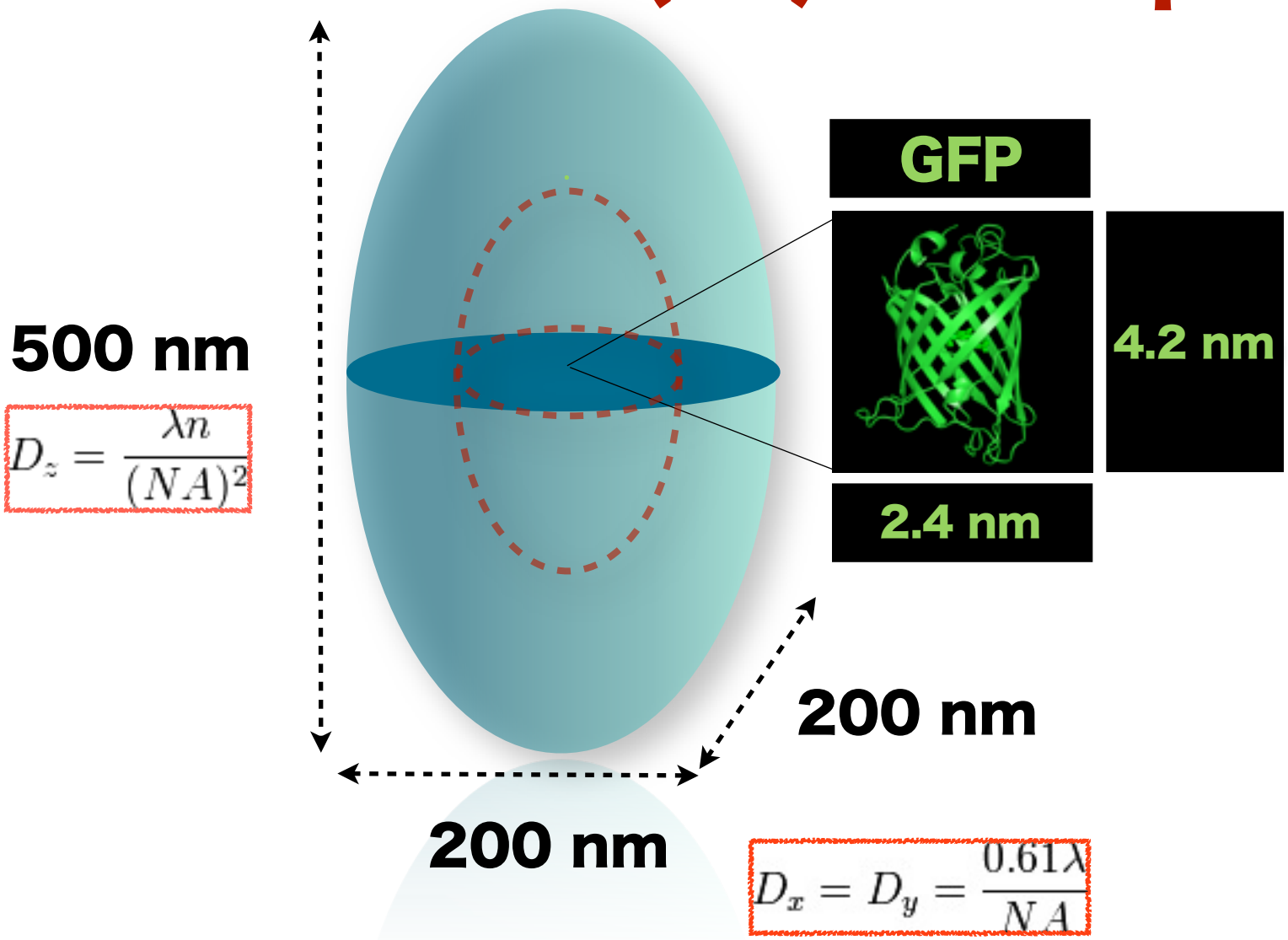


Breaking the resolution limits

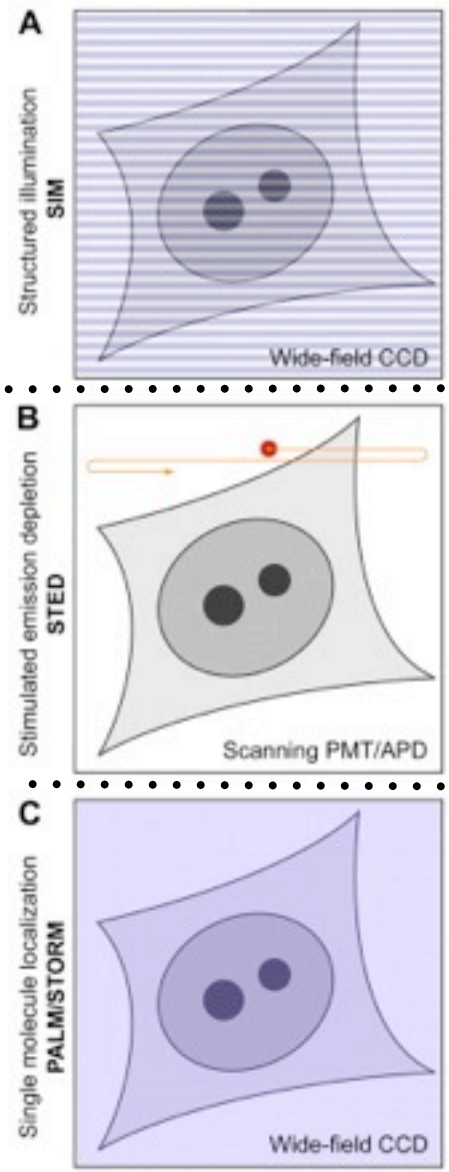


Breaking the resolution limits

Super-Resolution (SR) techniques !!



Super-Resolution (SR) techniques



Structured **I**llumination **M**icroscopy
> SIM

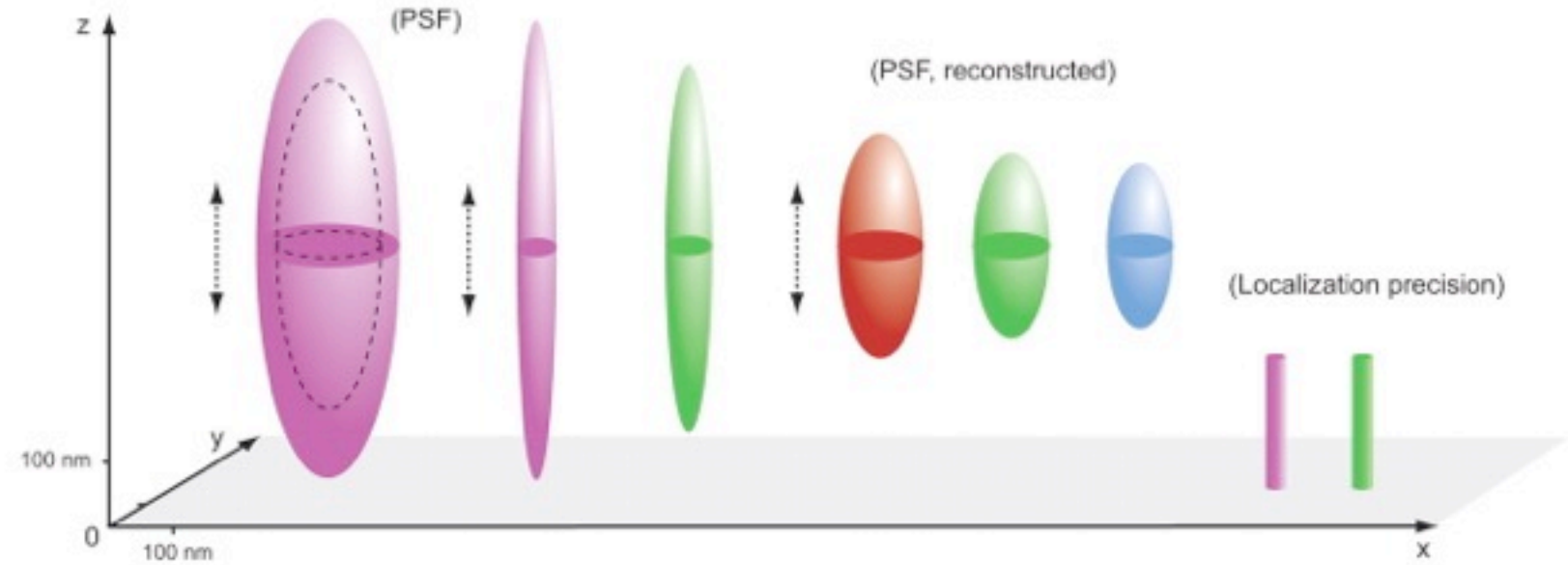
Stimulated **E**mission **D**epletion
> STED

Photo**a**ctivated **L**ocalization
Microscopy **> PALM**

Stochastic **O**ptical **R**econstruction
Microscopy **> STORM**

Resolvable volumes obtained with current commercial SR microscopes

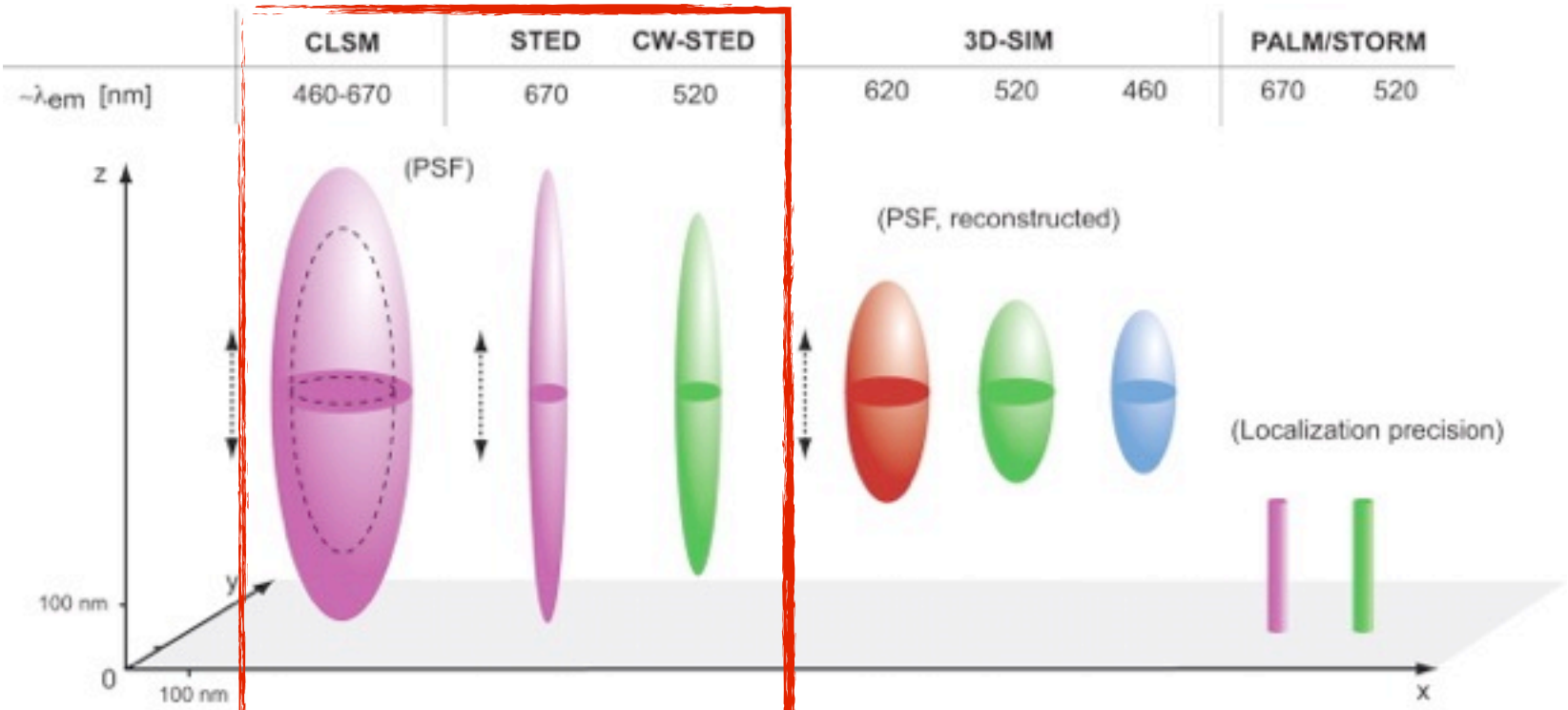
	CLSM	STED	CW-STED	3D-SIM			PALM/STORM	
~λ_{em} [nm]	460-670	670	520	620	520	460	670	520



~$D_{x,y}$ [nm]	180-250	60	70	130	110	100	30
~D_z [nm]	500-700	700	560	340	280	250	140 (TIRF-range)
~$V_{x,y,z}$ [*10^{-3} μm^3]	10-23	1.3	1.5	3.0	1.8	1.3	0.1

Schermelleh L et al. JCB 2010;190:165-175

Resolvable volumes obtained with current commercial SR microscopes



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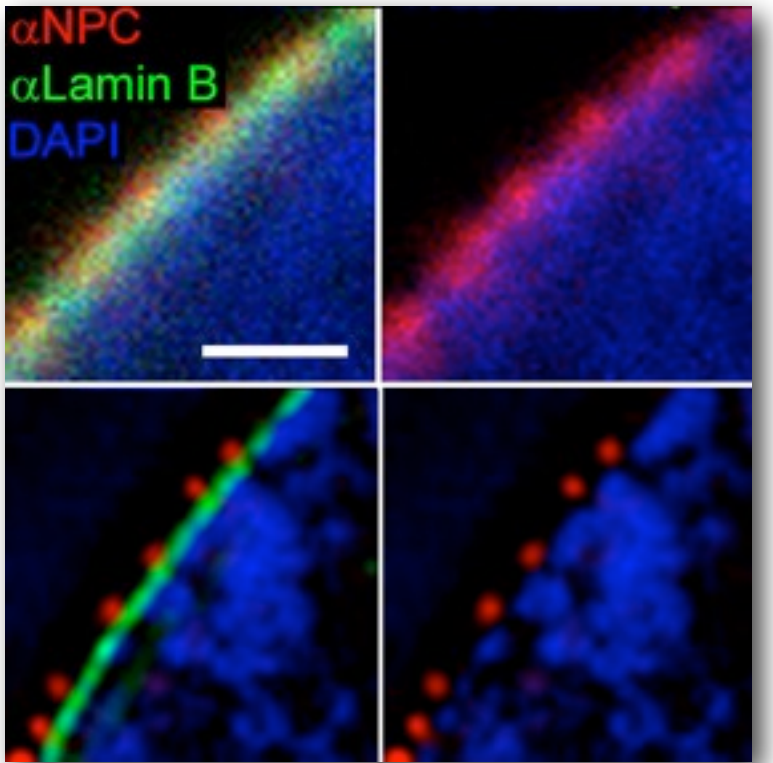
Structured Illumination Microscopy (SIM)



Schermelleh et al. Science (2008)

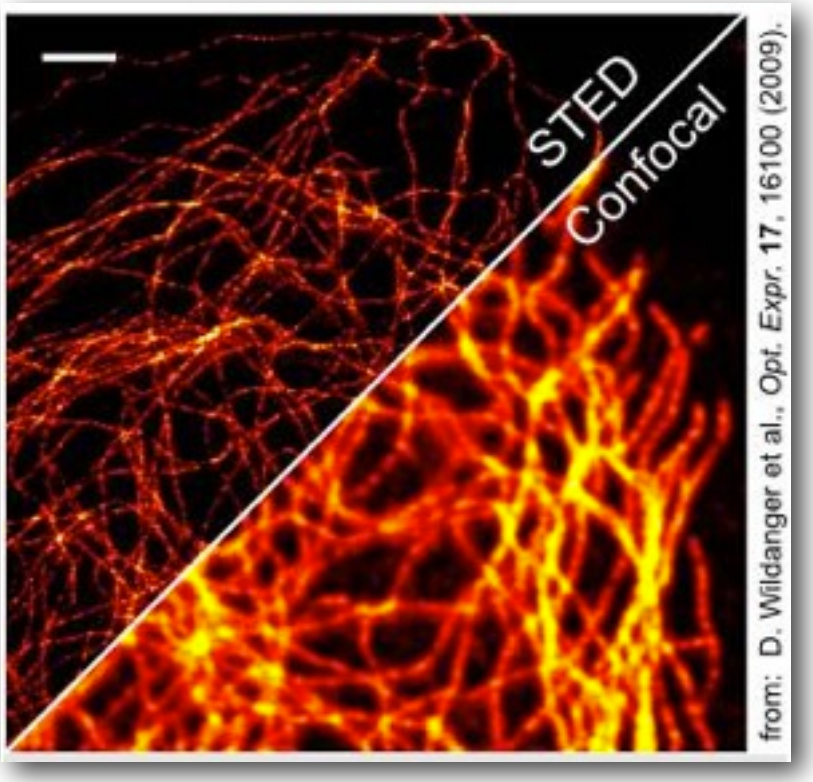
3D-SIM (pros & cons)

+ up to 4 colors, standard dyes (e.g. Alexa, GFP, …)
+ improvement of the lateral resolution in XY (2x) and axial resolution in Z (8x volumetric)
+ Optical sectioning over larger volumes (10 um in Z)

° Only moderate xy- resolution improvement

- Mathematical reconstruction > artifact proness: requires 9-15 images
- Sample quality required and system calibration

Stimulated Depletion Emission (STED)



STED (pros & cons)

+ What you see is what you get → no math required!
+ High xy-resolution (50-70 nm, Leica TCS STED)
+ > 20 μm depth

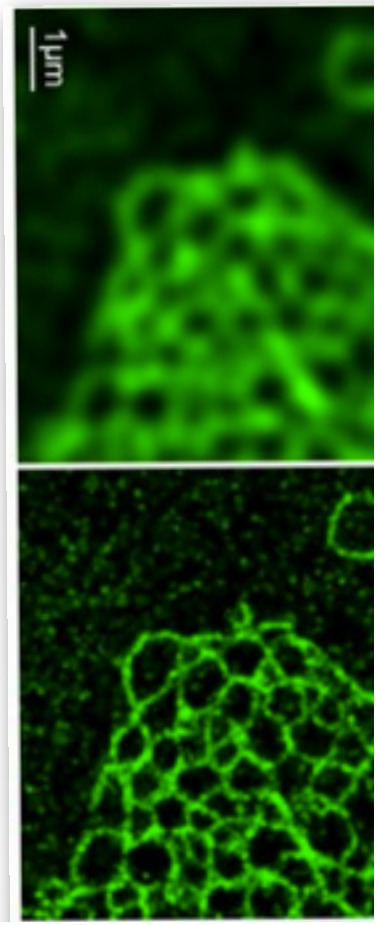
o Maximum 2 colors (no UV! no red!))
o Special dyes required for optimal performance

- Only confocal axial resolution (600-800 nm)
- Relative high energy load → photodamage
- Not ideal for 3D and live cell imaging
- Complex instrumentation, price tag

Photoactivated Localization Microscopy (PALM)

CLSM

PALM



(from: http://www.micron.ox.ac.uk/microngroup/research/localization-microscopy.shtml)

Localization Microscopy (pros & cons)

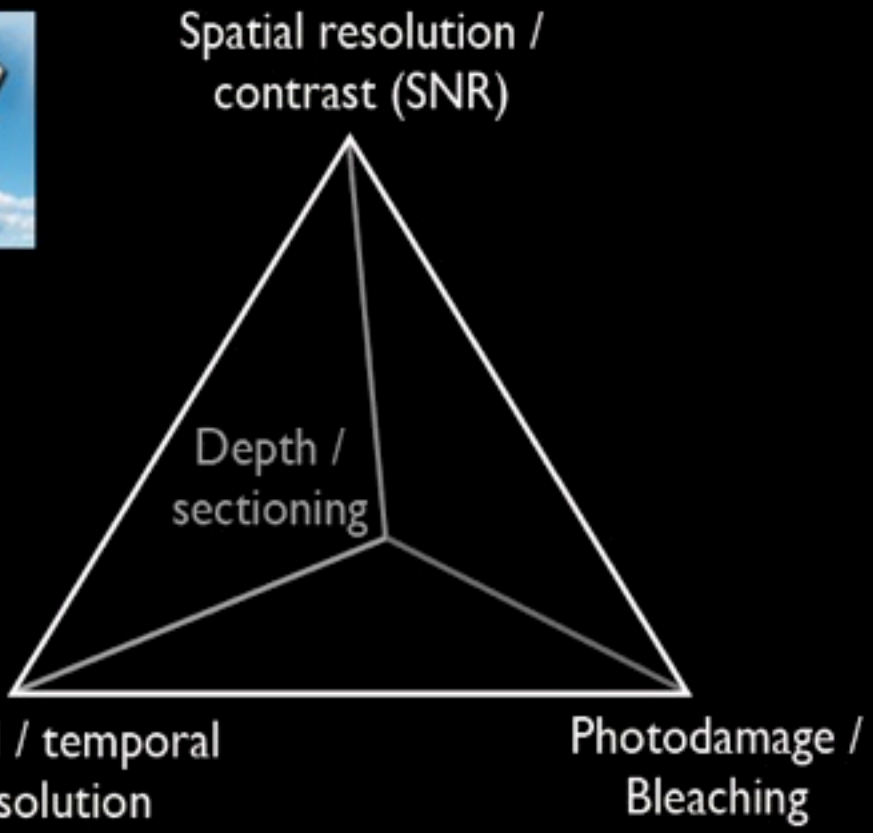
+ High localization precision (± 20 nm)
+ Quantification of single molecules
(e.g., cluster analysis, single particle tracking)

o Only single plane, best with TIRF, fxed samples
o Dye/embedding restrictions (photophysics)

- Slow (several minutes)
- Requires 5,000 -20,000 images
- Not suited for z-extended 3D structures

SR microscopy comes with costs !

Photon budget



The acquisition of the future SR-technique will be determined by the demands of the future R'equip application (in 2014)!

Bioimaging Core Facility

User Meeting

OPEN DISCUSSION

- 1) Identify ways to optimize the facility's efficiency
- 2) Evaluate common needs
- 3) Discuss ways to fund new equipments & scientific contributions


Thanks for your participation!