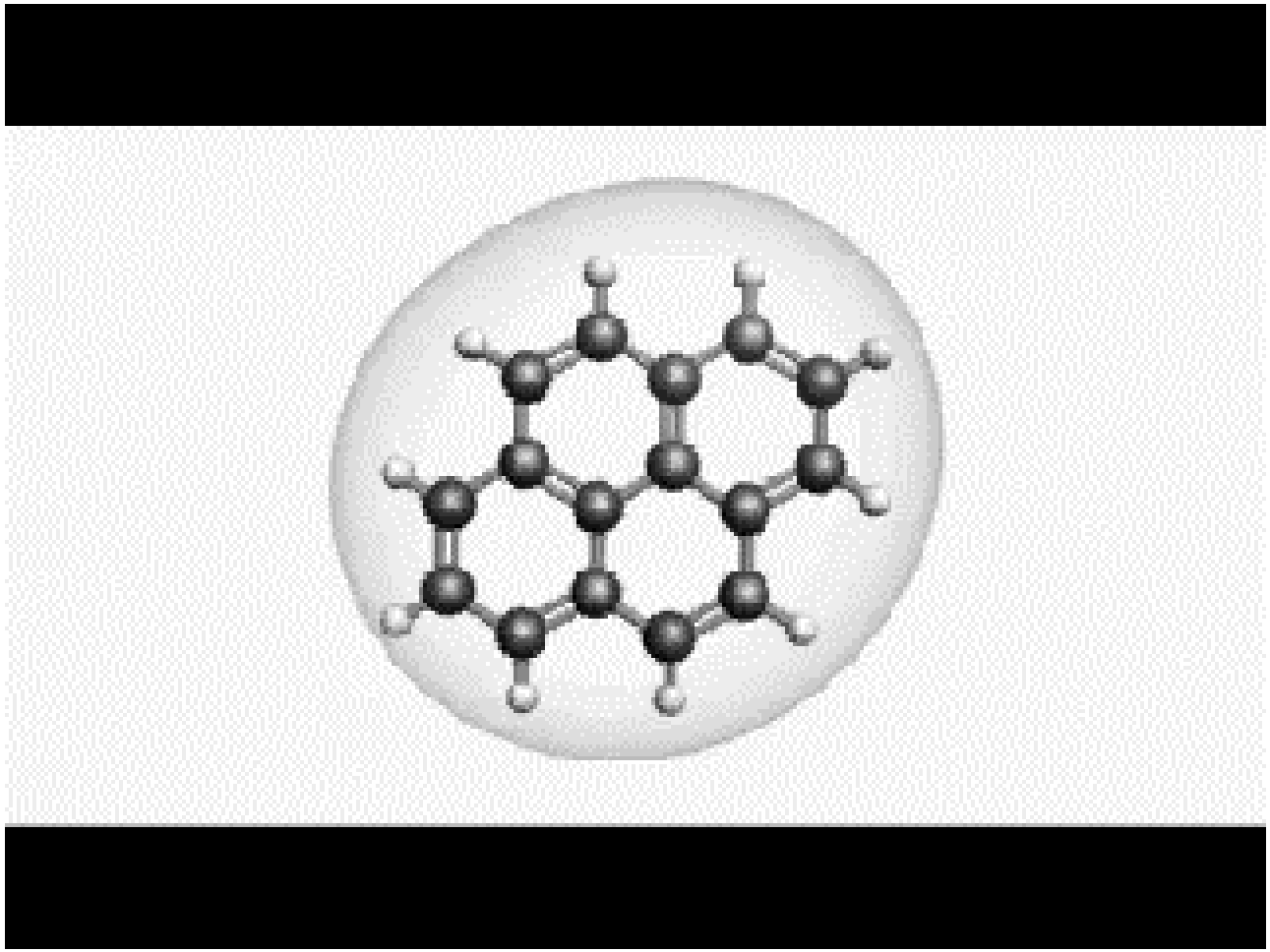
Современные методы флуоресцентной микроскопии

к.б.н. Владимир Черкас


SEEiNG IS
BELiEViNG

Флуоресценция



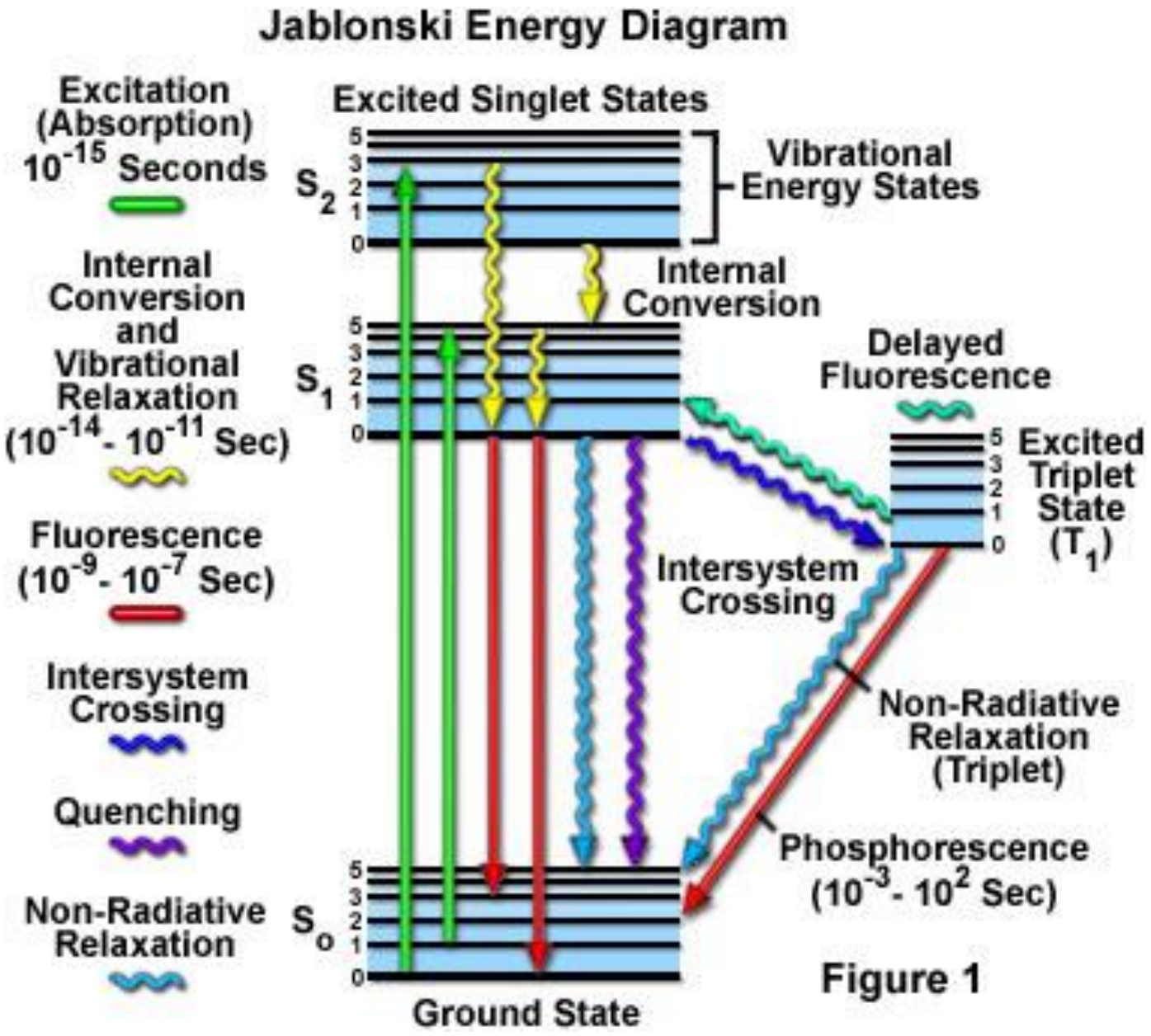
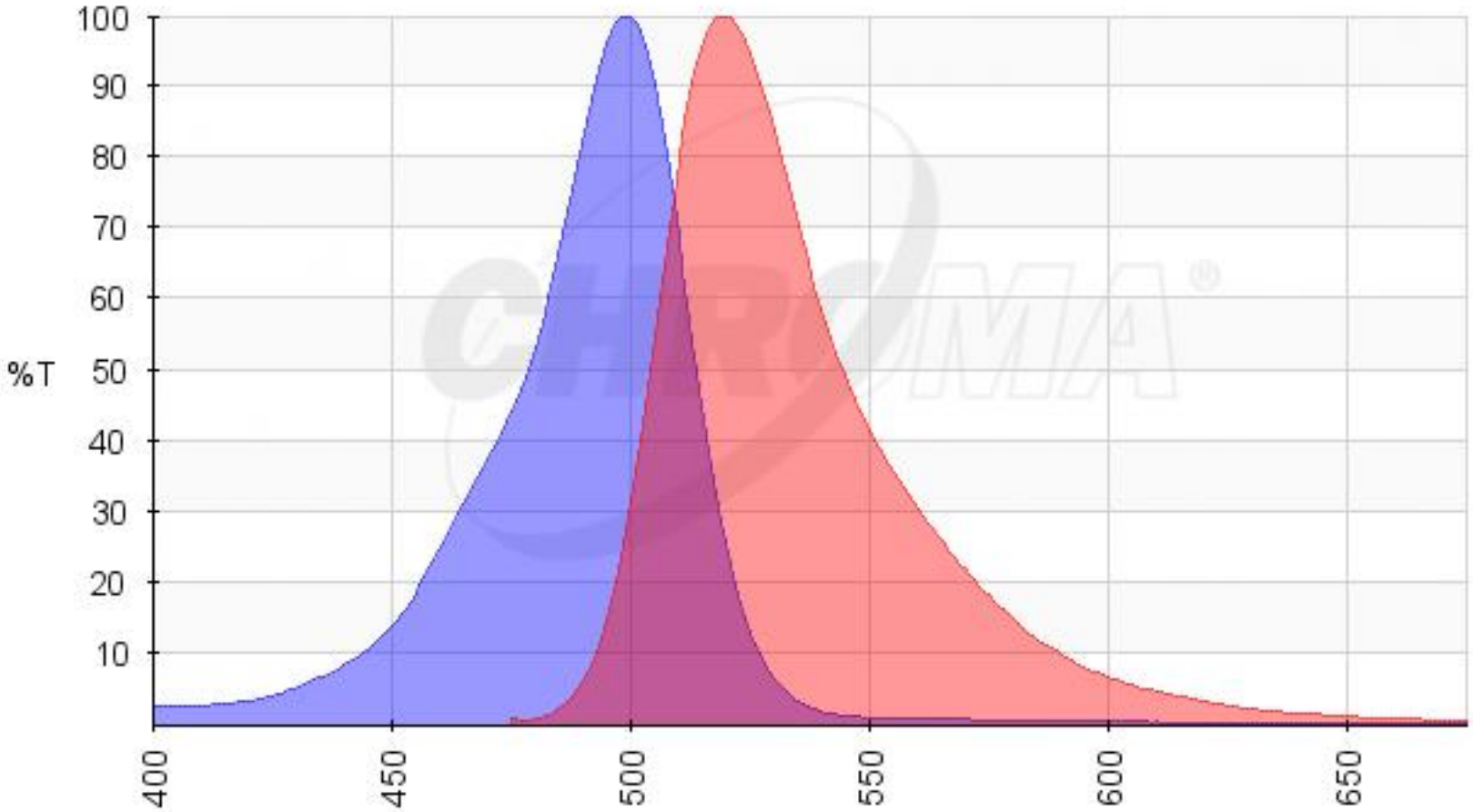


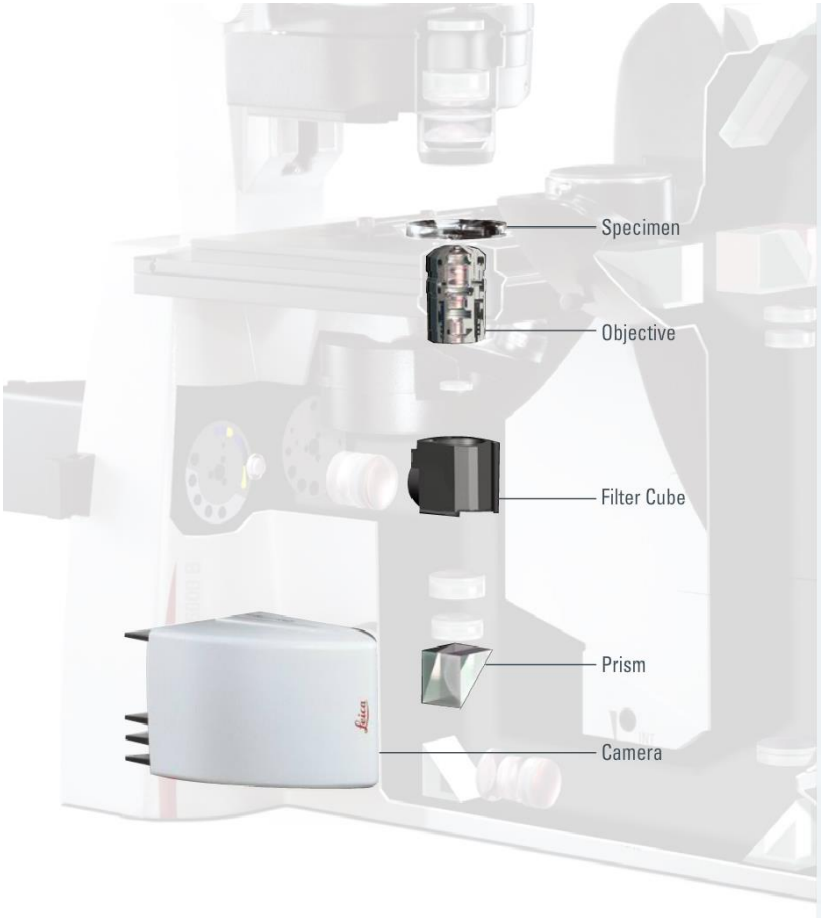
Figure 1

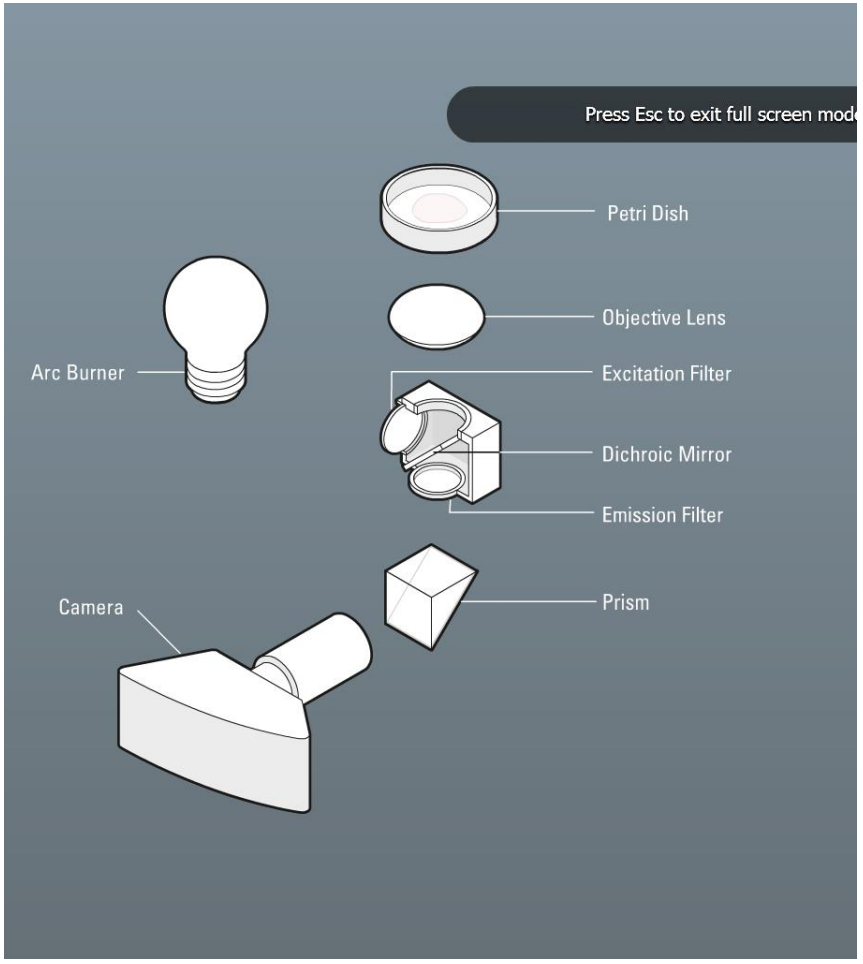
http://www.olympusconfocal.com/theory/

Fluorescence spectrum

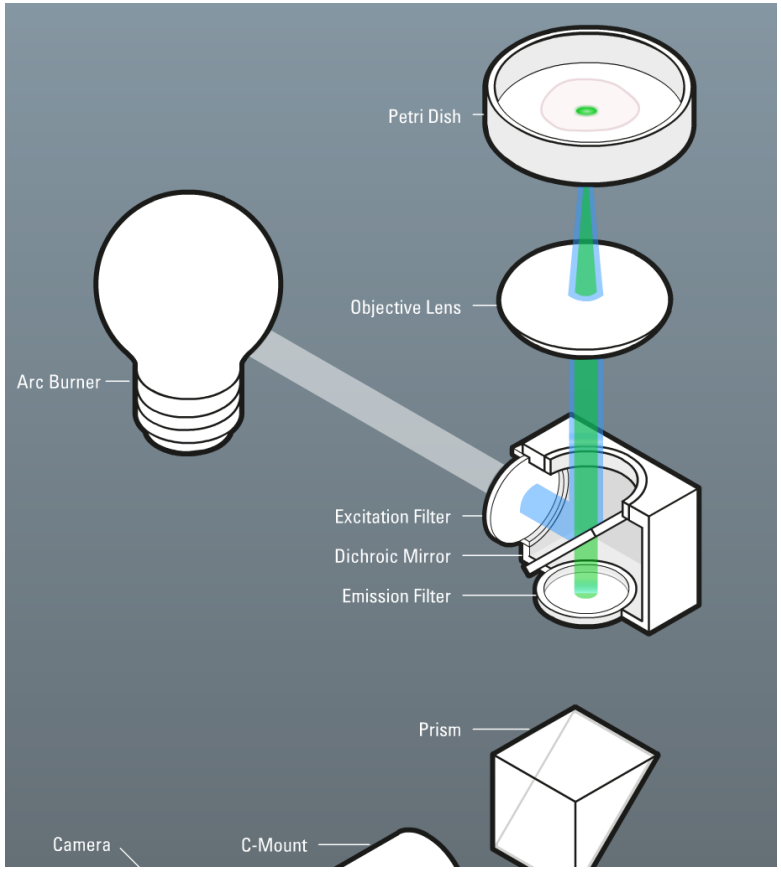


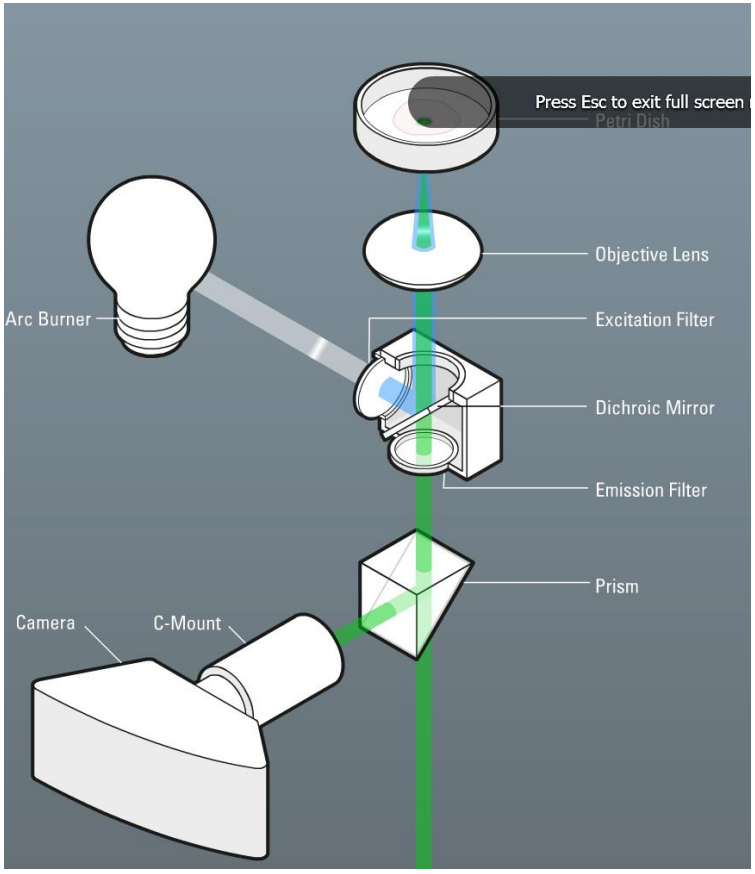
Базовые методы: Widefield





http://www.leica-microsystems.com/science-lab/step-by-step-guide-to-fluorescence-microscopy/





http://www.leica-microsystems.com/science-lab/step-by-step-guide-to-fluorescence-microscopy/

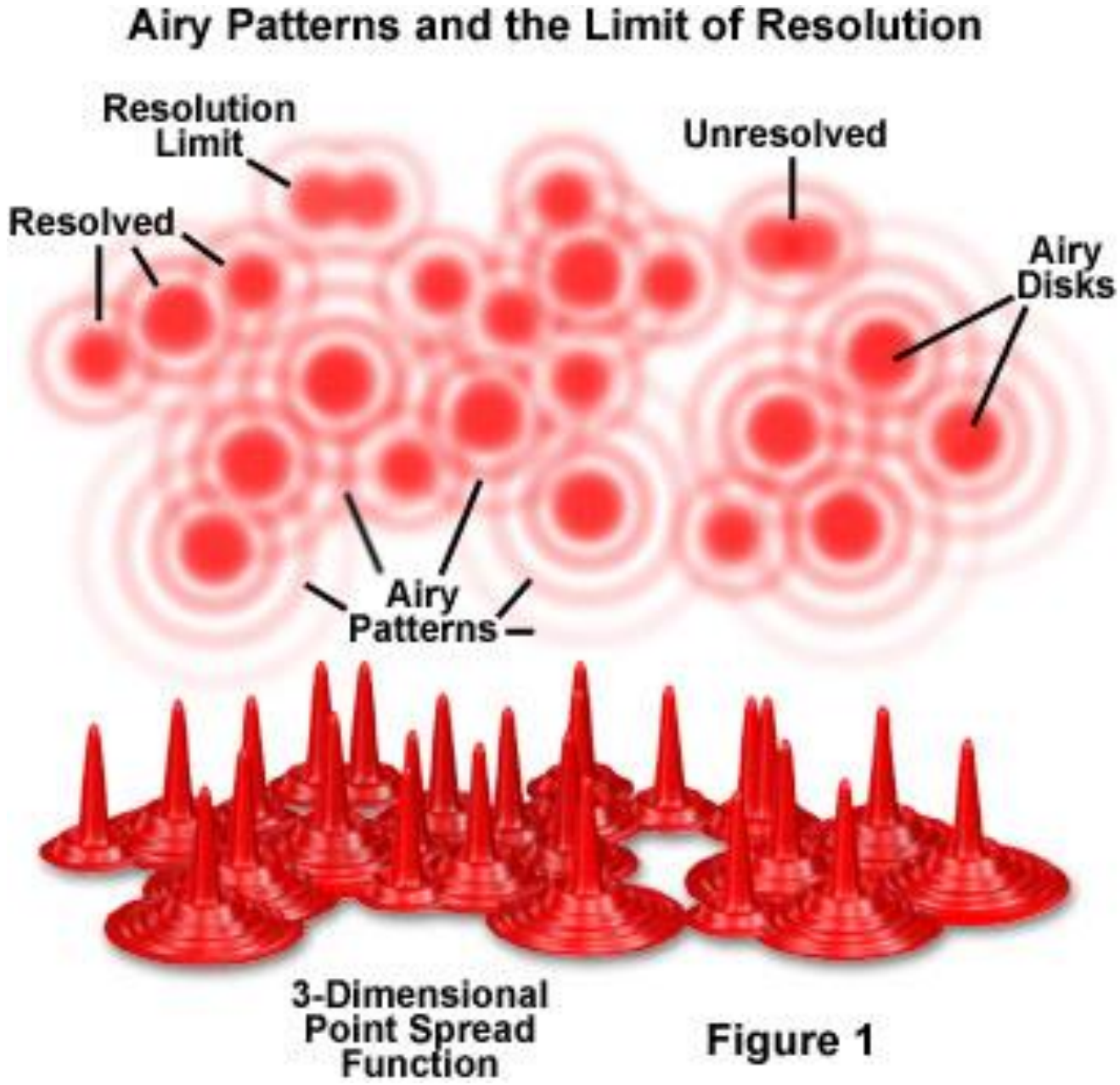
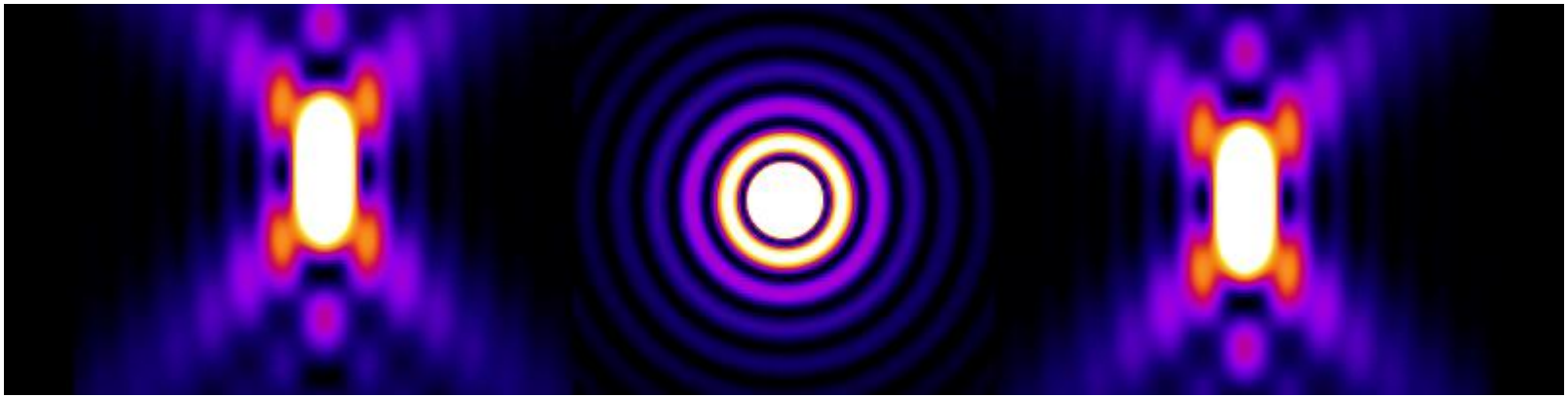
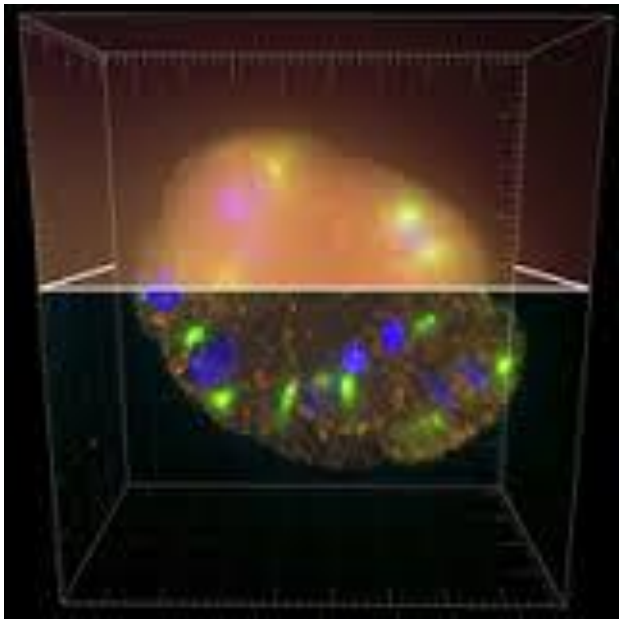

Airy Patterns and the Limit of Resolution
Resolution Limit
Unresolved
Resolved
Airy Disks
Airy Patterns
3-Dimensional Point Spread Function

Figure 1

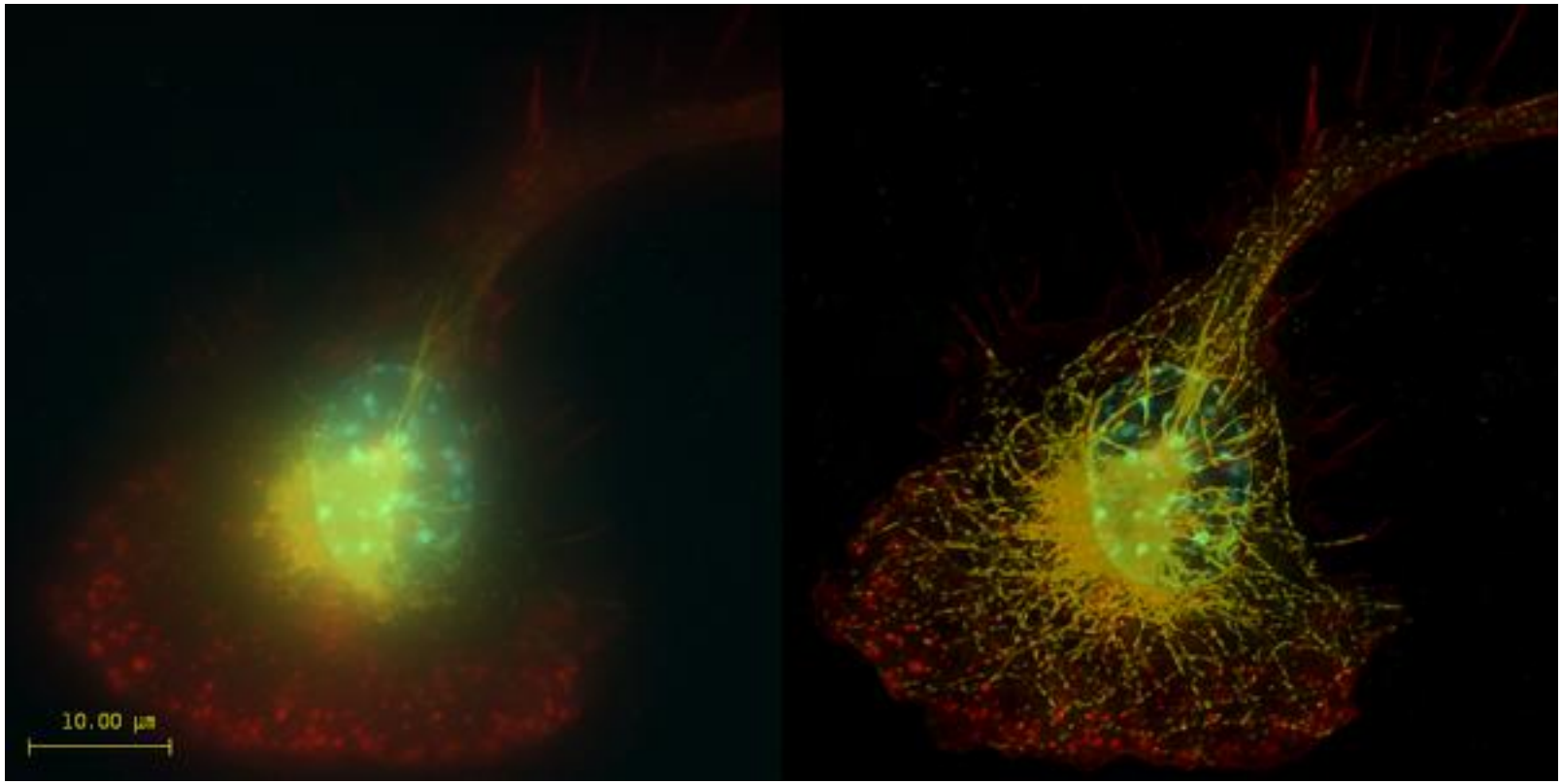
3D Deconvolution

Point spread function



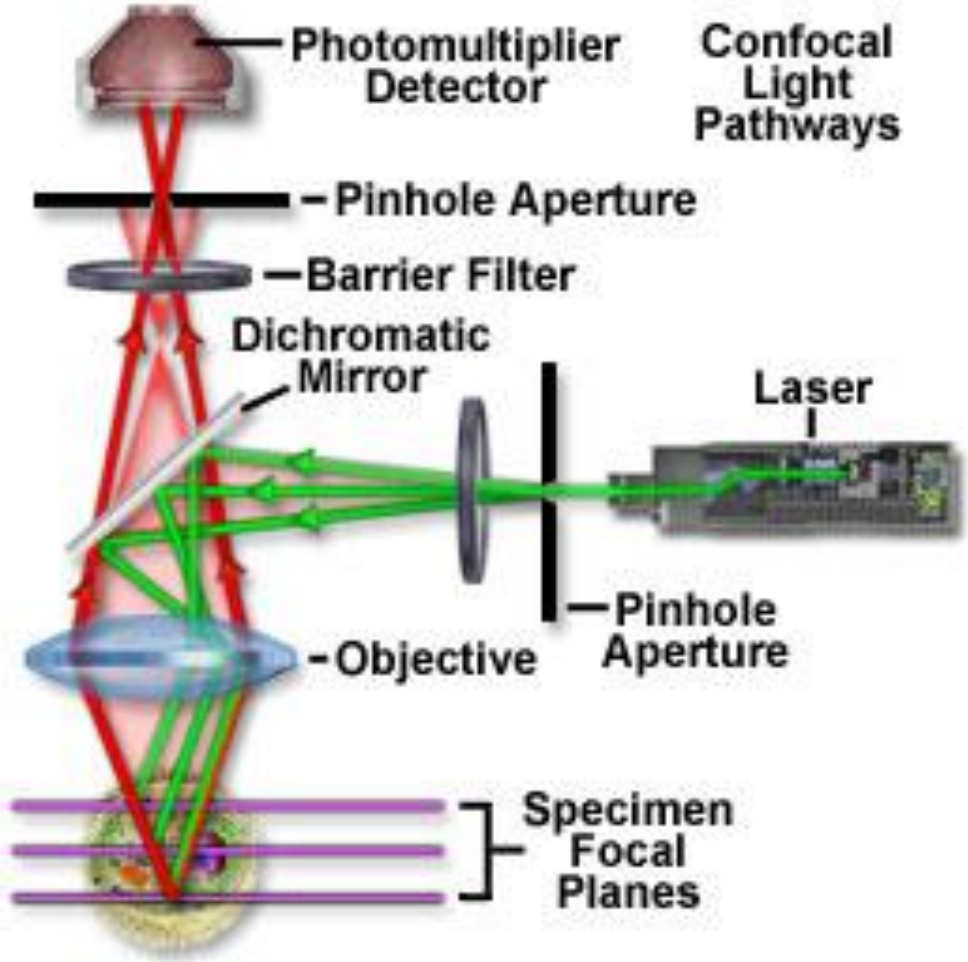


Widefield RAW vs Deconvolved



http://www.svi.nl

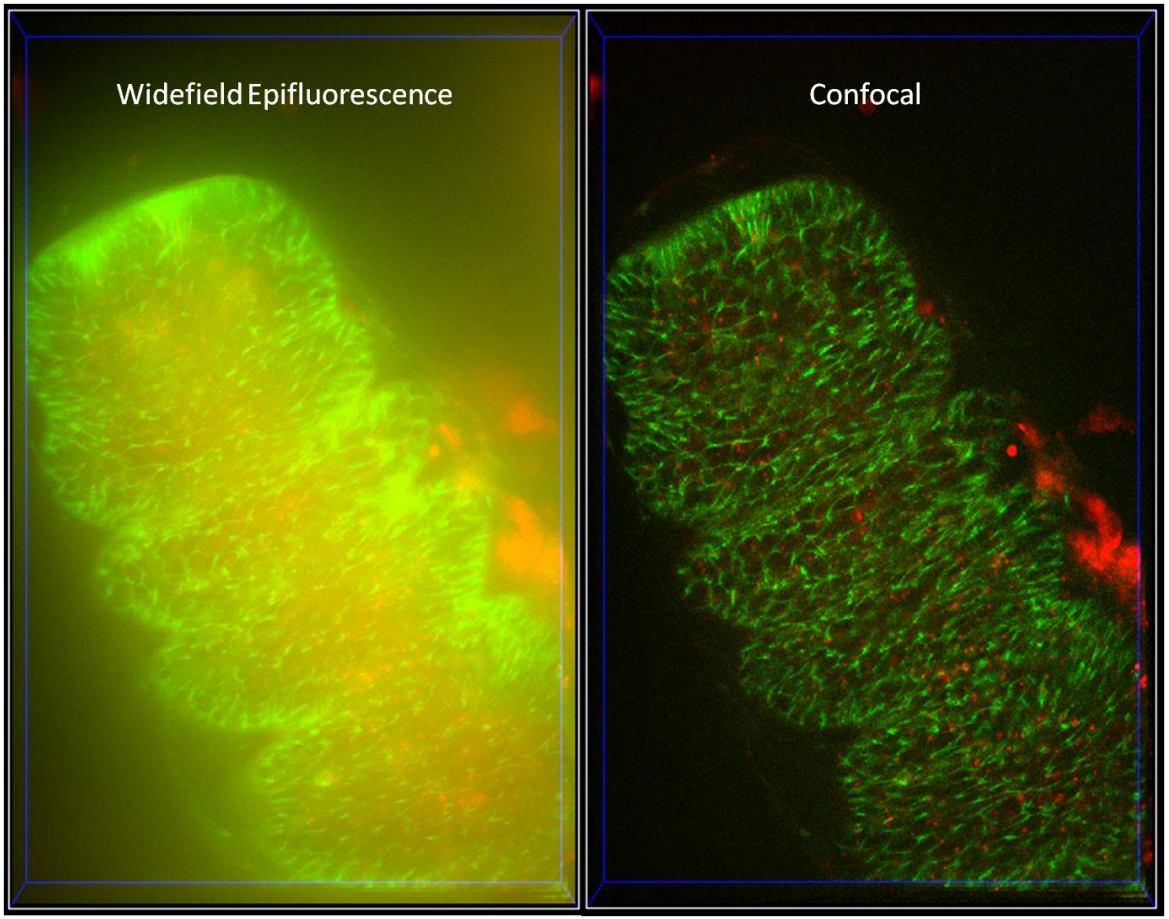
Confocal Laser Scanning Microscope (CLSM)



http://www.olympusconfocal.com/theory/

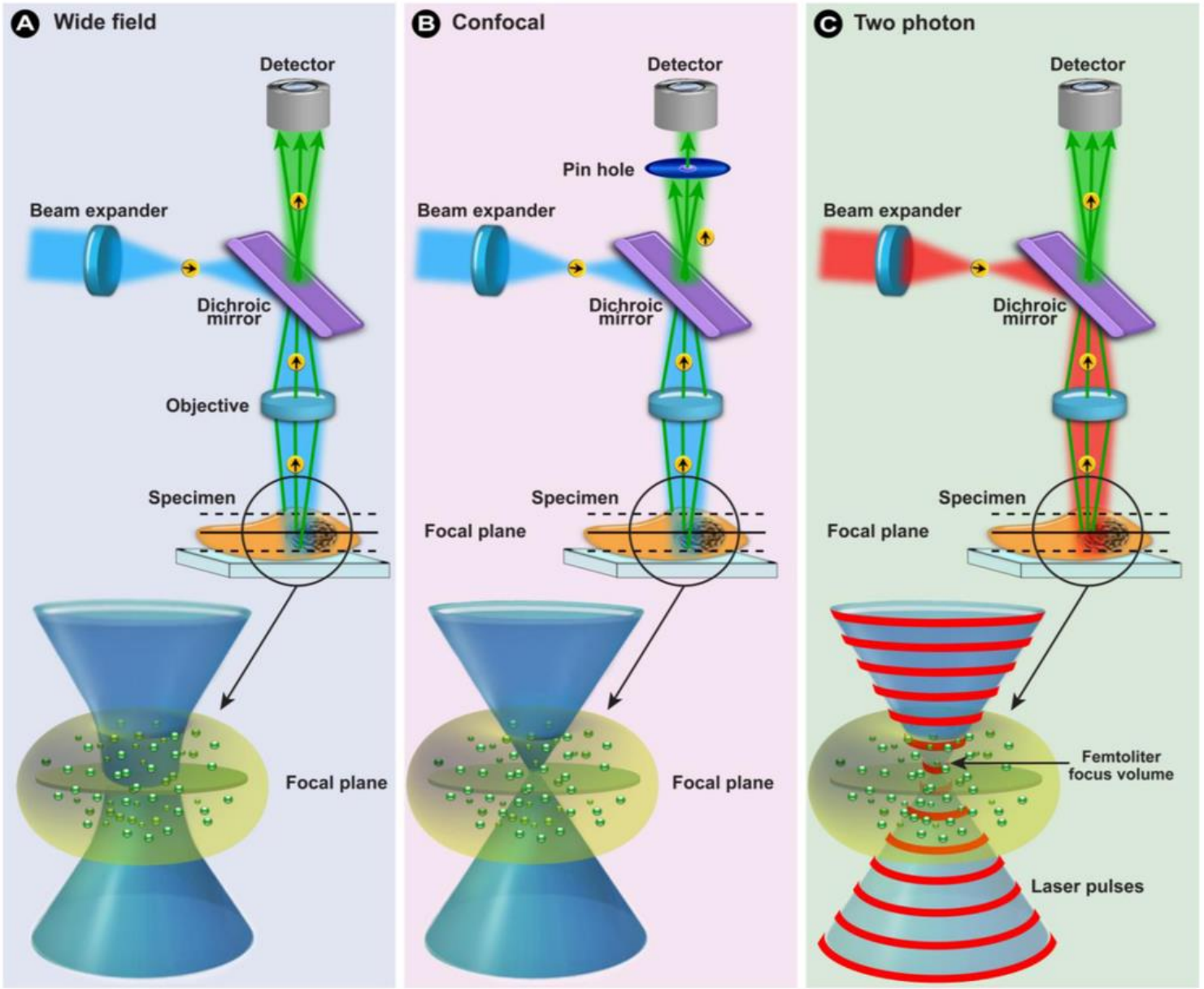
Universal confocal/STED

Widefield vs Confocal



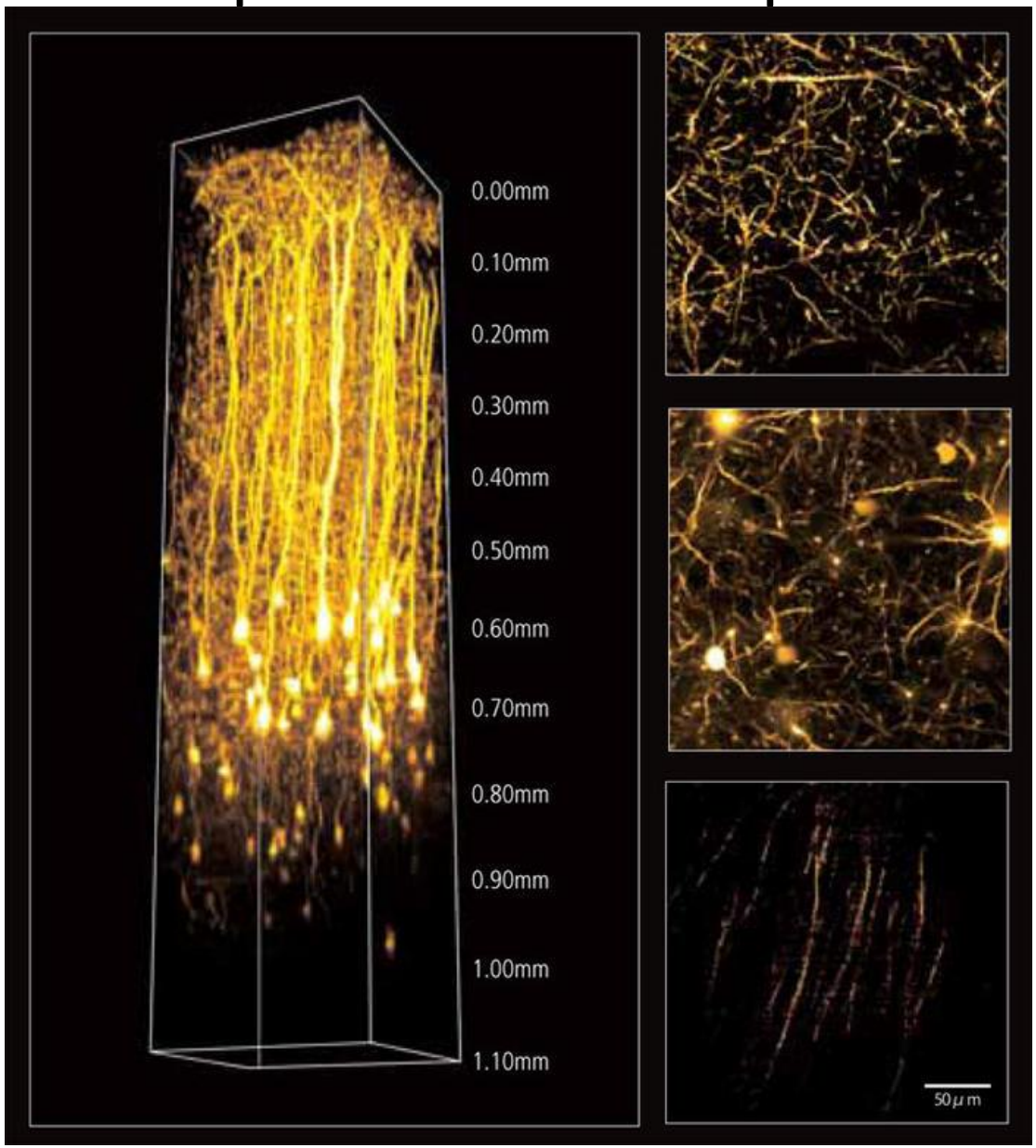
http://www.andor.com

Multiphoton



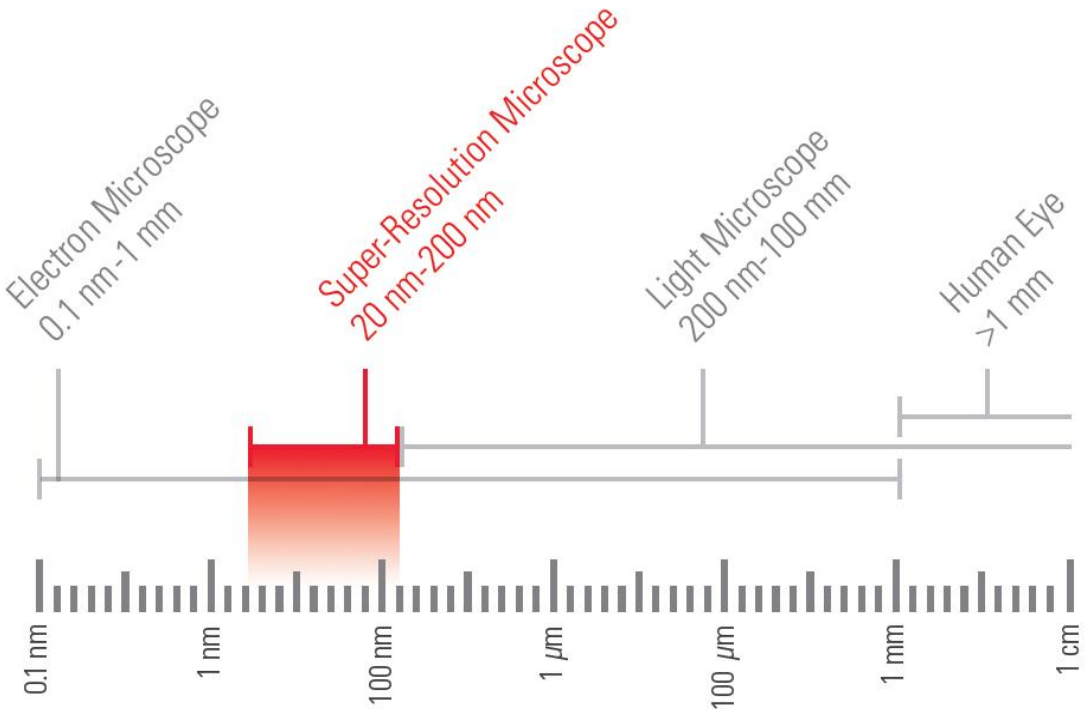
Hellen C. Ishikawa-Ankerhold, *Molecules* **2012**

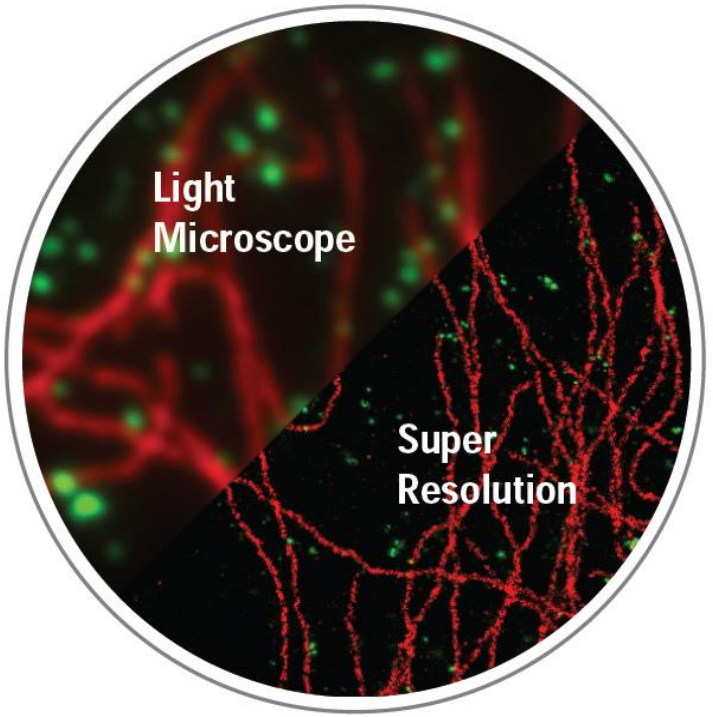
Multiphoton z-depth



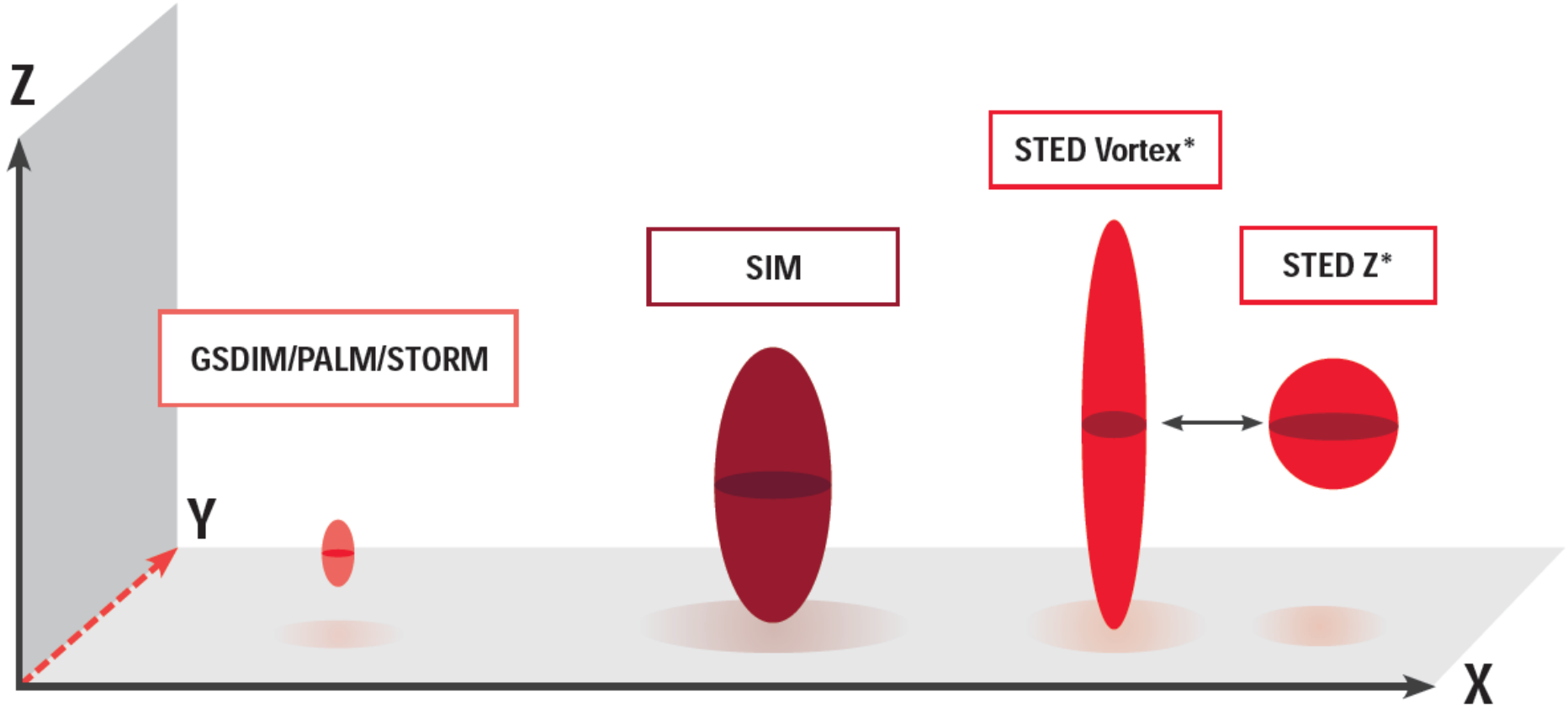
www.nikoninstruments.com

Superresolution





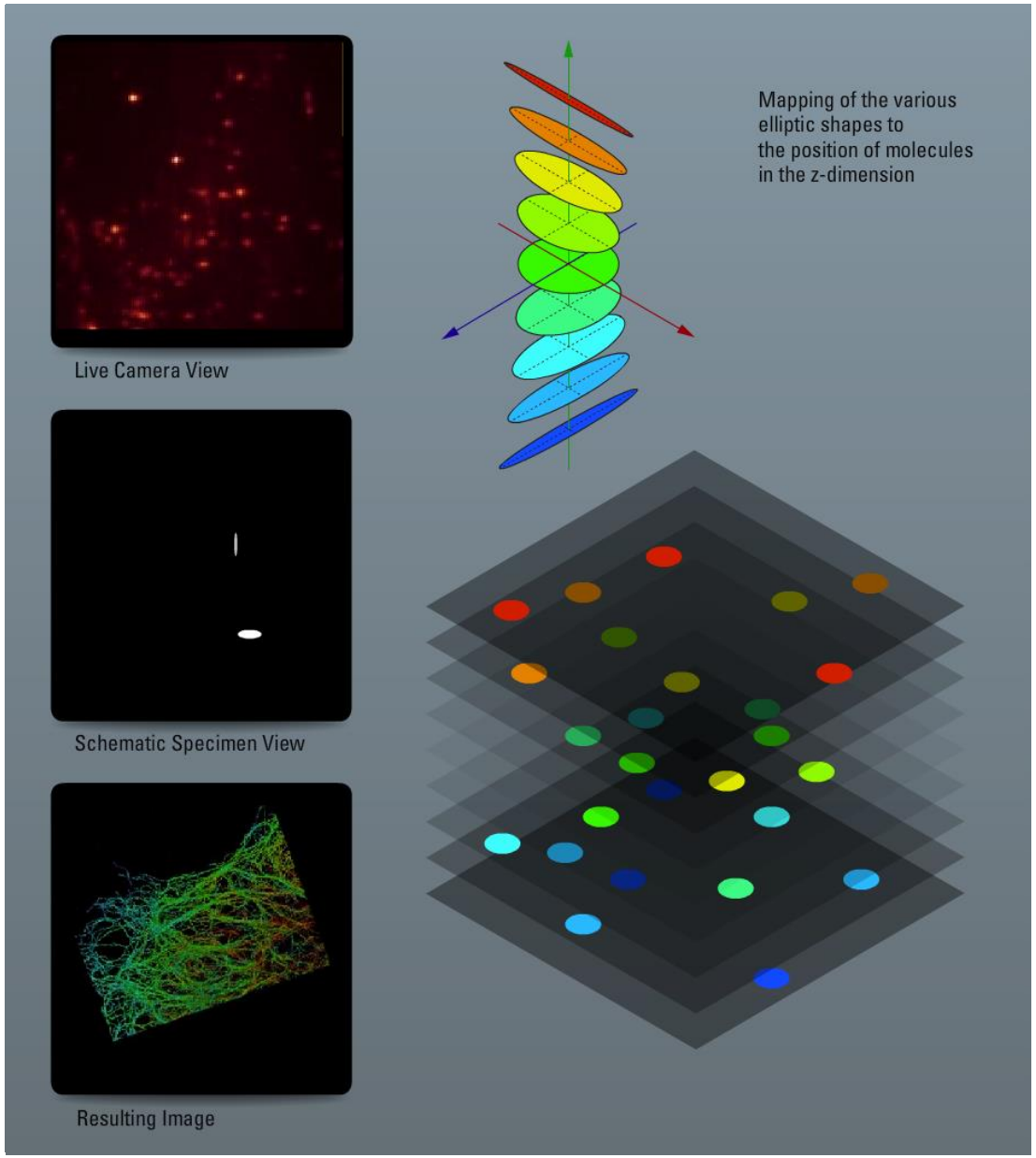
http://www.leica-microsystems.com/superres-infographic/



Dx,y	20 nm	100-130 nm	50-80 nm	150 nm
Dz	50 nm	250-340 nm	500-700 nm	170 nm

http://www.leica-microsystems.com/superres-infographic/

GSDIM/PALM/STORM - стохастические



Важно:
Флуорофоры должны «мигать», т.е. одновременно не должны излучать два флуорофора, расстояние между которыми меньше 300нм.
Картинка получается путем вычисления координат центра пятна.
Для хорошего изображения нужно снять от 10 до 100 тысяч кадров.

Разрешение(XYZ):
20x20x50 нм

Confocal vs STORM

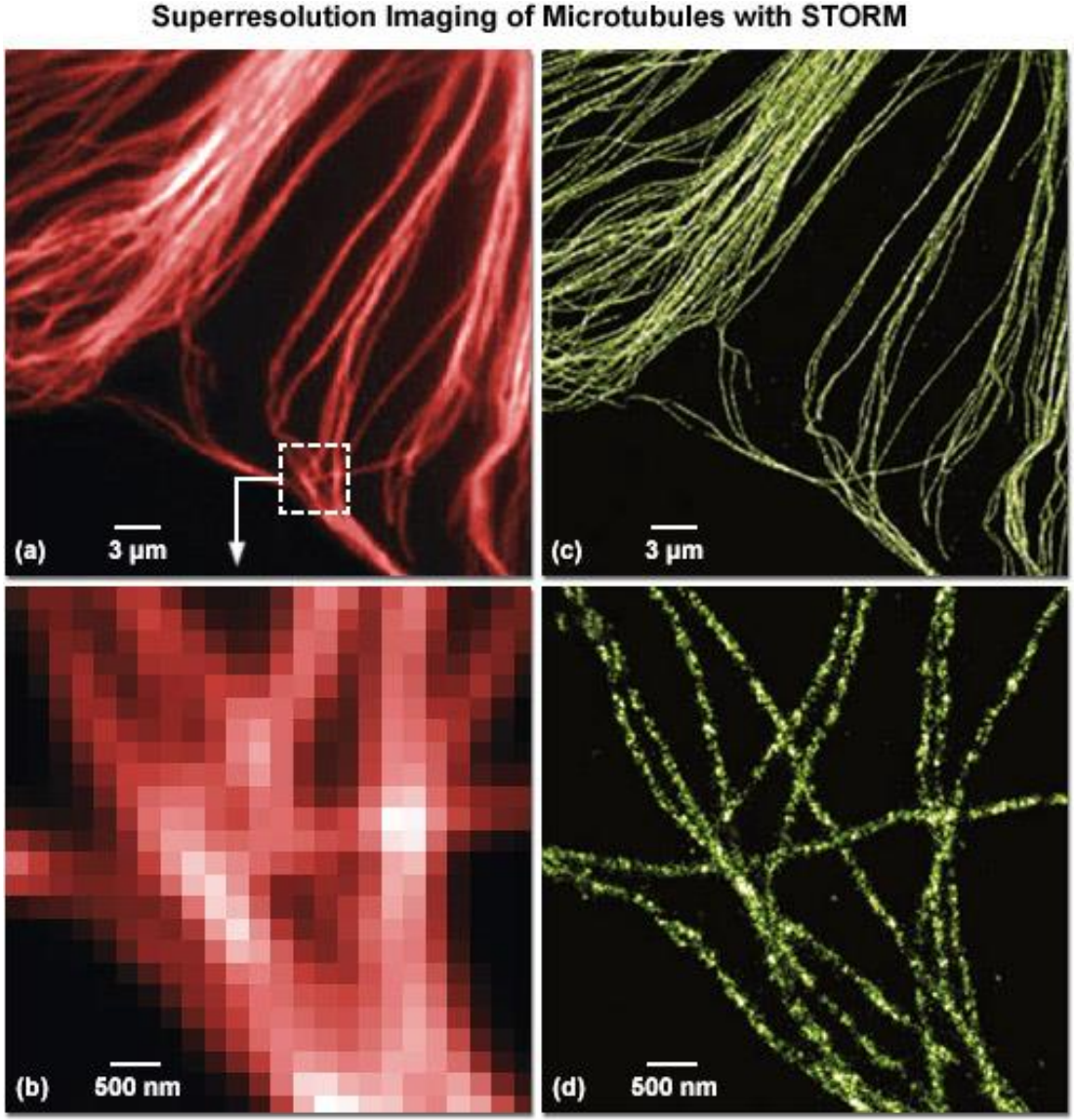


Figure 4

www.nikon.com

STED – Stimulated Emission Depletion

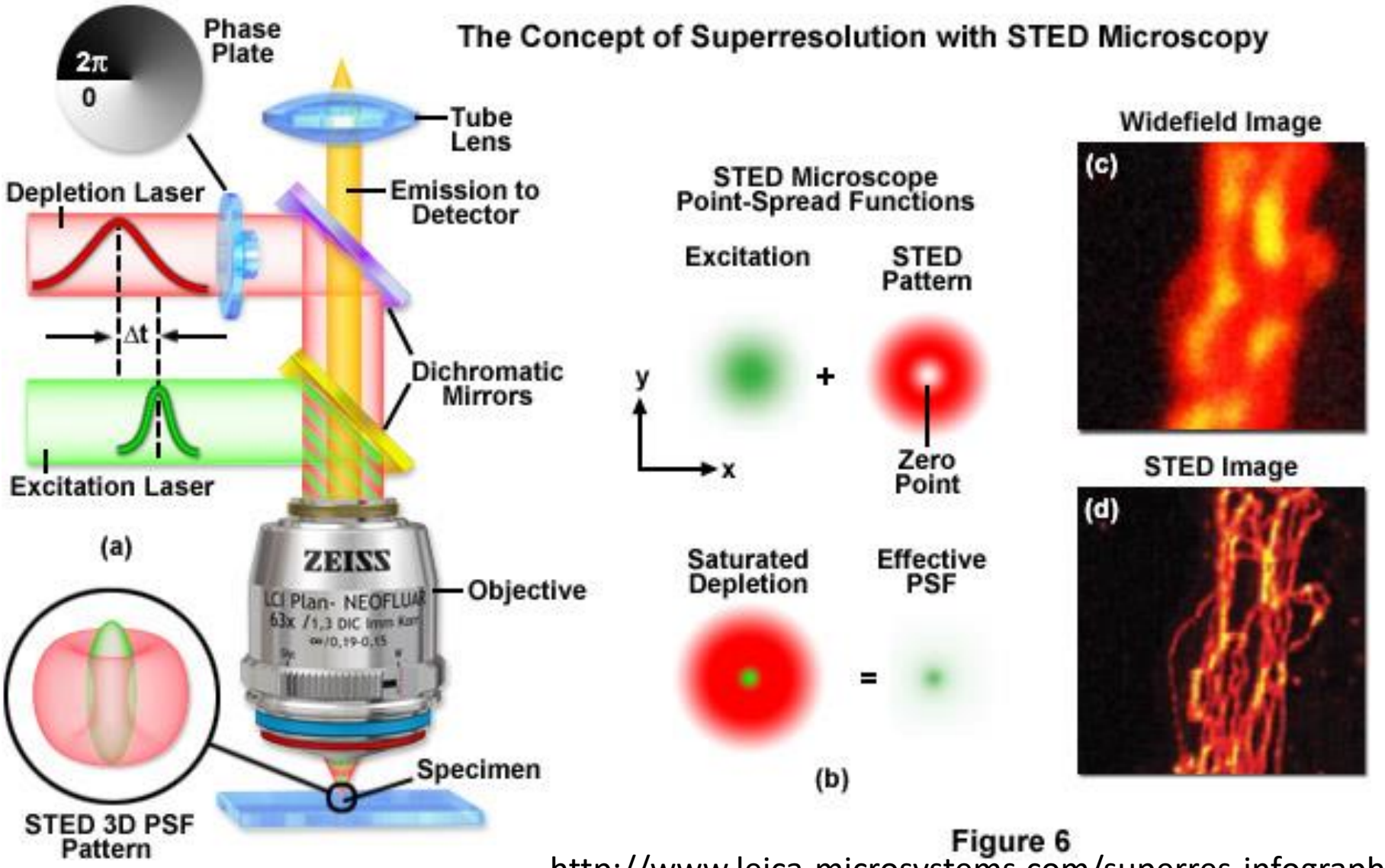
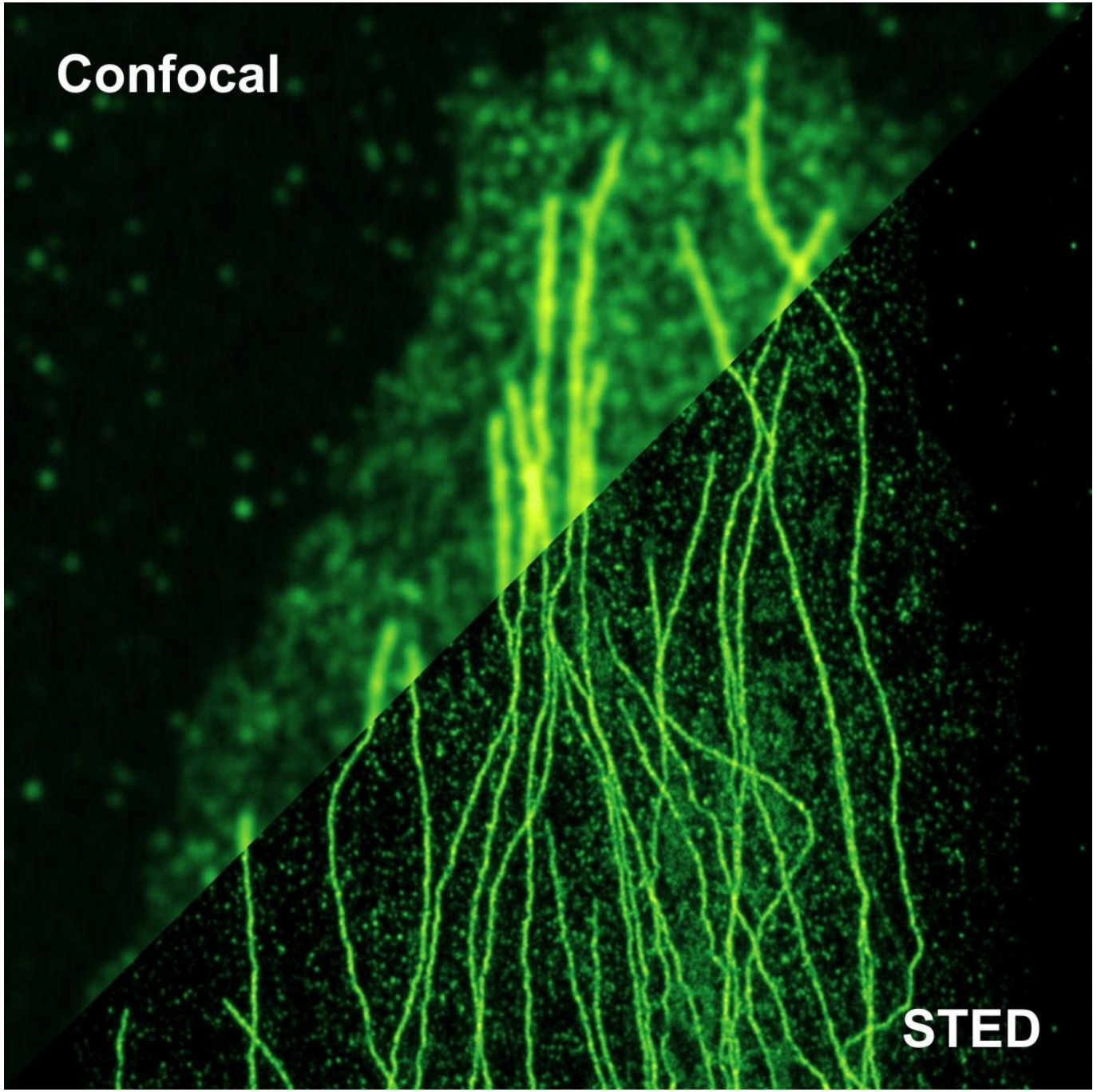
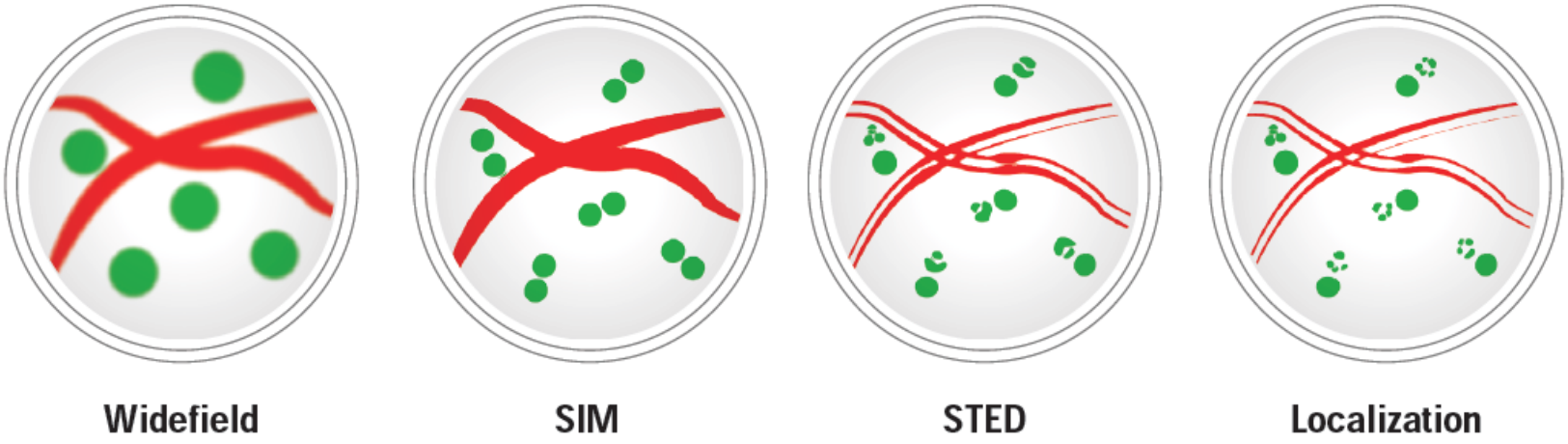


Figure 6

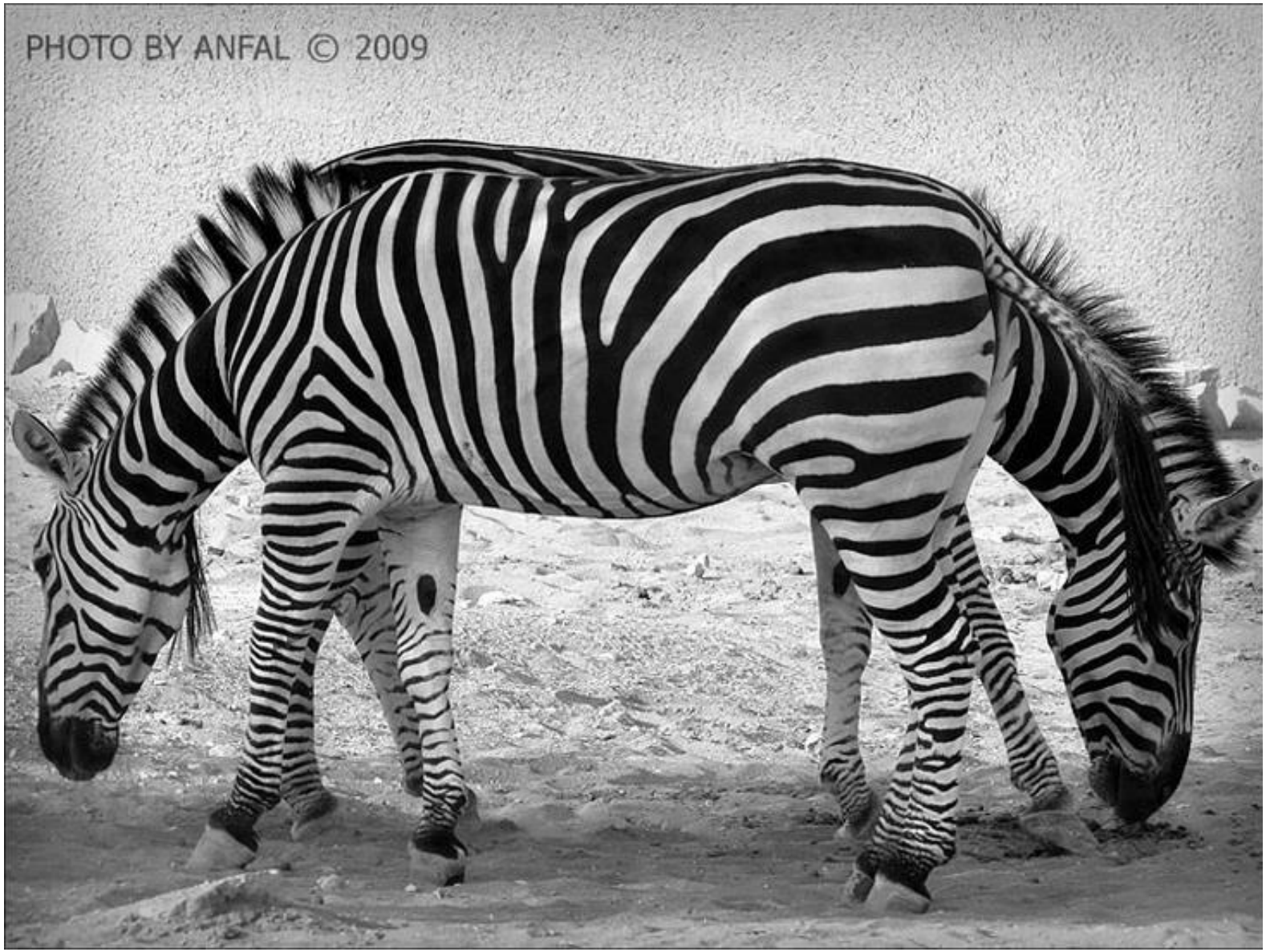
http://www.leica-microsystems.com/superres-infographic/


Confocal
STED

У всех методов есть преимущества и недостатки, зная их, нужно оптимально выбирать метод исследования для конкретной цели.



Seeing is not always believing!



Спасибо за внимание