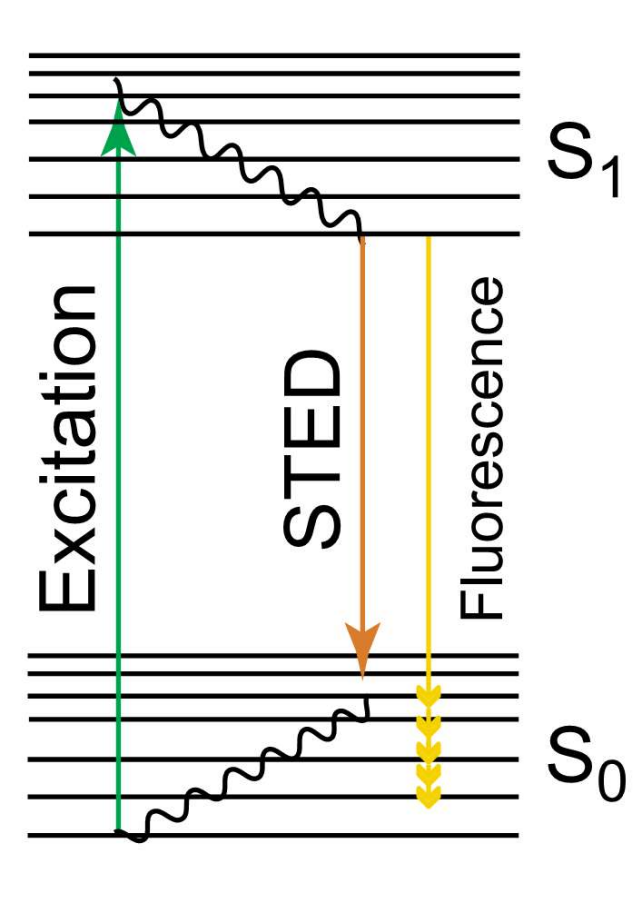
Lecture-12

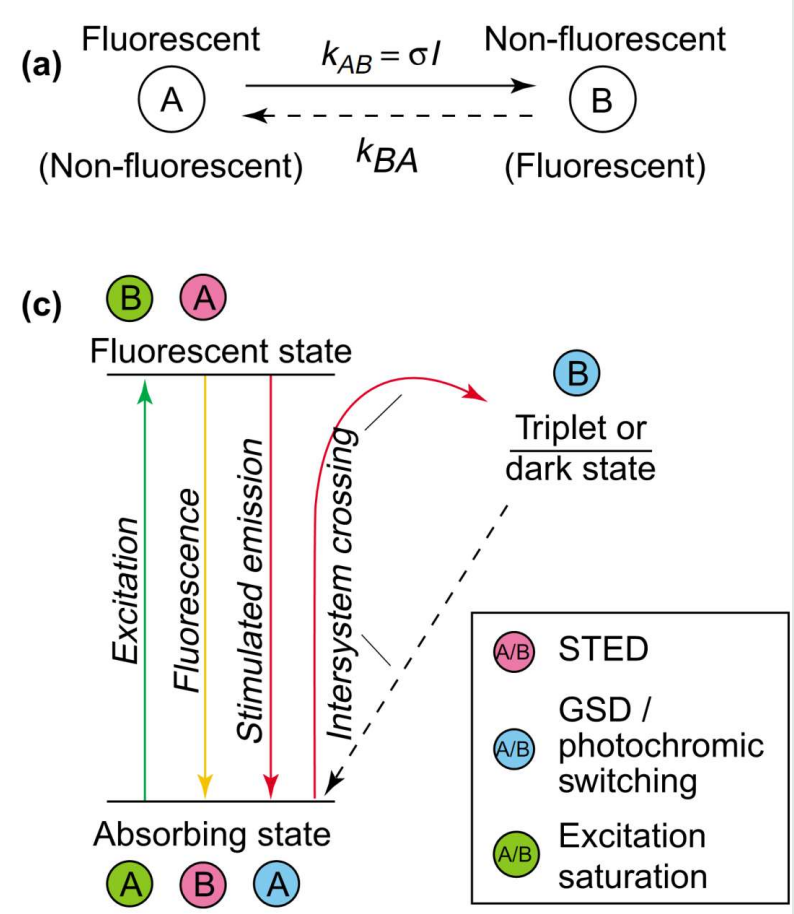
CSO202: Atoms, Photons & Molecules

Debabrata Goswami

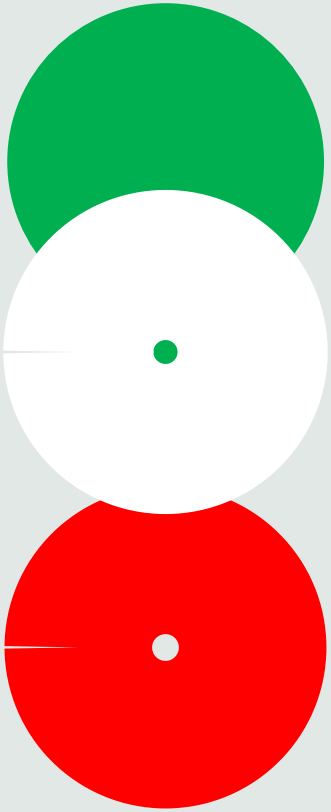
Stimulated Emission Depletion Microscopy (STED)

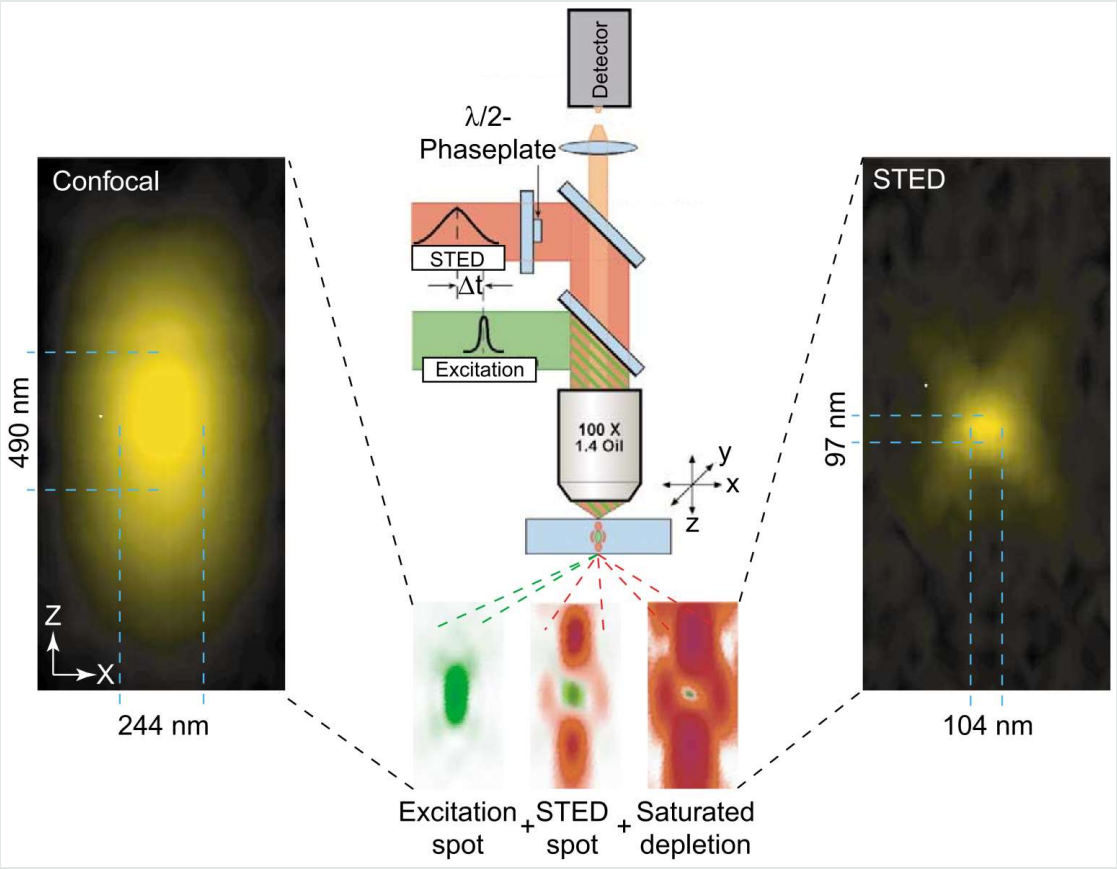
Basics of STED



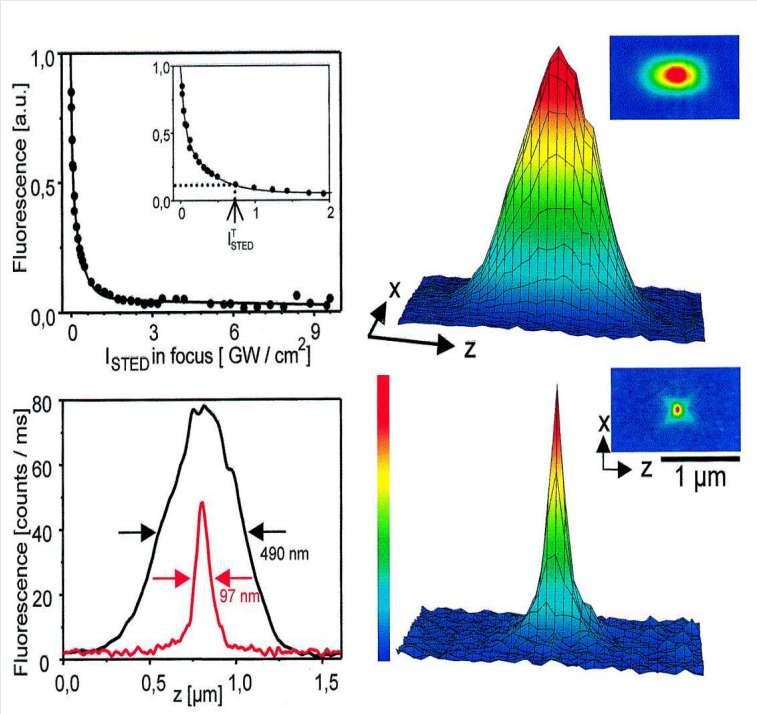


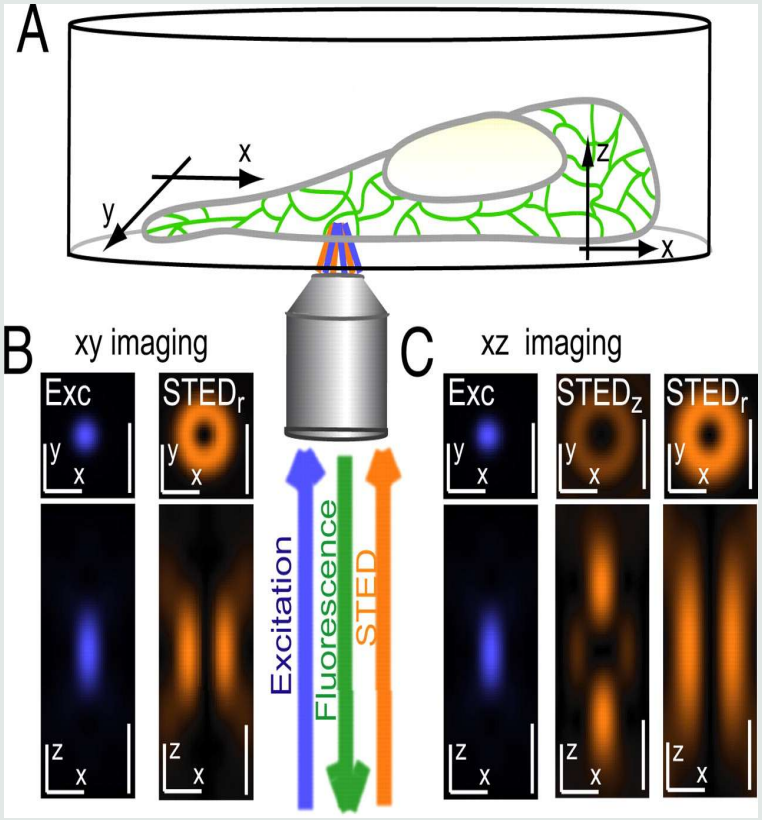
Basics of STED Microscopy





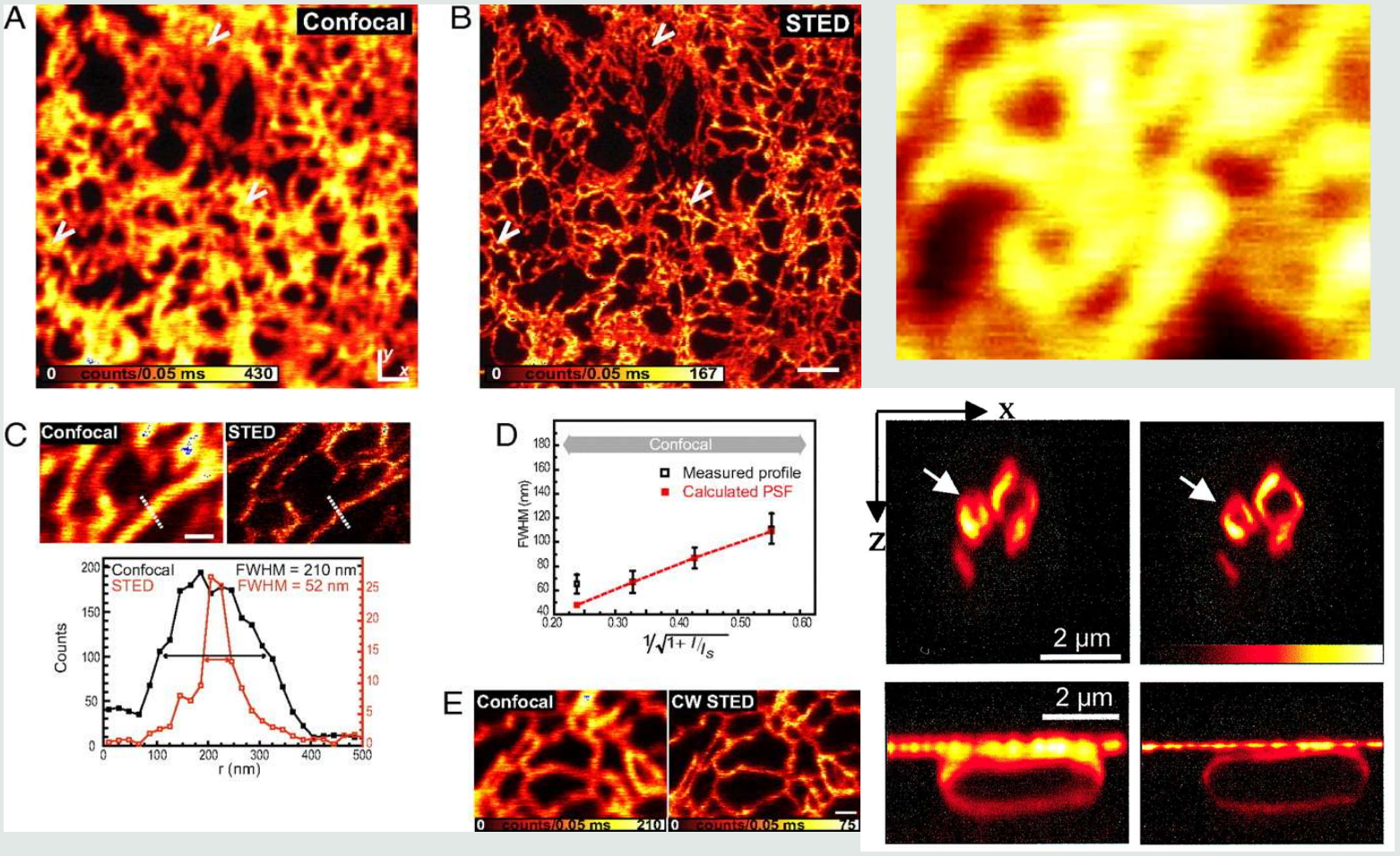
Basics of Resolution Enhancement in STED Microscopy



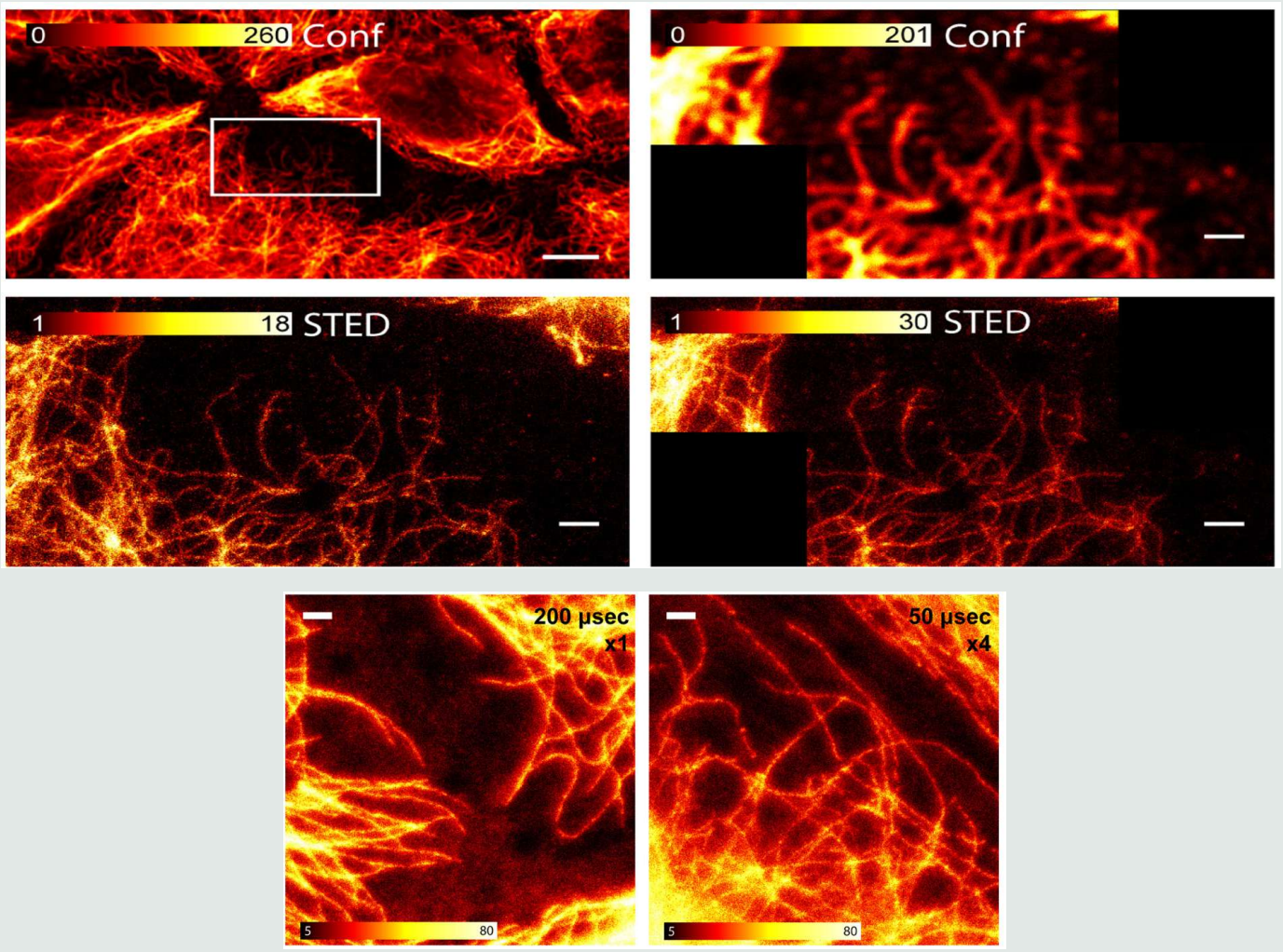


$$d_{STED} = \frac{\lambda}{2n \sin\alpha \sqrt{1 + {I_0}/{I_{sat}}}}$$

STED Microscopy Images



STED Microscopy Images



Multiple Doughnut STED Microscopy Images

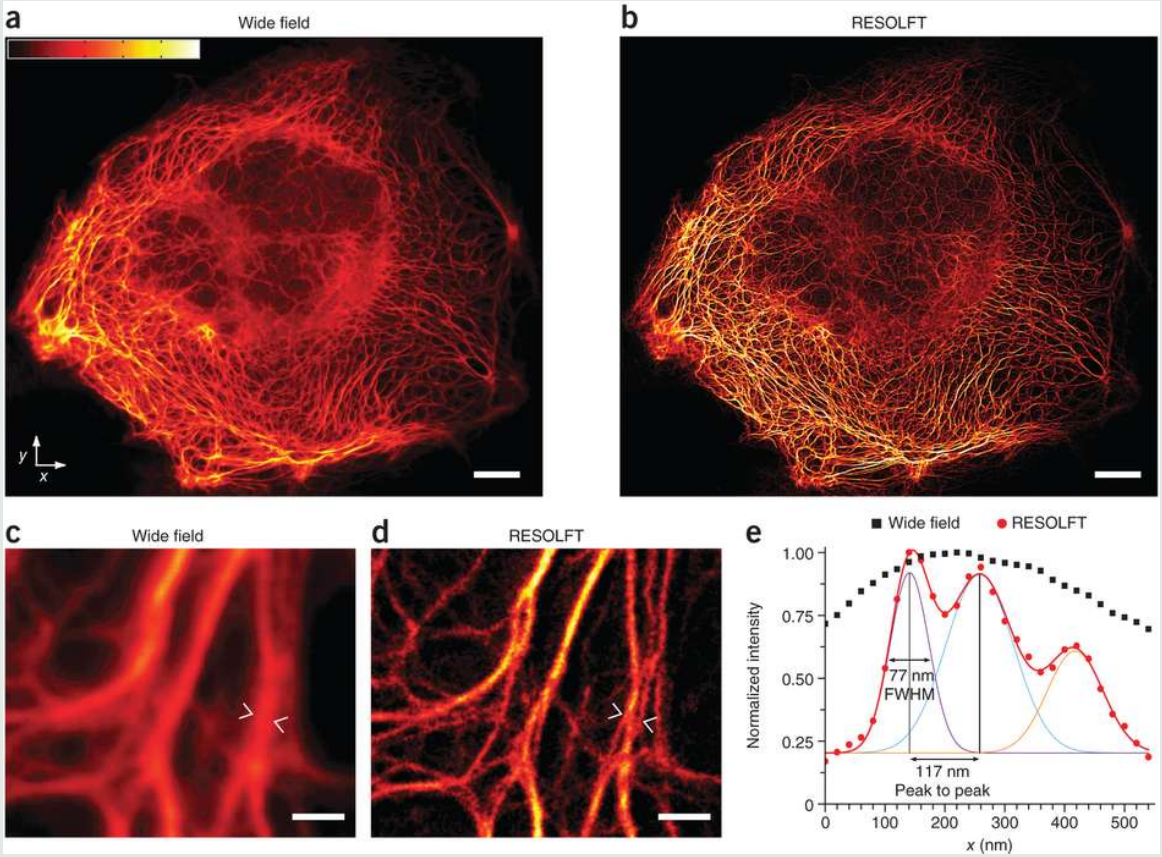


Image acquisition time: 2 sec

STED Microscopy Summary

❖Using fluorescence depletion to illuminate small spot to increase resolution (theoretically Infinite)

❖Product: Leica STED System $1.3M

❖Advantage

➢Can get high resolution (100nm) in 3D

➢Combining with 4Pi, Z resolution can be 33nm

➢No computation require to construct image

❖Disadvantage

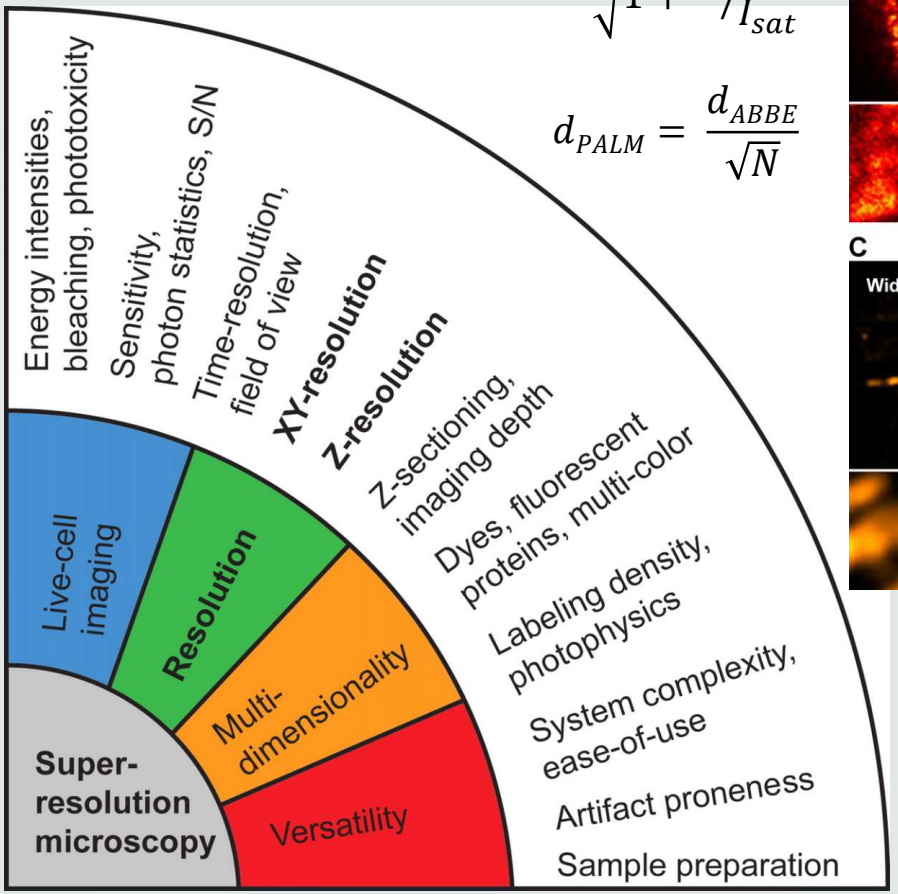
➢Expensive

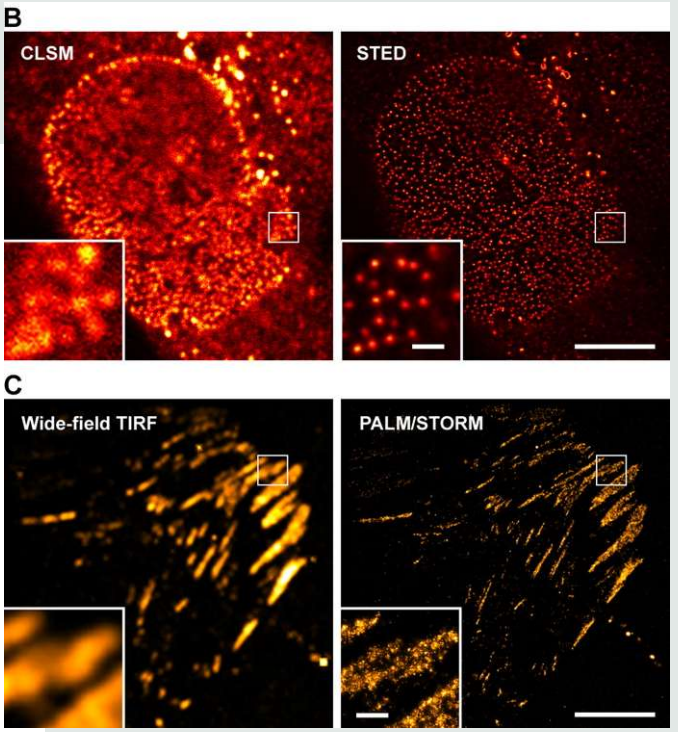
➢Difficult to image multiple dye

In a Nut Shell …

$$d_{ABBE} = \frac{\lambda}{2n \sin \alpha} \qquad d_{STED} = \frac{d_{ABBE}}{\sqrt{1 + {I_0}/{I_{sat}}}}$$

$$d_{PALM} = \frac{d_{ABBE}}{\sqrt{N}}$$





References

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