

# ISCORE Experience - Confocal and STED Microscopy -



**Momta Bhujel &  
Candyce Seger**



**Radu Moldovan**



Office of International Affairs  
University of Colorado  
Denver | Anschutz Medical Campus

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SCAN ME **iSCORE Experience**  
- Confocal and STED Microscopy -

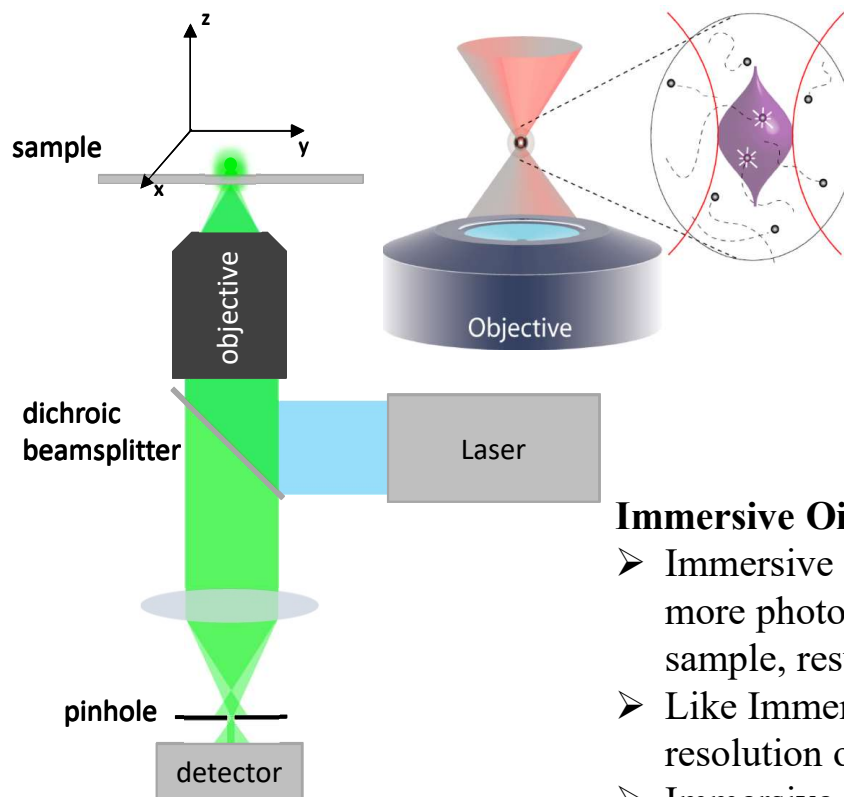
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Momta Bhujel and Candyce Seger  
with Radu Moldovan



# What is Confocal Microscopy?

Diffraction limited resolution in a laser scanning confocal microscope



A laser is used to excite fluorescent markers in a sample, which then emits lower energy and longer wavelength fluorescent signal, that is detected through a pinhole to discard out of focus signal.

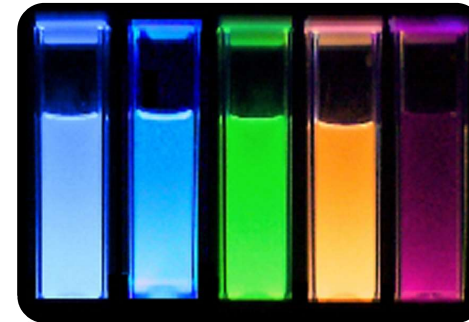
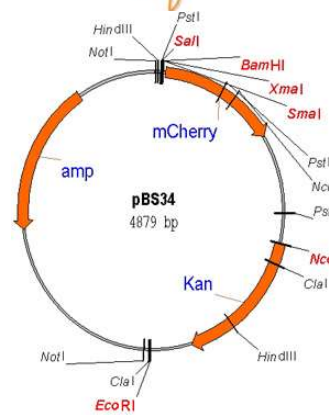
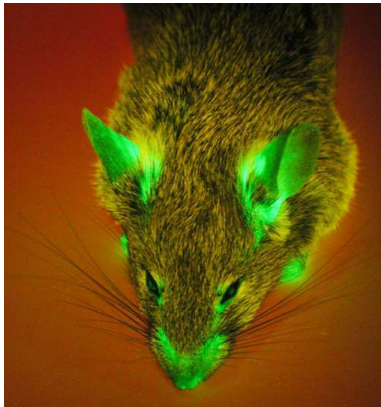
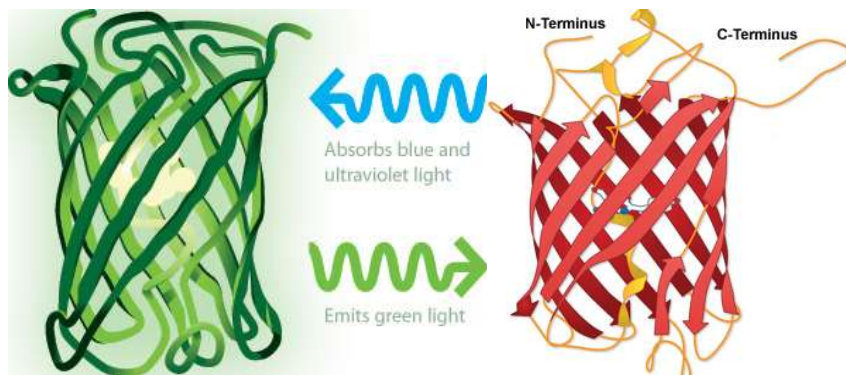
Resolution<sub>XY</sub> for different NA, n and  $\lambda$

$\lambda$	NA 0.5 $n_{\text{air}}=1$	NA 1 $n_{\text{H}_2\text{O}}=1.33$	NA 1.4 $n_{\text{oil}}=1.52$
405 nm	494 nm	248 nm	176 nm
543 nm	662 nm	331 nm	236 nm
633 nm	772 nm	386 nm	276 nm

## Immersive Oil vs. Water Vs. Dry

- Immersive oil objective can bend the light more efficiently, allowing more photons to be collected from the fluorescent emission of the sample, resulting in a higher resolution.
- Like Immersive oil, Water Immersion is also used to increase the resolution of the image by bending the light.
- Immersive Oil objective resolution is better than Water objective resolution, which is better than Dry objective resolution.

# Fluorescence dyes & Proteins



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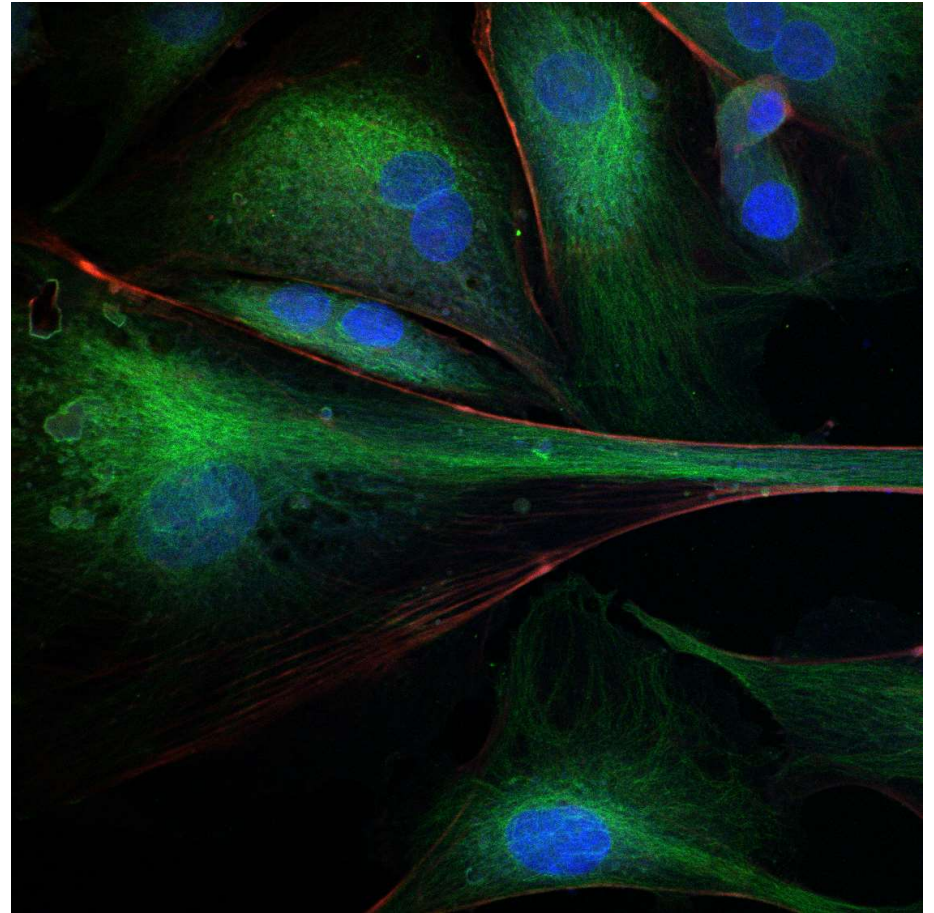
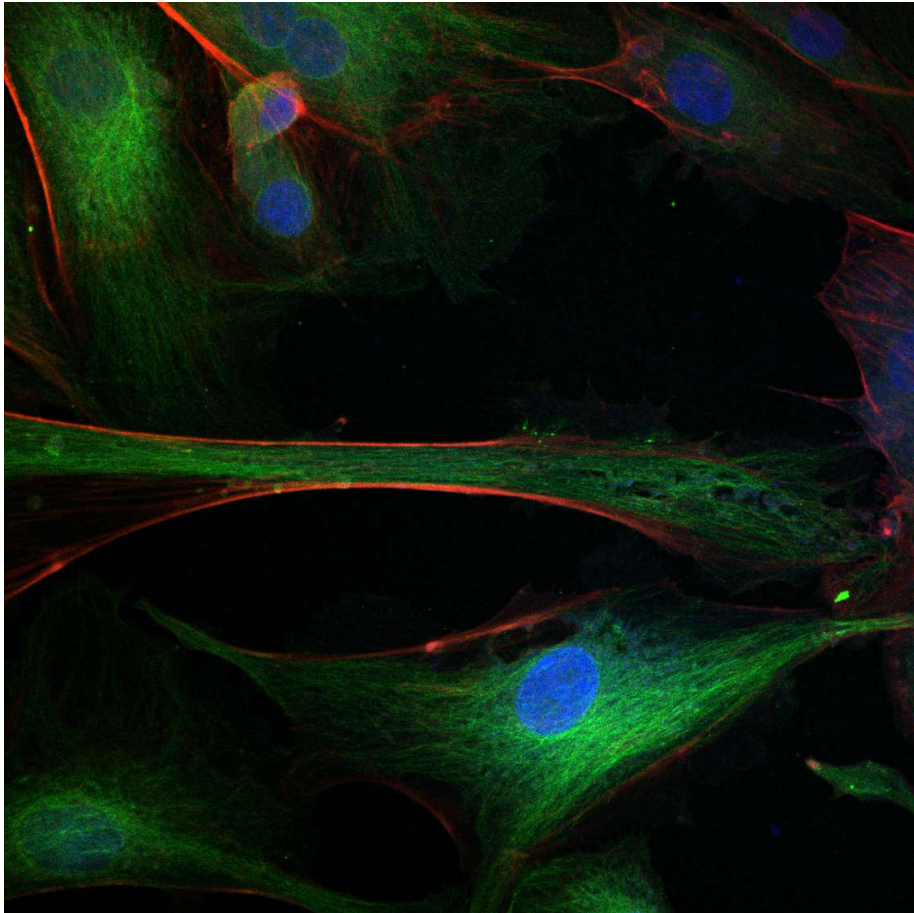
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hcCP     MAGLLKESMRIKMTMEGTVNGHYFKCTGEGEGGNPFEGTQSMRIHVTEGAPLFFAFDI
eqFF611  MNSLIKENRDMVMVMEGSVNGYQFKCTGEGEGGNPFEGTQSMRIHVTEGAPLFFAFDI
DaRed    MRSSKNVIEKEMRFKVMEGTVNGHFEIEGEGGRPYEGHNTVYKLVKVGKGGFLPFAFDI
GFP      MSKGEELFGVVPFILVELDGDVNGHKFSVSGEGEGDATYKLTIKFLCTTG-KLPVWFPT
          10      20      30      40      50

aeCP597  LAPCCNYGSKTIFIKHVSQIP--DYFKESFFEGFTWERTQIFEDGGGLTIRGQTSLSQQNN
asFF595  LSTSCNYGSKTIFIKHVSQIP--DYFKQSFFEGFTWERTTYEDGGGLTIRGQTSLSLDQC
hcCP     LAPCCNYGSKTIVHSTALIP--DYFKQSFFEGFTWERTTYEDGGGLTIRGQTSLSLDQC
eqFF611  LATSIFYGSKTIFIKHVSQIP--DYFKQSFFEGFTWERTTYEDGGGLTIRGQTSLSLDQC
DaRed    LSPQFYGSKVYVHPADIP--DYKLSFFEGFKMERVMNFDGGVVTYQSSLSLQGC
GFP      LVTTFSYGVQCFSRYPDIDMKQHDFFKSAMPEGTYVQRTIFFKDDGNYKTRAEVKEGDT
          60      70      80      90     100s   110

aeCP597  FIFKVNIVIGANFFANGPVMQKKA-GWEPQVELEYP--DGVLCGQSLMALKCTDGRHLT-
asFF595  LVYKVKILGNFFADGPMQNKAG-RWEPATEIVVYV--DGVLRGQSLMALKCPGGRHLT-
hcCP     LIYKVKVLGNFFADGPMQNKAG-GWEPQTEVYVPE--NGVLCGRNVMALKV-GDRRLI-
eqFF611  LVYHAKVTVGNFFSNGAVMOKKTK-GWEPNTEMLYPA--DGLRGRYSQMALNVDDGGYLSR
DaRed    FLYKVKVIGVNFSDGPMQKKTG-GWEPNTEMLYPR--DGVLRGQSLMALKKIDGGHYL-
GFP      LVNRIELKGIIDFKEDGNILGHKLEYNYSNHYIMADKQNGIKVNFKIRINIEGDSVQL-
          120     130     140   s   150     160     170

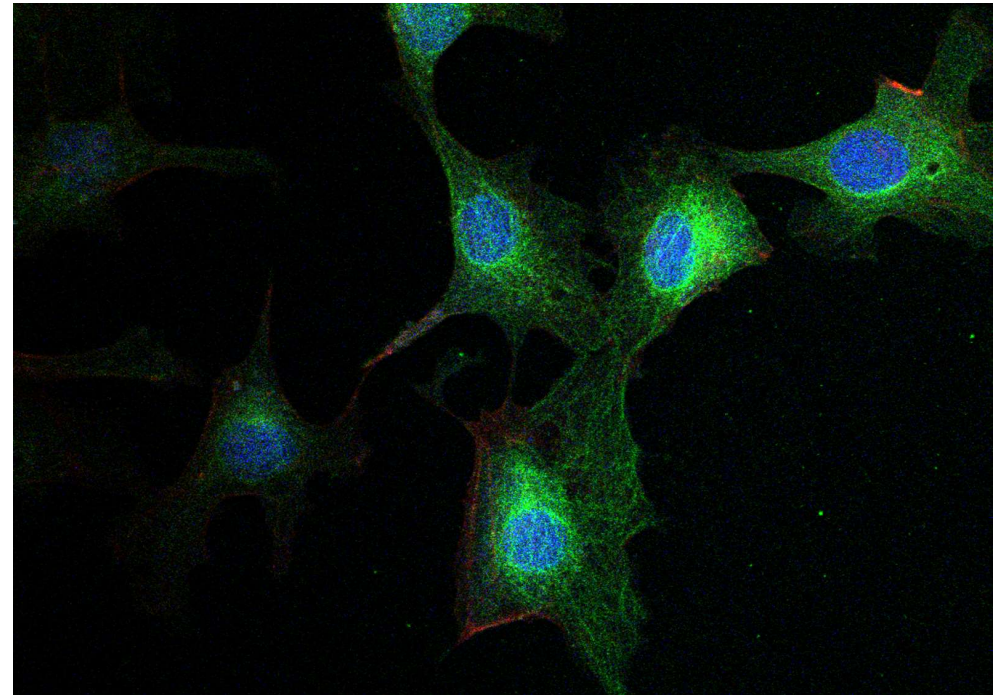
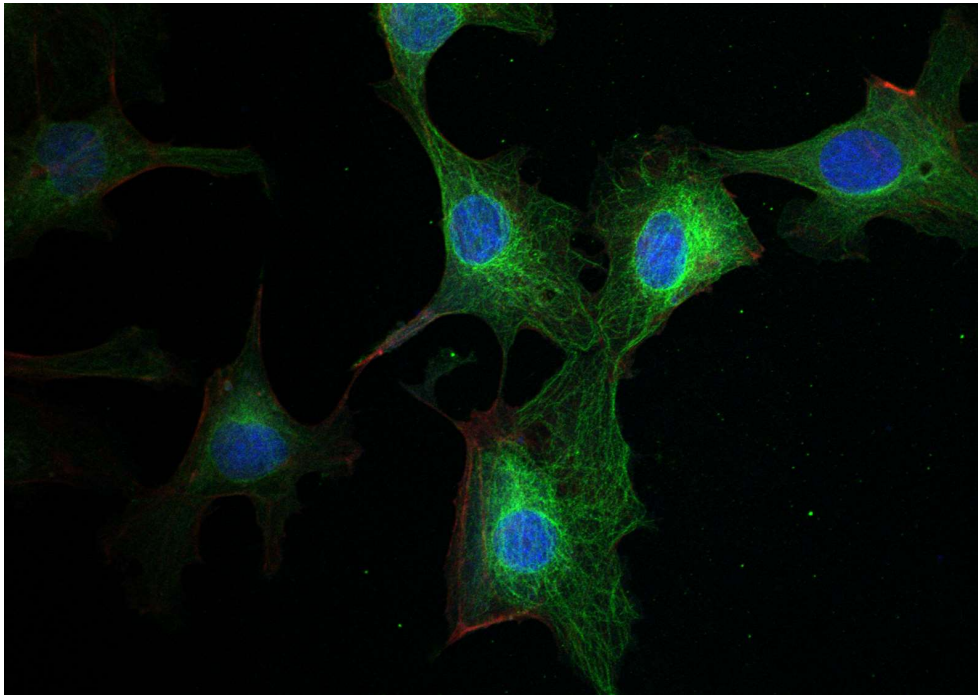
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hcCP     CHLRTTYRSKKAVALTMPGFHFDIRLQMPRK--EKDEYFELYEASVARYSDLPKAN
eqFF611  CSFETTYRSKKTVENFMPGFHFVDRLELLEE--SDKEMFVQHEHAVAKFDLPKSLGRL
DaRed    VEFKSIYMAKKP---VQLPGYTYVDSKLDITSH-NEDYTYVEQYERTEGRHRLFL
GFP      ADHYQNTPIGD-GPVLDPNHYLSTQALSXKDPNEKRDRHMLLEFVYTAAGITRMDDELYK
          180     190     200     210     220     230
    
```

# Confocal Imaging

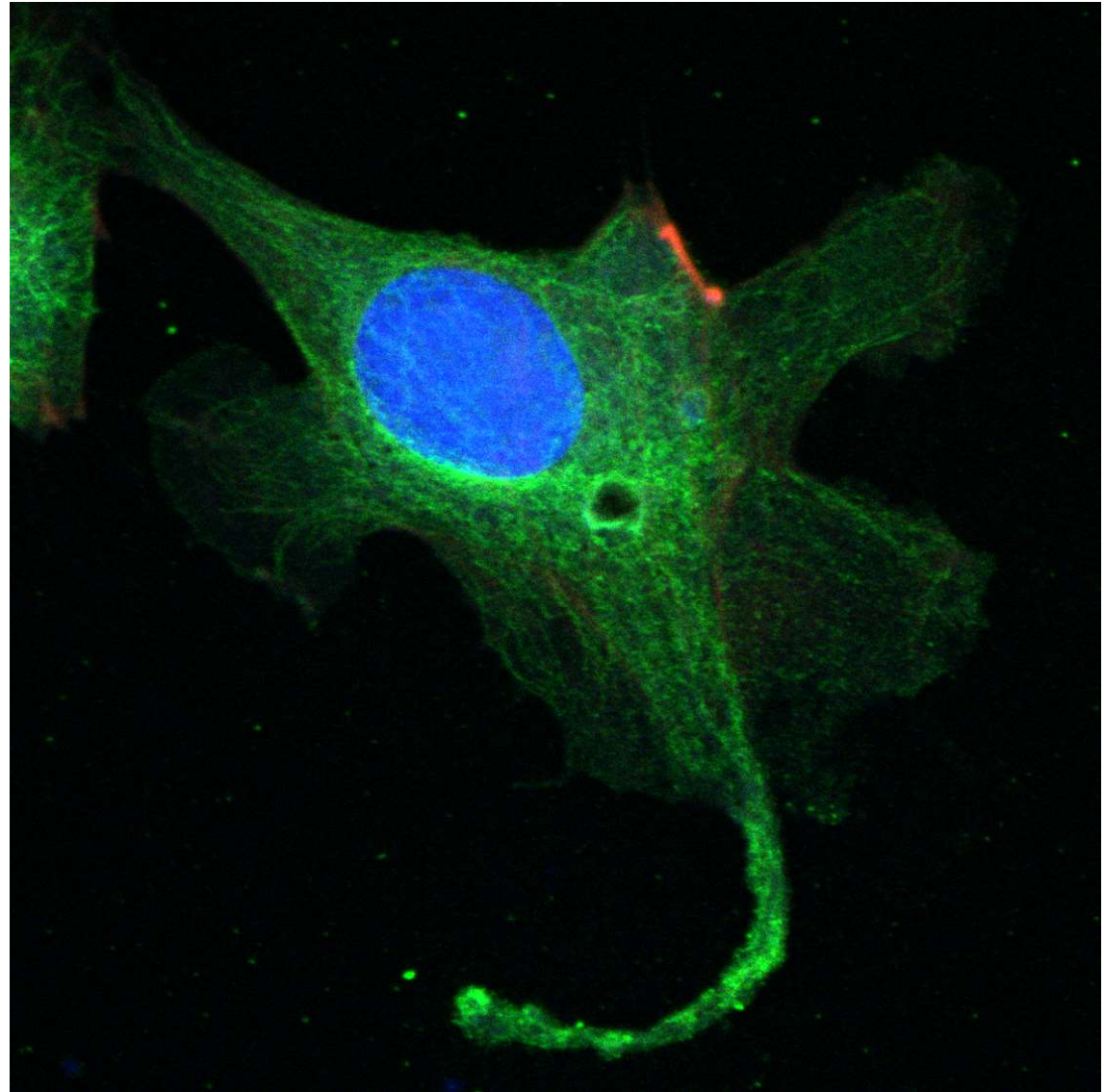
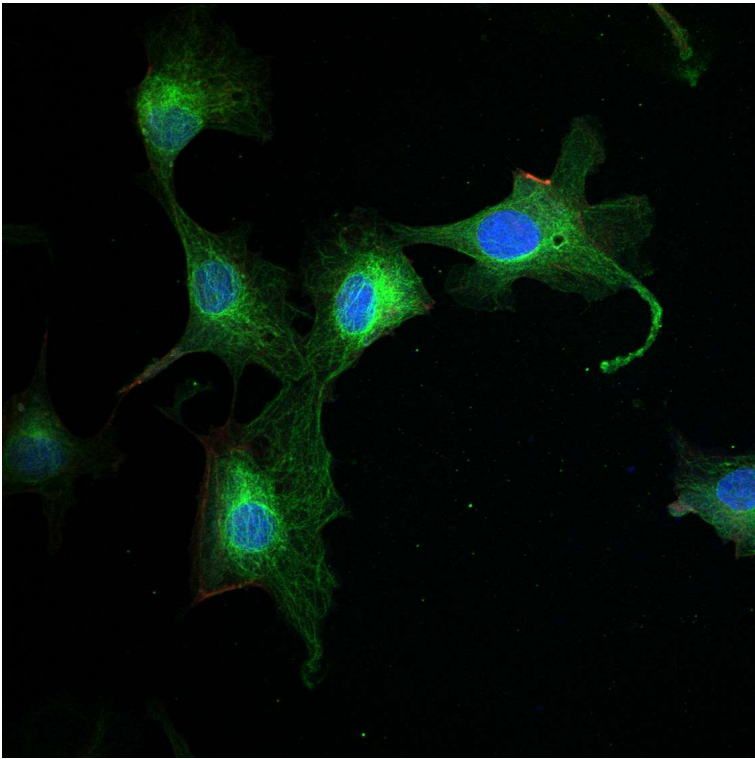


# Slow vs Fast Acquisition

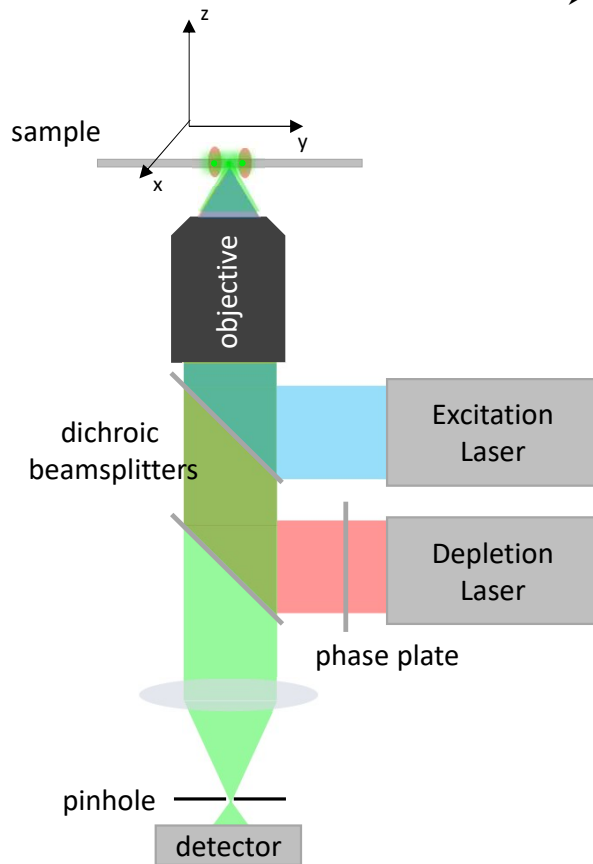
- How long it takes the laser scanning confocal microscope to acquire a single optical section of the sample.
- several acquisitions are averaged to improve signal to noise ratio



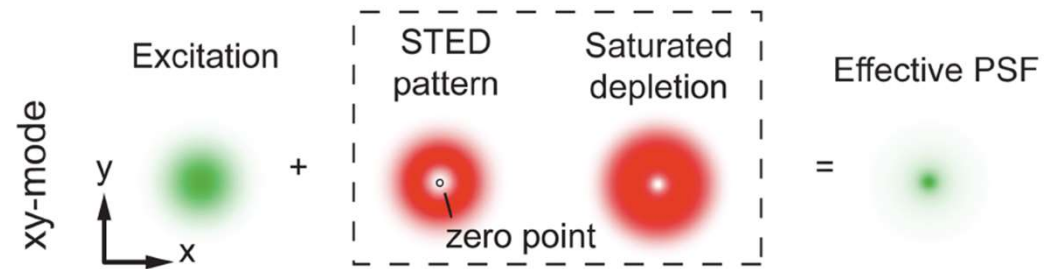
Cropped and Reoriented



# STED schematic

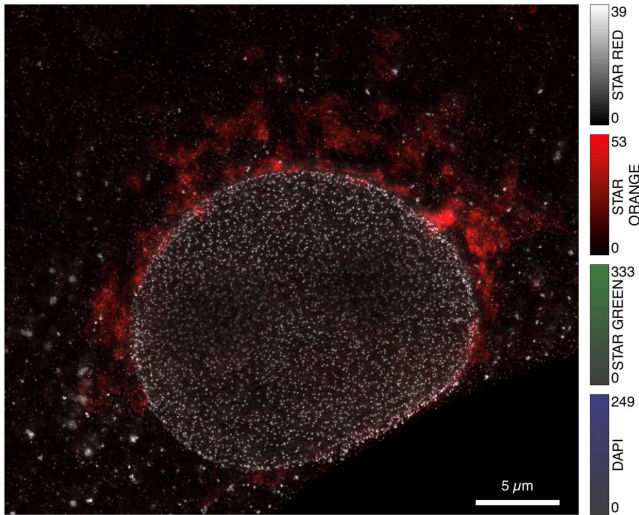


- another laser is added (depletion laser) in the shape of doughnut which will turn off the fluorescent molecules in the donut area.

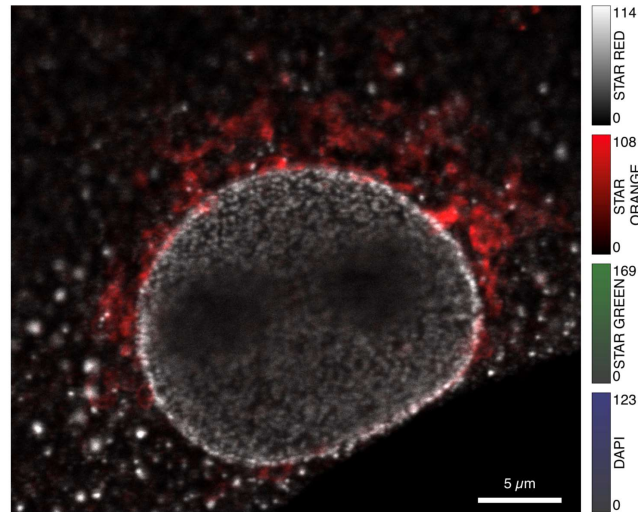




Session name - Sample name - overlay (STED)



Session name - Sample name - overlay (confocal)

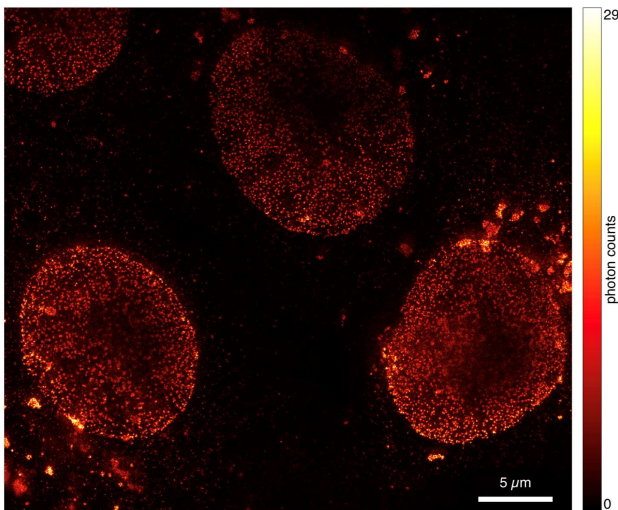


# STED vs Confocal

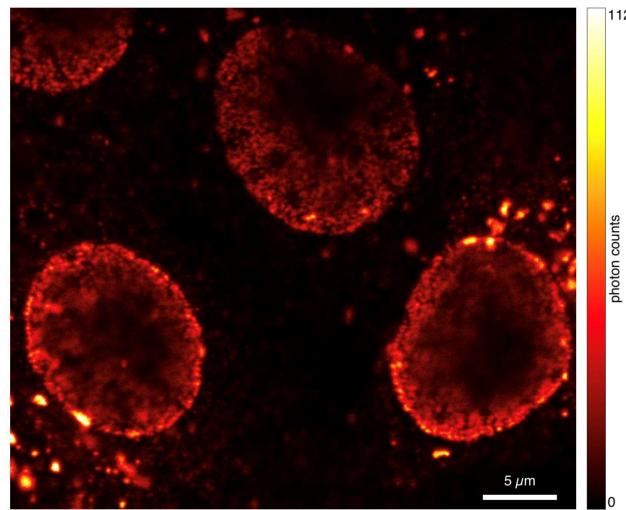
Both use dichroic mirrors  
Both use pinholes

STED uses two lasers, one in the shape of a doughnut for turning off fluorescent molecules in the outer area of the PSF (focal volume). Therefore, the apparent PSF is smaller = better resolution.

Session name - Sample name - STAR RED (STED)

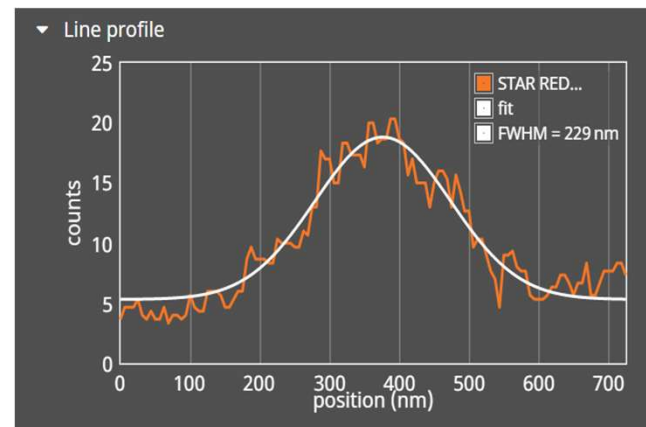
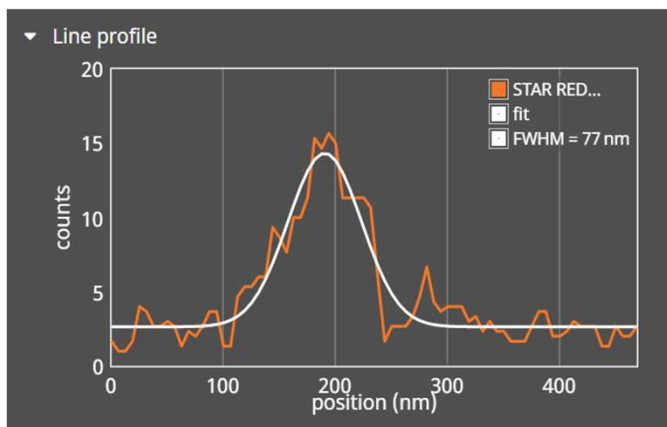
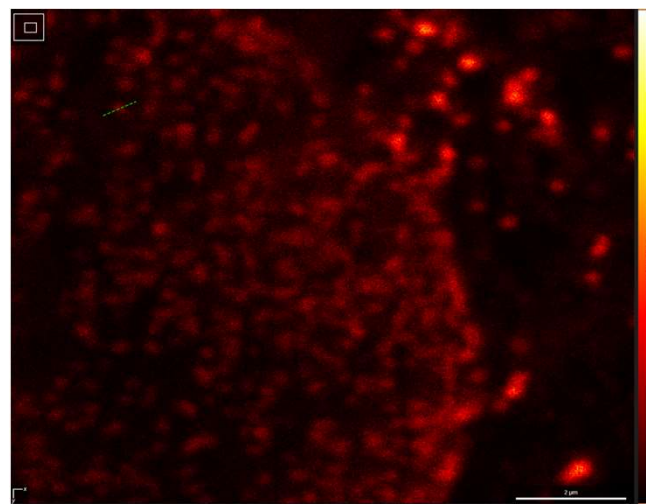
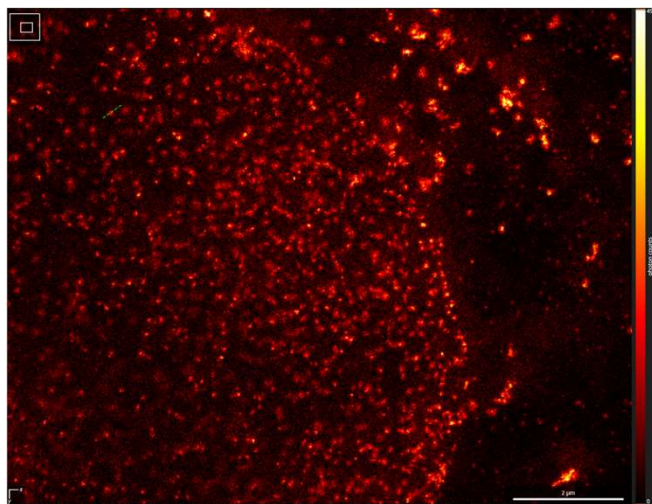


Session name - Sample name - STAR RED (confocal)



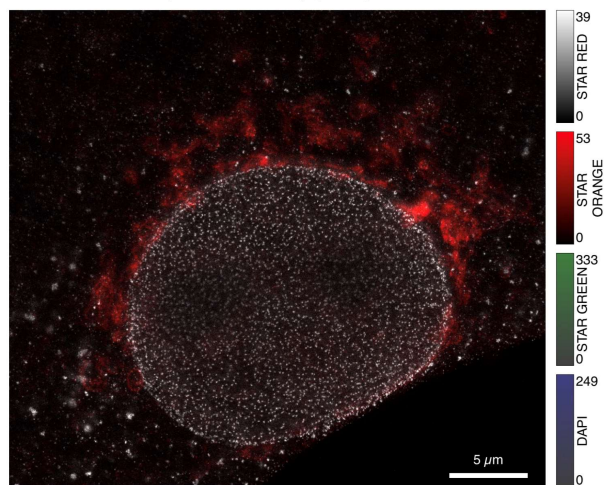
# Line Profiles

STED vs Confocal- STAR RED channel

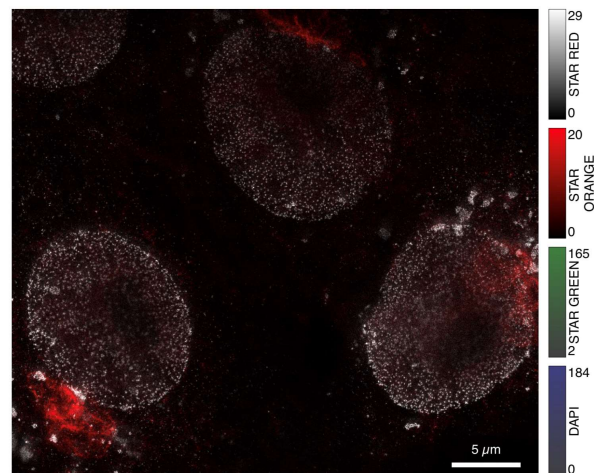


# Slow vs Fast Acquisition

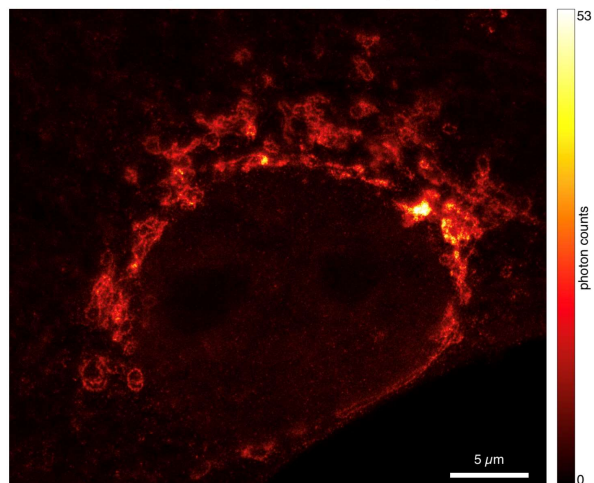
Session name - Sample name - overlay (STED)



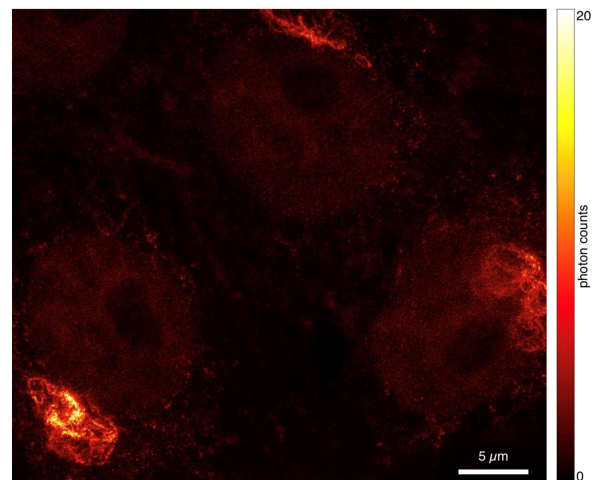
Session name - Sample name - overlay (STED)



Session name - Sample name - STAR ORANGE (STED)

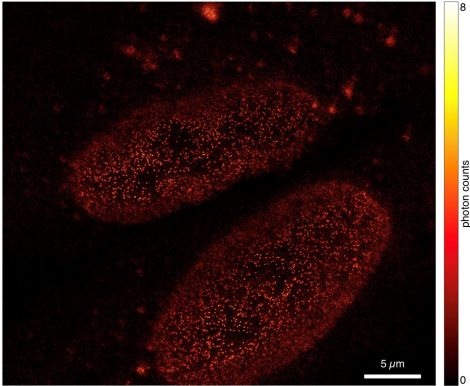


Session name - Sample name - STAR ORANGE (STED)

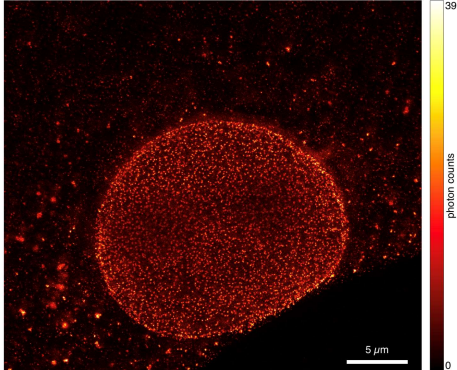


# Low vs High Laser Power for the STED donut

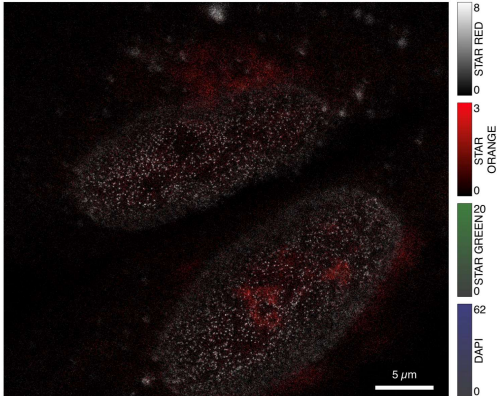
Session name - Sample name - STAR RED (STED)



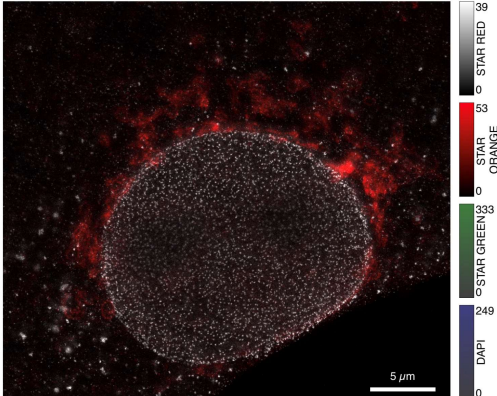
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Session name - Sample name - overlay (STED)



Session name - Sample name - overlay (STED)

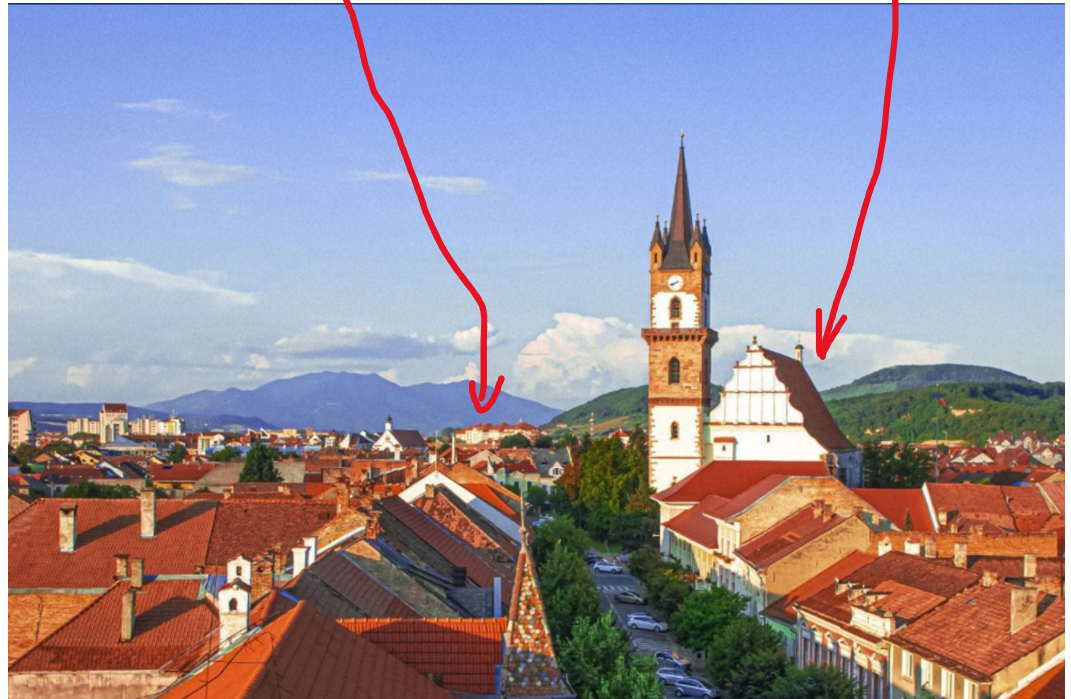


# Cultural Experience

- Language
- Childhood stories
- Hometown
- Jordan Peterson

Childhood home (block of flats)

Dangerous sliding on church roof



THANK YOU