ISCORE Experience
- Confocal and STED Microscopy -

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SCAN ME

iSCORE Experience
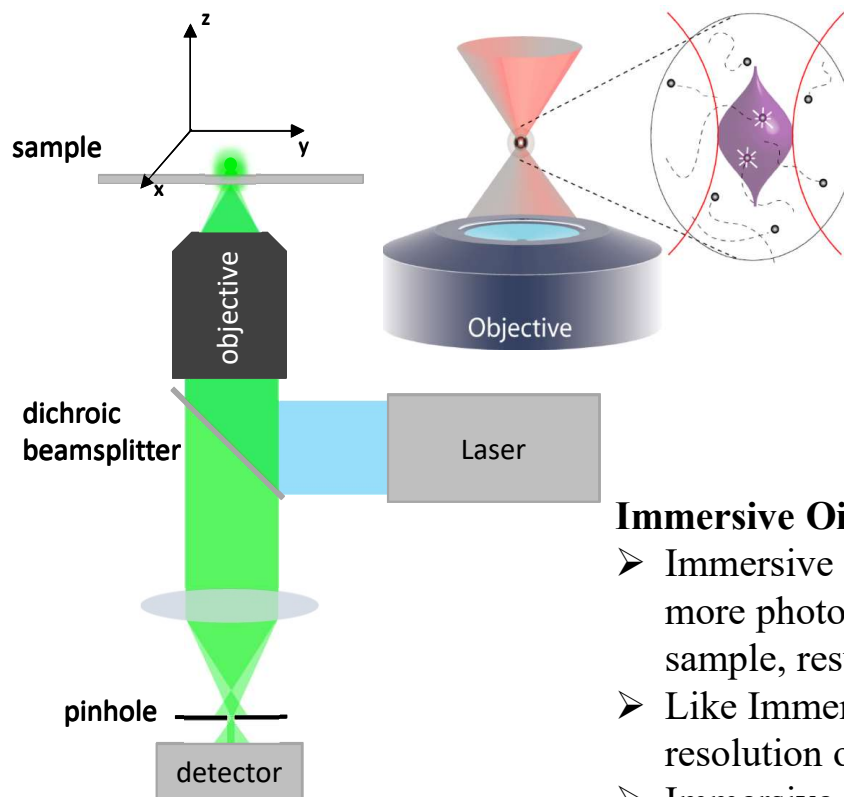- Confocal and STED Microscopy -

Momta Bhujel and Candyce Seger

with Radu Moldovan

5 μm

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.
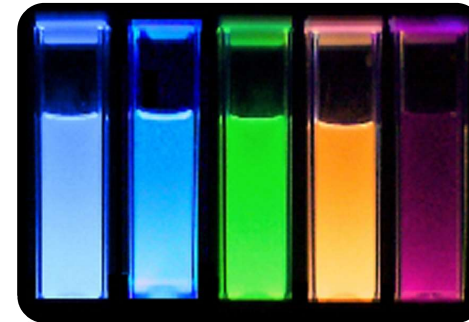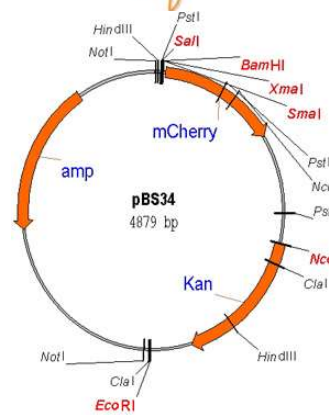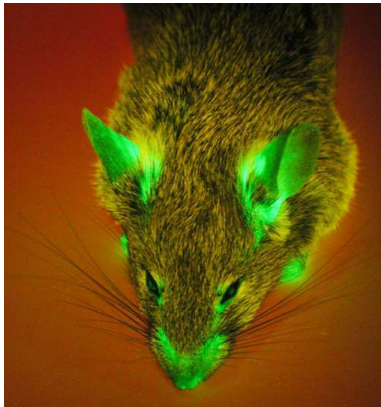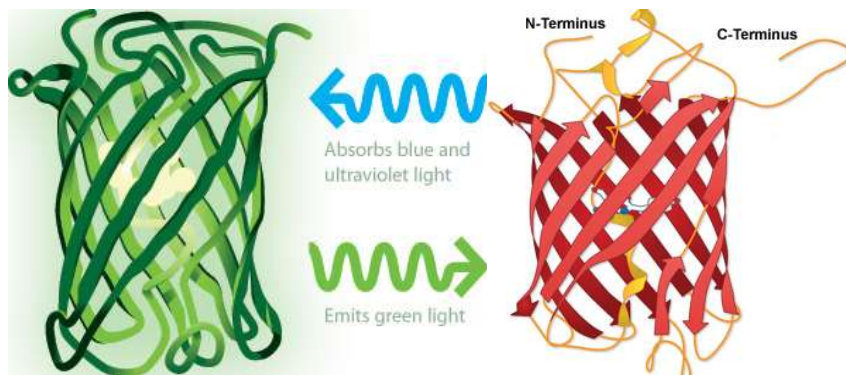

Resolution$_{XY}$ for different NA, n and λ

λ	NA 0.5 n_{air}=1	NA 1 n_{H2O}=1.33	NA 1.4 n_{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.
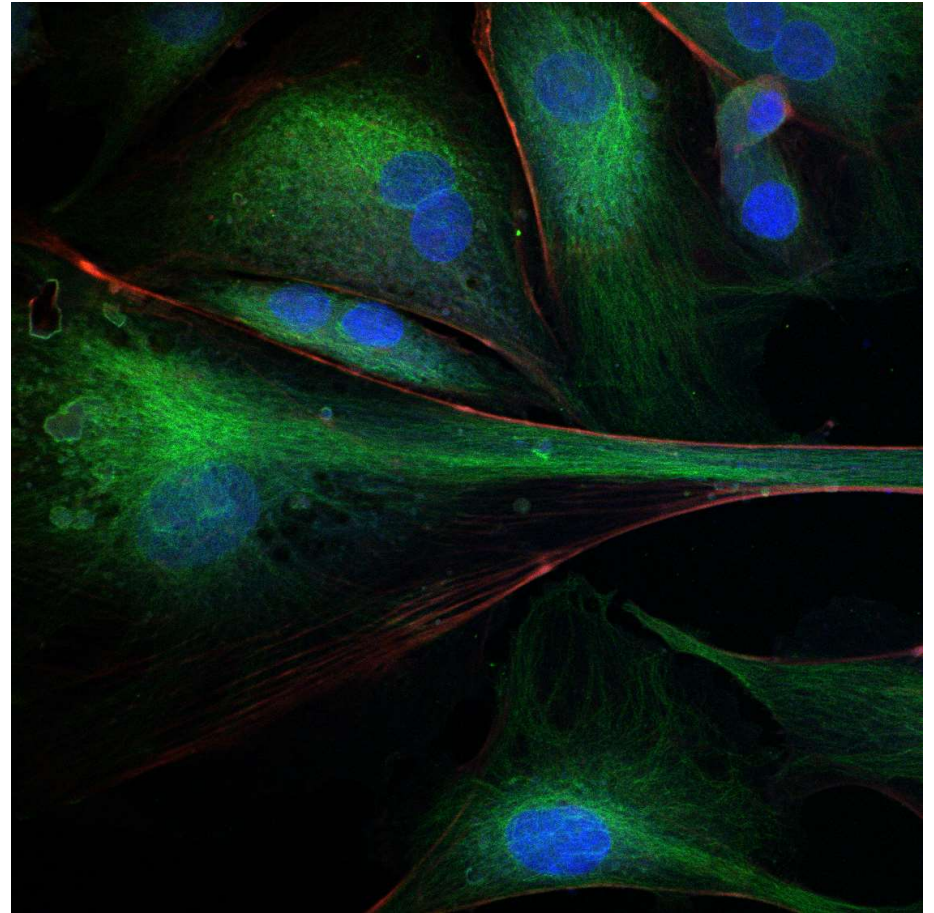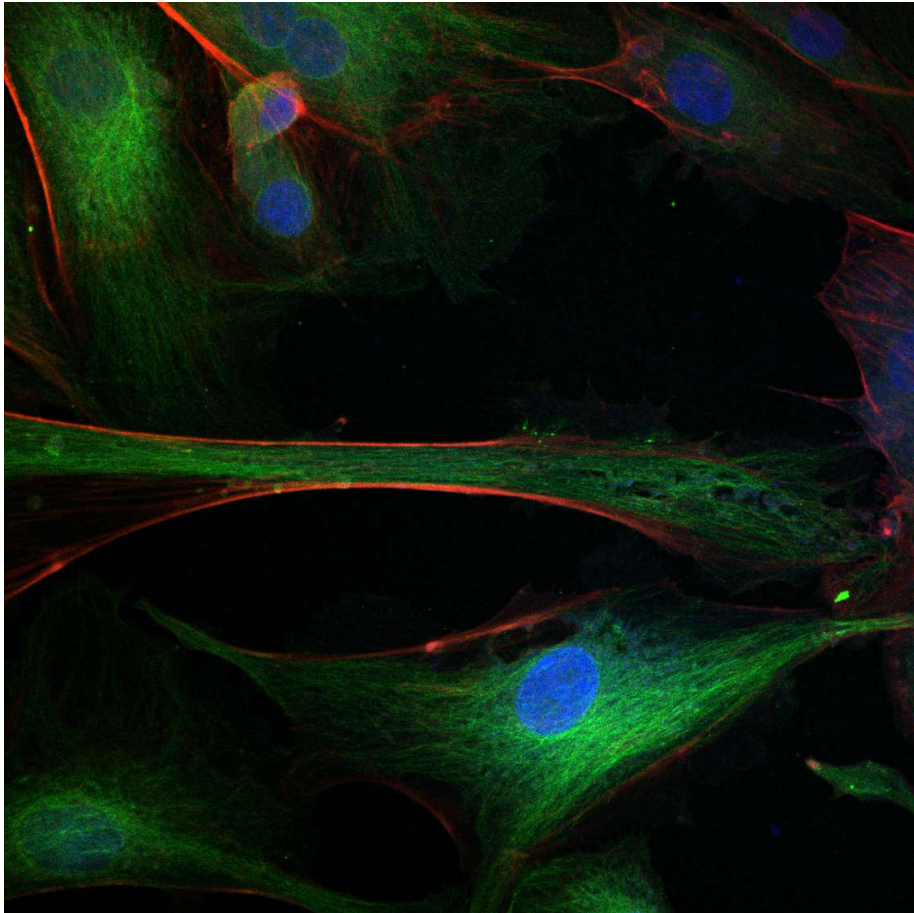
Fluorescence dyes & Proteins
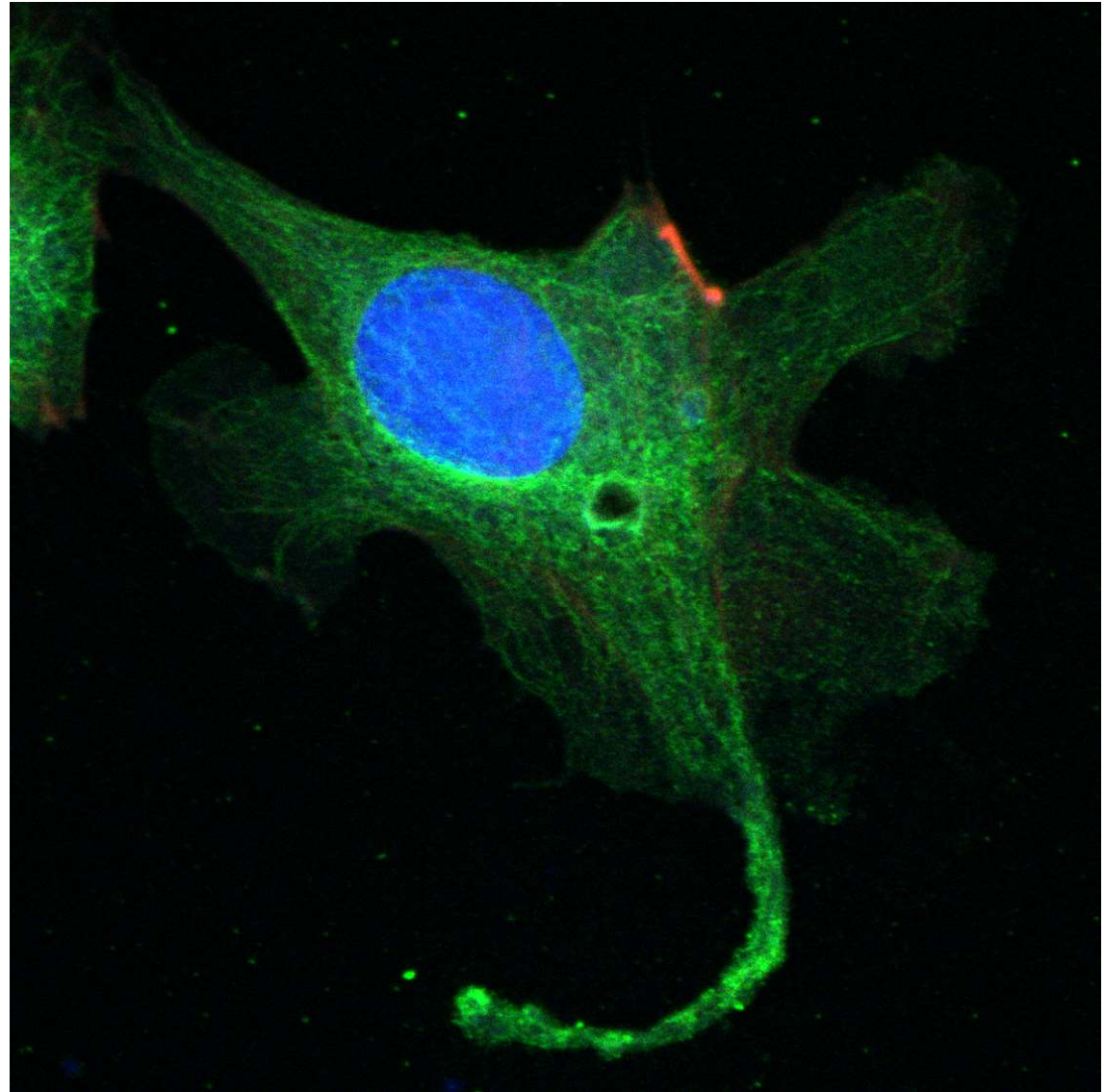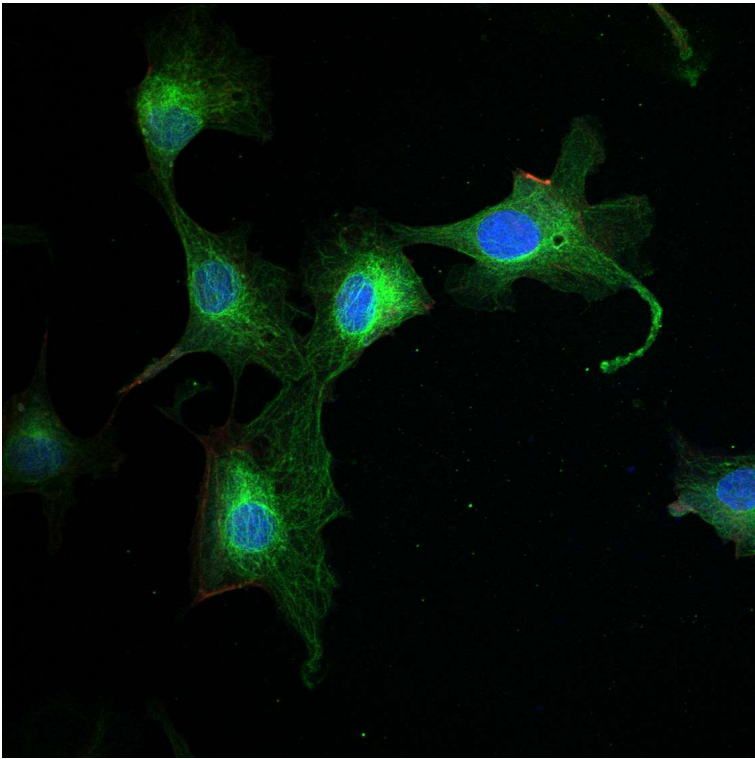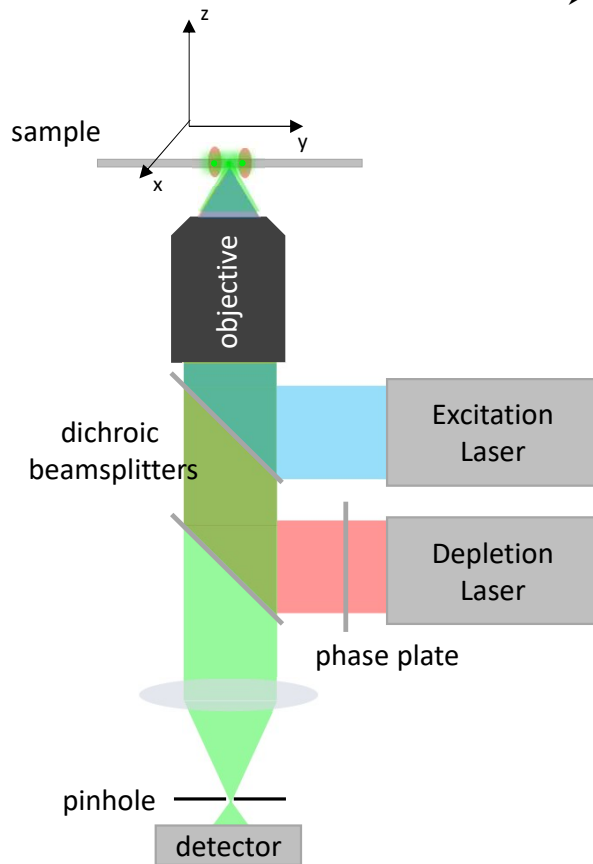
Confocal Imaging

Slow vs Fast Acquisition

➢ How long it takes the laser scanning confocal microscope to acquire a single optical section of the sample.

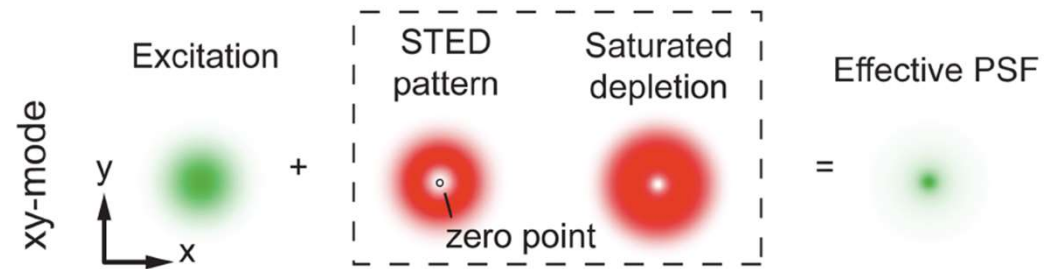➢ several acquisitions are averaged to improve signal to noise ratio
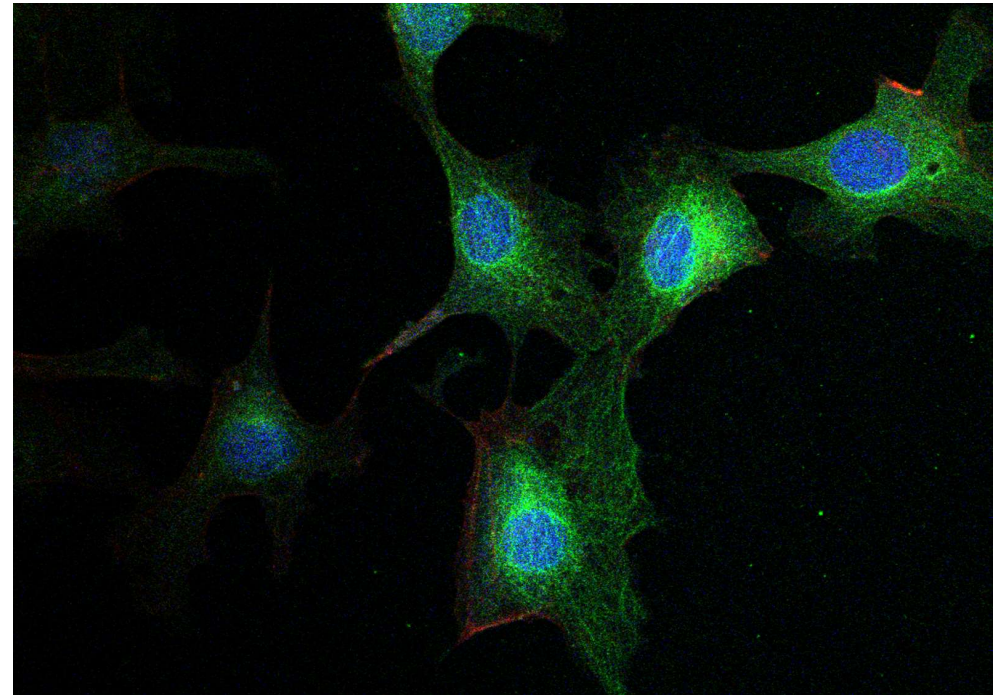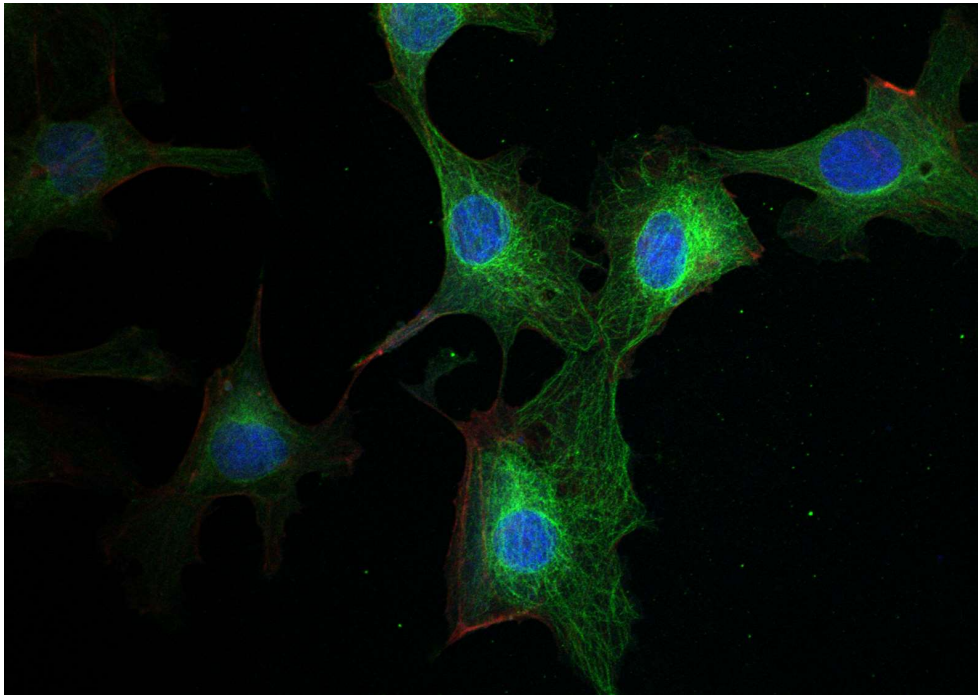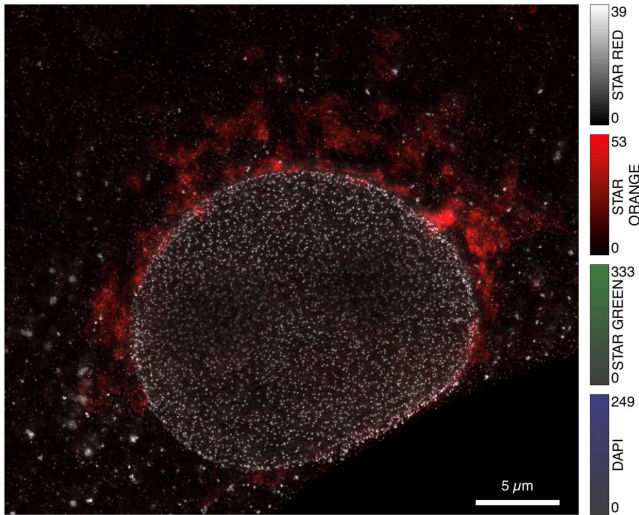
Cropped and Reoriented

STED schematic

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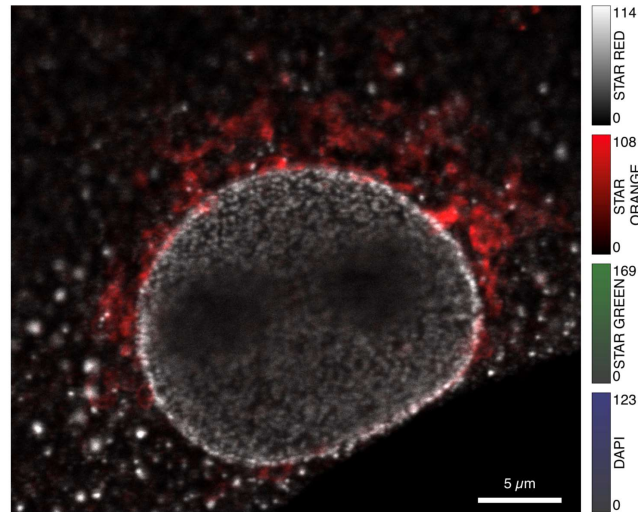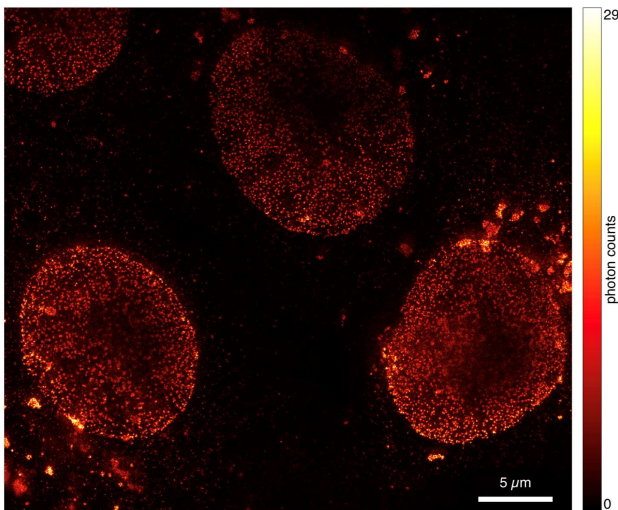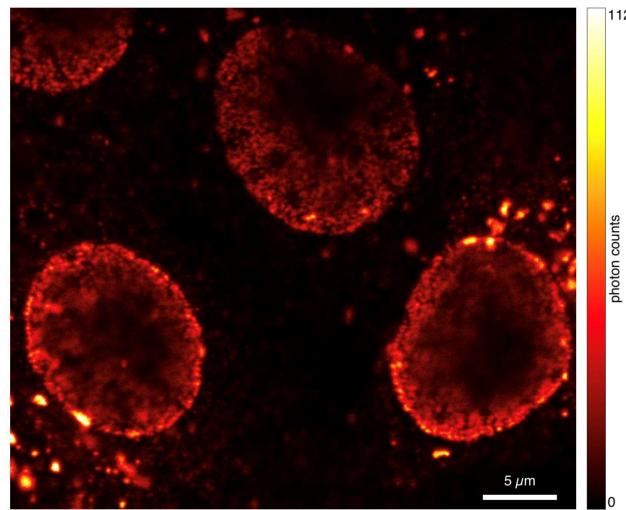
STED vs Confocal

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.
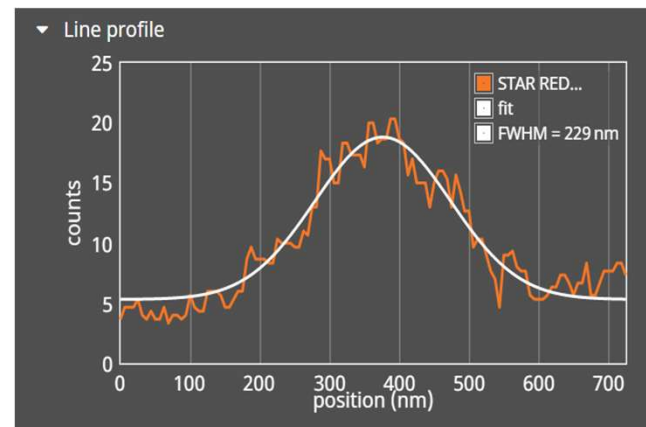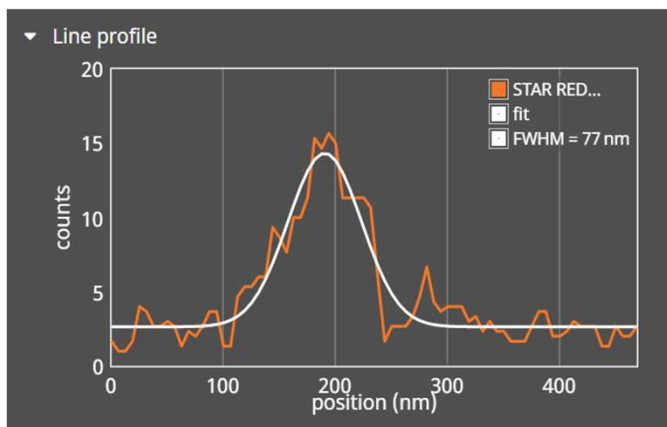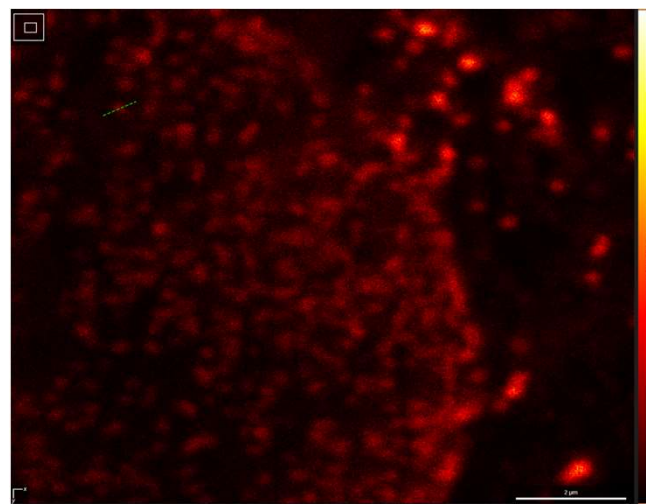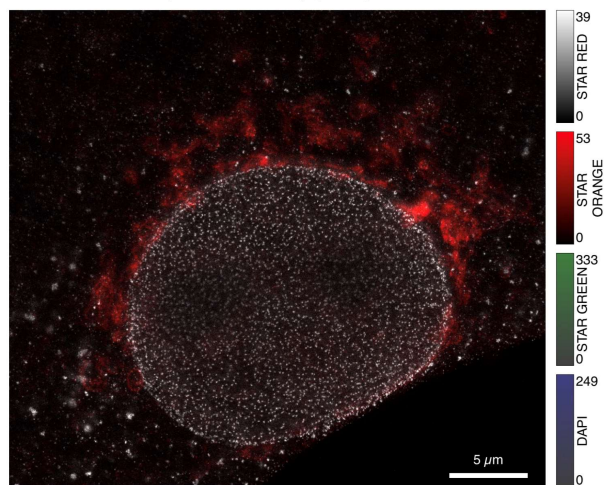
Line Profiles
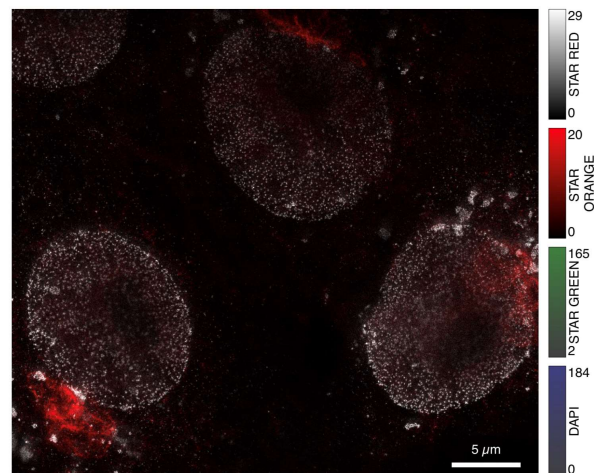## STED vs Confocal- STAR RED channel
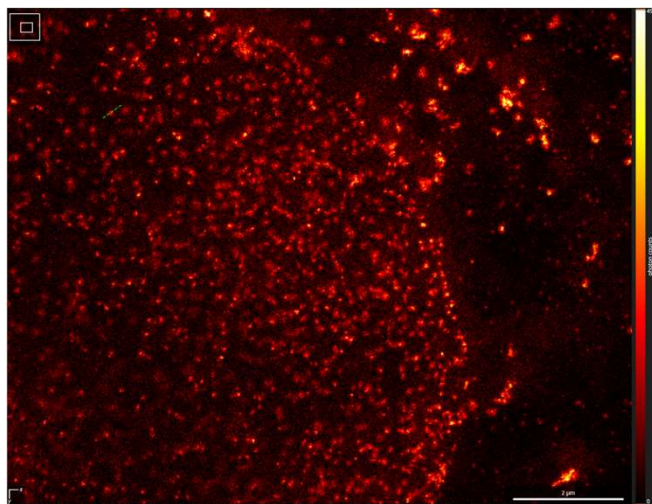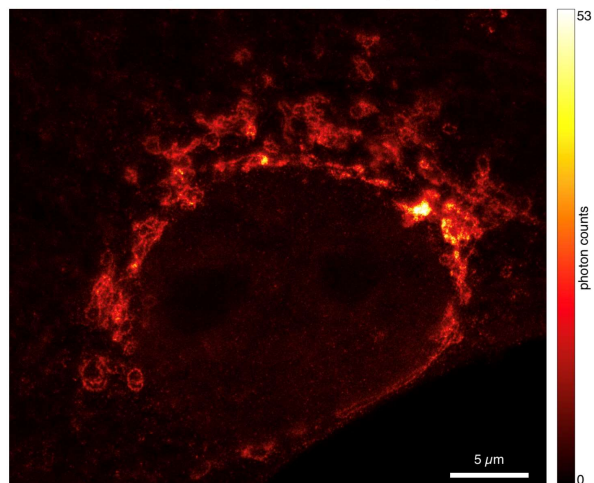
Slow vs Fast Acquisition
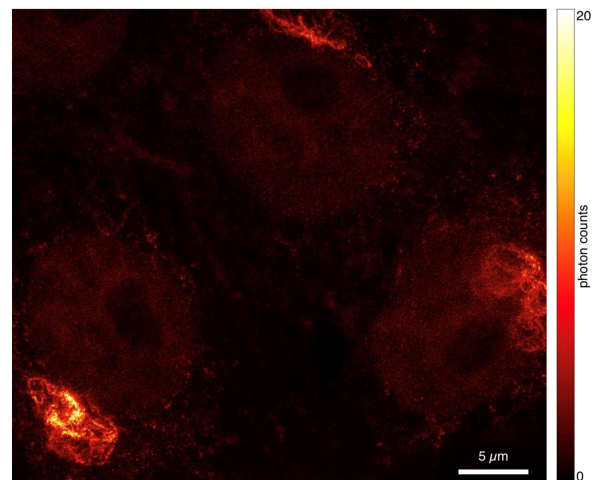

Session name - Sample name - overlay (STED)

Session name - Sample name - overlay (STED)
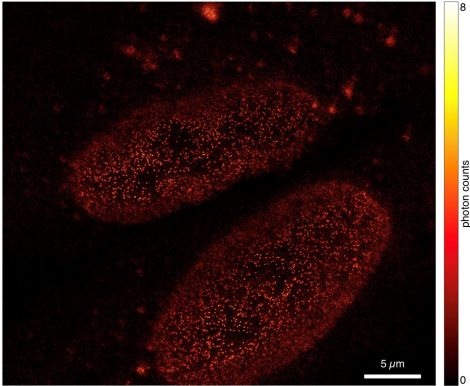
Session name - Sample name - STAR ORANGE (STED)

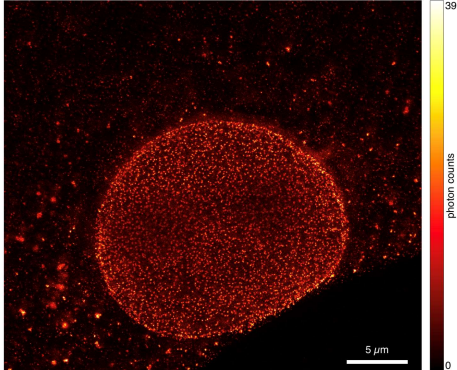Session name - Sample name - STAR ORANGE (STED)

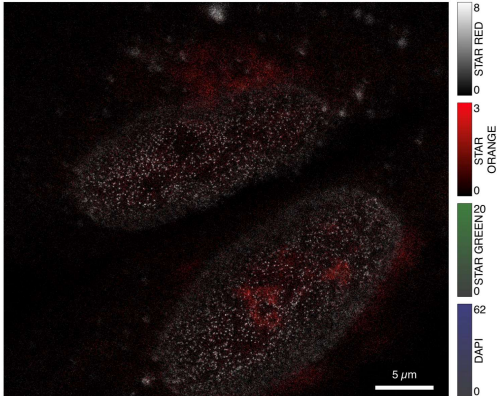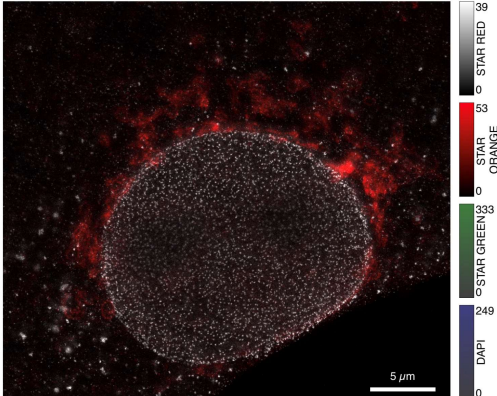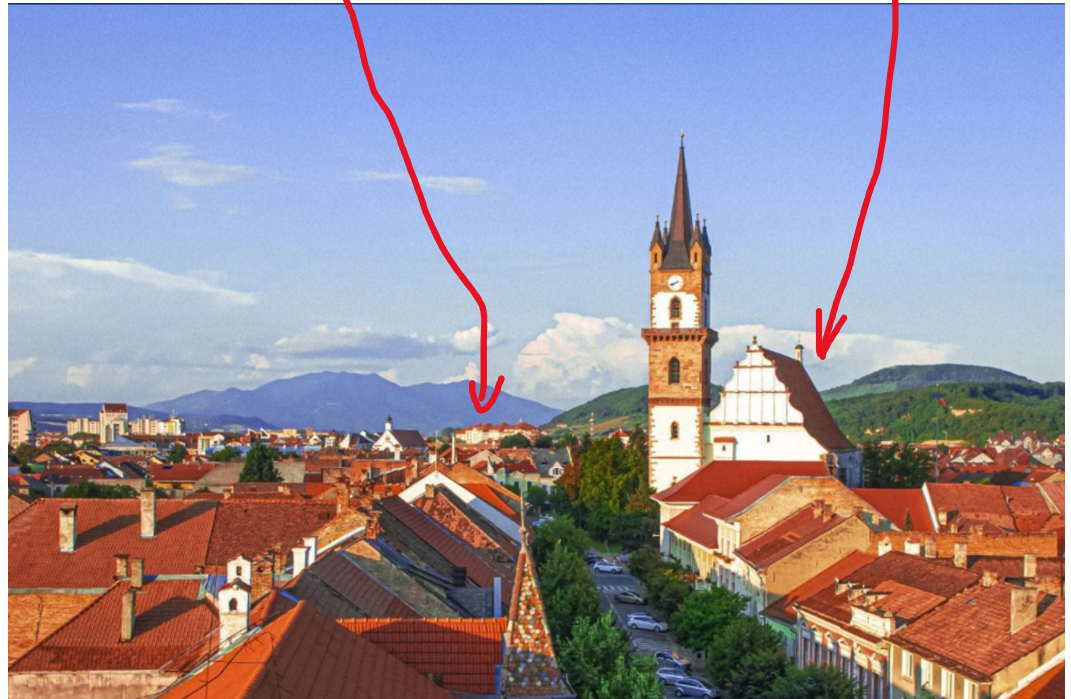# Low vs High Laser Power for the STED donut

Cultural Experience

- Language
- Childhood stories
- Hometown

- Jordan Peterson

Childhood home (block of flats)

Dangerous sliding on church roof

THANK YOU