STED microscopy with single light source

Teodora Şcheul

Dr. Iréne Wang, Dr. Jean-Claude Vial

LIPhy, Grenoble, France

Summary

I. Introduction to STED microscopy

II. STED with one laser source

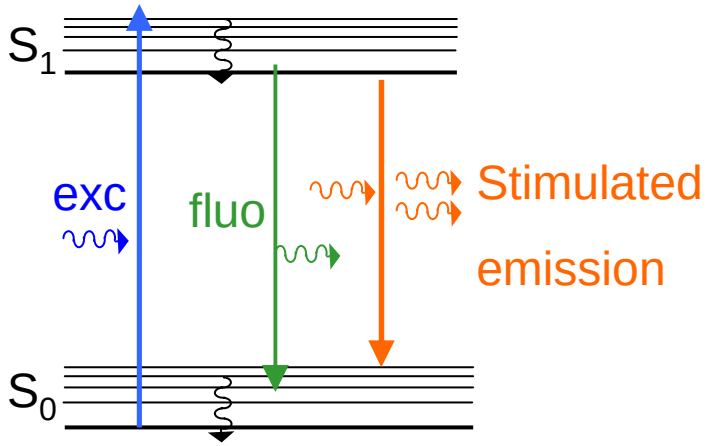
1. Two-photon single wavelength STED

2. Two colours for excitation and depletion - one source

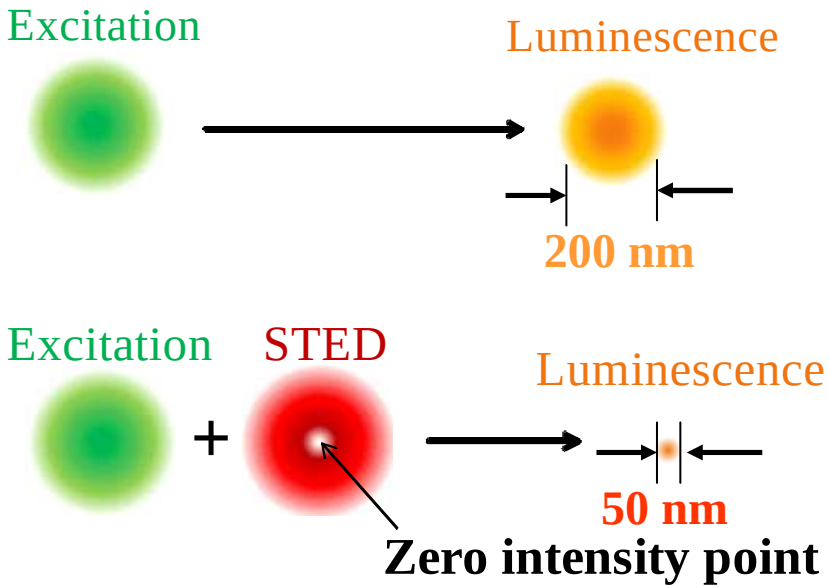
- Experimental set-ups
- Results
- Conclusions and future work

STED microscopy - principle

Stefan W. Hell et al., *Opt. Lett.,* **1994**, *19*, 780-782

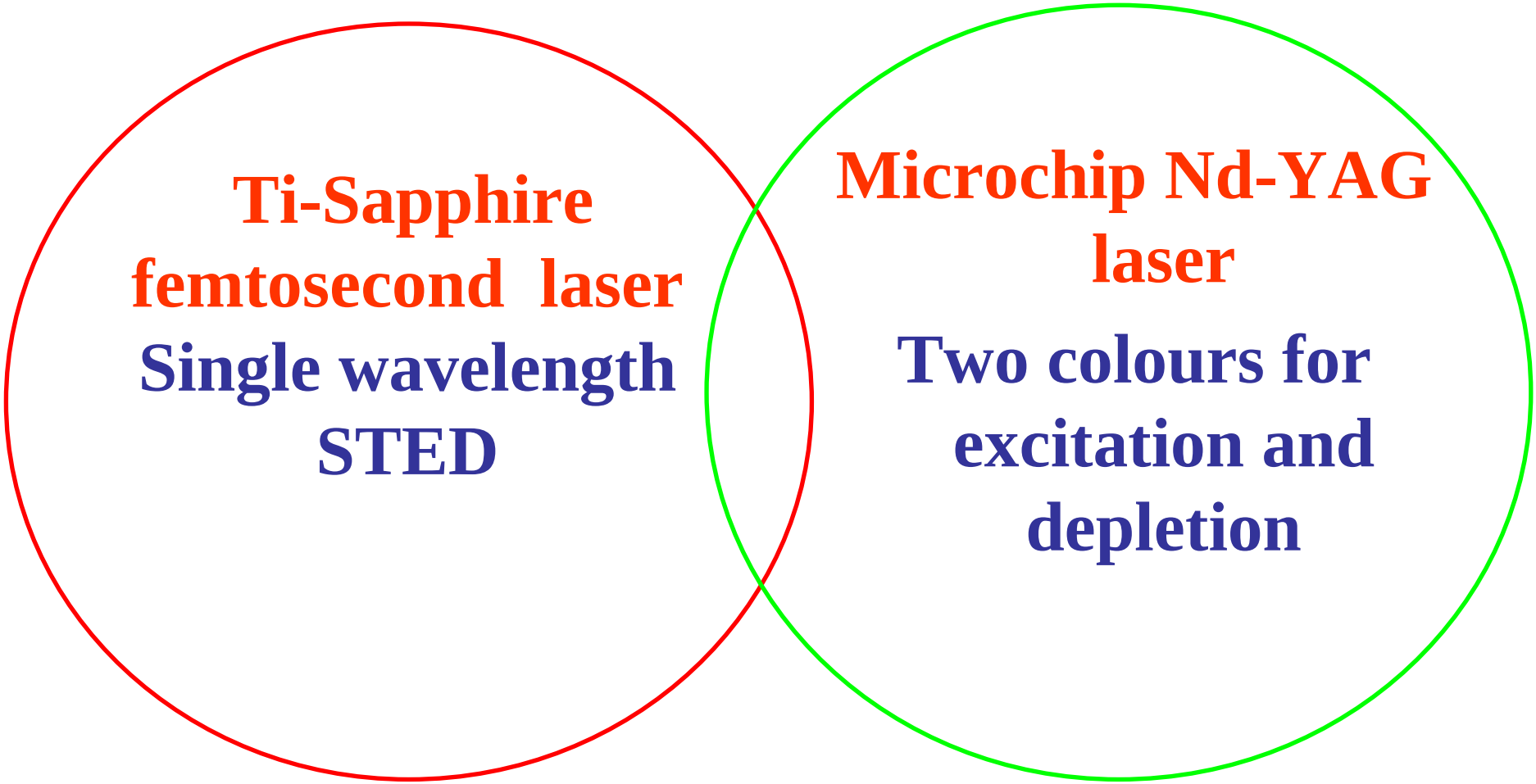


Energy levels and optical transitions involved in STED

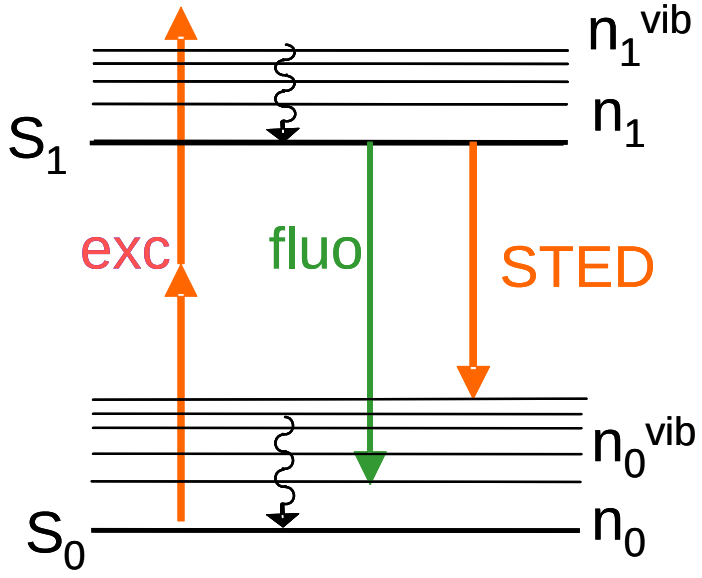


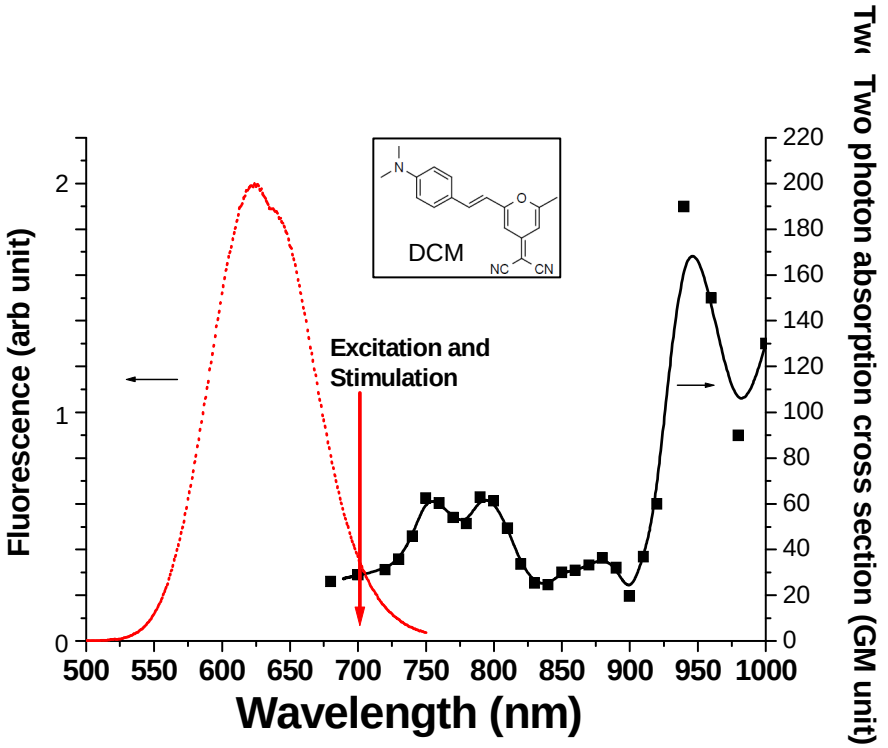
Switching off the fluorescence ability of the molecules in the periphery off the focus shrinks the fluorescing area

STED microscopy – one laser source?



I. Two-photon excitation and stimulated emission depletion by a single wavelength



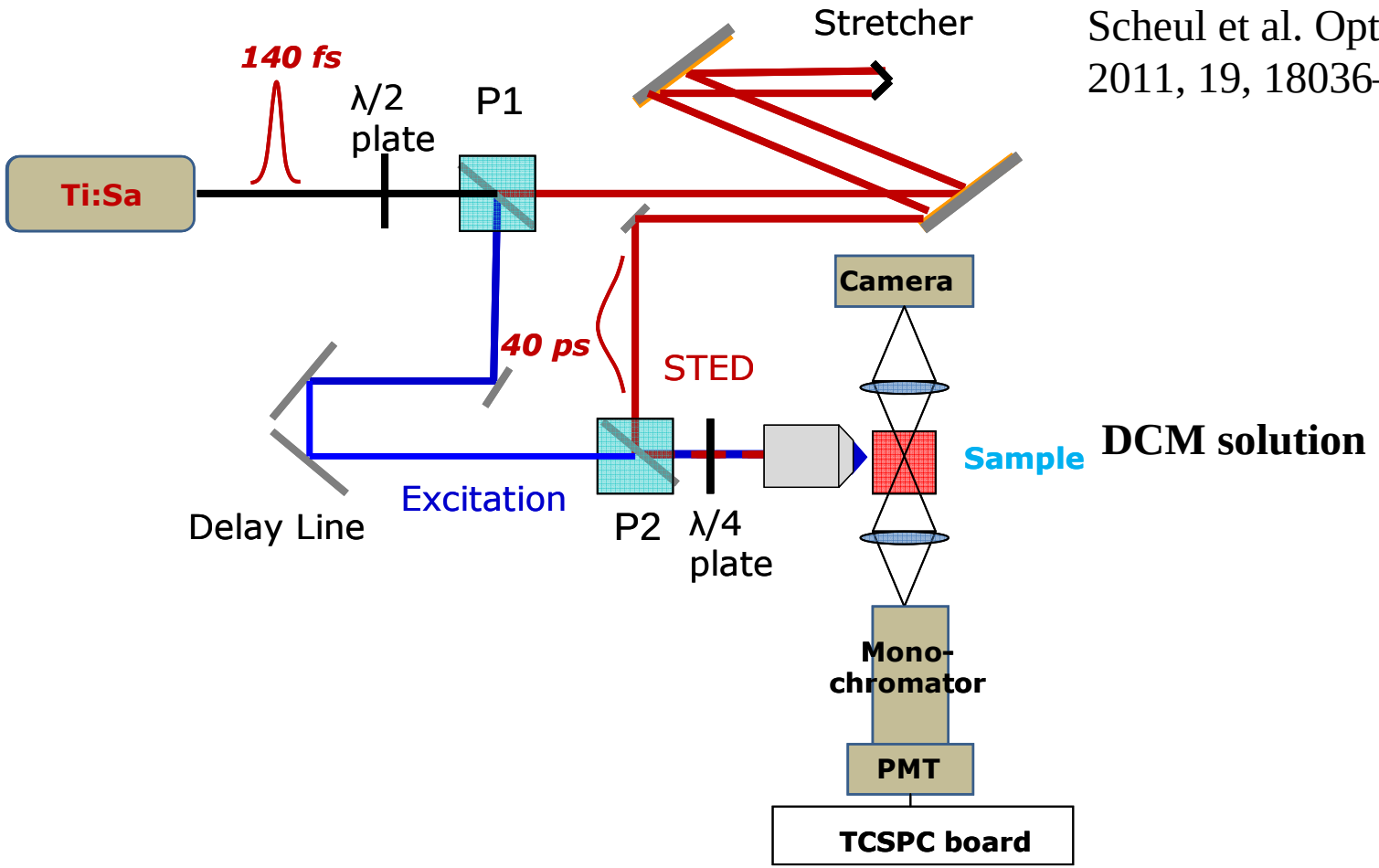


$2h\nu$ excitation ~ I^2 (fs pulse)

STED ~ I (ps pulse)

Two-photon absorption and emission spectra of DCM dye

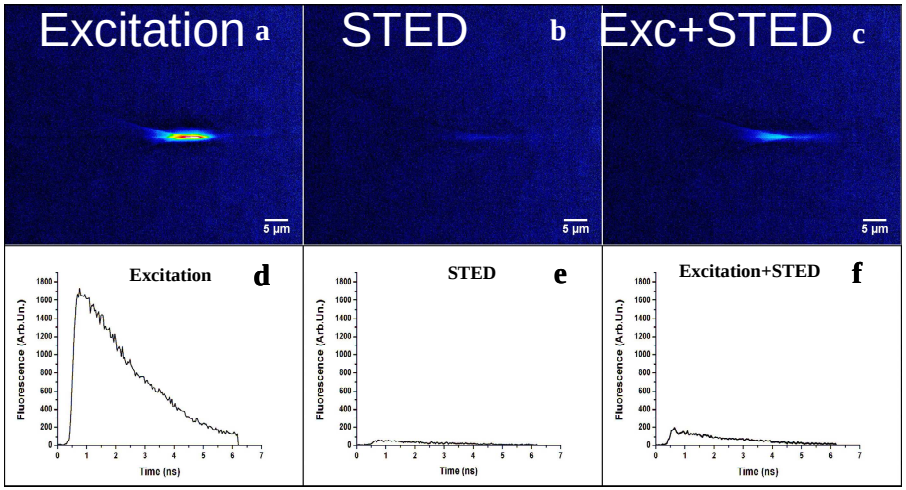
Experimental setup: proof of principle in solution



Scheul et al. Opt. Express, 2011, 19, 18036–18048

- Laser source: mode-locked Ti:Sapphire laser (Chameleon CoherentTM , Ultra II): pulse duration 140 fs, repetition rate of 80 MHz.
- The duration of the stretched pulse: ~40 ps

Experimental results



Scheul et al. Opt. Express, 2011, 19, 18036–18048

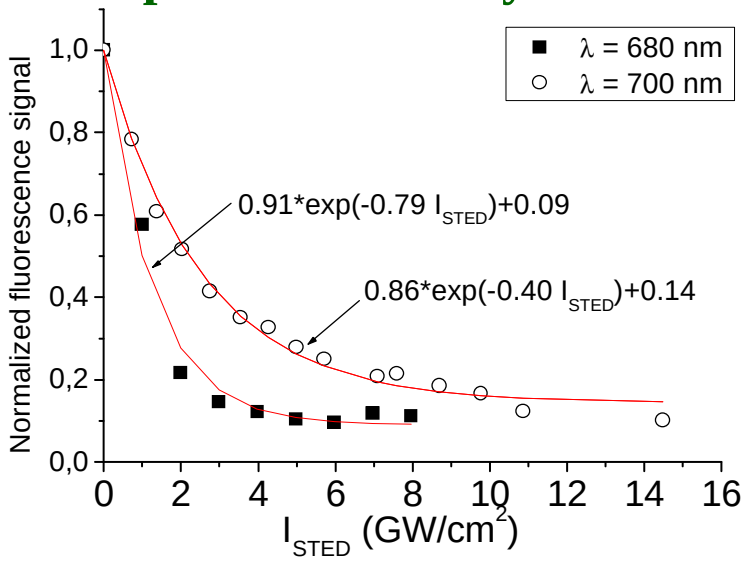
CCD Images

Fluorescent decays

*Average powers: 25 mW for excitation beam, 80 mW for STED beam

➡ **The fluorescence is quenched when the two beams overlap**

Fluorescence depletion efficiency increases with STED intensity

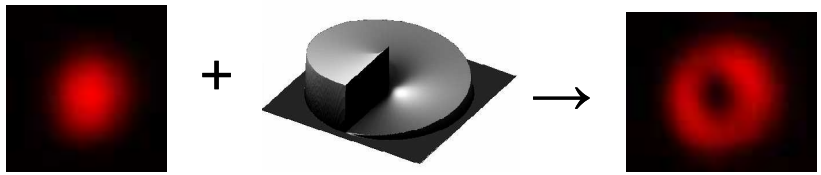


Conclusions of Part 1

- We have demonstrated the possibility to quench two-photon excited fluorescence by stimulated emission with a single wavelength
- Standard two-photon microscope into STED microscope

Outlook

- We are building Single wavelength STED microscope by implementing the vortex phase plate

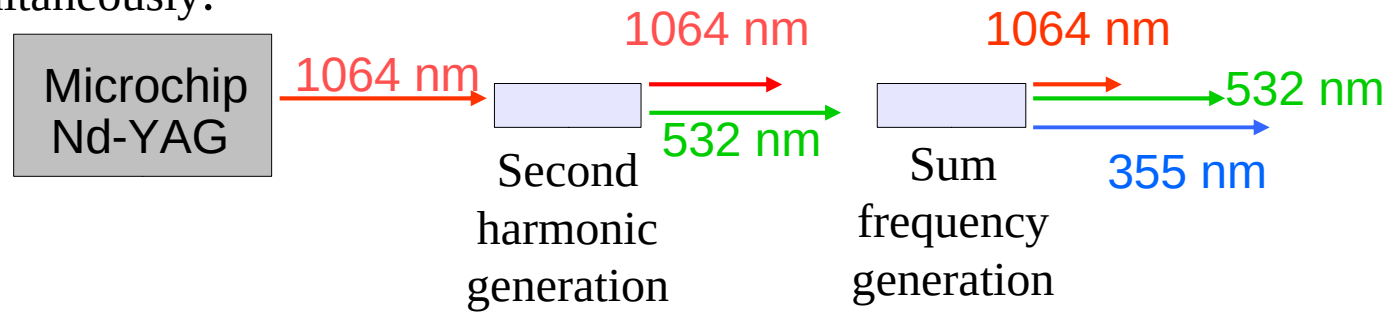


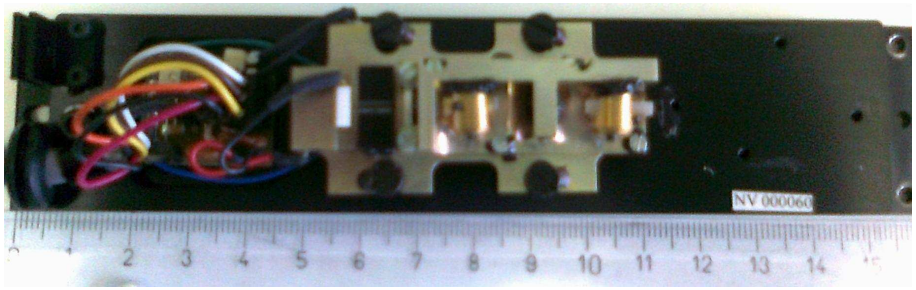
Gaussian beam **Vortex phase plate** (RPC photonics) **Doughnut shaped beam**

- Test different dyes: Pyridines and Styryl dye families, for live cell imaging - large Stokes-shift red fluorescent proteins

II. STED with a two color microchip laser

- **Goal**: STED microscopy with a single laser source that delivers two wavelengths simultaneously.





- **Q-switched Nd-YAG laser**
- **Repetition rate of 8 kHz to 150 kHz).**
- **Pulse duration ~1 ns**

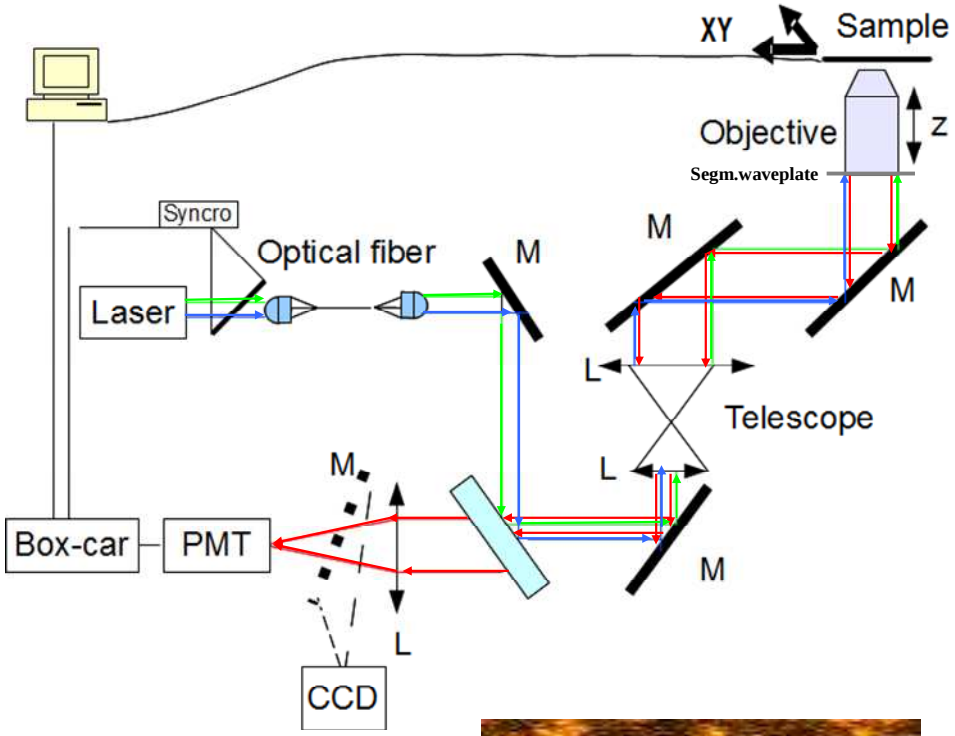
- **Advantages:**

- Two beams are intrinsically aligned and synchronized.
- Low cost

- **Disadvantages:**

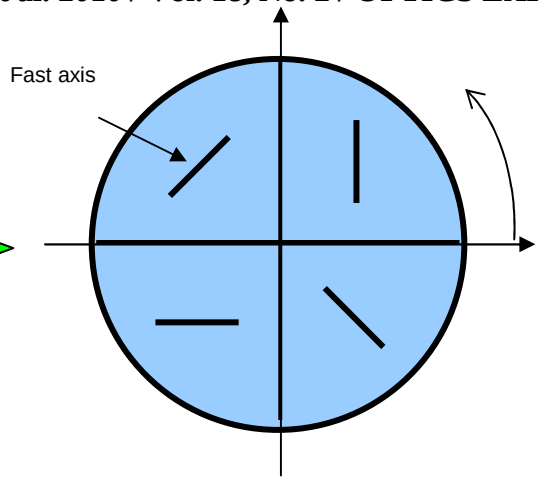
- Restricted dye choice (large Stokes shift and UV absorbing dyes)
- The need of achromatic optics for the two wavelengths simultaneously

Experimental setup: confocal towards STED microscope



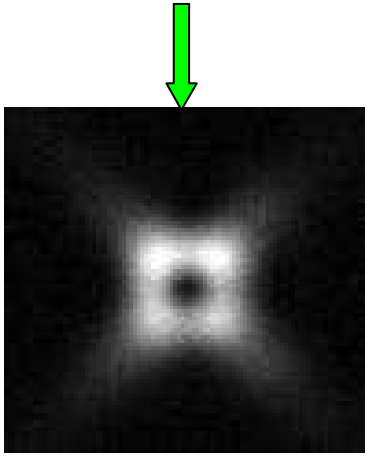
STED

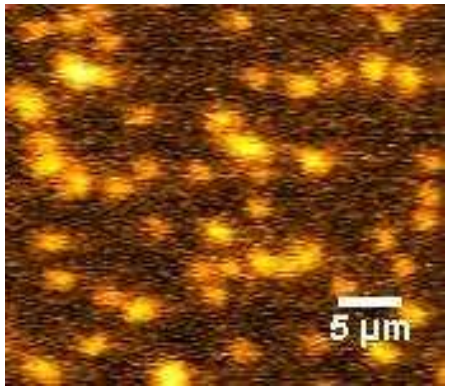
Reuss et al. 2010 / Vol. 18, No. 2 / OPTICS EXPRESS.



Segmented waveplate:

λ/2 for 532 nm λ at 355 nm

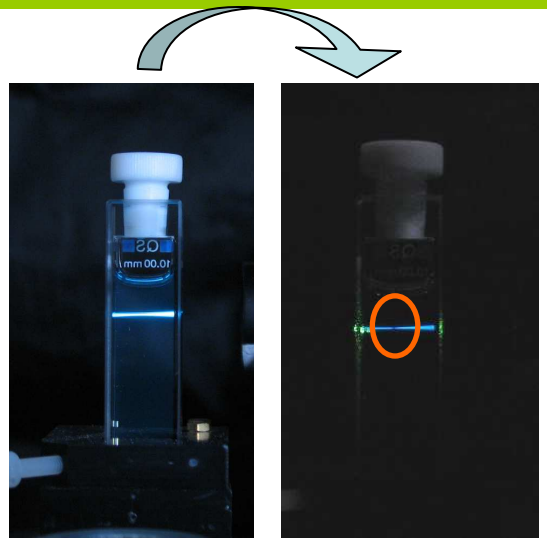




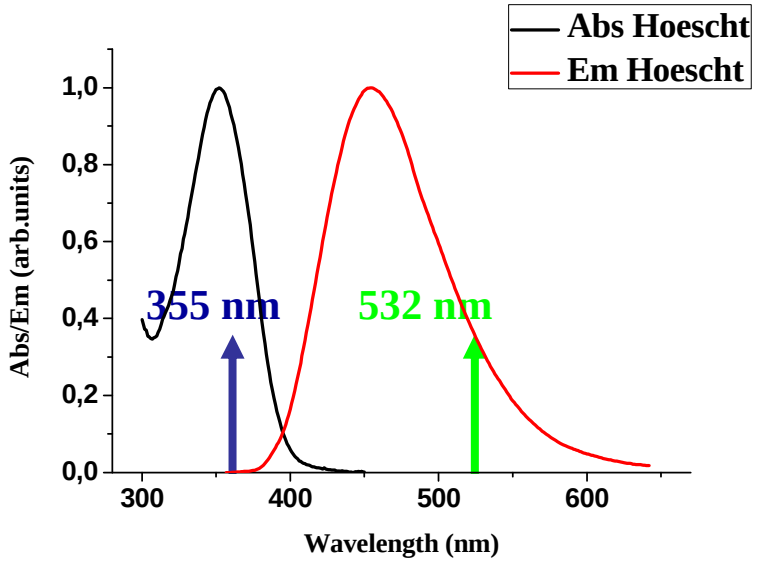
Confocal image of fluorescent beads

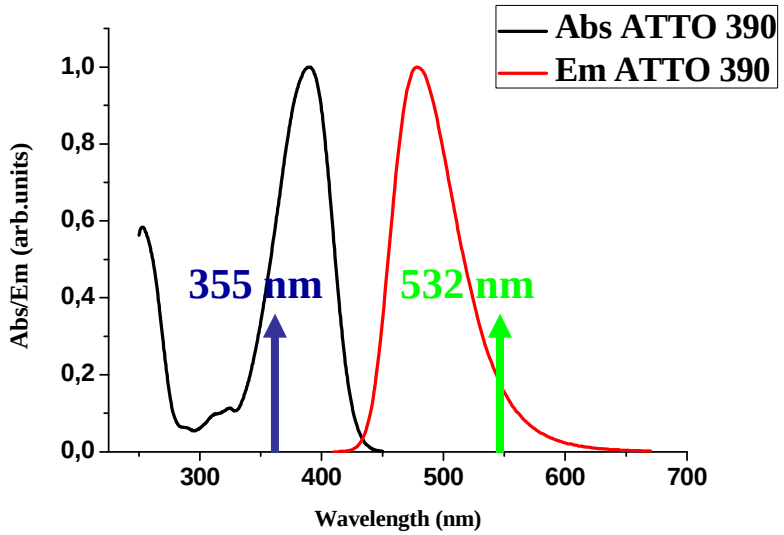
Experimental – results

Exc (355 nm)



Exc (355 nm)+
STED (532 nm)





Conclusions of Part 2

- We have build a confocal microscope with a bicolor laser source that we believe it can be turned in a STED microscope by adding a chromatic birefringent device for the beam shaping
- Several dyes proved to be adequate to excitation at 355 nm and stimulated emission at 532 nm

Outlook

- Future work will be done on fluorescent beads and fixed stained cells.

Acknowledgements

Irène Wang

Jean-Claude Vial

Ciro d'Amico

nanoSCIENCES FONDATION

LIPhy - MOTIV

Thank you!