Confocal laser-scan-microscopy
# Nanoporous guest / host systems

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Phys. Chemie, Ak Bräuchle

1. Microscopes for flurescence
    Conventional
    TIR
    Confocal
    Use at low temperature
2. Porous sample systems
3. 3 dimensional images
4 Polaristion
5. Fluorescence spectra
6. Fluorescence lifetime
7. Sensitivity
8. Summary

Conventional

Leitz Monocular
Microscope
(circa 1930s)

Image

Optics

Object

$d \sim \lambda \ / \ N.A.$

Light

Total internal refexion (TIR)

Image

Optics

Evalescent field
1/e decrease
few nm thickness

Object

Prism

Laser

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Confocal microscopy

Point detector

Pinhole

Optics

Scan object
or spot

Object

Optics

Pinhole

Point light source
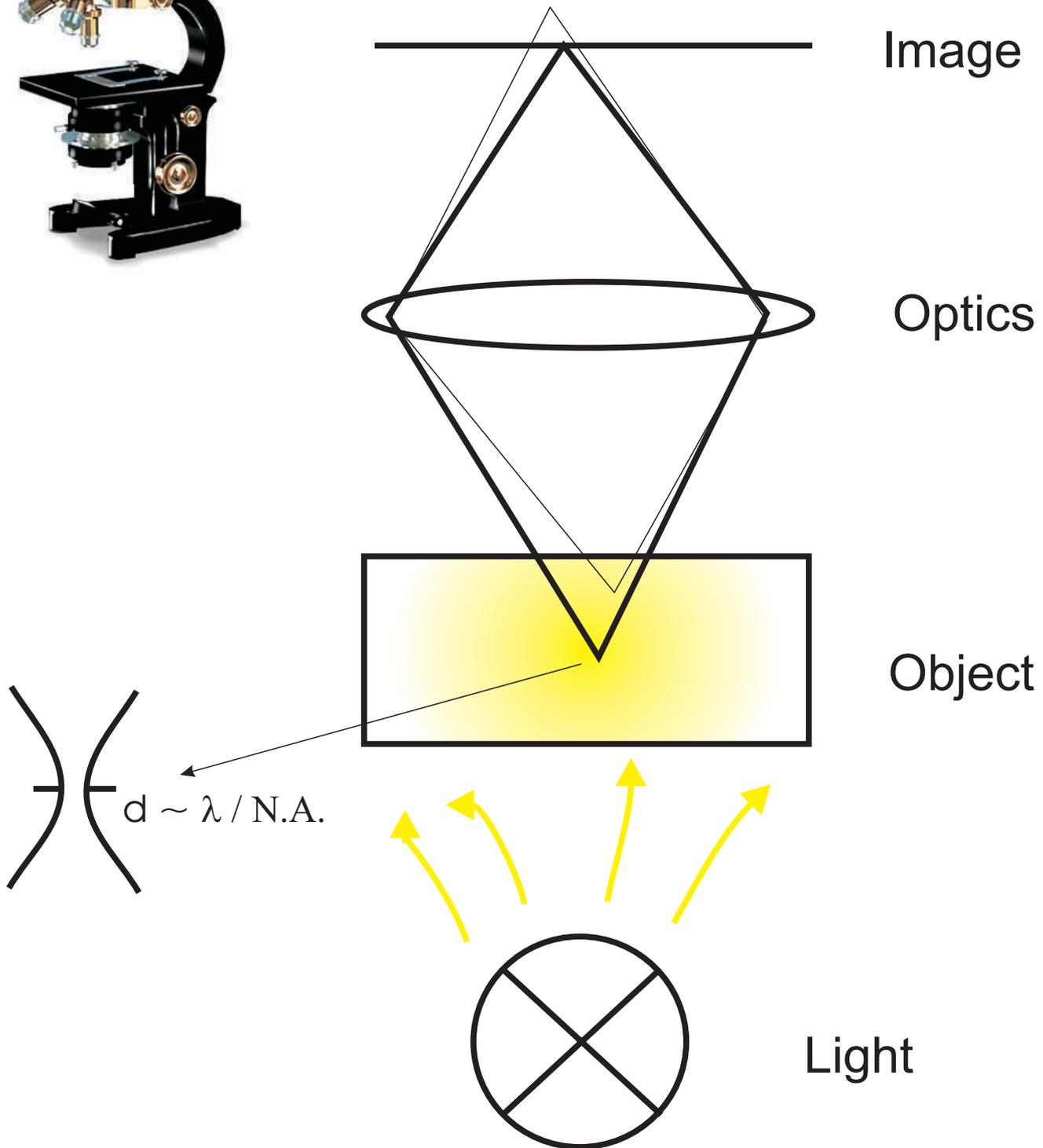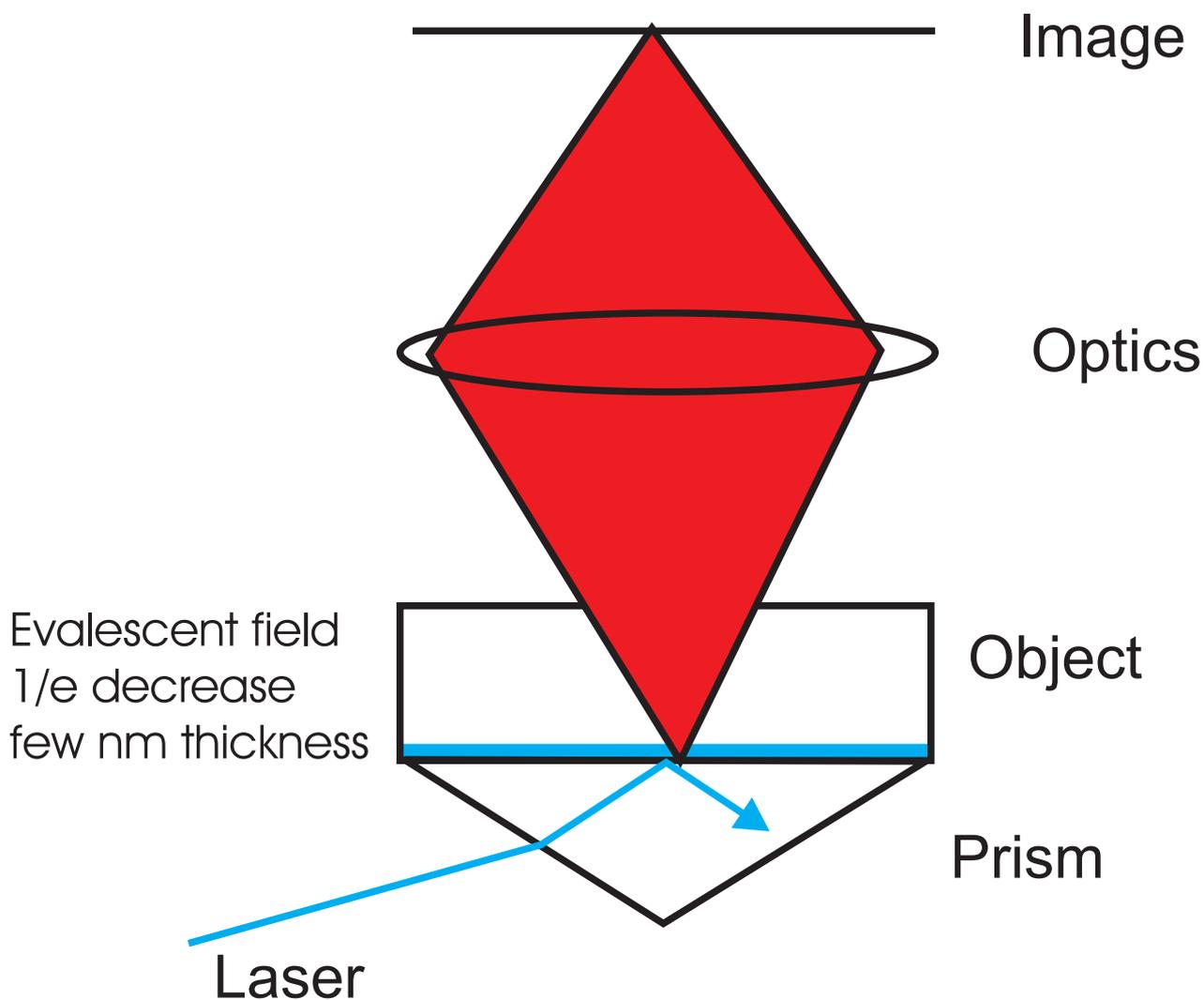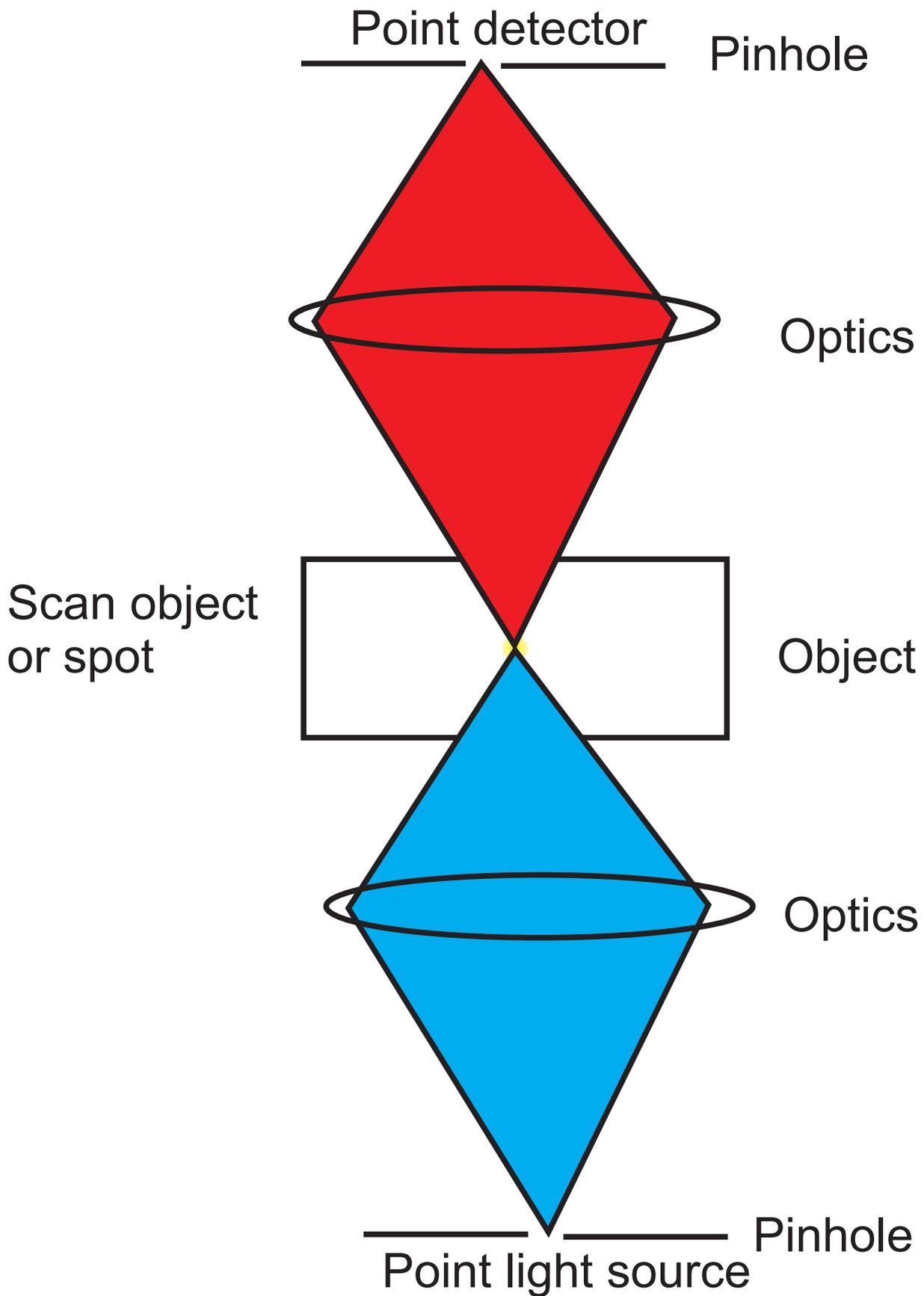
Confocal microscopy: Resolution

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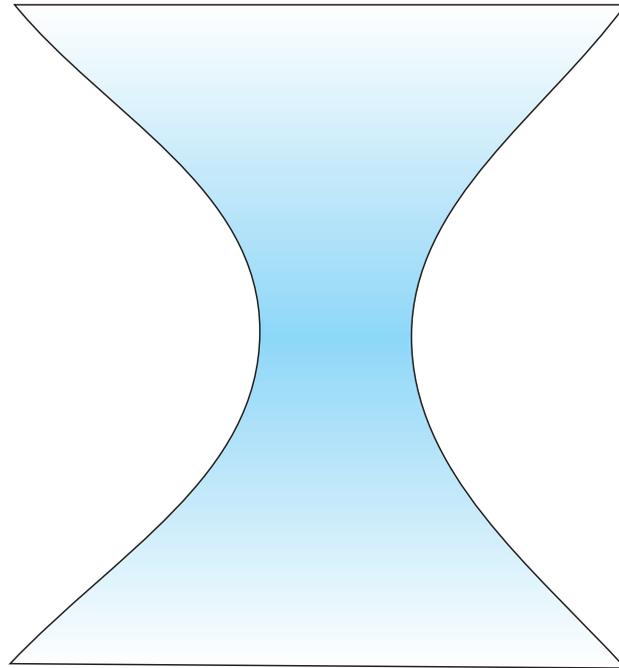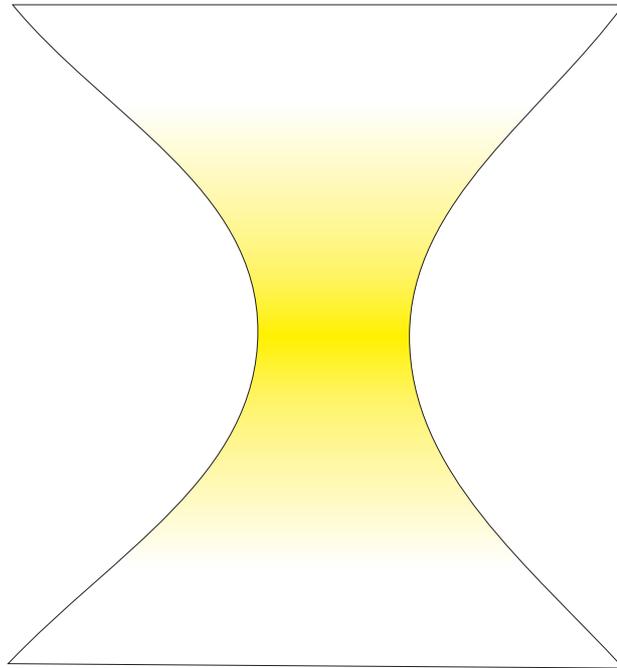
Theory

$xy = 0.4 \lambda / N.A.$

$z = \lambda / N.A.^2$

Example 633 nm, N.A. 1.3

200 nm

380 nm

Experiment

Resolution at 633 nm
(N.A. 1.3, single molekule)

xy scan   xz scan

0.3 µm xy Resolution

1.2 µm z Resolution

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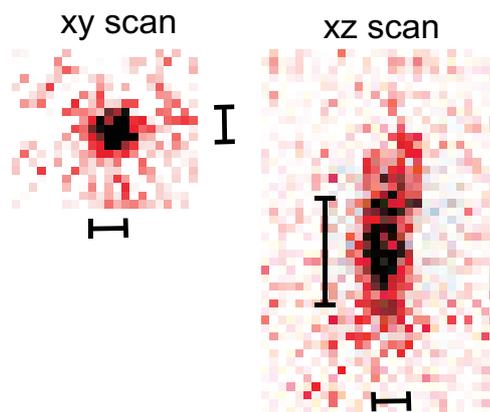
Konfokales Raumtemperatur Laser - Scan - Mikroskop

Okular

Filter

offen

Spiegel

Z - Scan

Probe

Polarisations-
filter Griff⊥E

0°

90°

Halogenlampe

Klappspiegel

Objektiv

externer Laser

He-Ne Laser

Detektor

Bis zu 10 Bilder / sec

XY-Scanner
Kippwinkel ca. 6°

FT 655

80/20

FT 455

30/70

offen

Spiegel

Anregung
+
Detektion

Detektion

1,5%

A ca 0,6°

f = 8 cm

Pinhole

Ausgang

Pinhole

Aufweitungslinse

Langpass

interner
Photomultiplier

Detektor

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Low- temperature

Images

Fluorescence
Excitation Spectra

Fluorescence [1/s]

Laser detuning [GHz]

Ar⁺-Laser

Control
Scan

DYE

WM SA

Fiber

ST

A

APD

Counter

Pinhole

CCD

Spectrograph

LPF

Dichroic
Beamsplitter

X-Mirror

Y-Mirror

Telecentric
System

Cryostat

Sample

z

Cool down to 1.3 K

No imersion oil possible
low N.A. or no Z reolution

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Wavelength selection

Lasers

Detectors

Optic difficult

CW Ion

CW Dye

Pulsed YAG

Pulsed Ti Sa

Dyes

Photomultiplier

APD, CCD

400nm

instable

30%

500nm

600nm

good

80%

700nm

NIR

800nm

big

Optical switch

XY-Site $\lambda_0 = 579.428$ nm

X$_1$-Site $\lambda_0 = 580.375$ nm

$h\nu_i$

$h\nu_i{'}$

position [microns]

laser detuning [MHz]
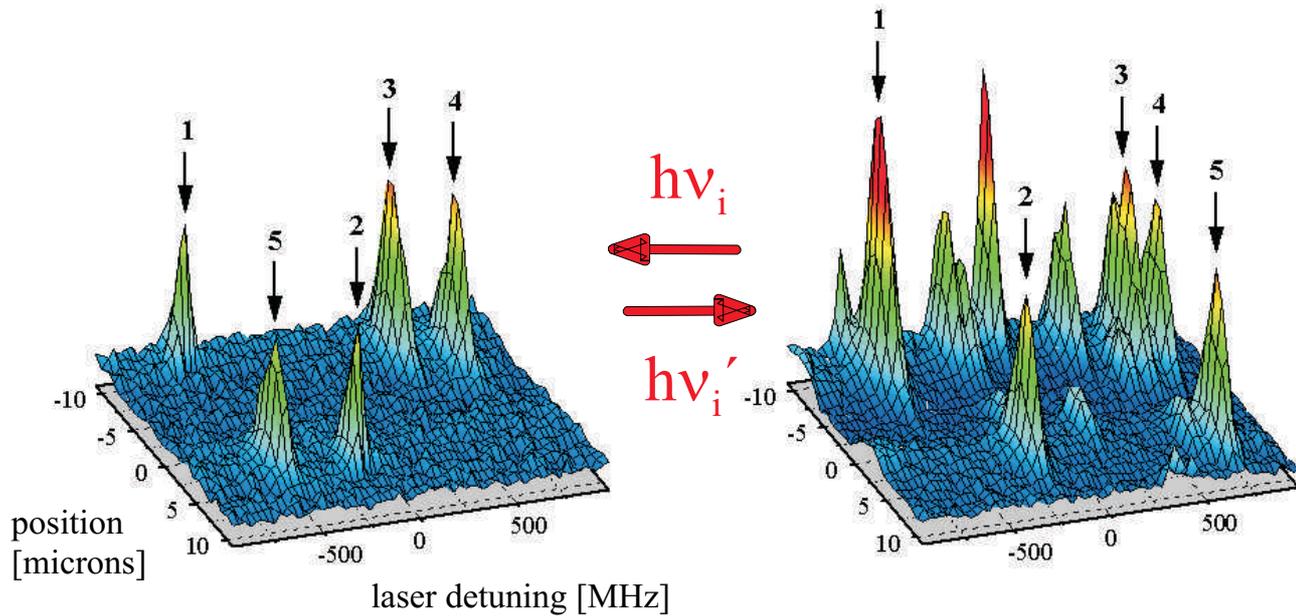
Spectral single molecule jumps (Terrylene in p-Terphenyl)

$h\nu_i$

$h\nu_i{'}$
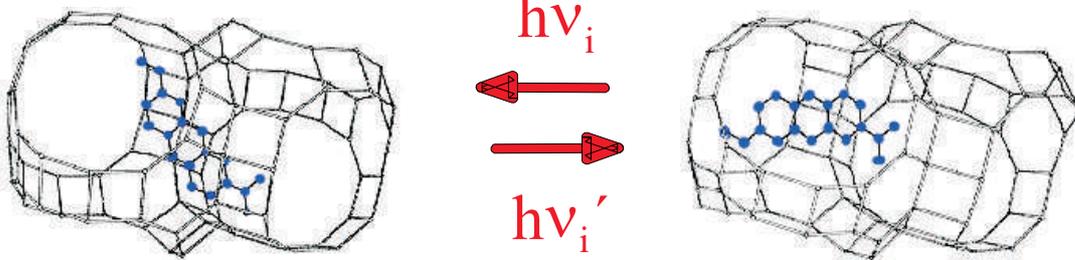
Optical switch: Thionine in Faujasit

Sample systems

AlPO$_4$ 5
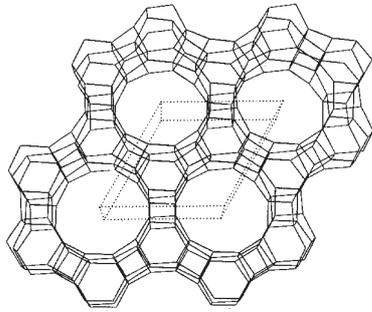
25 μm

MCM 41

20 - 80 Å

TEM - Image

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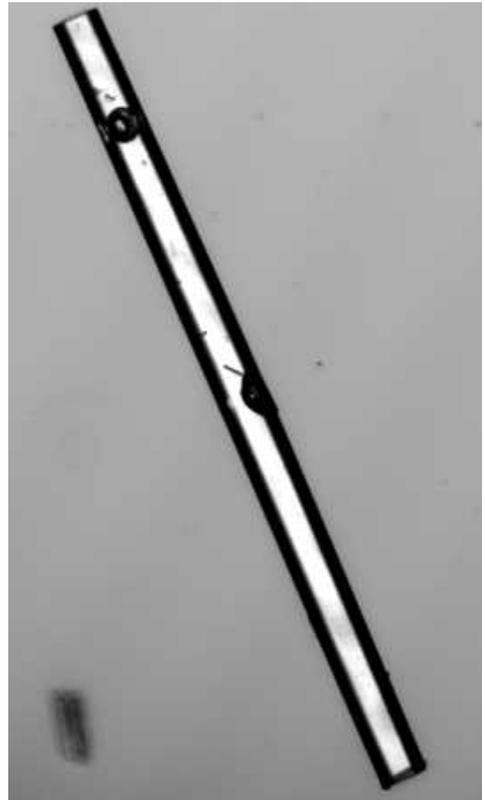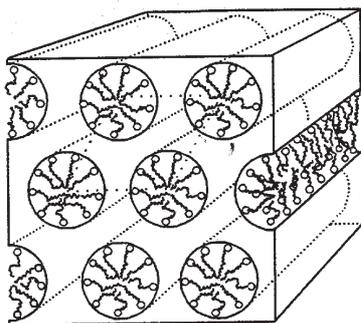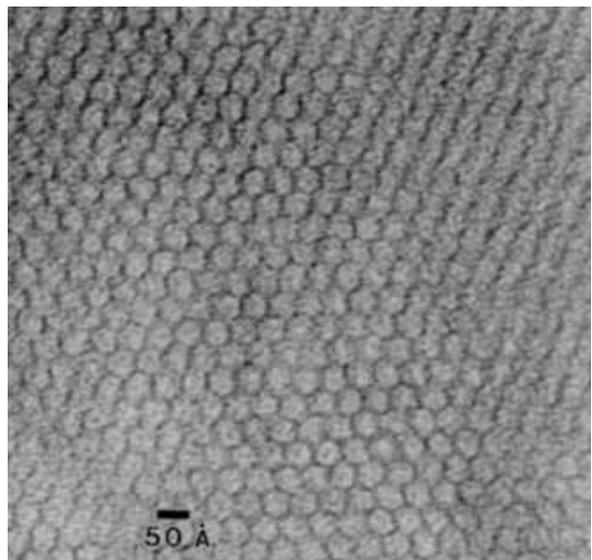
Chemical design of host / guest

Host:

Size

DDR 4.4 3.6

MFI 5.3 5.6

UTD-1 10.0 7.5
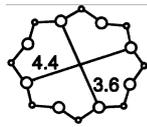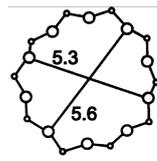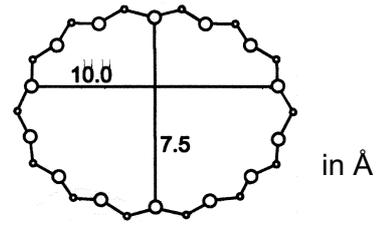
in Å

Topology
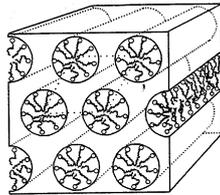
MCM 41

MCM 48

MCM 50

Polarity

OH
O-
OH
O-

CH₃
CH₃
CH₃
CH₃

Guest:

Size

1.10 nm Perylen
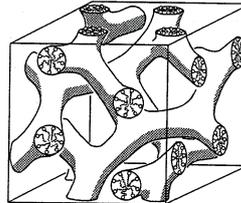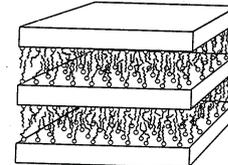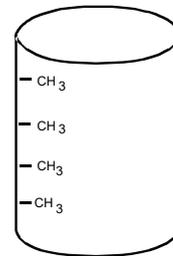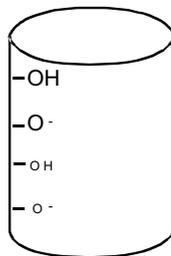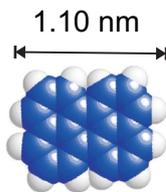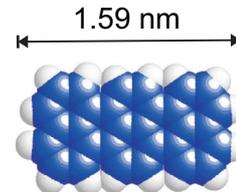
1.59 nm Terrylen

**Shape**
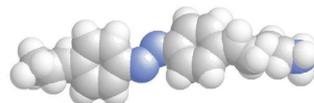
trans-Azofarbstoff
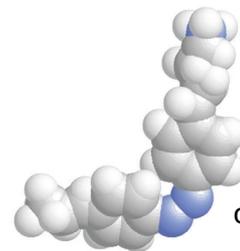
cis-Azofarbstoff

Polarity
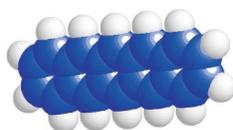
Pentacen
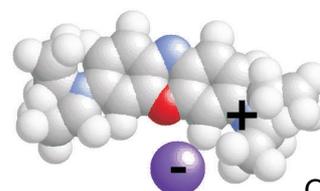
Oxazin1 + −

# 3 dimensional scans

Calculate the slices into a rotation
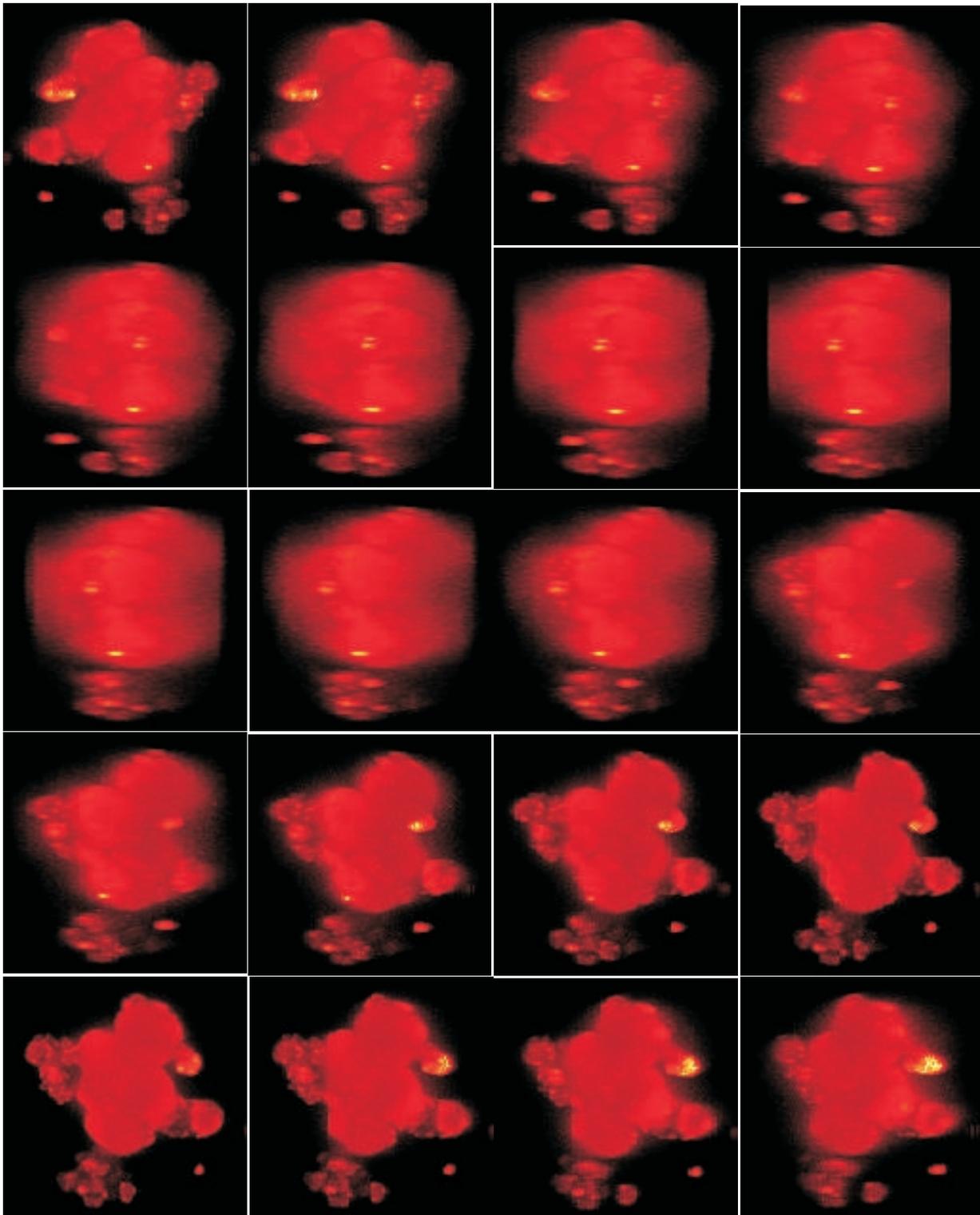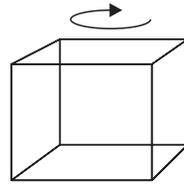

MCM 41 Cluster

25 µm

Polarisation dependent pictures

Transmission

5µm
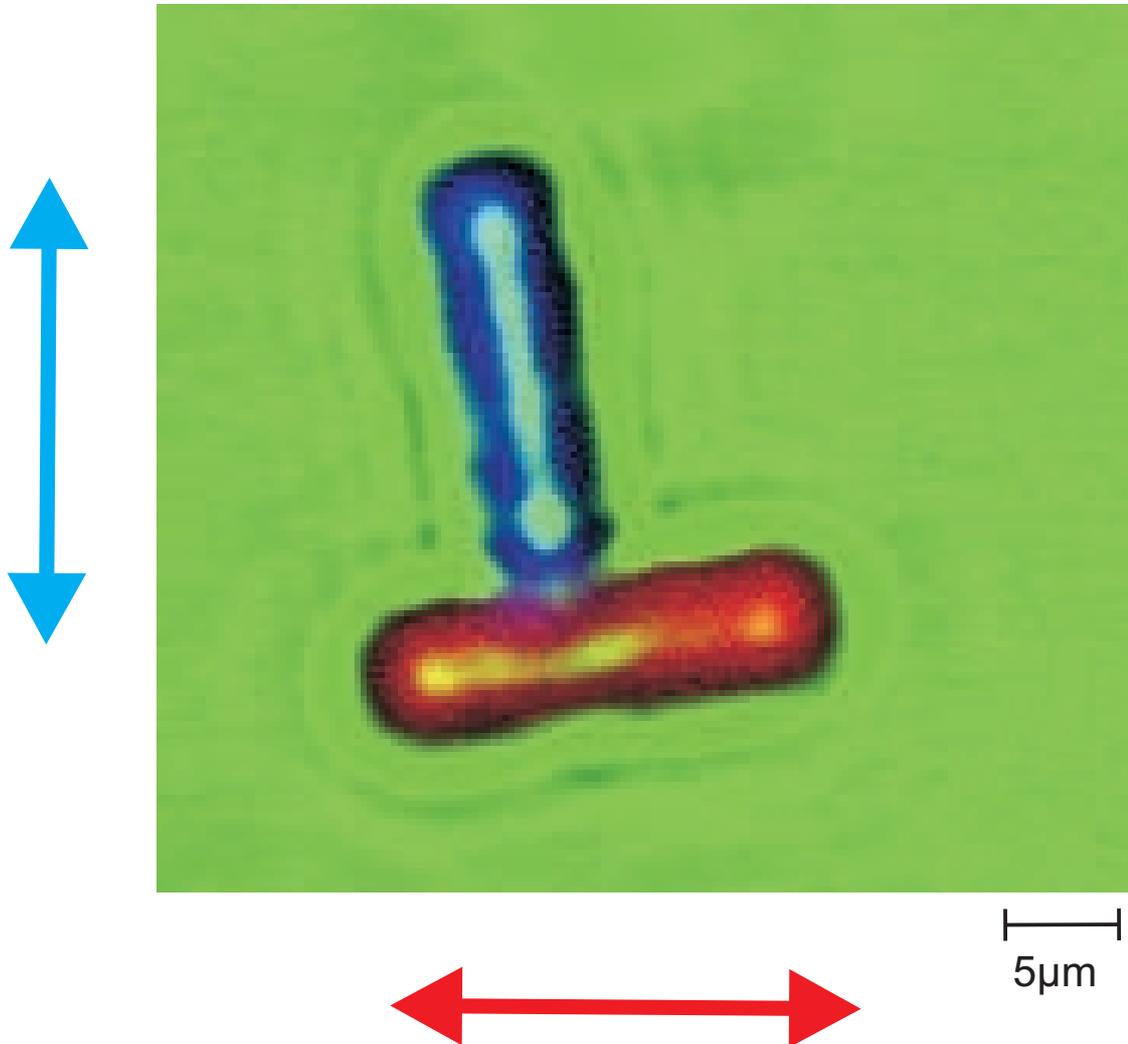
AlPO$_4$ 5
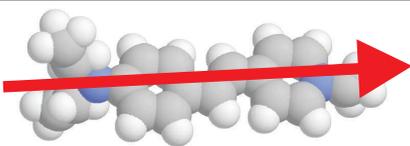
Transition dipole / molecule parallel to pore

Fluorescence spectra

From microscope

Dispersing element

CCD-line detector

Azo (50) in AlPO$_4$ 5
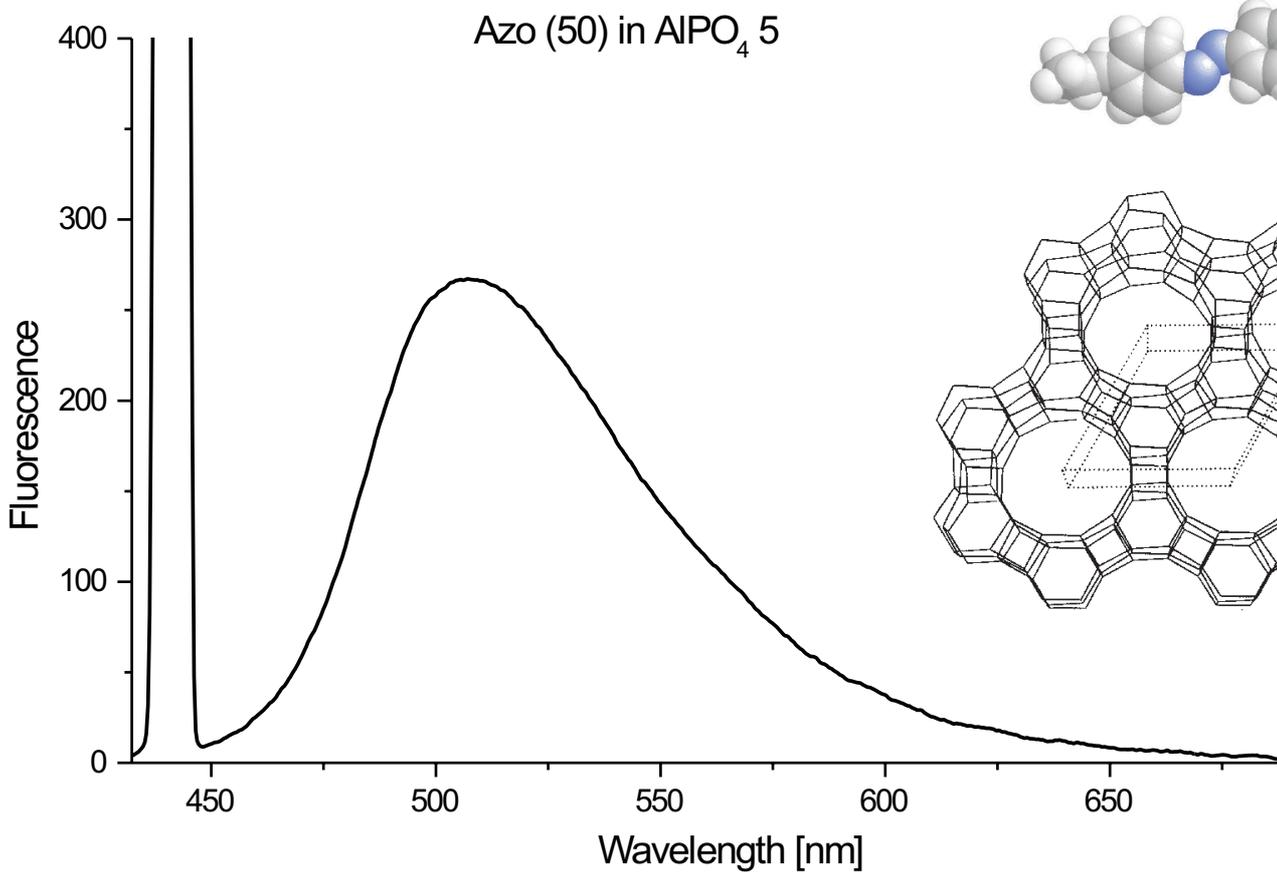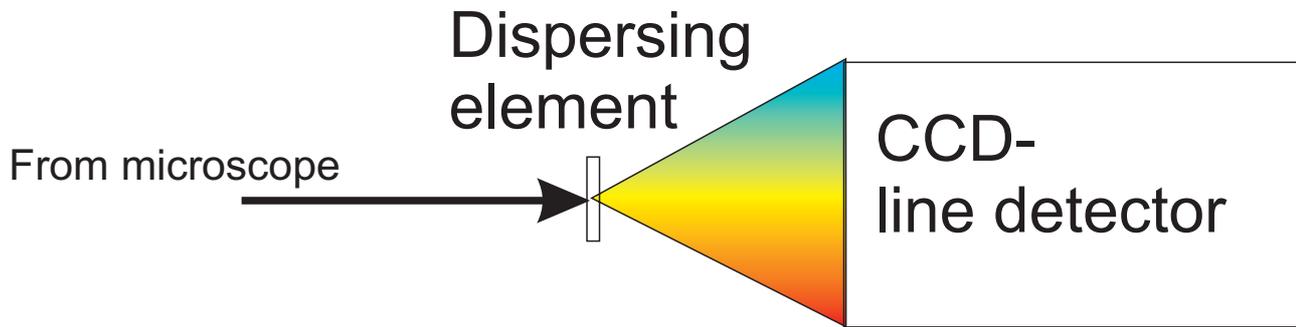
needs >1000 Photons

Fluorescence lifetime

Time correlated single photon counting

needs >1000 Photons
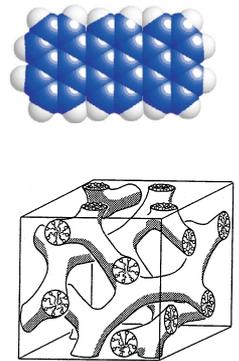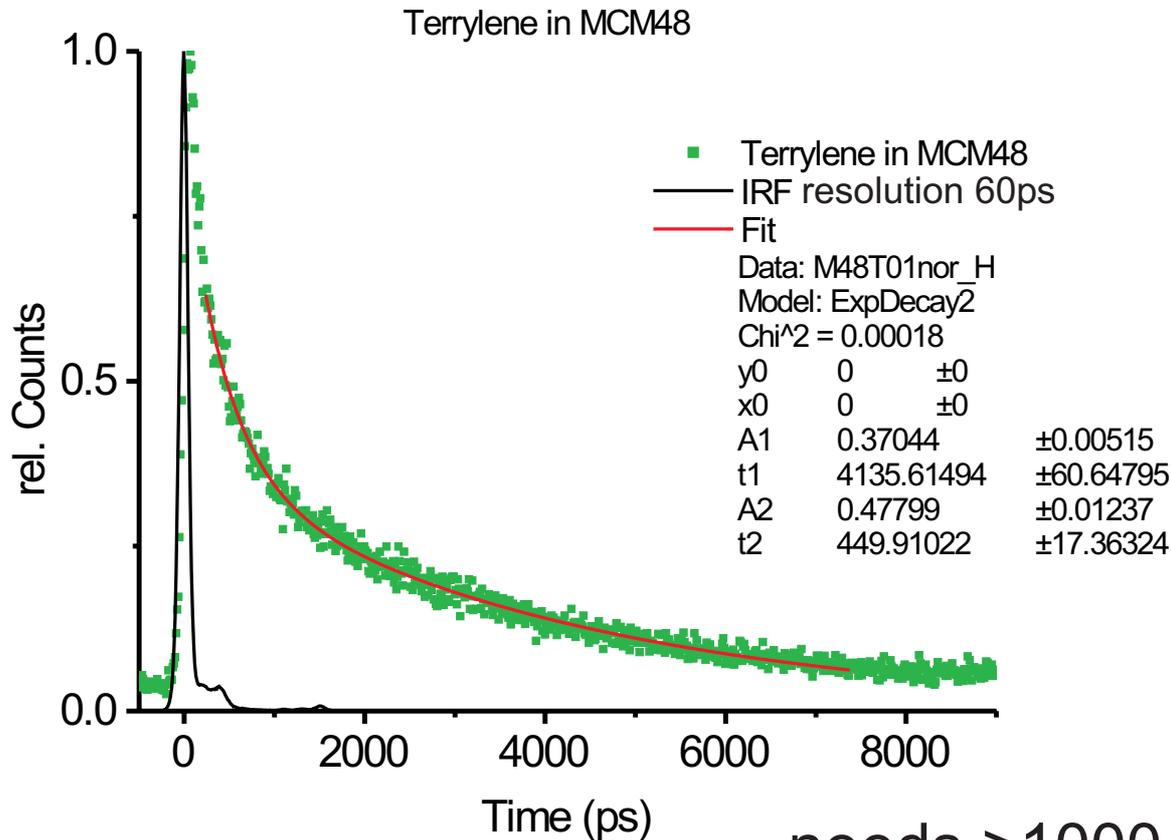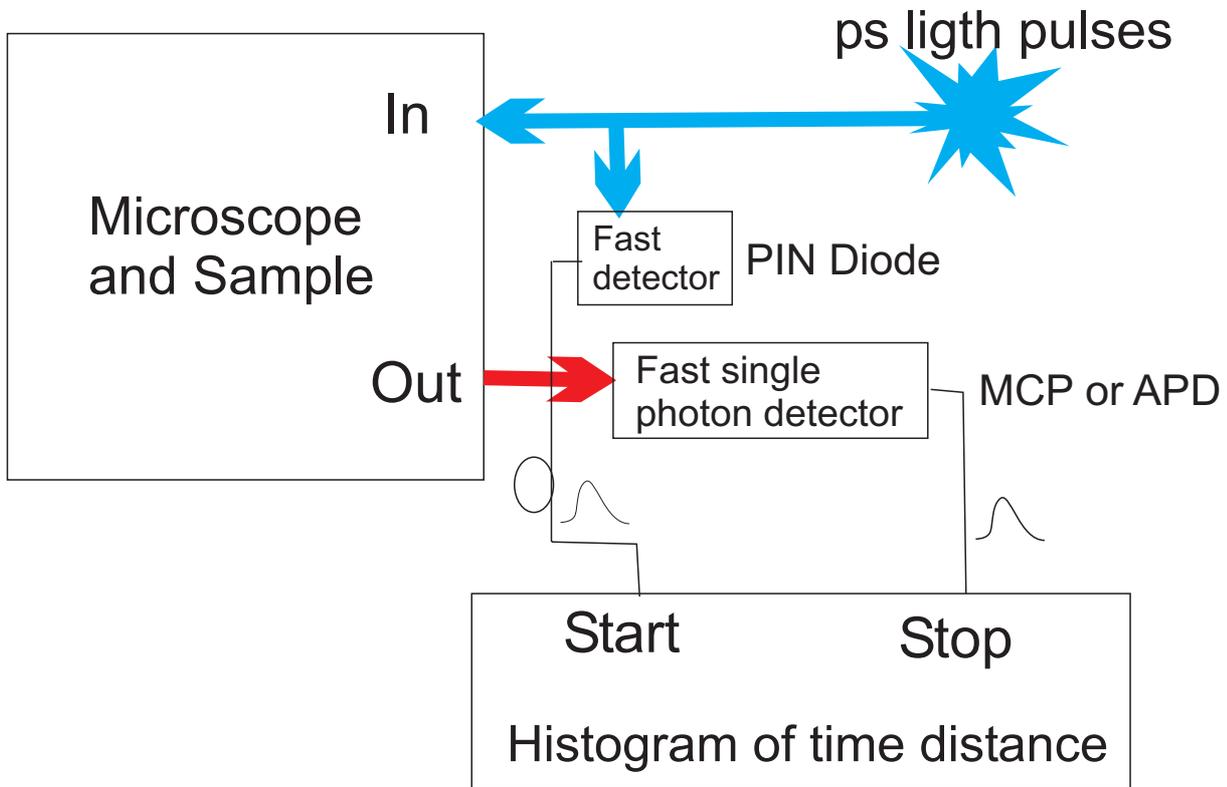
Sensitivity for single molecules

TDI in PMMA

high concentration

5 µm

low concentration

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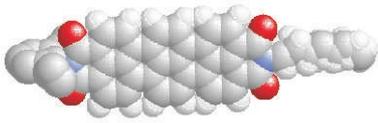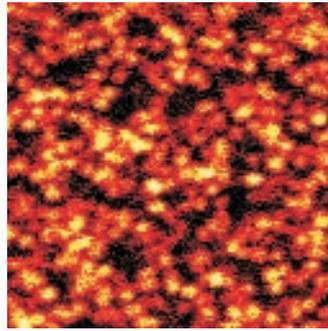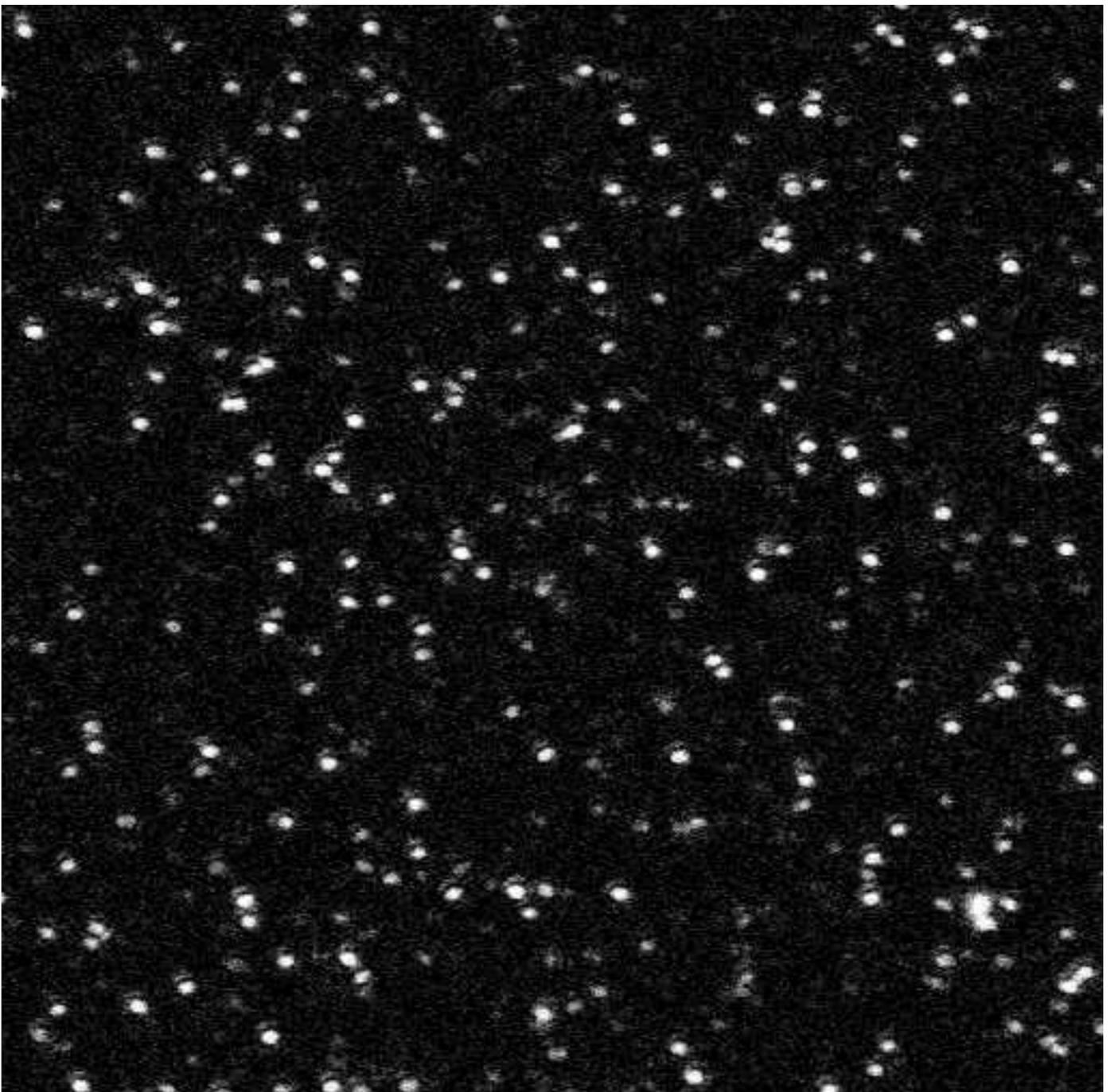
Summary confocal microscopy
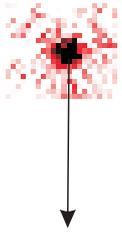
3 dimensinal spatially resolved
(0.3 µm x 0,3 µm x 1µm) microscopy

Combination with spectroscopy
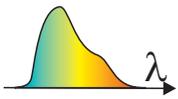
Spectral informations
   Sensors: Polarity, pH..., heterogeneity
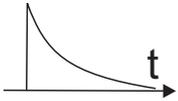
Dynamical information
   Photo induced reactions, heterogeneity
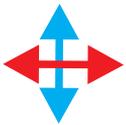
Structural information
   Molecular arrangement
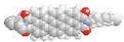
Needs:
stable fluorophor (visible or near IR)
no opague samples

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