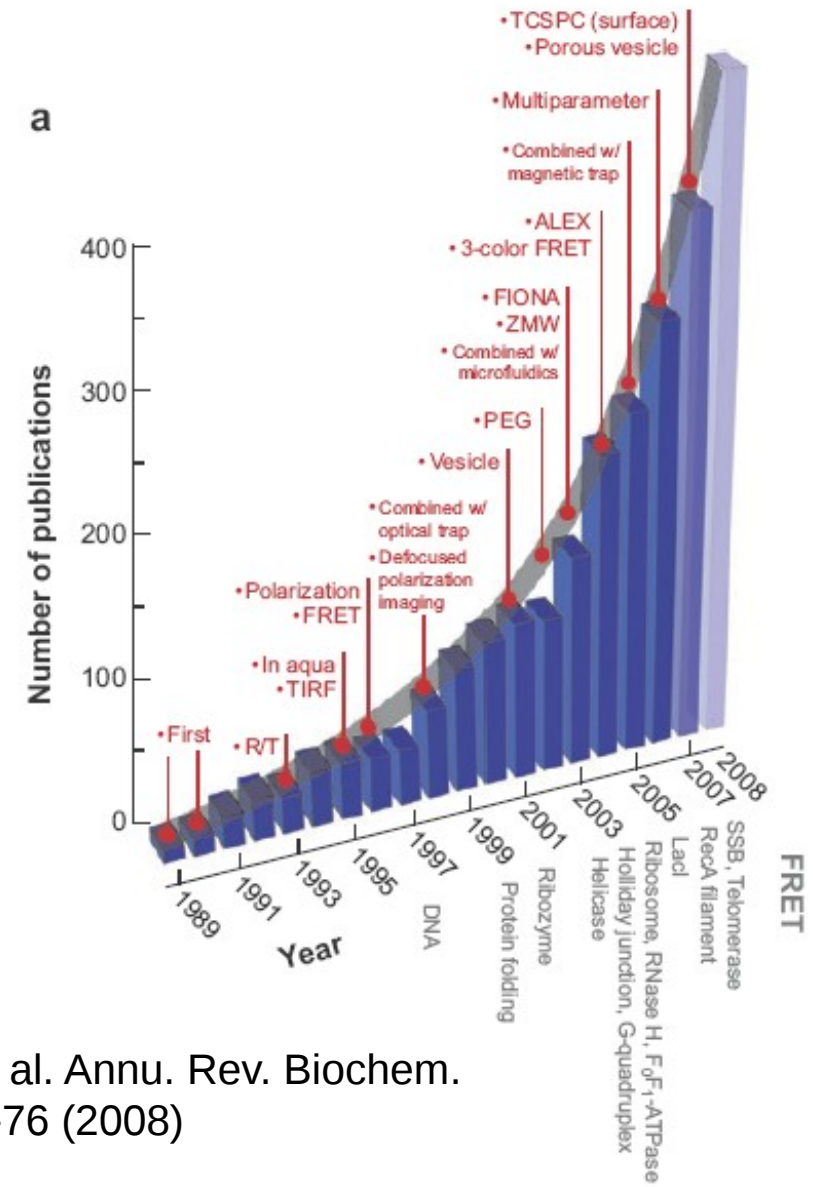
UNIVERSITY OF TWENTE.

Single-molecule fluorescence spectroscopy

Martijn Stopel – Nano biophysics group

Papers single molecule fluorescence

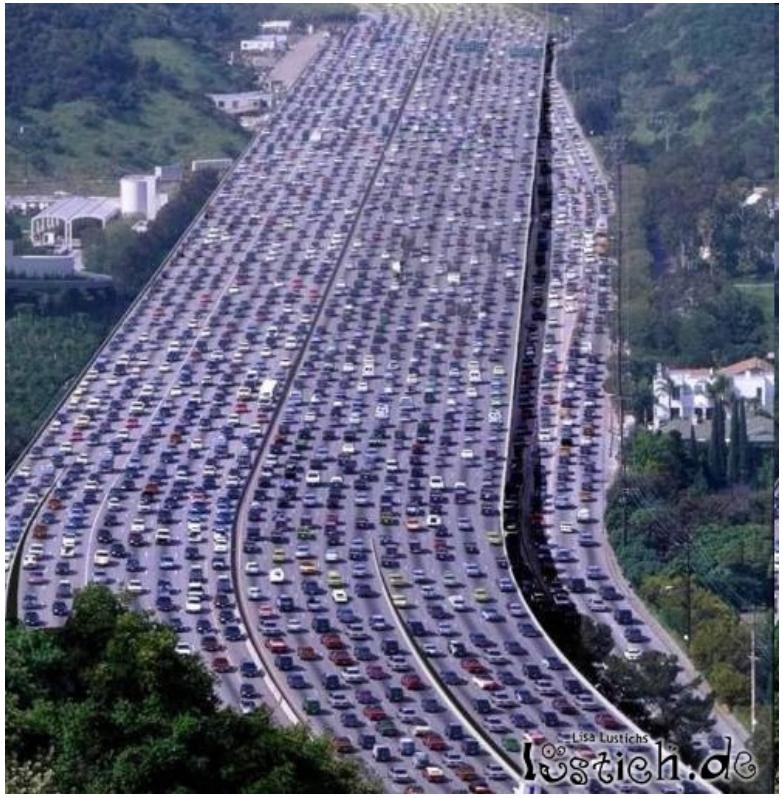


Ever increasing number of publications, especially in the life sciences

Joo et al. Annu. Rev. Biochem. 77:51-76 (2008)

Why study single objects?

Ensemble



Single molecule

The ensemble obscures the single object

What is it exactly? Where is it? What is it doing?

Properties of ensemble are composed of properties of single objects

Why study single molecules?

- The ultimate analysis tool

  Determination of „what“ is „where“

- Models are typically of „single molecule character“

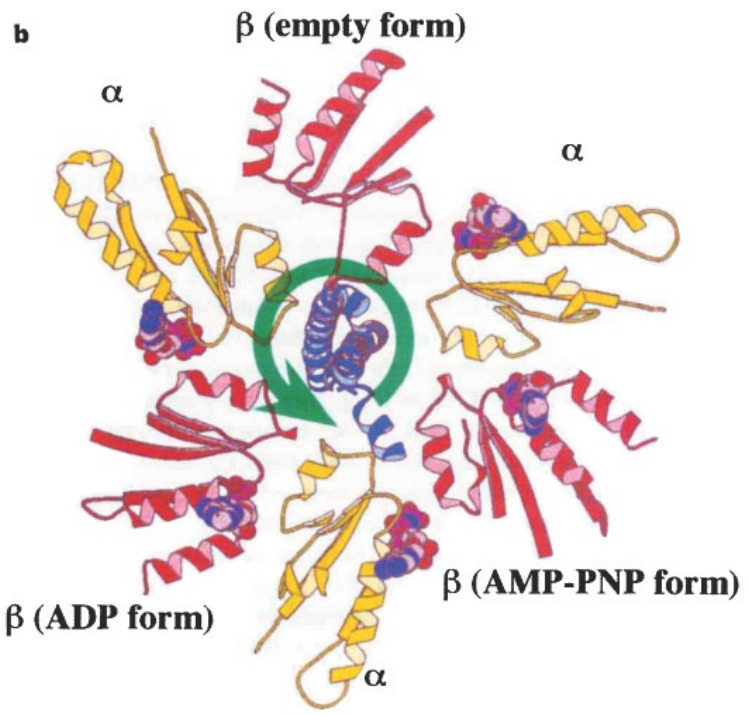
  A + B C

  but experimental detection is typically ensemble of molecules assuming ensemble of identical molecules

Why study single molecules?

- Removes ensemble averaging
  - Makes properties accessible that are hidden in bulk
  - Reveals information about distribution of parameters

Rotation of F1-ATPase



Noji et al. Nature, 386, 299 (1997)

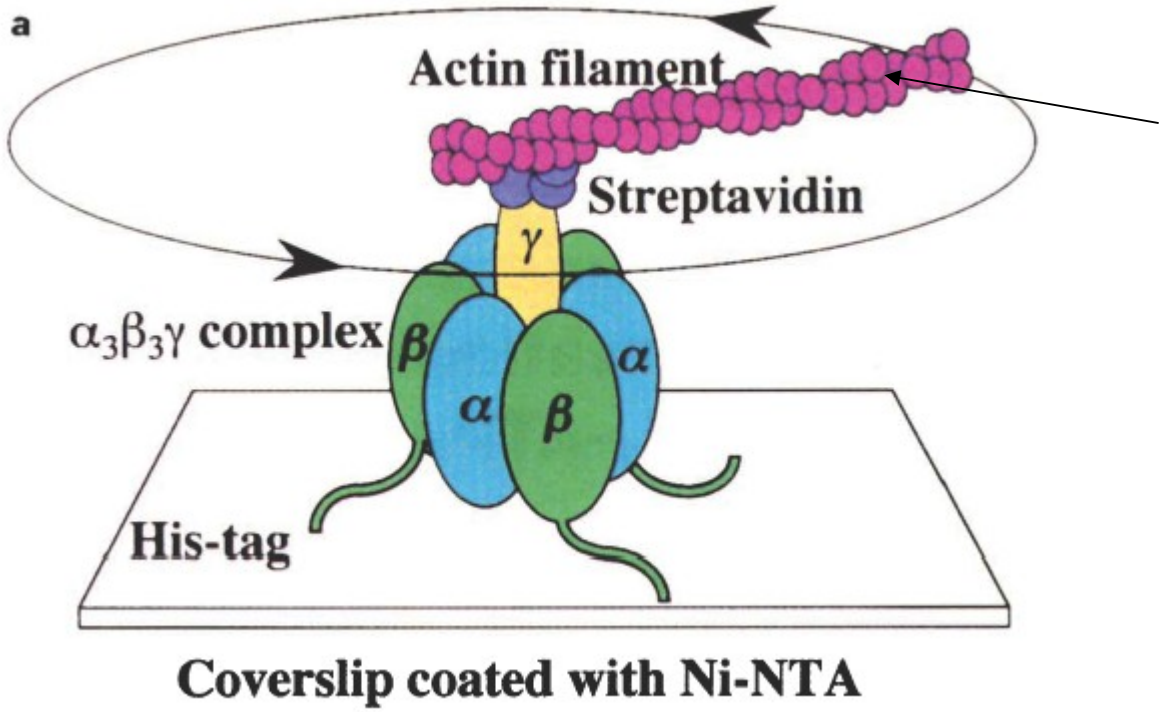
How does the rotary motor work?

- Continuous?
- Stepwise?
- Clockwise or anticlockwise?

Looking at the ensemble does not help

Why study single molecules?

The single molecule experiment:

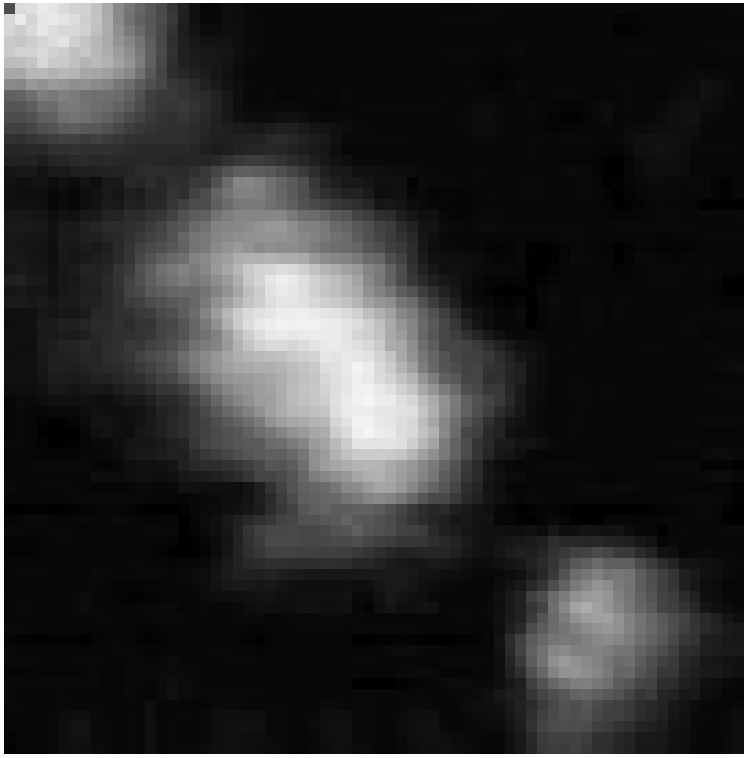


Labeling of the filament with fluorescent dye

Fluorescence Microscopy

Noji et al. Nature, 386, 299 (1997)

Why study single molecules?



- Anticlockwise rotation
- Steps of 120º

Noji et al. Nature, 386, 299 (1997)

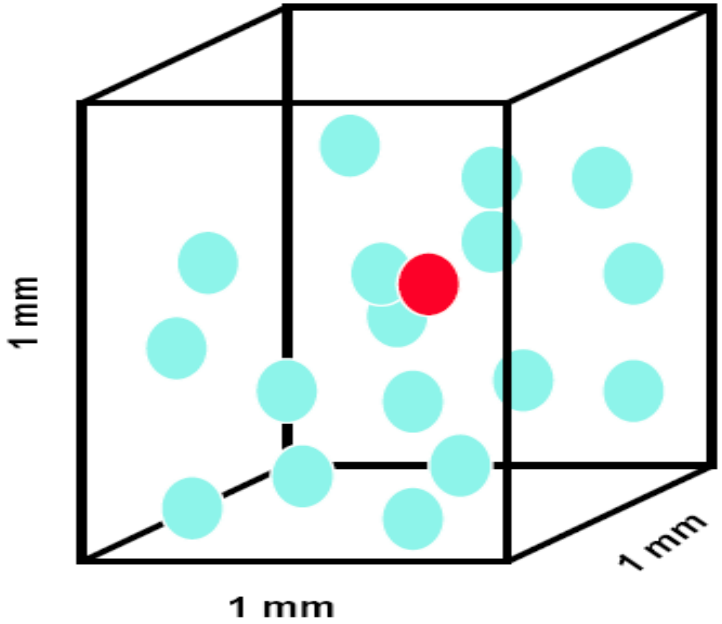
Why study single molecules?

- Single molecule detection is a scientists dream...

... but there is a number of difficulties

Problems

- A single molecule is small
- There are MANY single molecules
- The signal from a single molecule is weak
- Single molecules have neighbors



e.g. 1 target molecule in 1 l aqueous solution

1 target molecule
~ 3.3 x 1019 solvent molecules

Background/contrast problem!

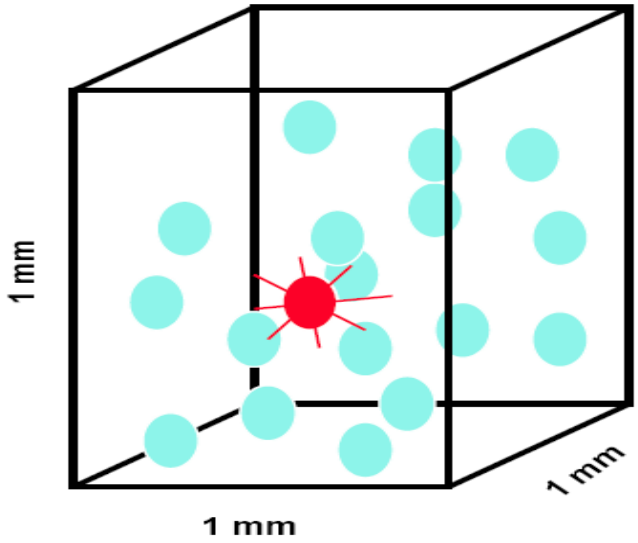
Possible solution

A method is needed that allows for

1. Looking only at molecules one is interested in
2. Sufficient signal from a single molecule
3. Small detection volumes

In Biophysics:

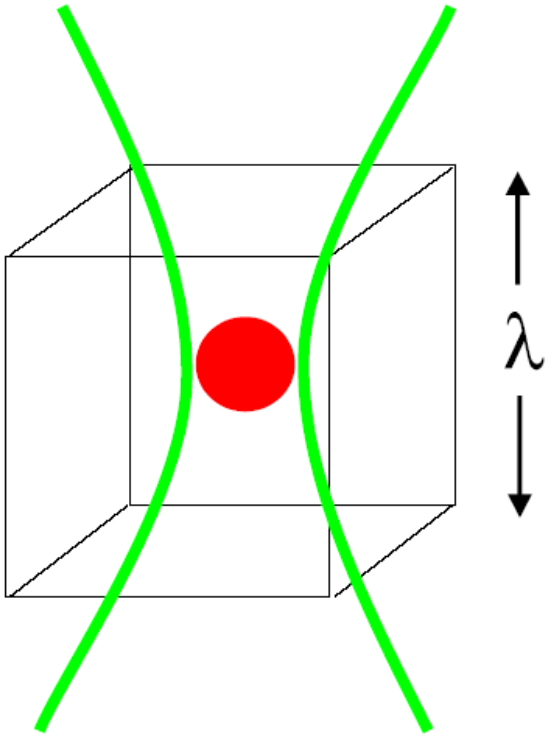
e.g. Single molecule fluorescence spectroscopy



Small detection volumes
AND
Low target molecule concentrations

Single molecule fluorescence spectroscopy

Detection volume limited by diffraction limit (Abbe limit)



$$D \approx \frac{\lambda}{2 \cdot NA}$$

Abb. 1: *Abbes Formel zur maximalen Auflösung eines Lichtmikroskops an einem von der Universität Jena errichteten Denkmal (vor dem Physiologischen Institut, Am Fürstengraben)*

Minimal volume:

~□3 = 0.1 fl

~ 109 solvent molecules

Single molecule fluorescence microscopy

- Small sampling volume

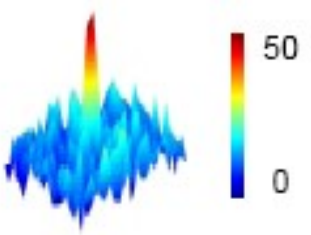
- Unfortunately most single molecules are not fluorescent

- Easy separation of single molecule signal from excitation light

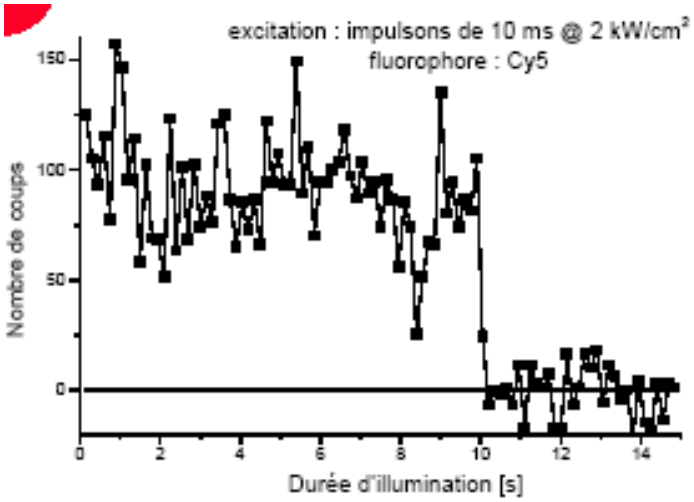
- *How does a typical single molecule behave?*

Signature of a single fluorophore

Low signal
(typically 20 counts/ms)

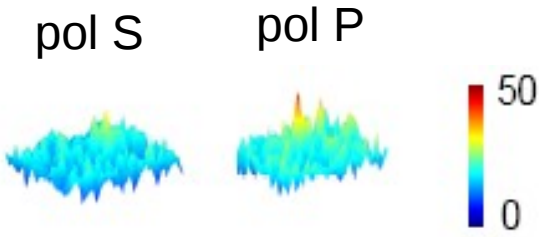


One step photobleaching

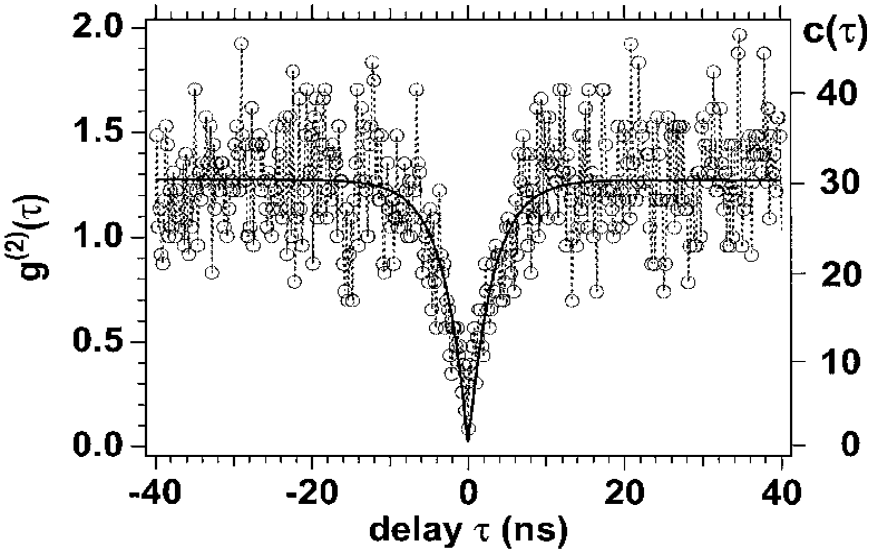


Signature of a single fluorophore

Fixed transition moment



Photon antibunching



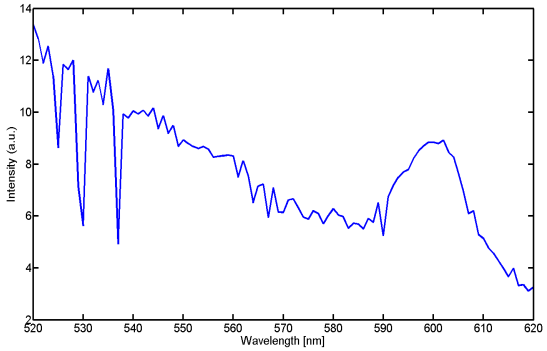
Treussart et. al. OPTICS LETTERS, 26, 19 (2001)

Single molecule fluorescence spectroscopy

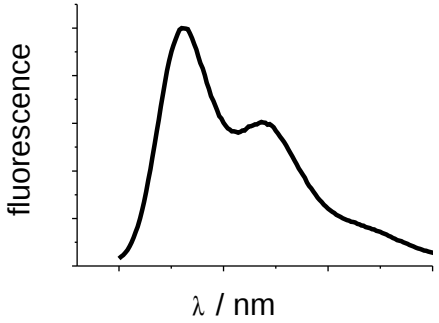
Different Observables:

- **Intensity**
- **Anisotropy**
- **Anti-bunching (Hanbury Brown and Twiss)**

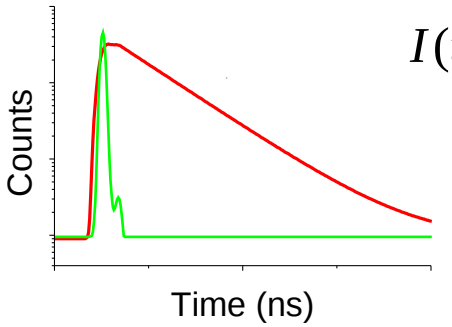
- **Excitation spectrum**



- **Emission spectrum**



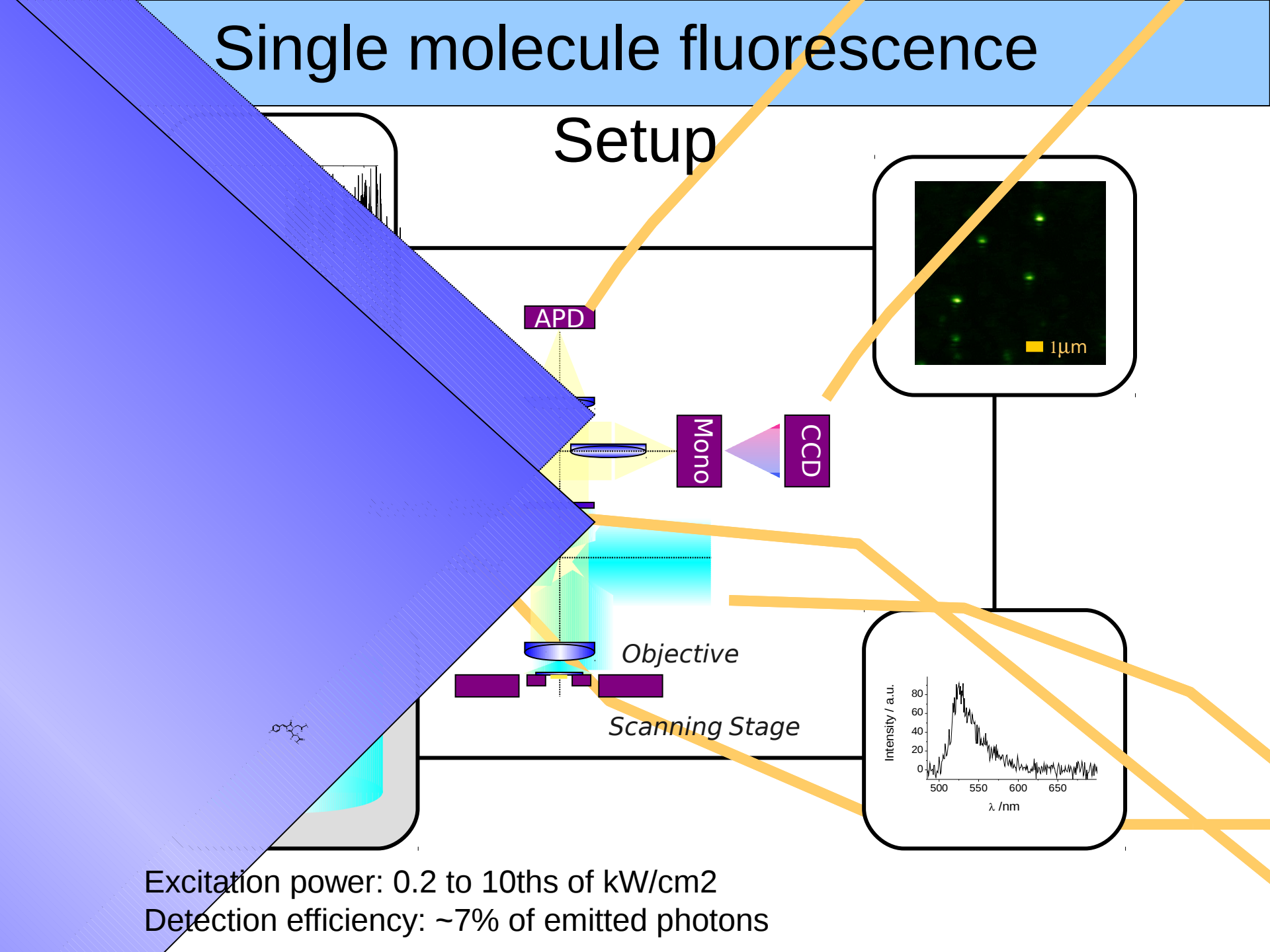
- **Lifetime**



$$I(t) = A \cdot \exp\left(-\frac{t}{\tau}\right)$$

Single molecule fluorescence

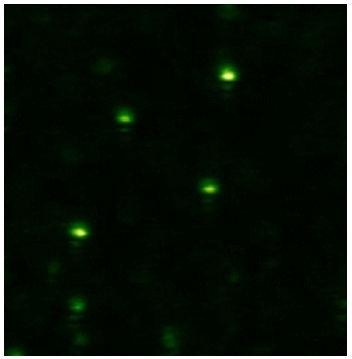
Setup



Excitation power: 0.2 to 10ths of kW/cm2
Detection efficiency: ~7% of emitted photons

Experimental approach

1. Fast localization of single molecules with APD detection

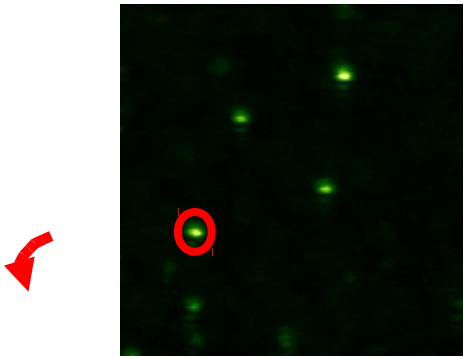


10□m

10□m

Observation of the emission intensity

1. Fast localization of single molecules with APD detection

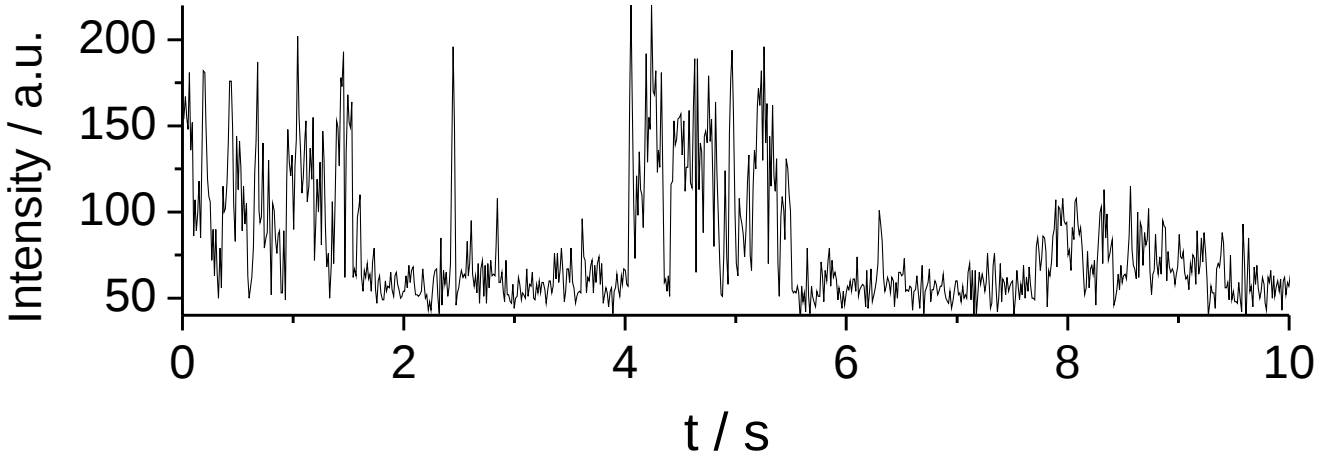


10□m

10□m

2. Observation of emission intensity evolution with APD

Blinking molecules



Summary

- Single-Molecule studies provide new insights
- Numerous difficulties, important to use fluorescence
- Single molecules show very specific behavior

Thanks for your attention