Quantum nano-plasmonics for biosensing and bioimaging on the level of single molecules and virions

Institute of Spectroscopy, Moscow, Russia

Structure of the talk

1. Detection of single molecules in biology and medicine

2. Optical microscopy and spectroscopy of single molecules

3. Detection of single molecules and virions

4. Quantum engineering of radiative properties of nanoscale emitters of light

I. Detection of single molecules in biology and medicine

Single-molecule experiment relying on fluorescence detection is dominating over all other optical techniques for the last 20 years

The Nobel Prize in Chemistry 2022 for the development of click chemistry and bioorthogonal chemistry

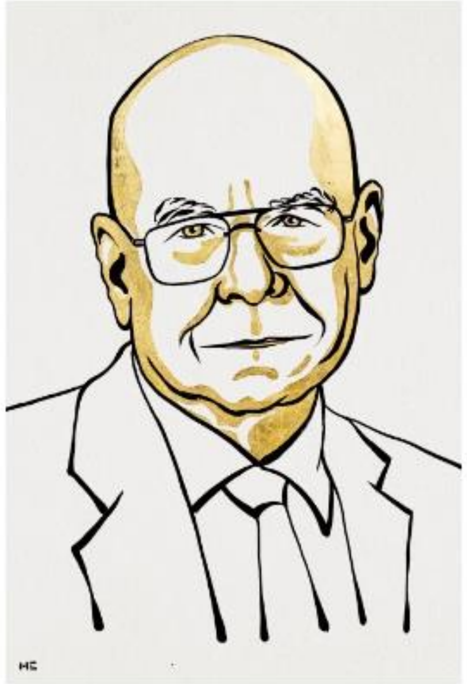
The Nobel Prize in Chemistry 2022

Ill. Niklas Elmehed © Nobel Prize Outreach

Carolyn R. Bertozzi

Ill. Niklas Elmehed © Nobel Prize Outreach

Morten Meldal



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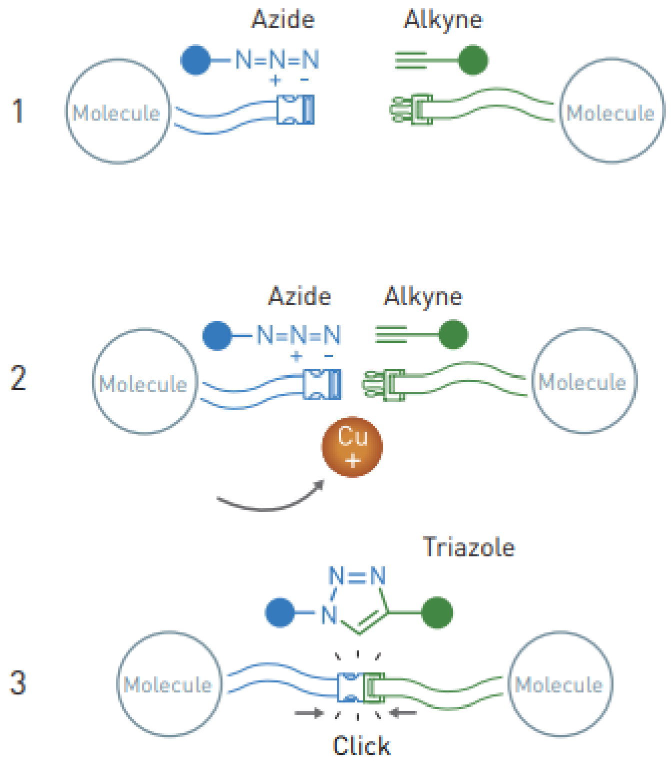
K. Barry Sharpless

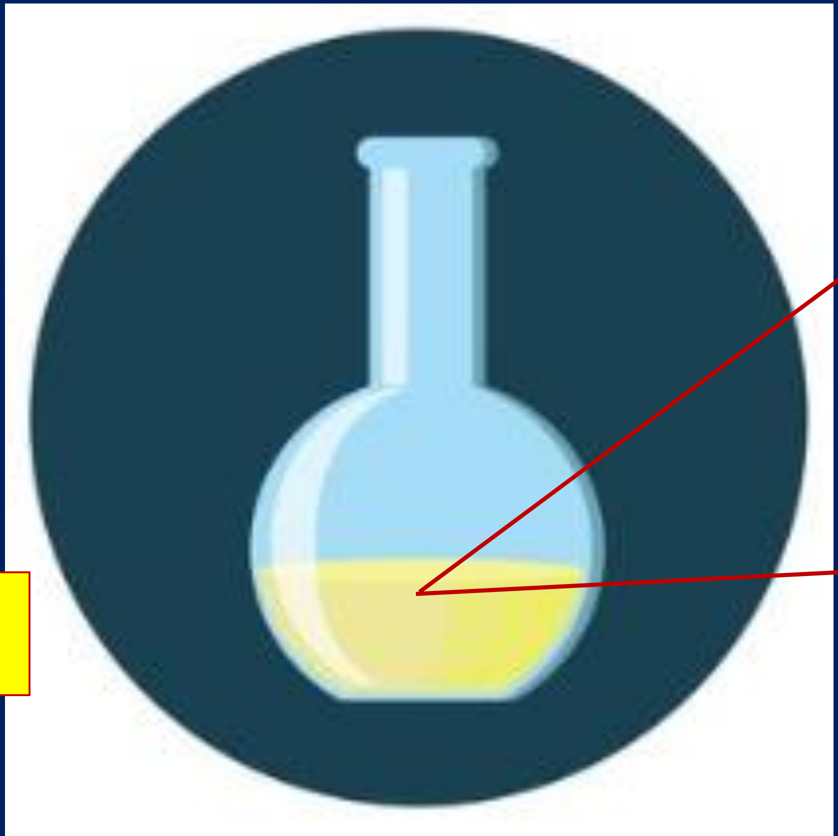
The click reaction that changed chemistry

Azides and alkynes react very efficiently when copper ions are added. This reaction is now used globally to link molecules together in a simple manner.

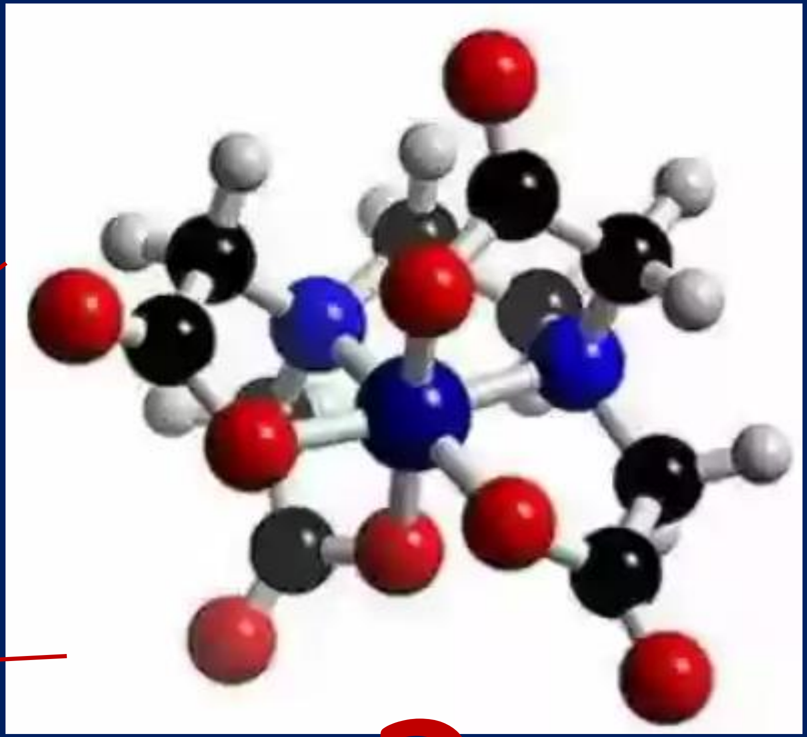


Is it possible to find a single specific molecule (analyte) in a big volume?

1. Yes/No
2. Concentration
3. Limit of Detection
4. Single molecule

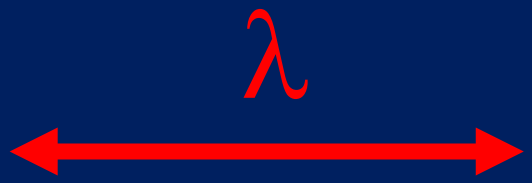


Bottle with liquid

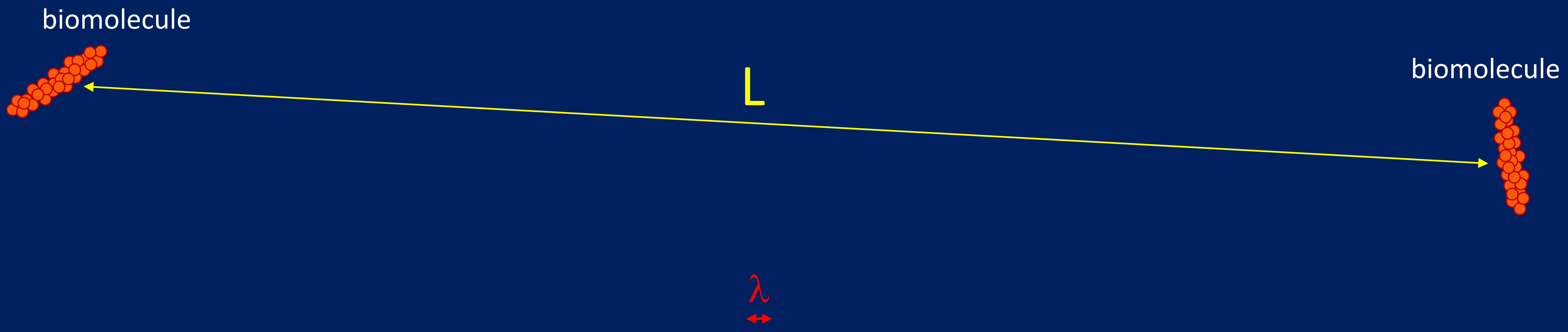


?

Ultra-low concentration in optics, how much low is it?



Ultra-low concentration in optics, how much low is it?

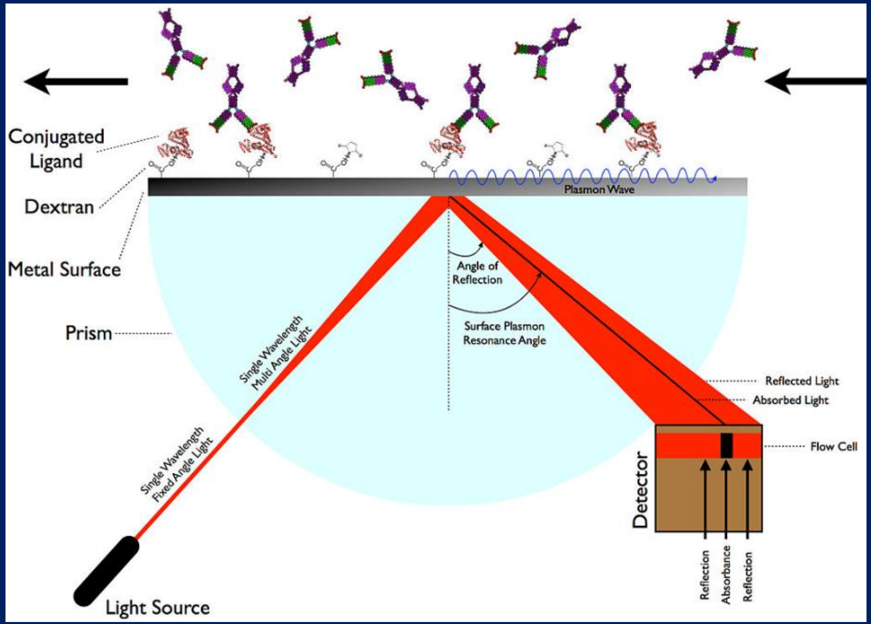


$$L >> \lambda$$

$N \sim 10^{7}\ cm^{-3}$

Optical Sensing approaches

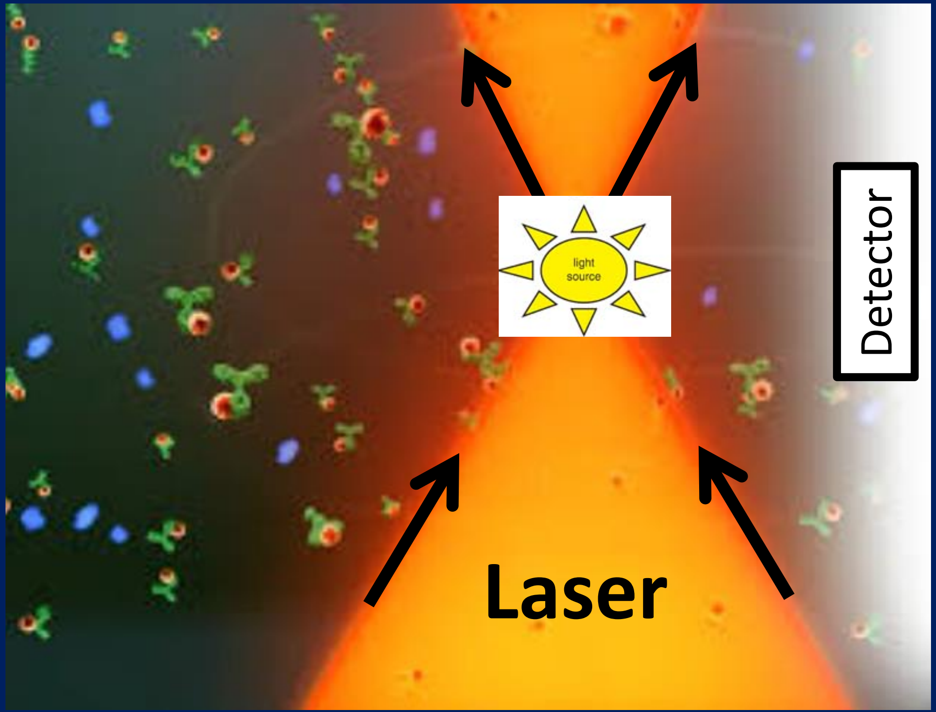
Refractive Index Measurements



Detection limit:

10^8 molecules (≈ 20 pM)

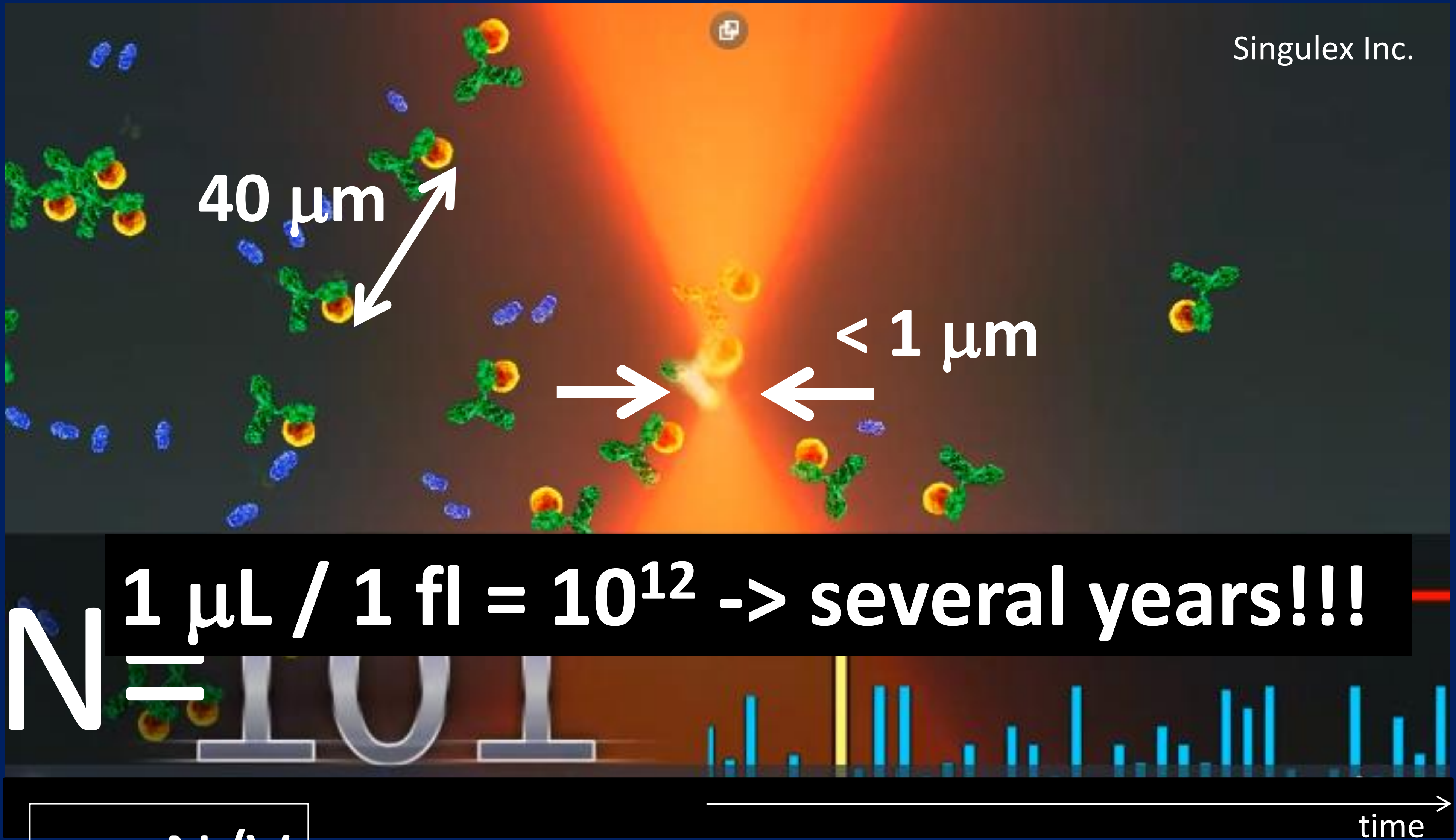
Single Molecules Detection



Detection limit:

1 molecule!!!

Single-Molecules-Counting Method



1 µL / 1 fl = 10^{12} -> several years!!!

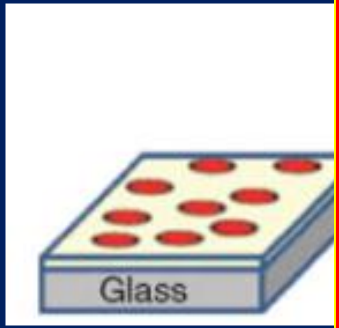
N=

$n = N/V$

Detection time = $N_{molecules} \times t_{single\ molecule\ detection}$

Concentration amplification in a sample

(I) Concentration of analyte molecules on the functionalized surface



Disad

- Techni

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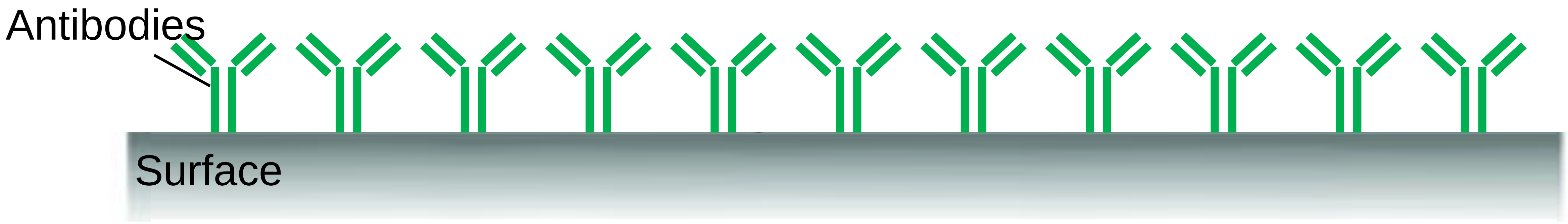
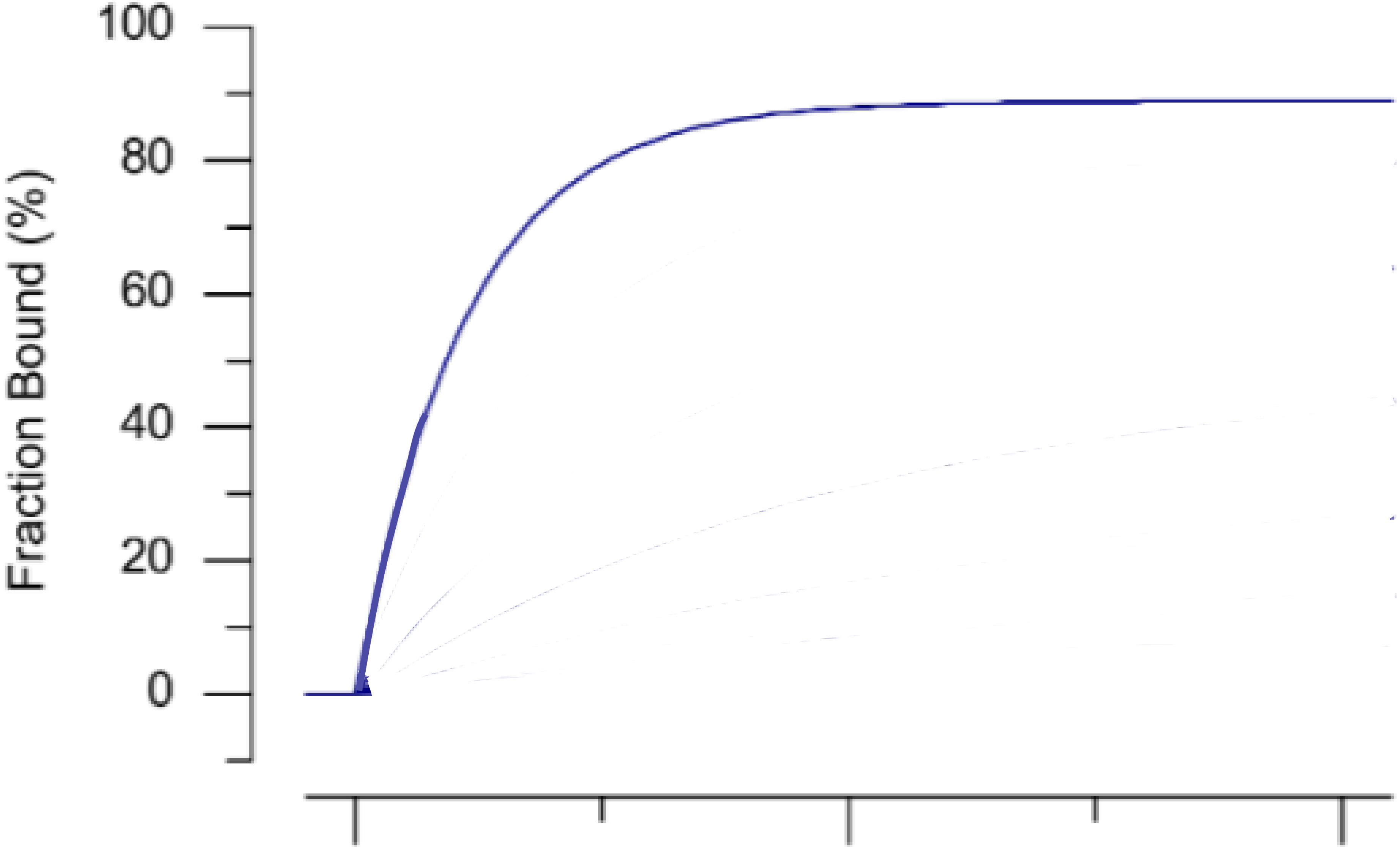
- Long sample

- Errors due to ad

“Catastrophe” of the Langmuir binding isotherm



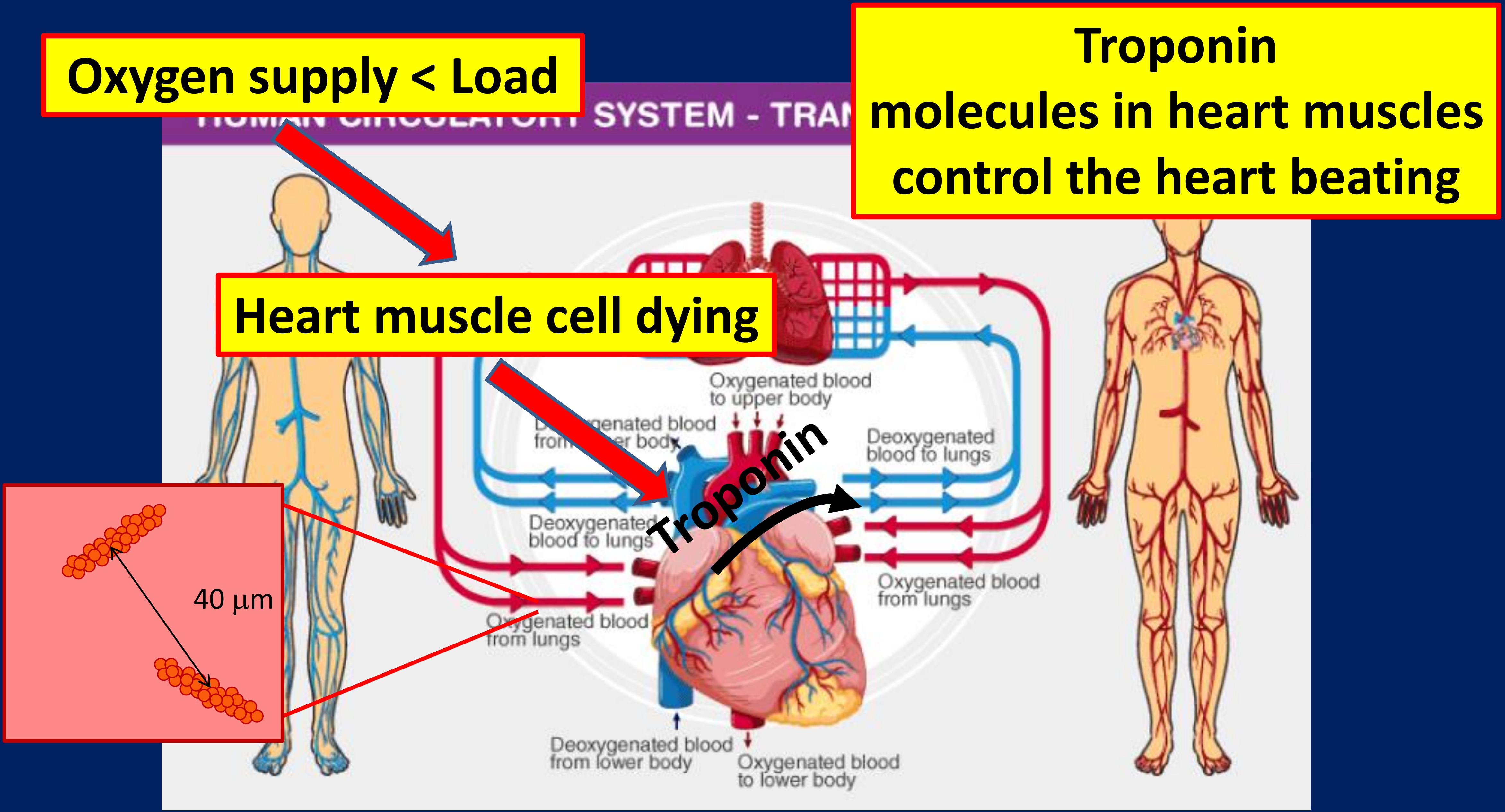
"Catastrophe" of the Langmuir binding isotherm



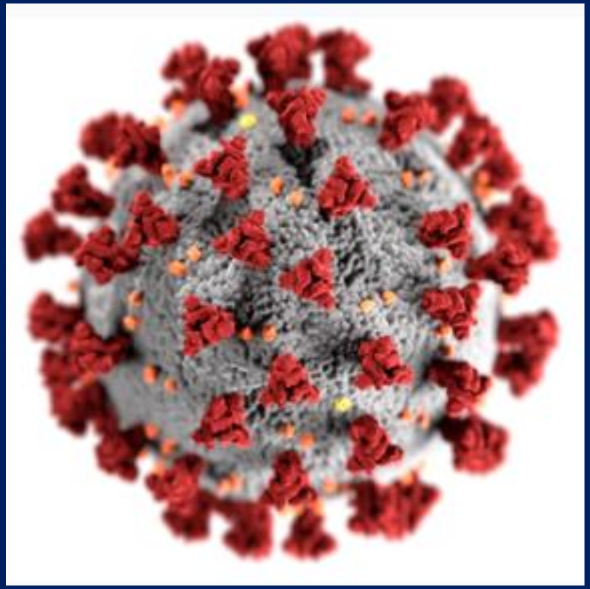
Latour, Robert A. "The Langmuir isotherm: a commonly applied but misleading approach for the analysis of protein adsorption behavior." *Journal of Biomedical Materials Research Part A* 103.3 (2015): 949-958.

Selected applications of fluorescence of single molecules in our lab

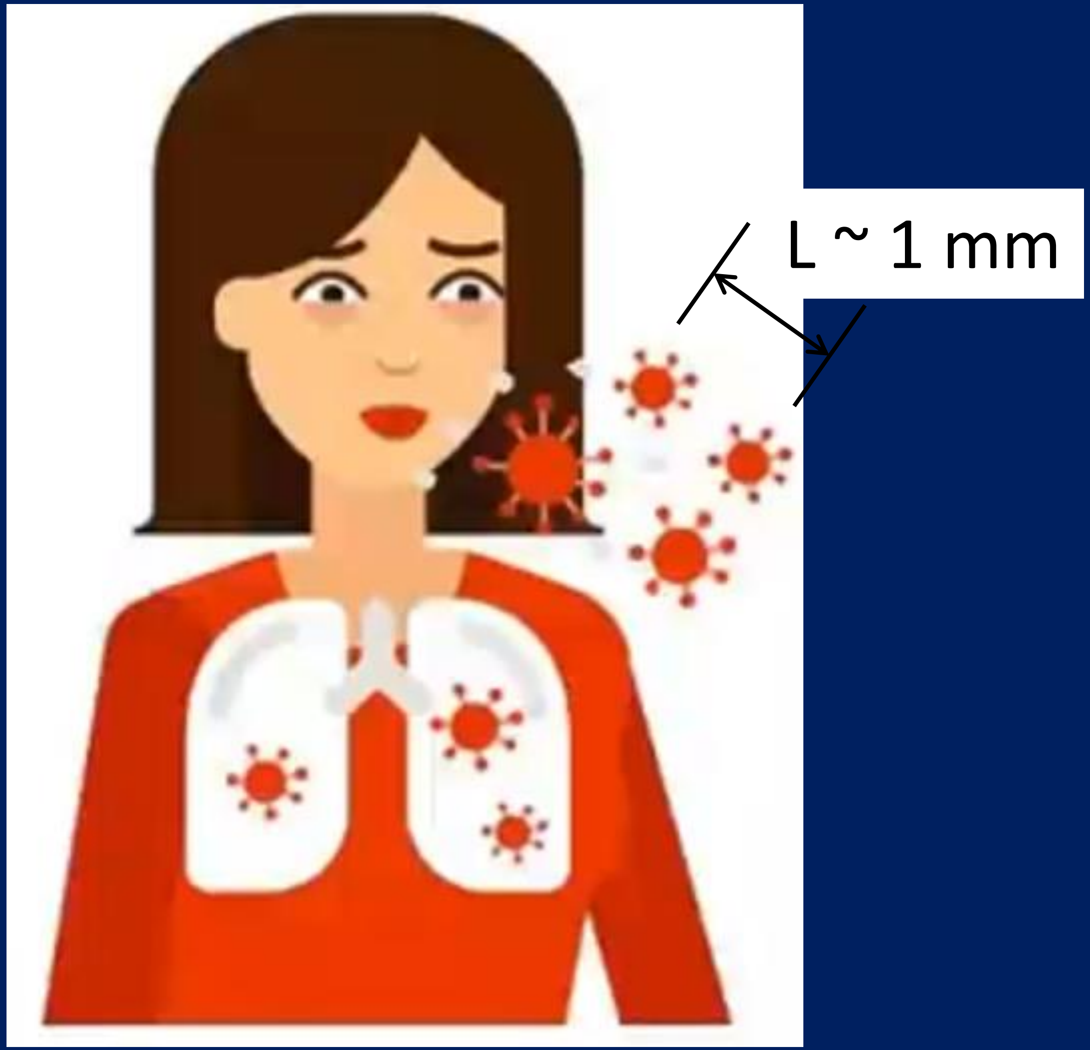
Example -1: control of Troponin molecules in blood



Example-2: detection of virus particles



Schematic of
SARS-CoV-2 virion



Example 3: Single DNA molecule sequencing

Real-Time DNA Sequencing from Single Polymerase Molecules
John Eid, *et al.*
Science **323**, 133 (2009);
DOI: 10.1126/science.1162986

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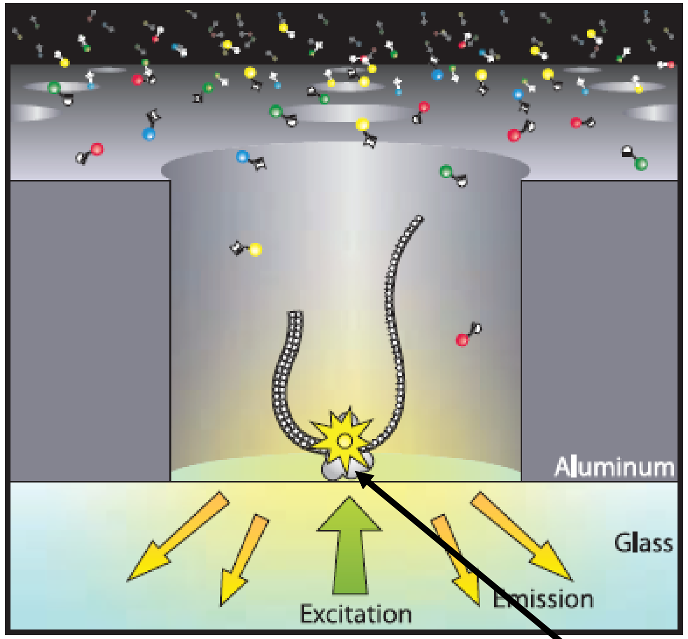
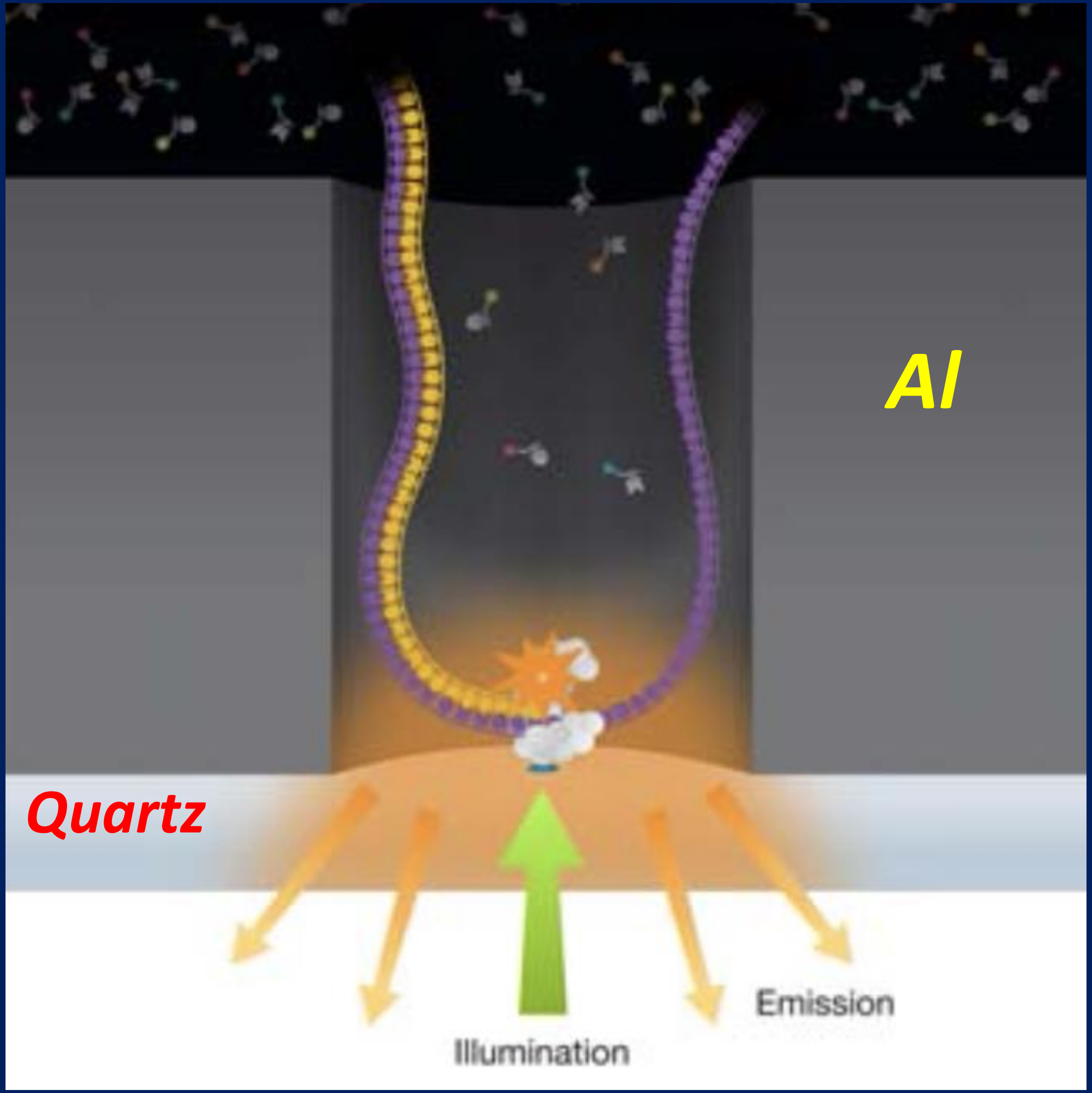
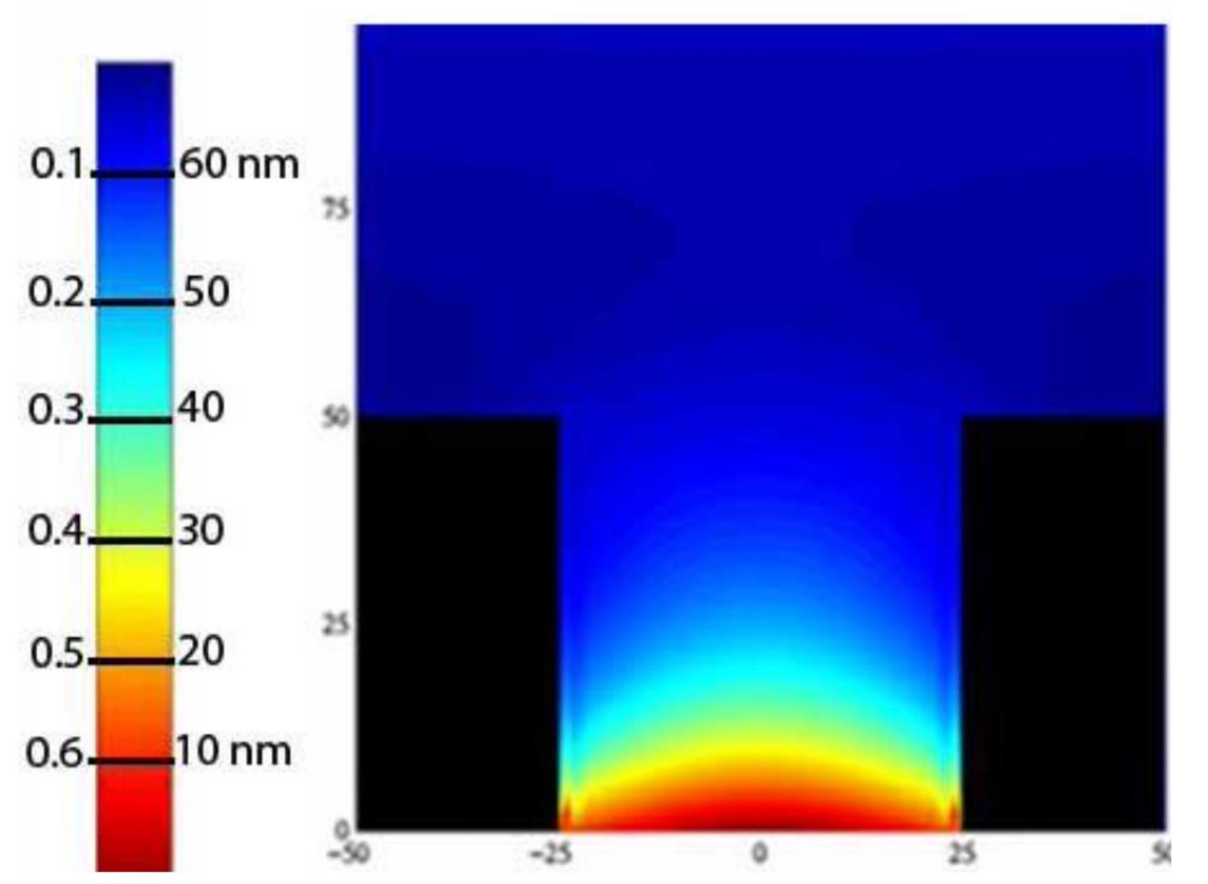
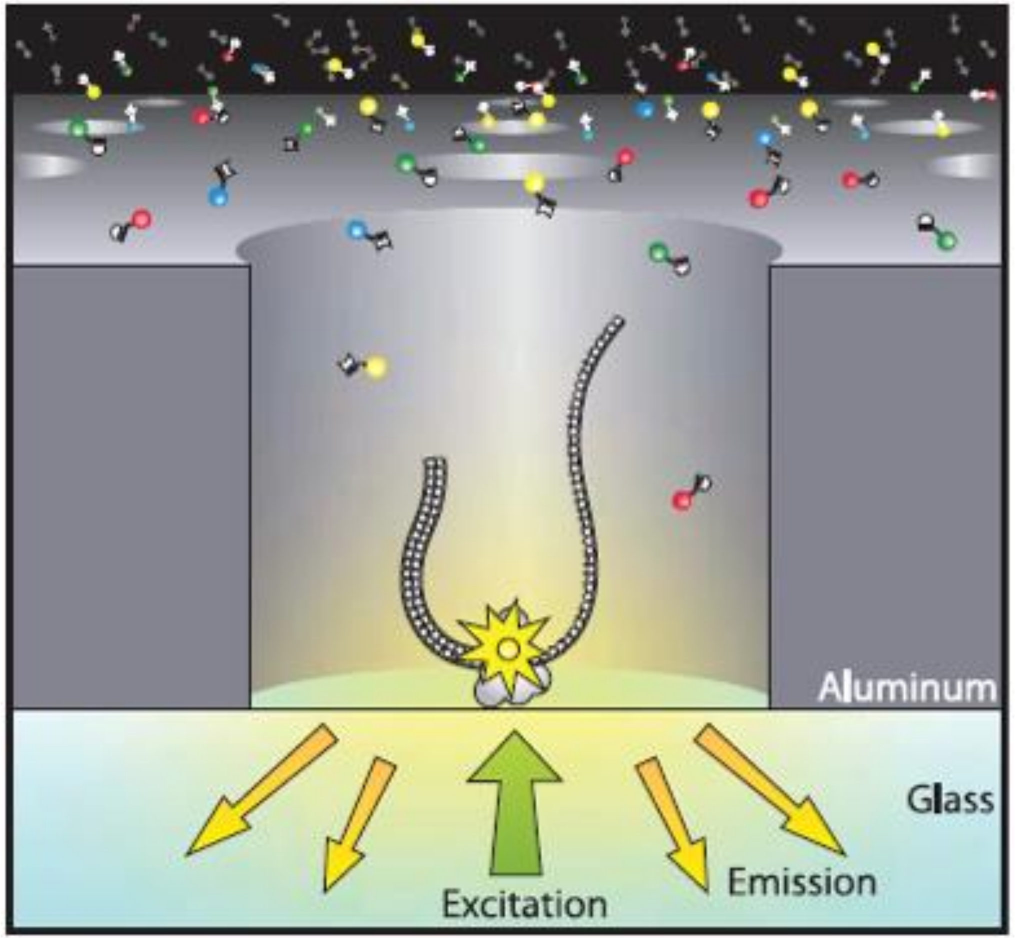


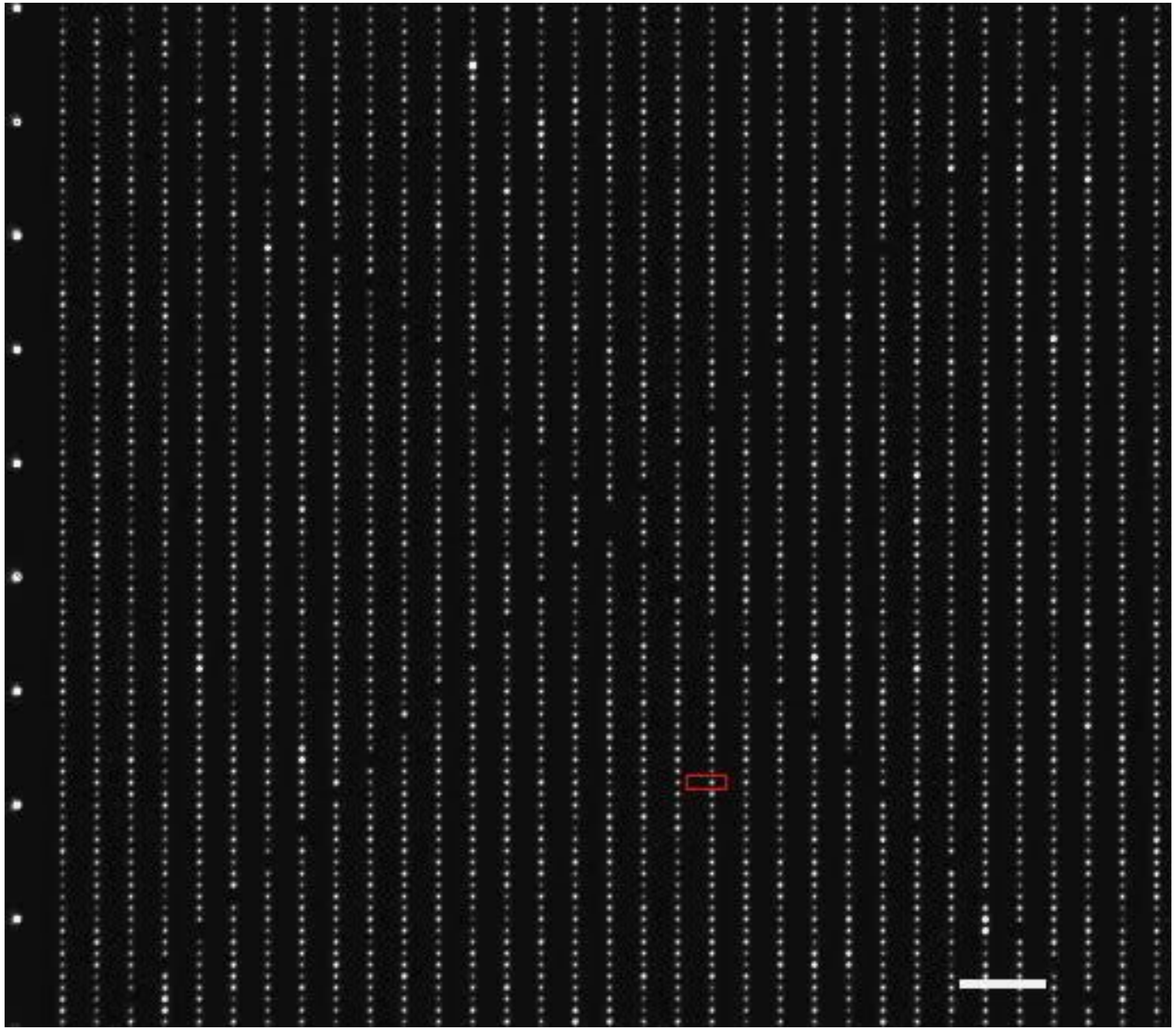
Polymerase molecule

Zero Mode Waveguide






Aluminum
Glass
Excitation
Emission



“THIRD” GENERATION SEQUENCING
(large fragment single molecule)

2. Optical microscopy and spectroscopy of single molecules

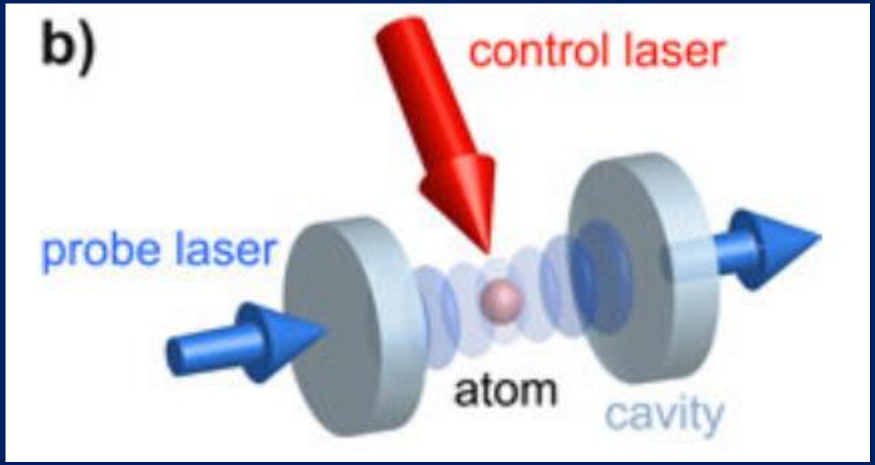
Marking of biomolecules via antibodies

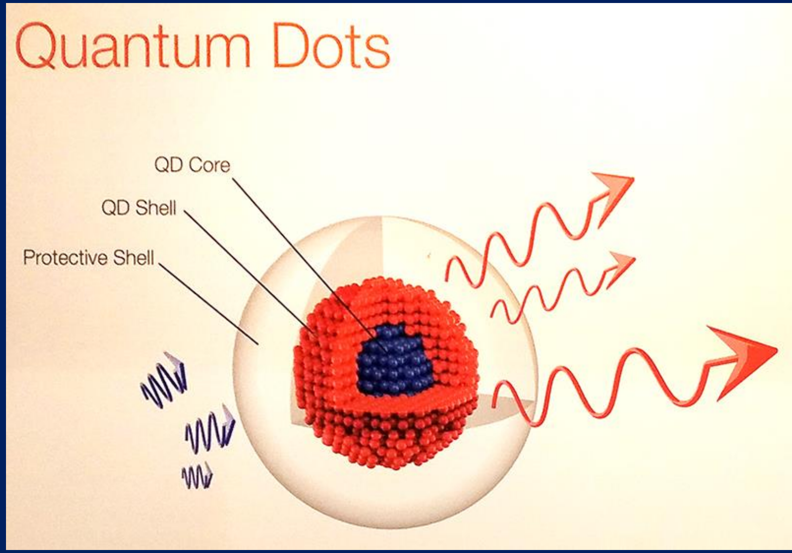
 - antibody +  - fluorescent marker =  - marked antibody

Nanolocalized sources of light– fluorescent markers

Nanolocalized sources of light

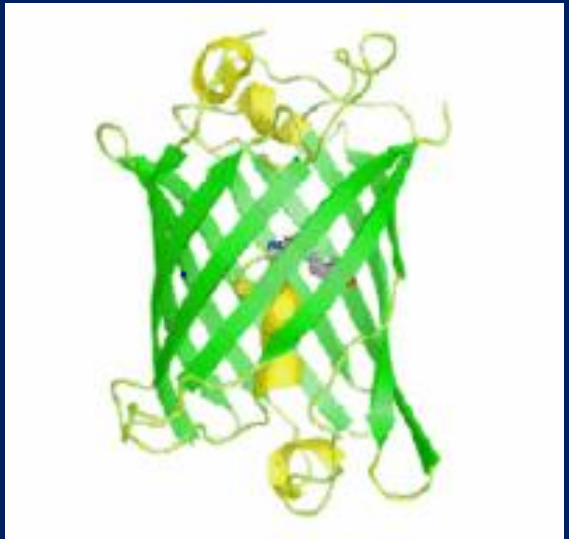
Single atoms and ions



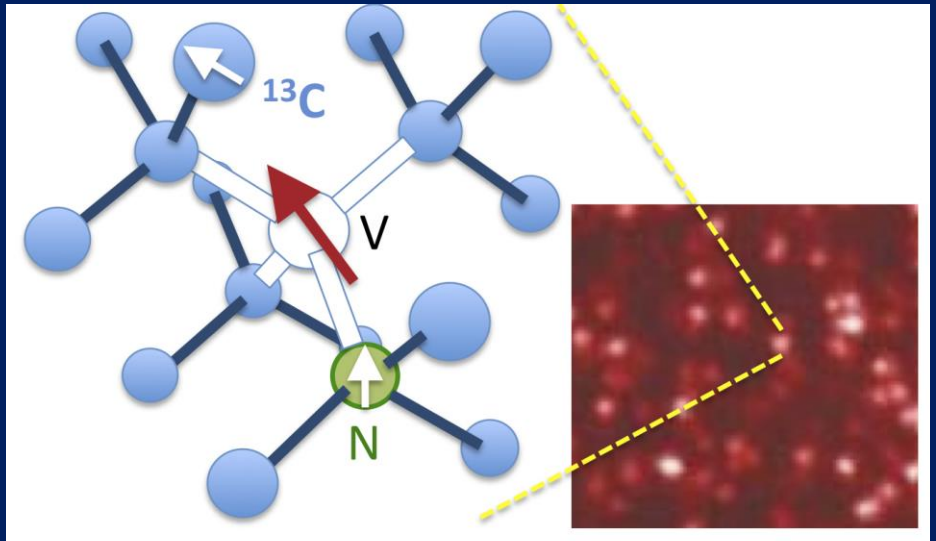


Dye molecule

Fluorescent proteins



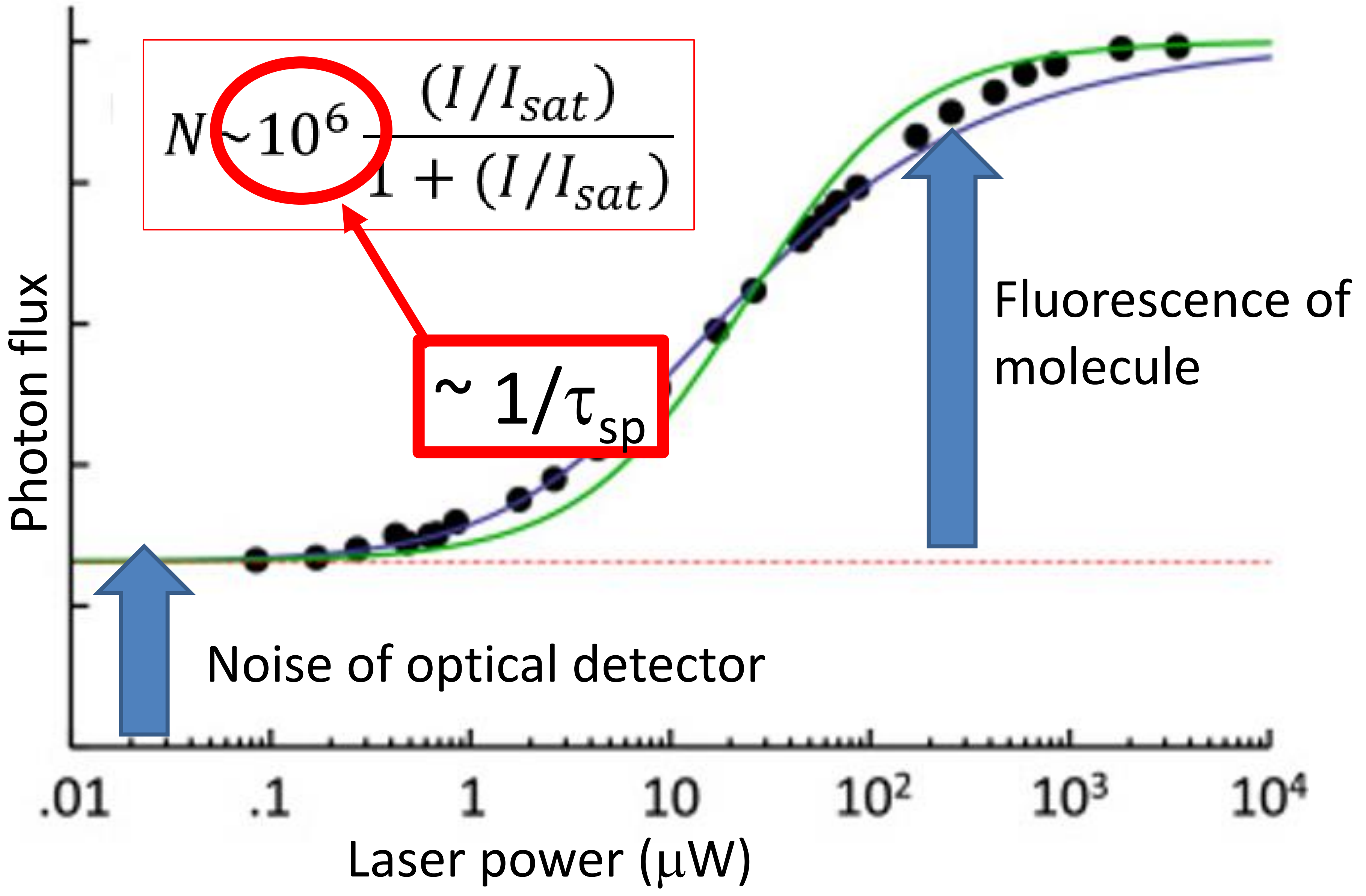
Defects in crystals



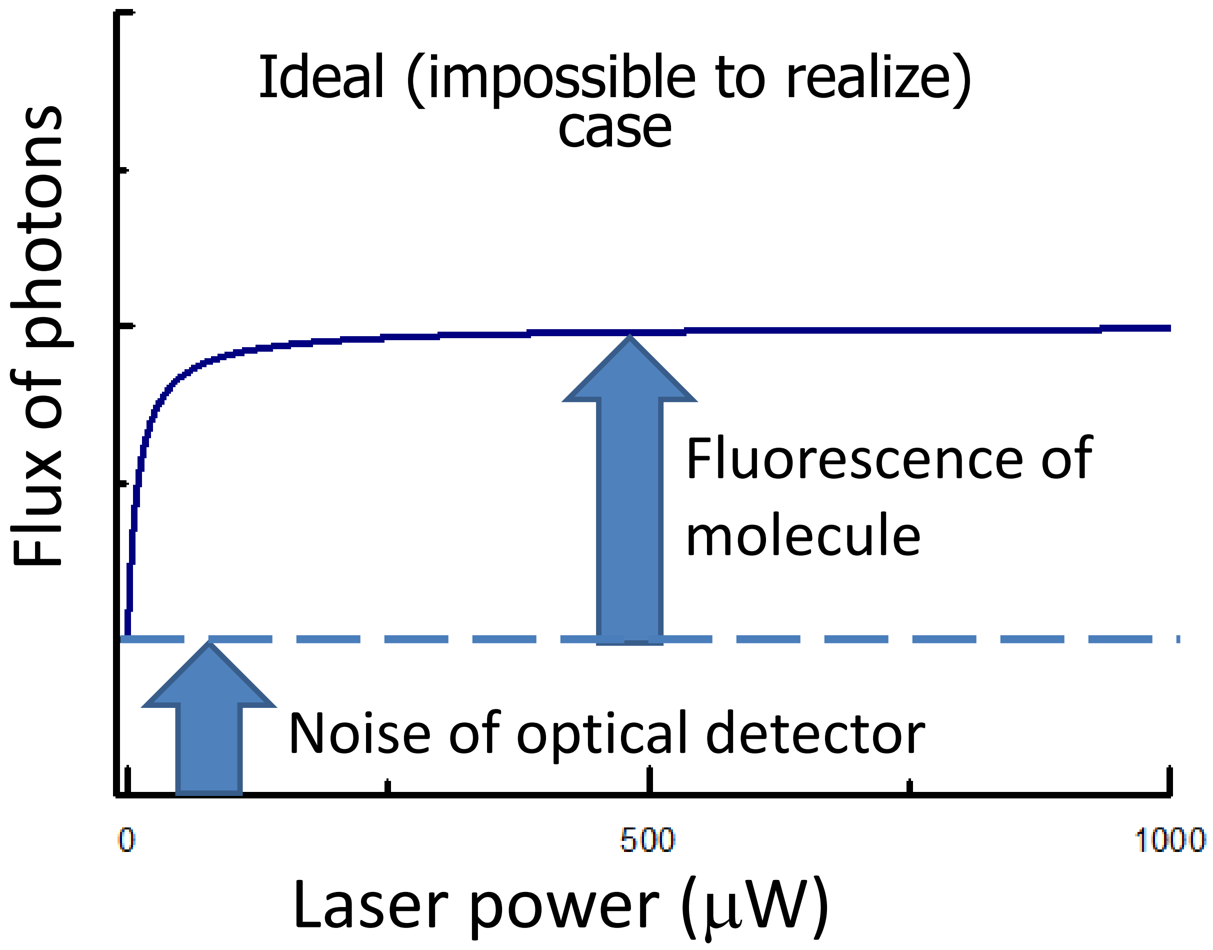
Comparison of different emitters of light

Emitter	FWHM (nm)	$I_{sat}\ (\frac{W}{cm^2})$	$\sigma_{rad}\ (cm^2)$	$\Phi_{max} = \frac{I_{sat} * \sigma_{rad}}{FWHM}\ (W/nm)$	Quantum yield	Size
Quantum dot	~ 40	$\sim 10^3$	$\sim 10^{-14}$	$\sim 2{,}5 * 10^{-13}$	30-50%	$\sim 5\ nm$
Dye molecules	~ 40	$\sim 10^6$	$\sim 10^{-16}$	$\sim 2{,}5 * 10^{-12}$	20-100%	~ 10 Å
Defects in diamond	~ 5 (ZPL, 300K) ~ 150 (PSB, 300K)	$\sim 10^4 - 10^5$	$\sim 10^{-16}$	$> 2 * 10^{-13}$	1-10%	$\sim 5\ nm - 100\ nm$
Single atom	$\sim 6\ MHz$	$\sim 10^{-3}$	$\sim 10^{-8}$	$\sim 10^{-5}$	100%	~ 2 Å

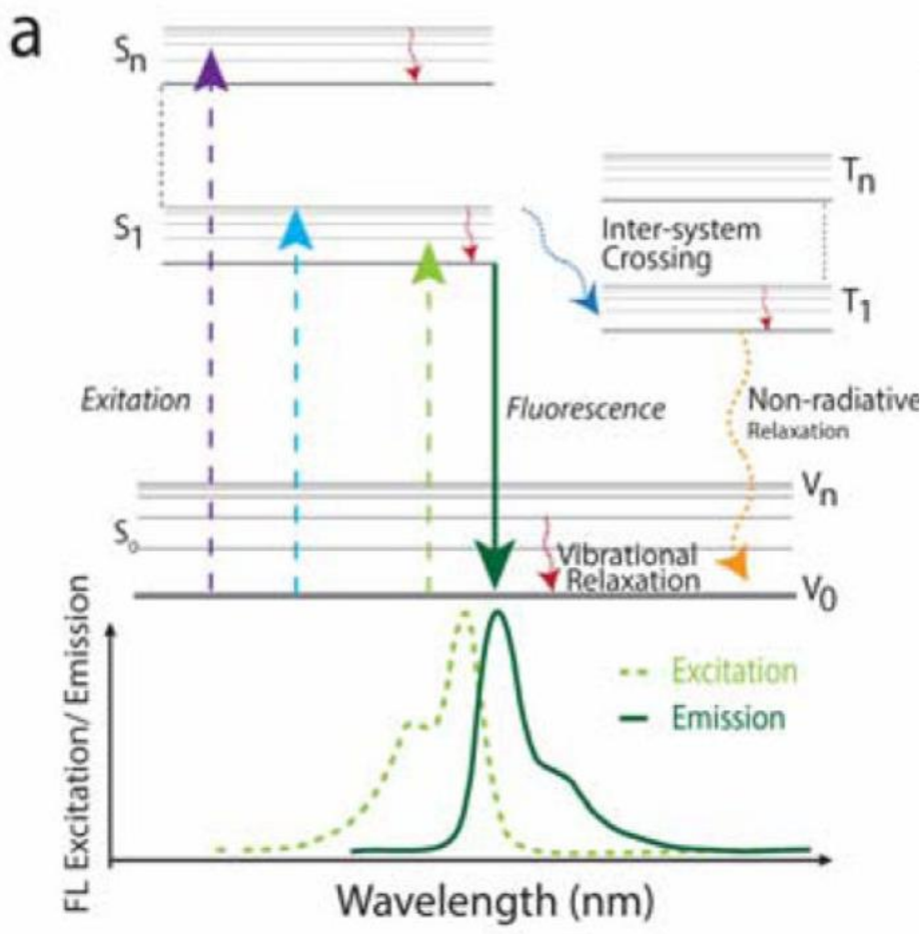
Saturation effect of the photon flux



Photon flux vs laser power



Fluorescence microscopy of single molecules



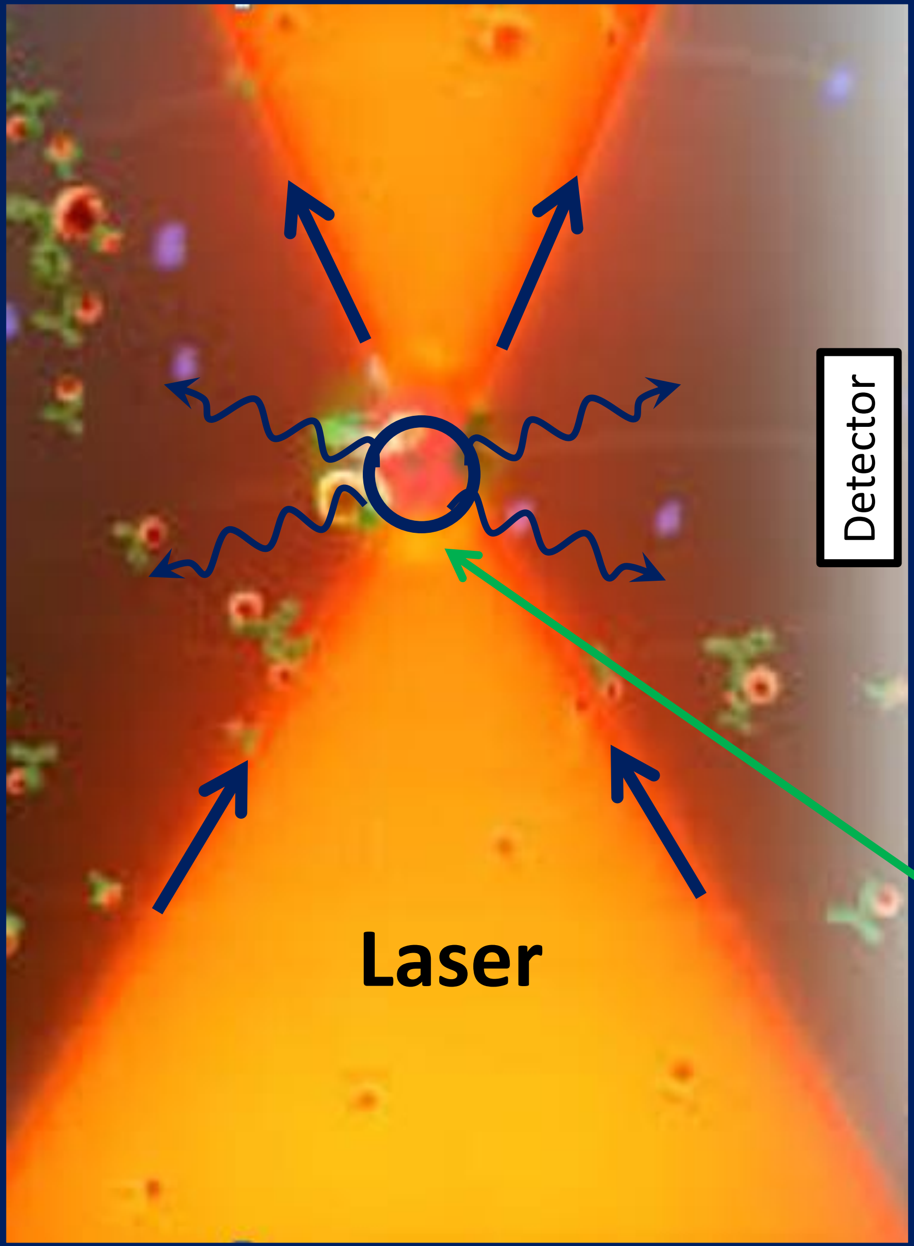
Problems of available quantum emitters of light

1. Low photon flux

2. Large spectral width

3. Photodegradation

Low photon flux and tight focusing of laser light



Detected signal =

$$\alpha\gamma \times I_{laser} + \beta V \times I_{laser}$$

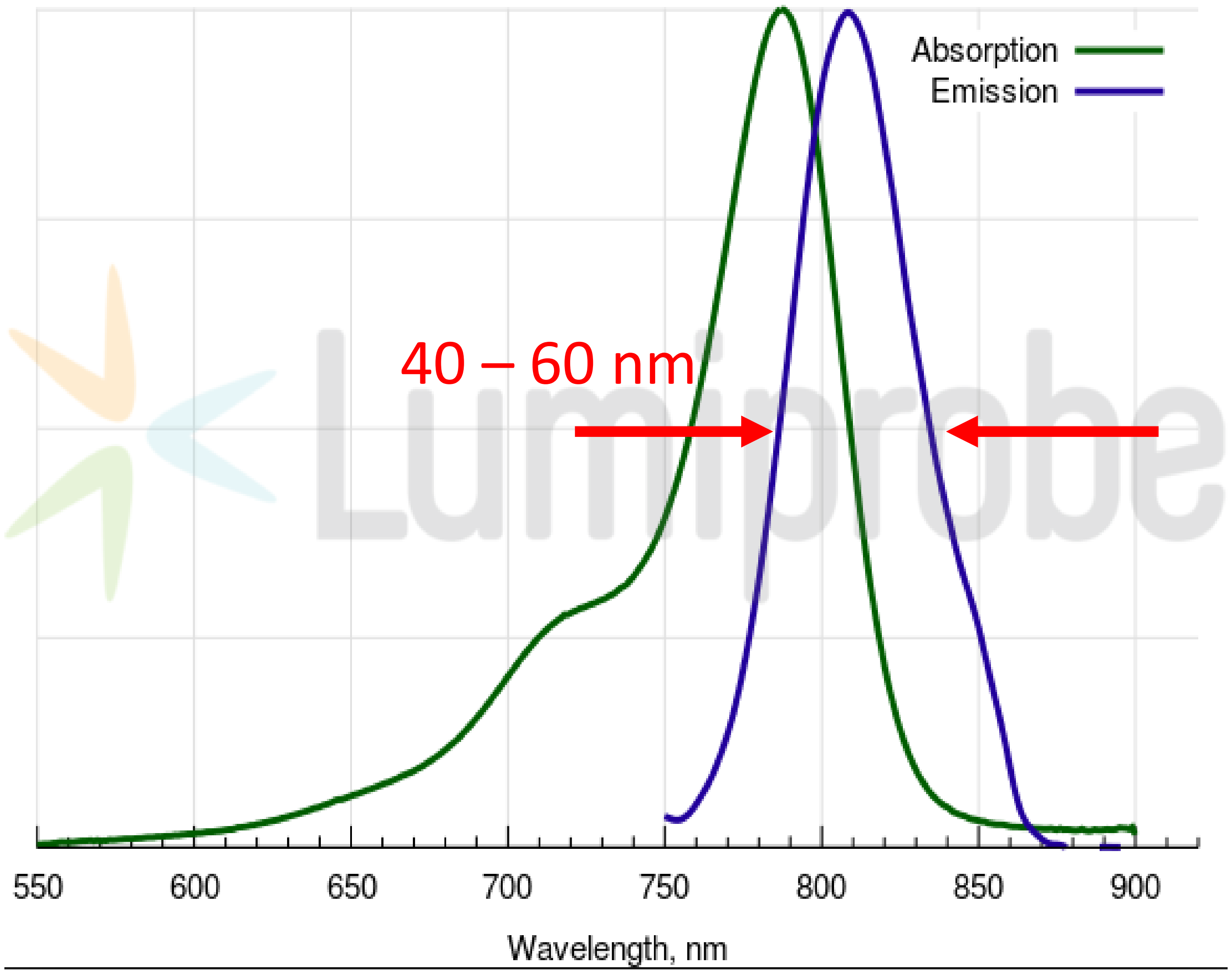
Fluorescence of nanolocalized source (single molecules)

Parasitic luminescence of surrounding media

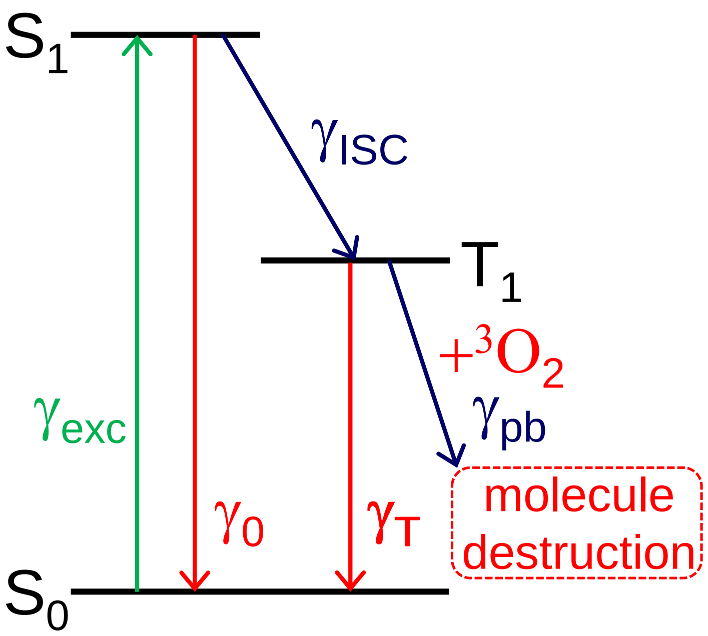
V – effective excitation volume

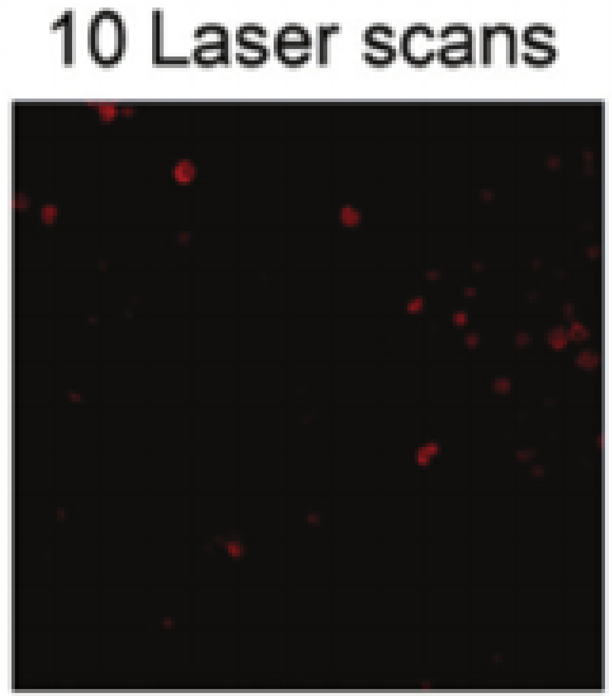
dx.doi.org/10.1021/ac3027178 | Anal. Chem. 2013, 85, 1258−1263

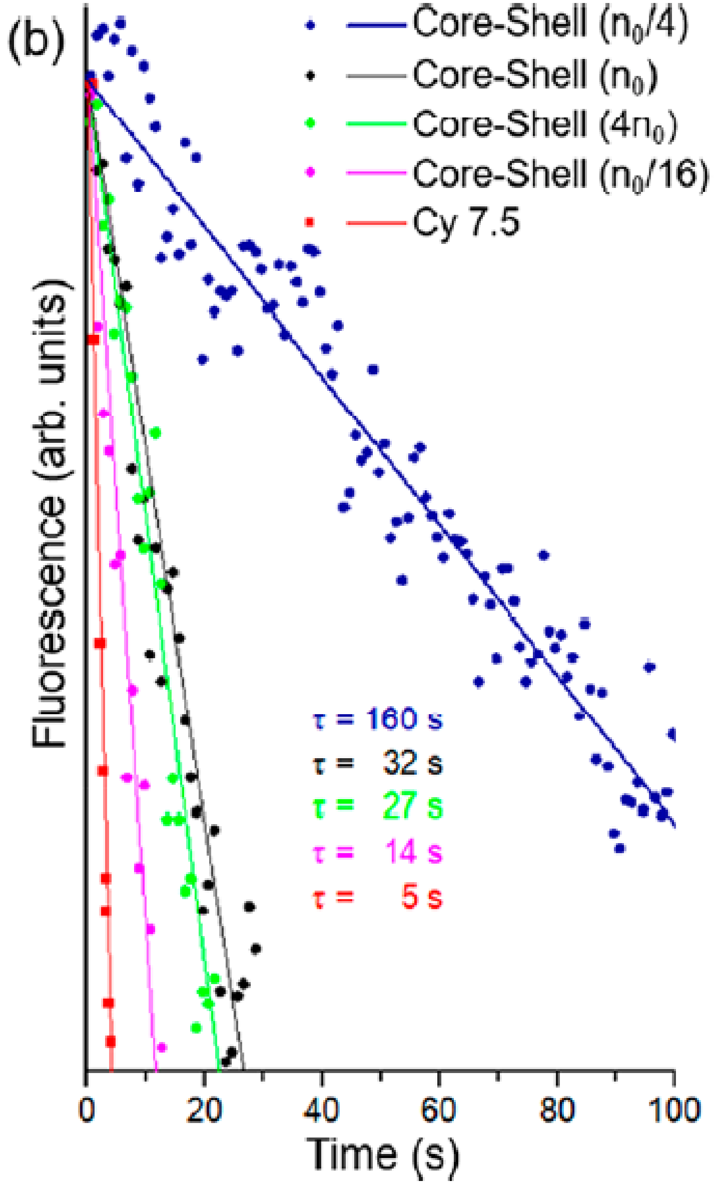
Why spectral width of fluorescence is important?



Photodegradation of dye molecules

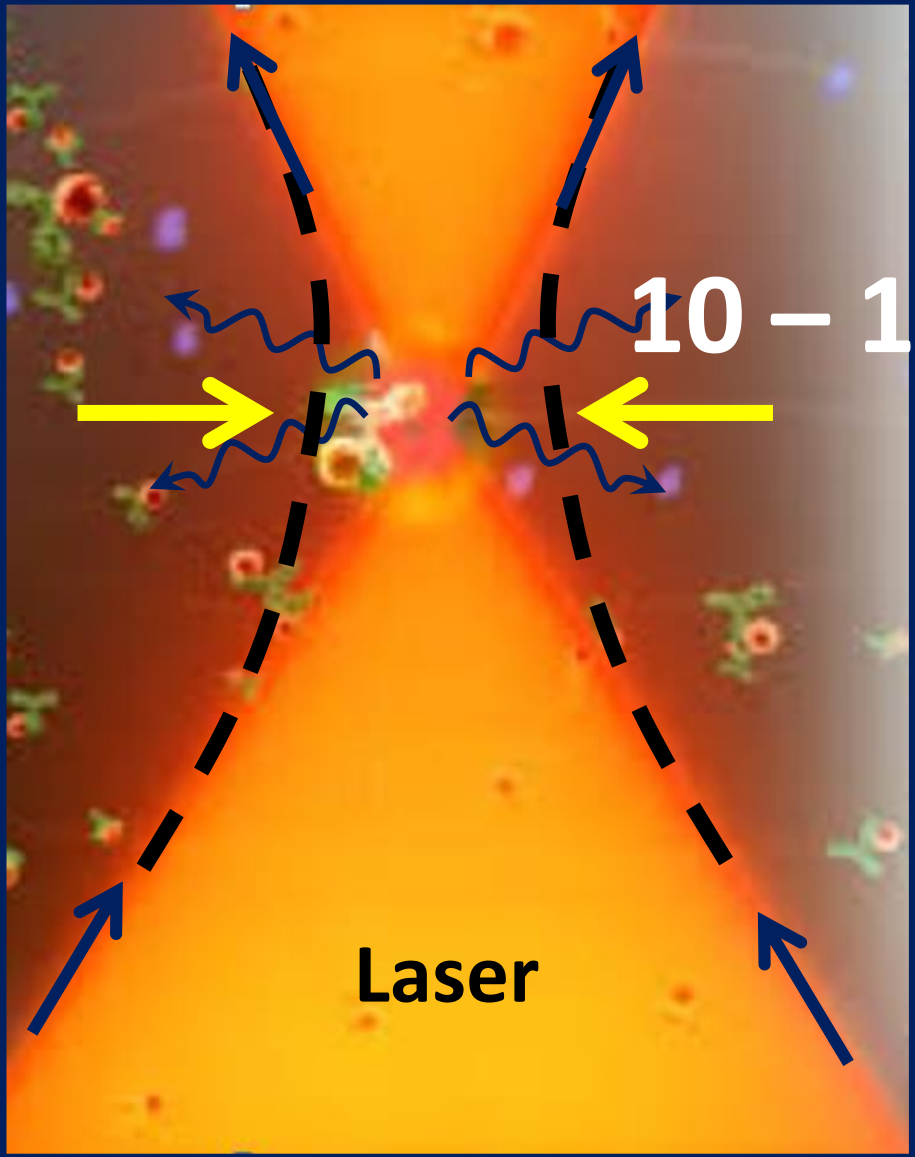






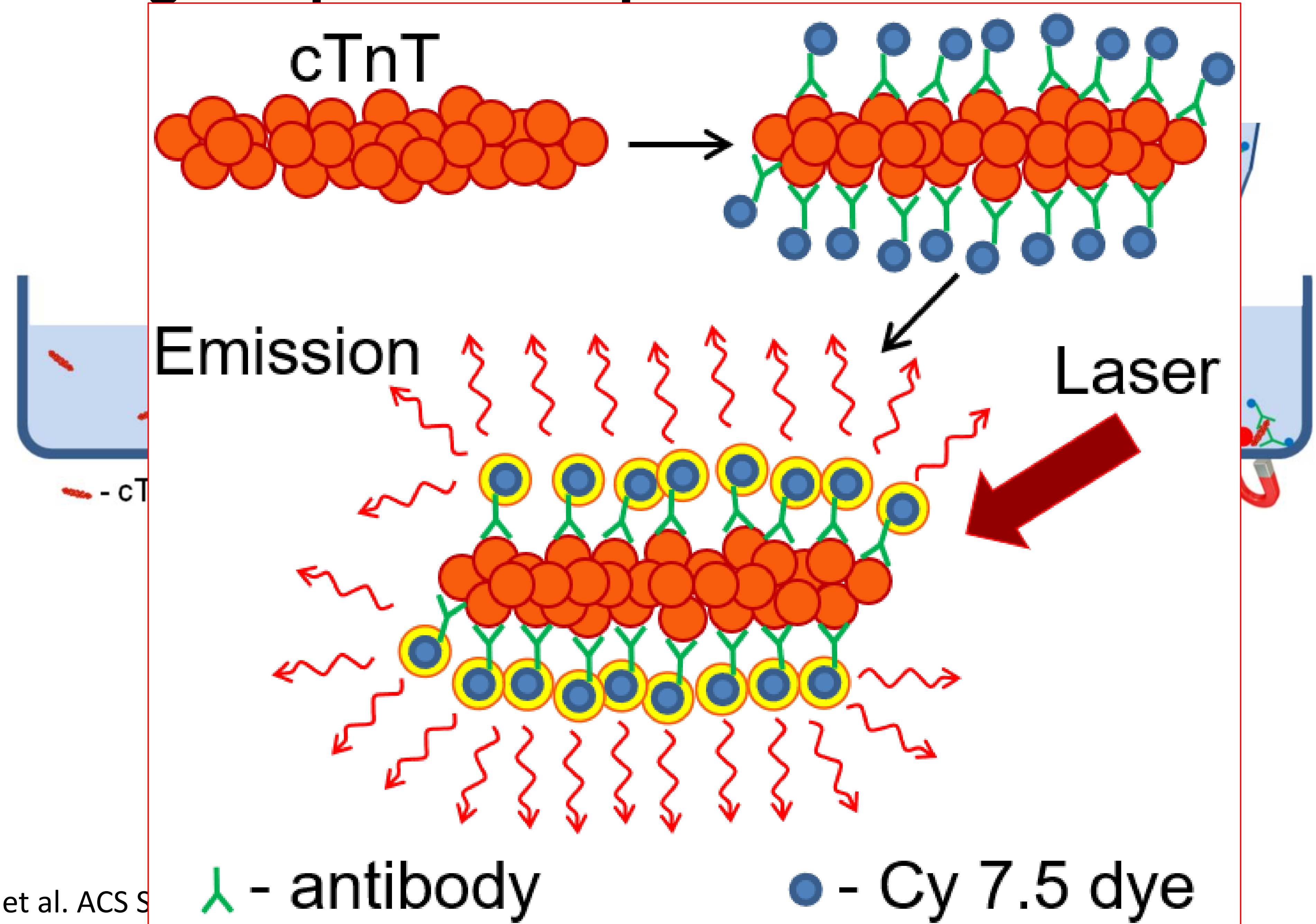
3. Detection of single molecules and virions

Single-Molecules-Counting Method with "unfocused" laser light



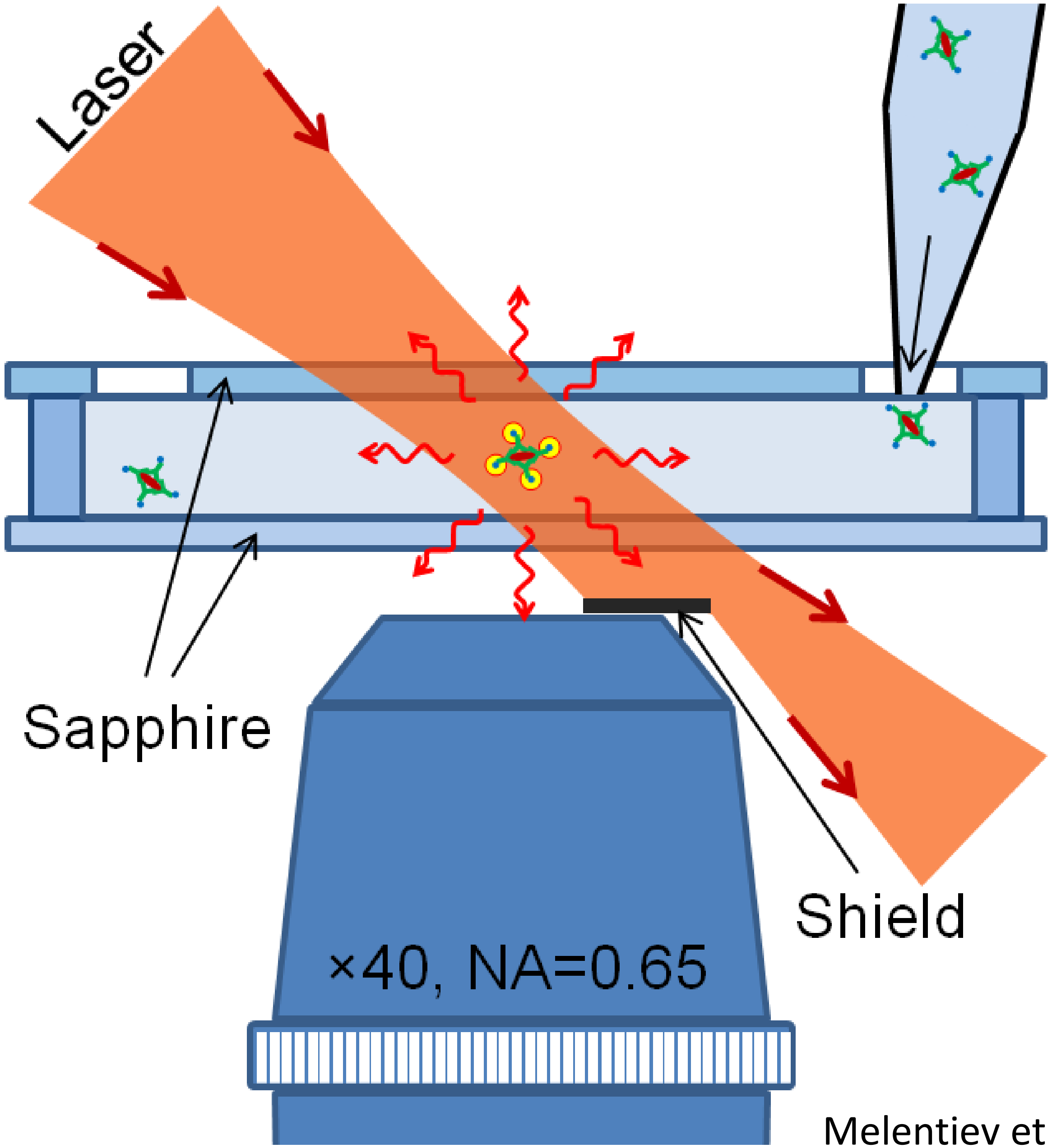
Detection of Troponin molecules

Making samples for Troponin molecules detection



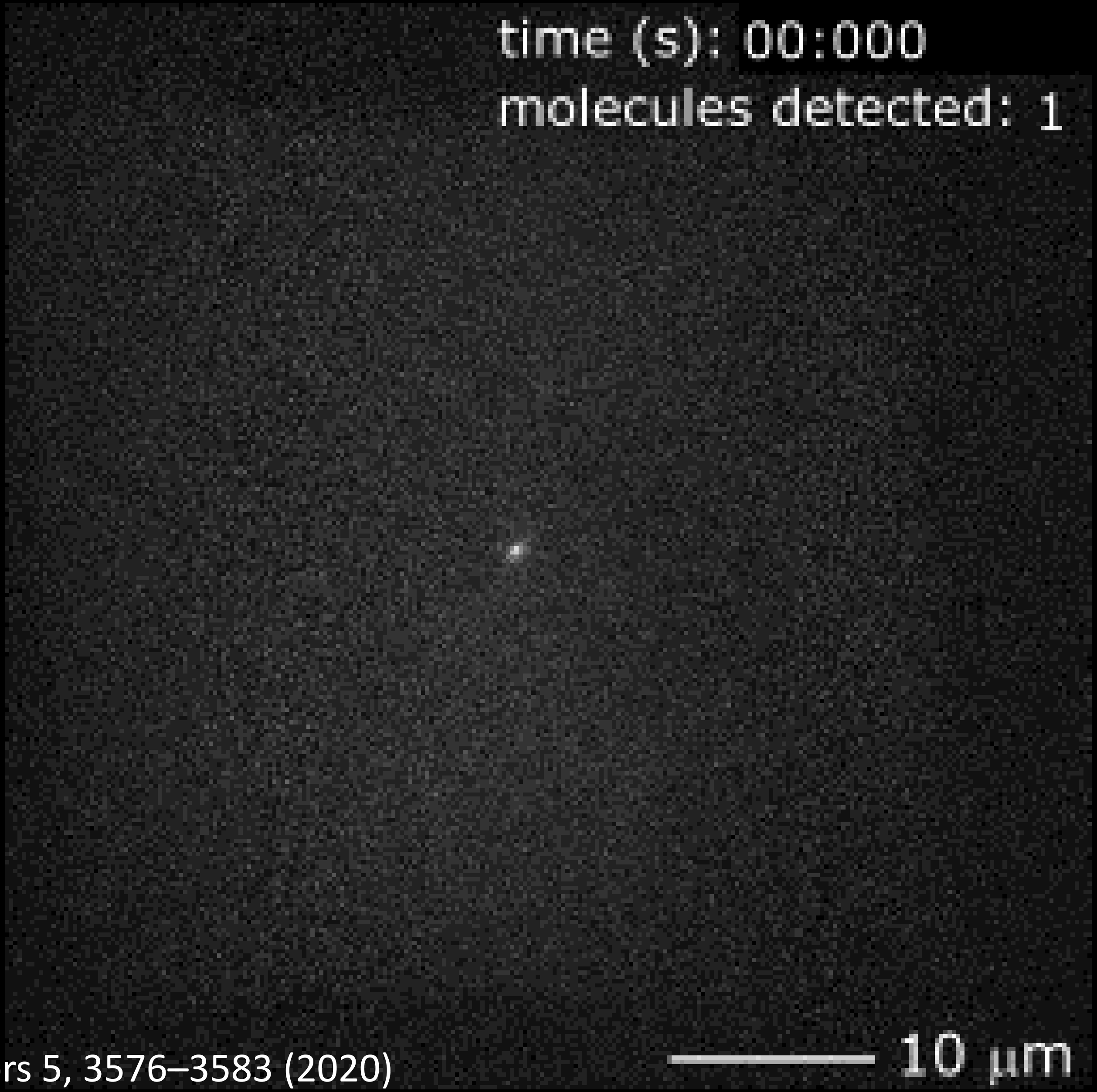
P.N. Melentiev et al. ACS S

Optical setup



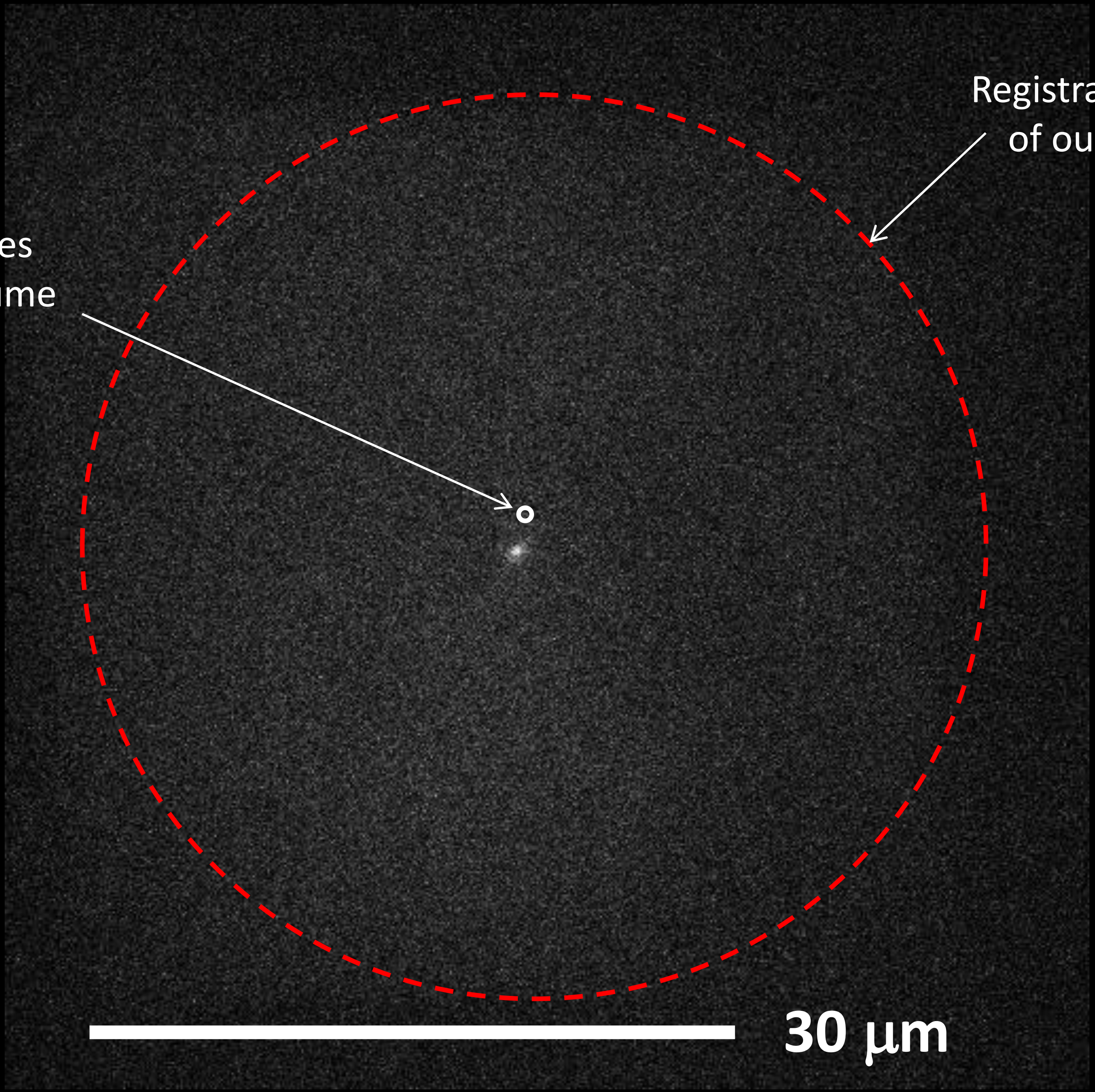
Melentiev et al. ACS Sensors 5, 3576–3583 (2020)

Single Troponin molecules visualization



P.N. Melentiev et al. ACS Sensors 5, 3576–3583 (2020)

Optical imaging of cTnT molecules



Troponin Detection

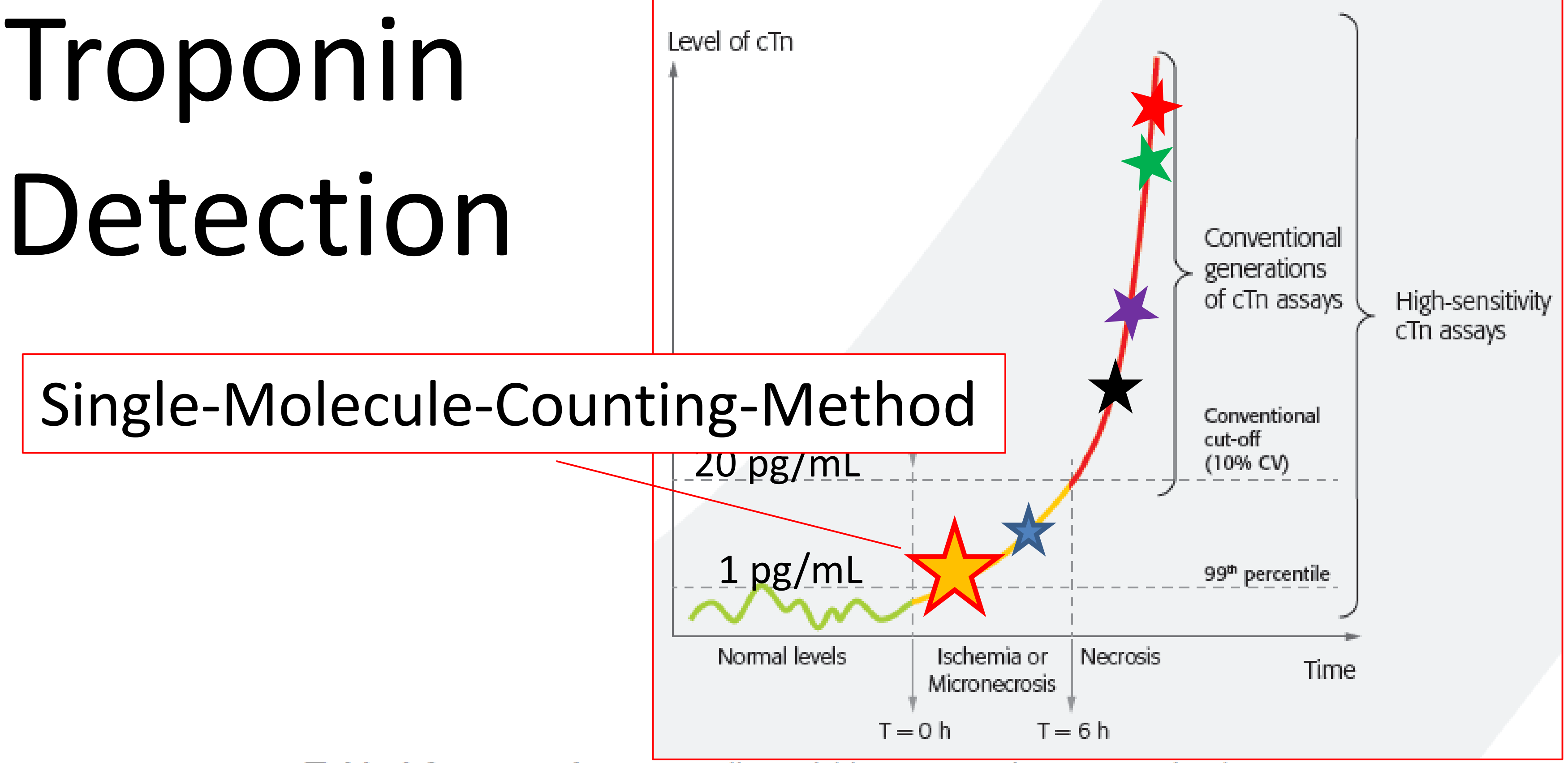


Table 1 Summary of commercially available troponin detection technologies

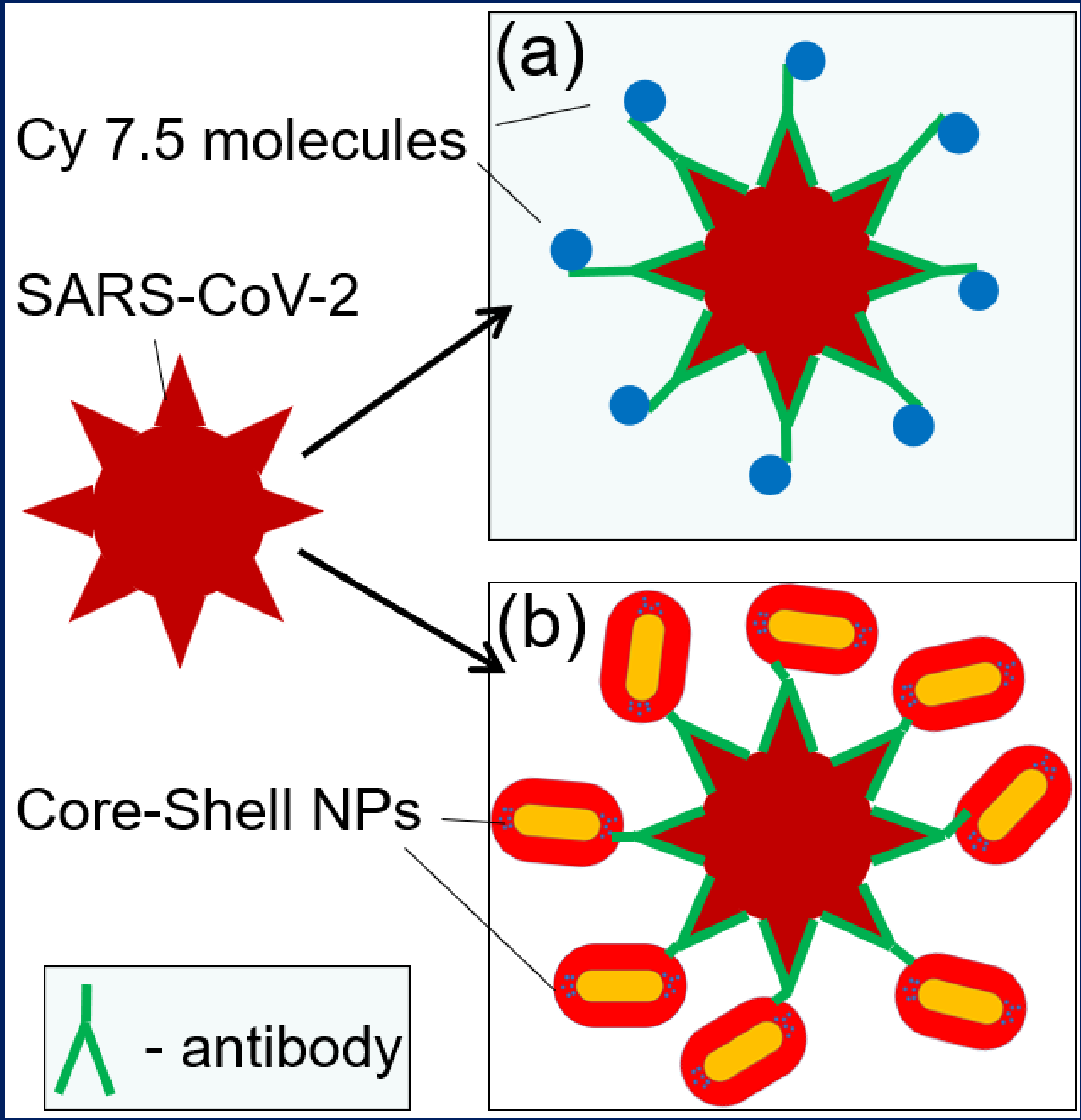
Commercially available troponin assays	99th percentile cut-off (ng/mL)	Time	Cost (USD)	Volume
CTnT				
Elecsys, third generation (Roche, Basel, Switzerland)	0.01	60 min	31,148	150 μL
cTnI				
i-STAT (Abbott Point of Care, Princeton, NJ, USA)	0.08	10 min	420	17 μL
ACS:180 (Bayer, Leverkusen, Germany)	0.07	24 h	1,200	10 mL
Dimension Vista (Siemens, Munich, Germany)	0.05	10 min	278,000	20 μL
Architect (Abbott, Princeton, NJ, USA)	0.04	10 min	19,000	62 μL

Abbreviations: CTnT, cardiac troponin T; cTnI, cardiac troponin I.

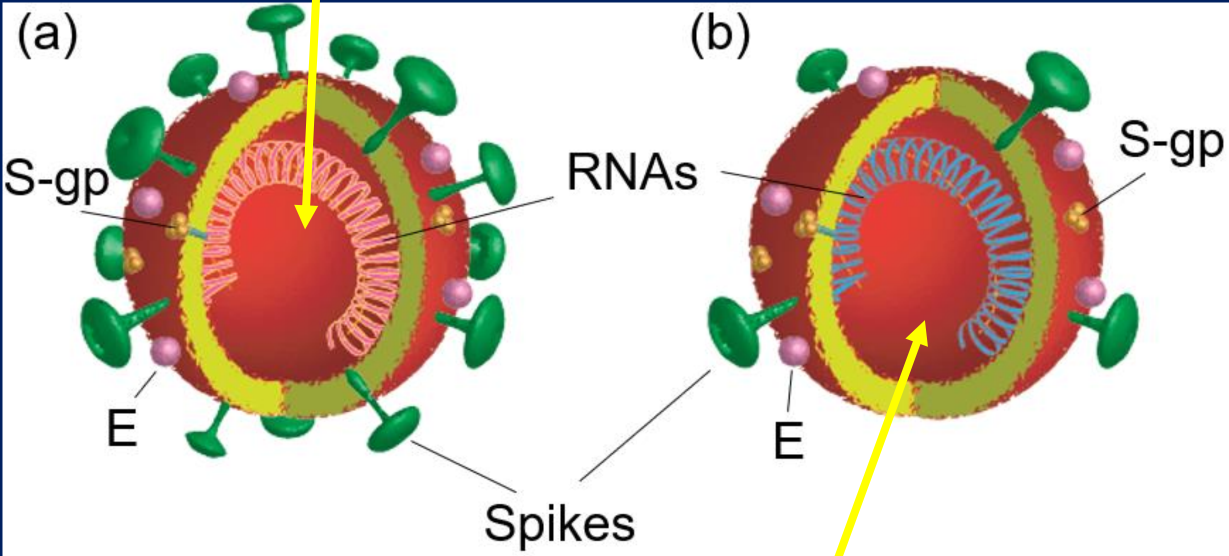
S. Upasham, A. Tanak, S. Prasad, Advanced Health Care Technologies 2018:4 1–13

Detection of SARS-CoV-2 virions

Two strategies for SARS-CoV-2 single virions detection

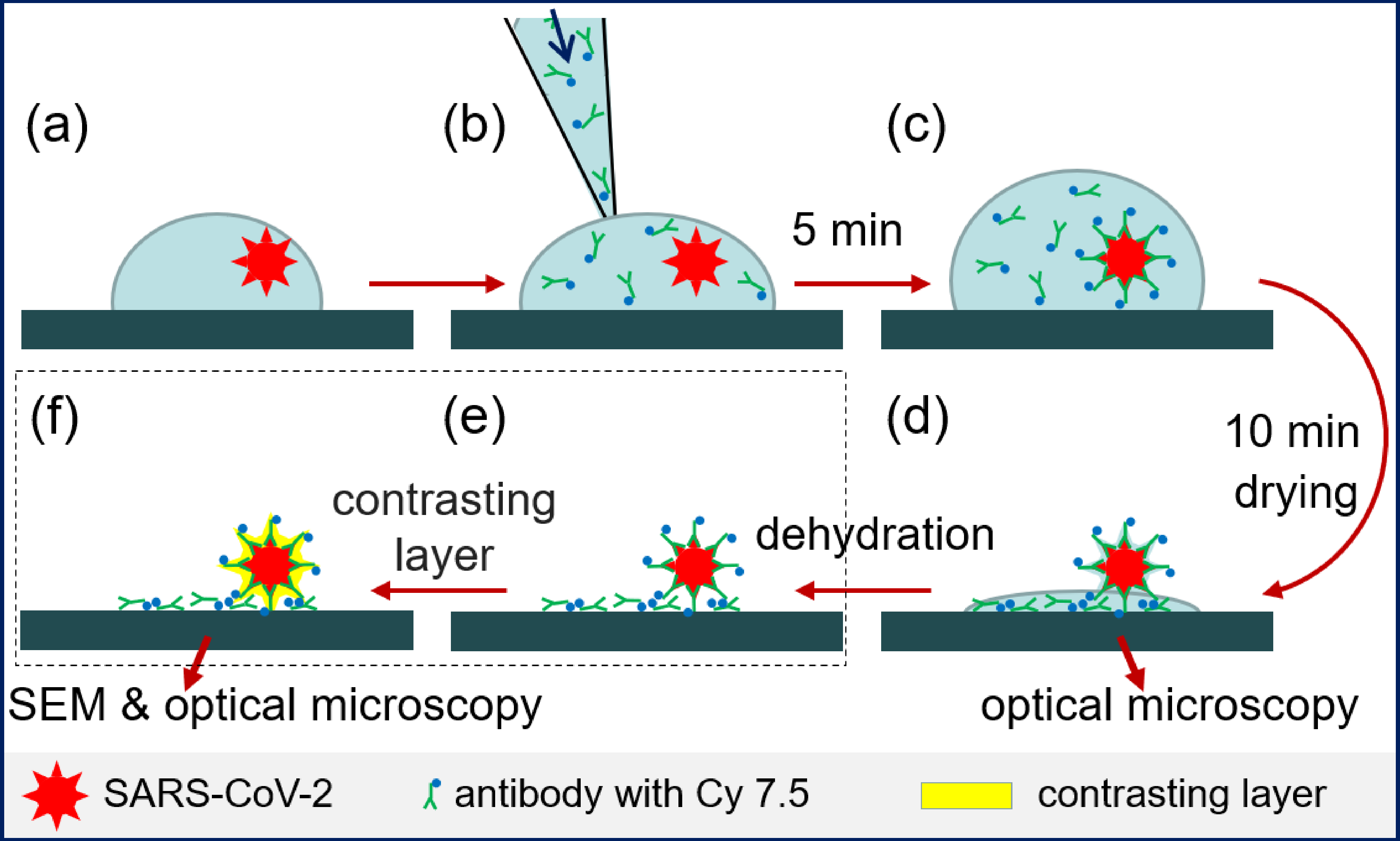


Viral particle



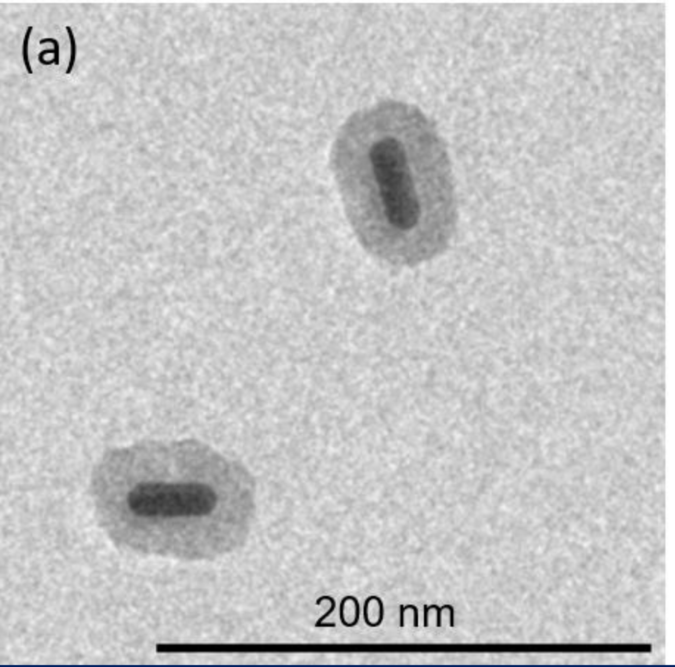
Pseudo-viral particle (not harmful)

Making samples for SARS-CoV-2 single virions detection



D. Kudryavtsev et al. Nature Photonics, submitting (2022)

SARS-CoV-2 virions detection with use of hubrid plasmonic nanoparticles (nanoscale mesoscopic systems)

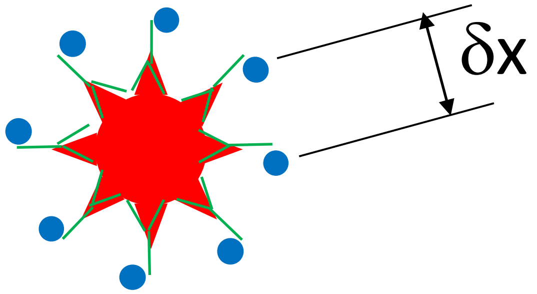


P. Melentiev et al. Nature Nanotechnology, submitting (2022)

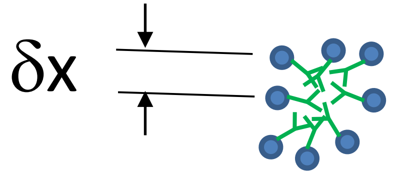
Viral particles vs aggregates of antibodies

- antibody + - fluorescent dye = - Marked antibody

SARS-CoV-2



Aggregate of antibodies



δx

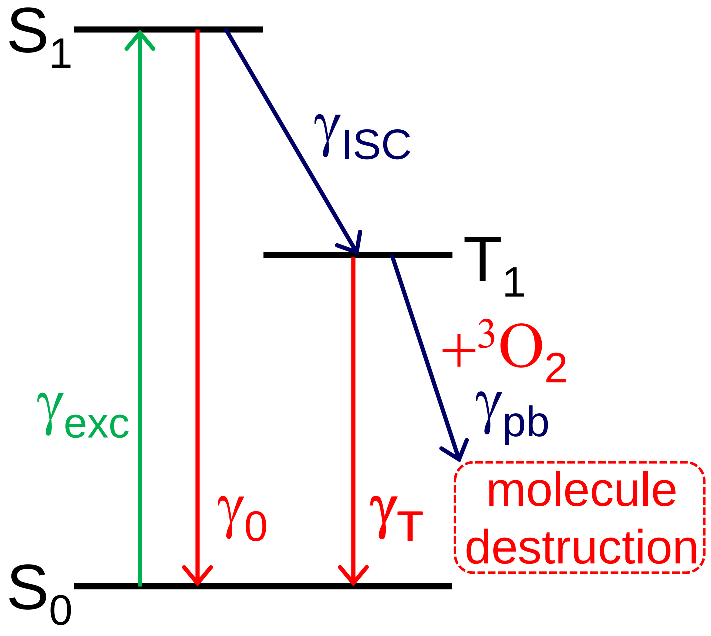
- Energy transfer
- Fluorescence efficiency
- Kinetics of pumping to the triplet sublevel

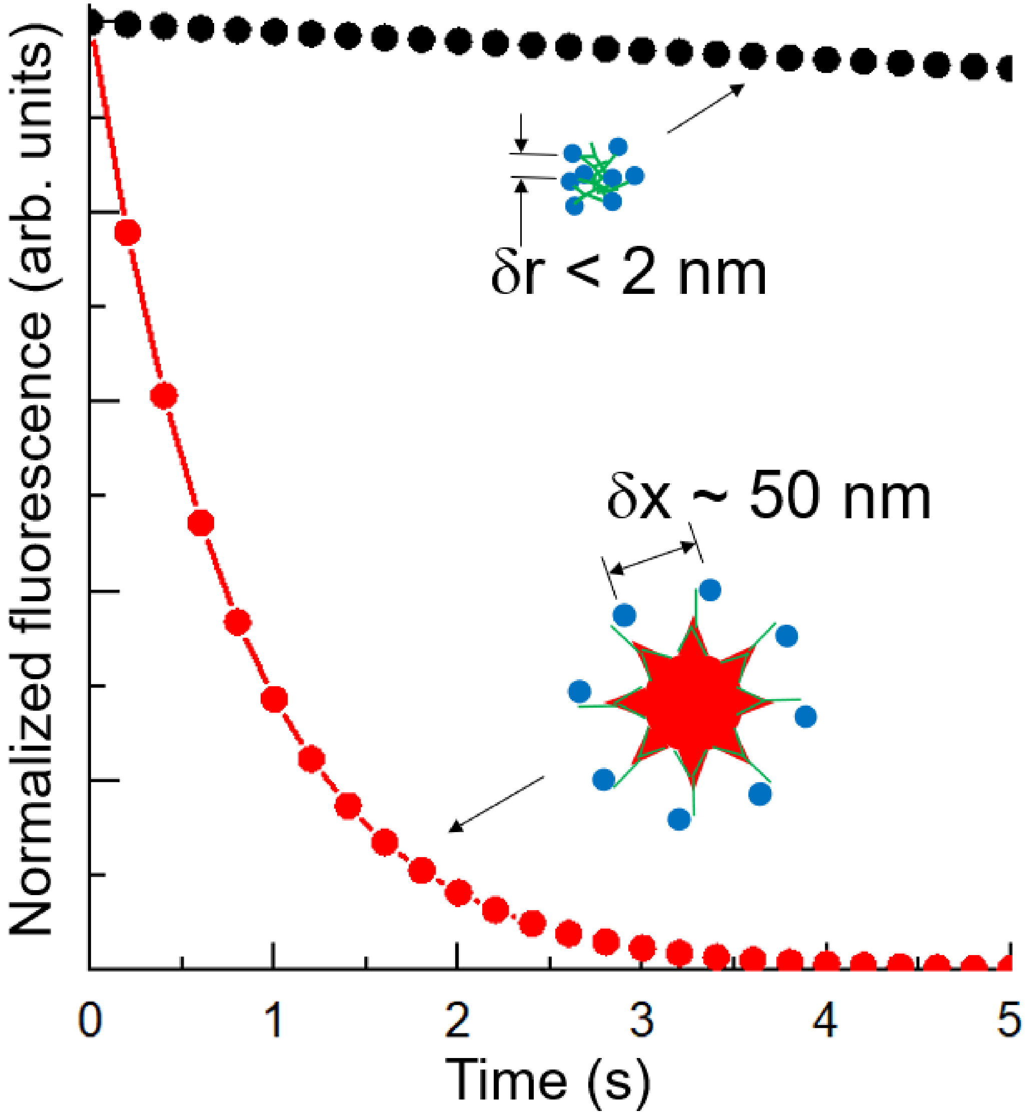
(1) Total flux of photons

(2) Kinetics of photoluminescence degradation

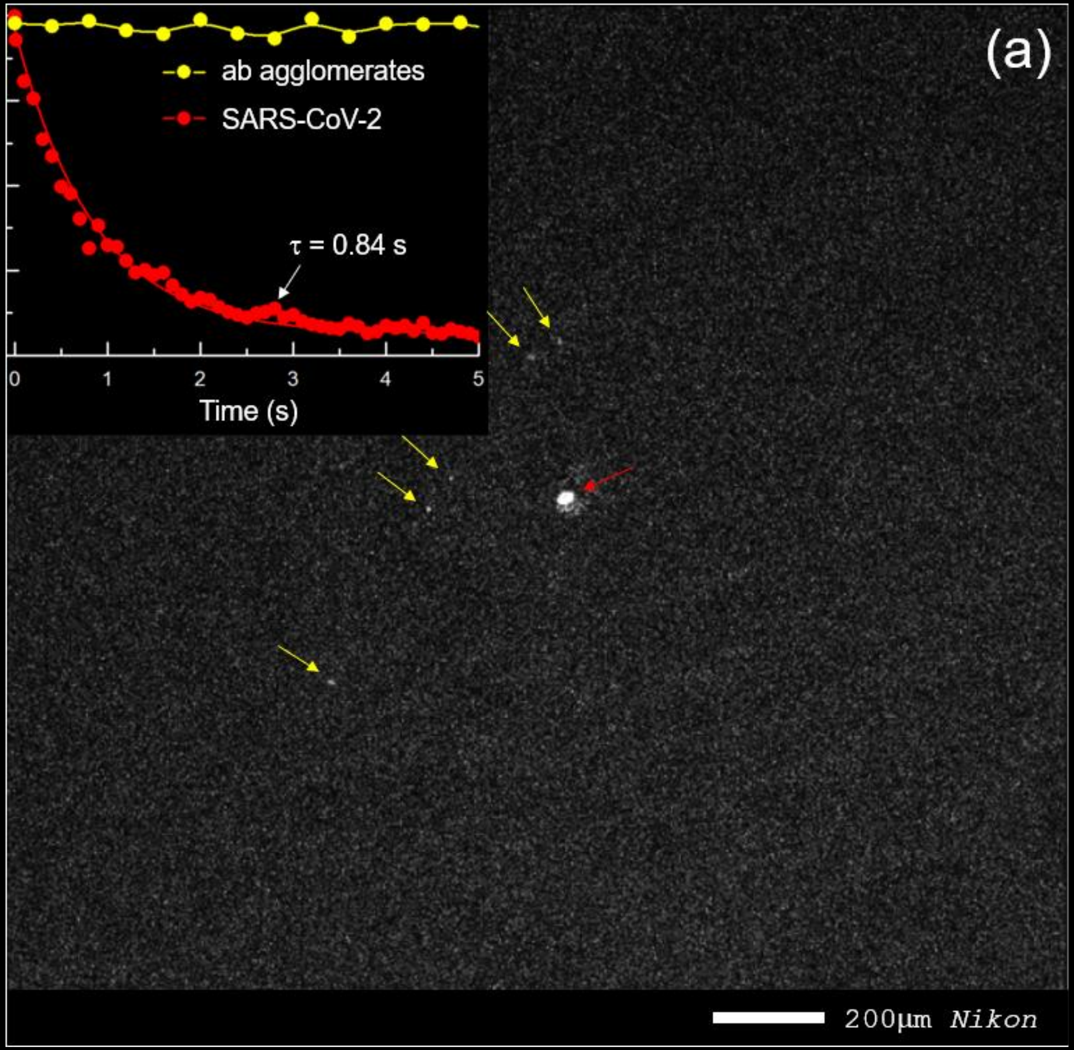
Viral particles vs aggregates of antibodies

- antibody + - fluorescent dye = - Marked antibody





Detection of SARS-CoV-2 virions directly in a swab



D. Kudryavtsev et al. Nature Photonics, submitting (2022)

4. Quantum engineering of radiative properties of a nanoscale emitters of light

Outline

- We show that it is possible to manipulate by the emission rate of fluorescent molecules in such systems by carefully designing the energy levels of a specific system formed using a strongly coupled dye molecules in the shell and a plasmonic nanoparticle.
-
-