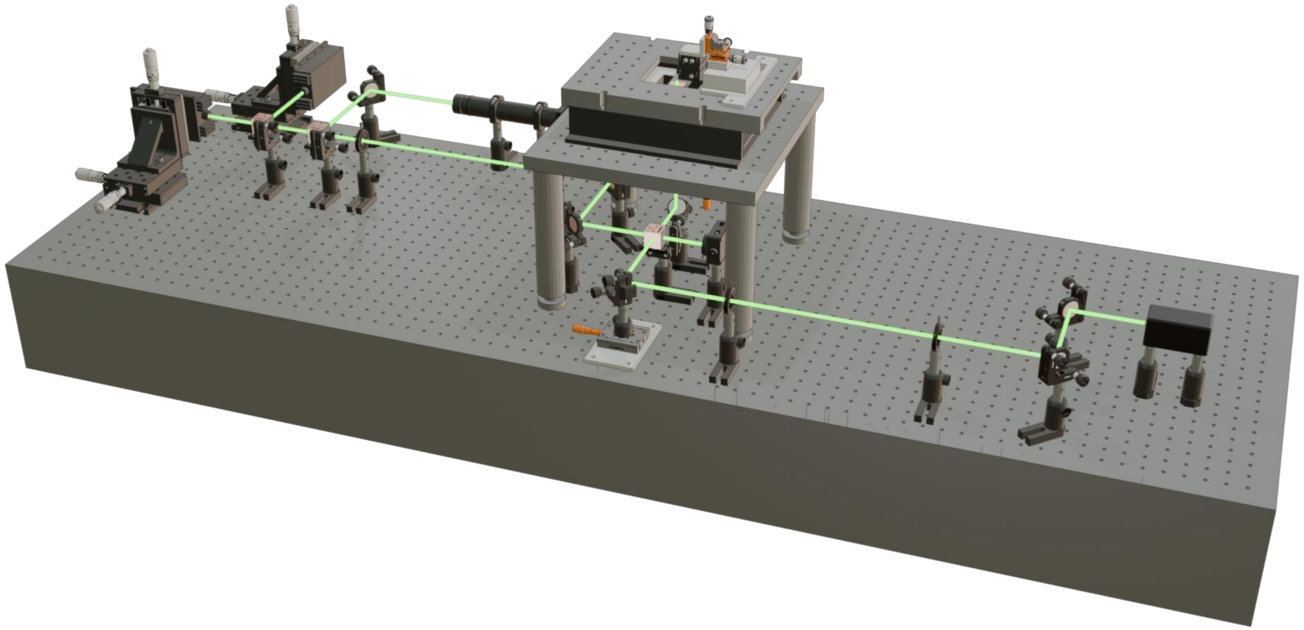
How to build a « Home-made » near-field optical microscope to observe single molecules

Simon Vassant (LEPO)

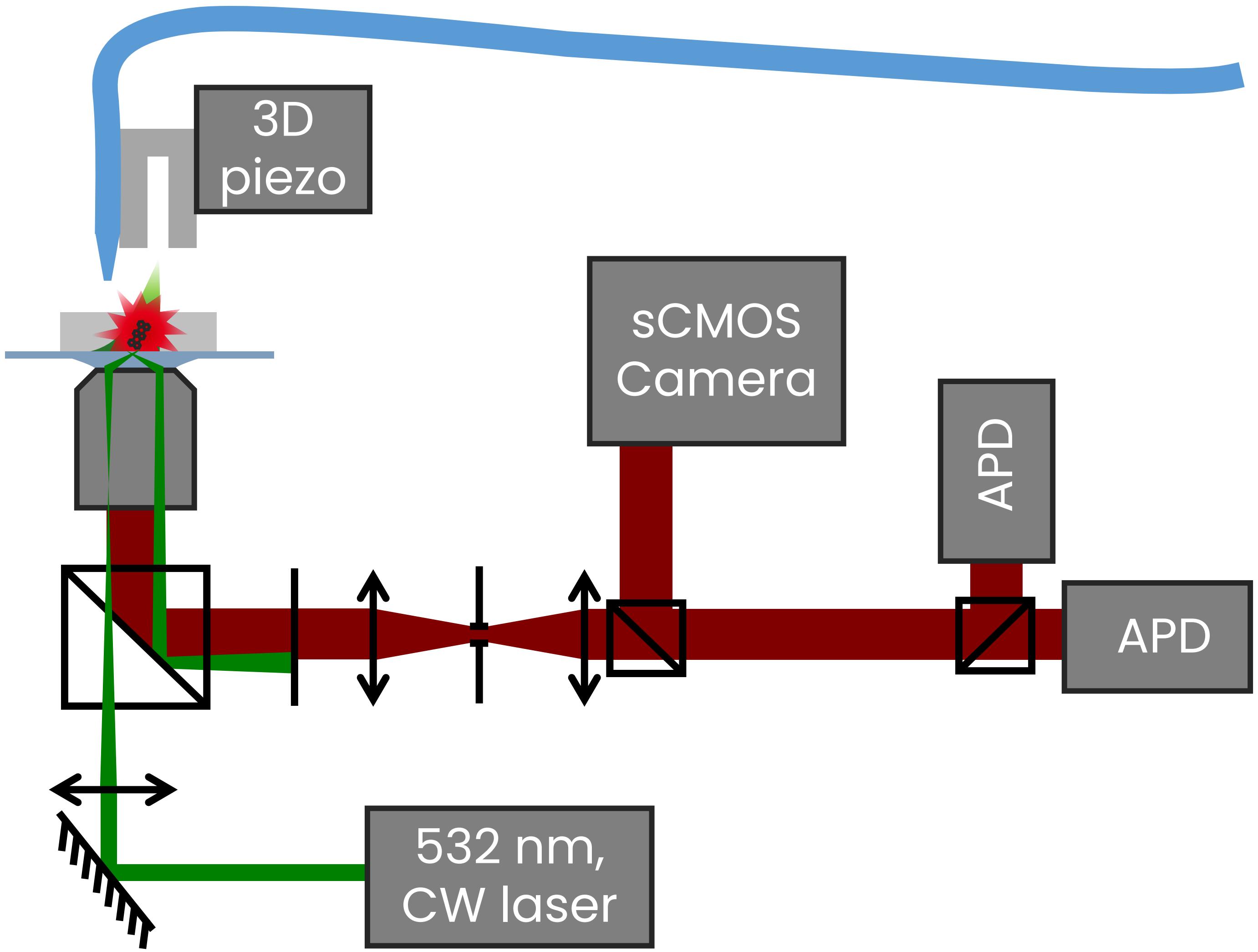
Remigiusz Trojanowicz (PhD 2023)



Projet ANR JCJC PlasmonISC

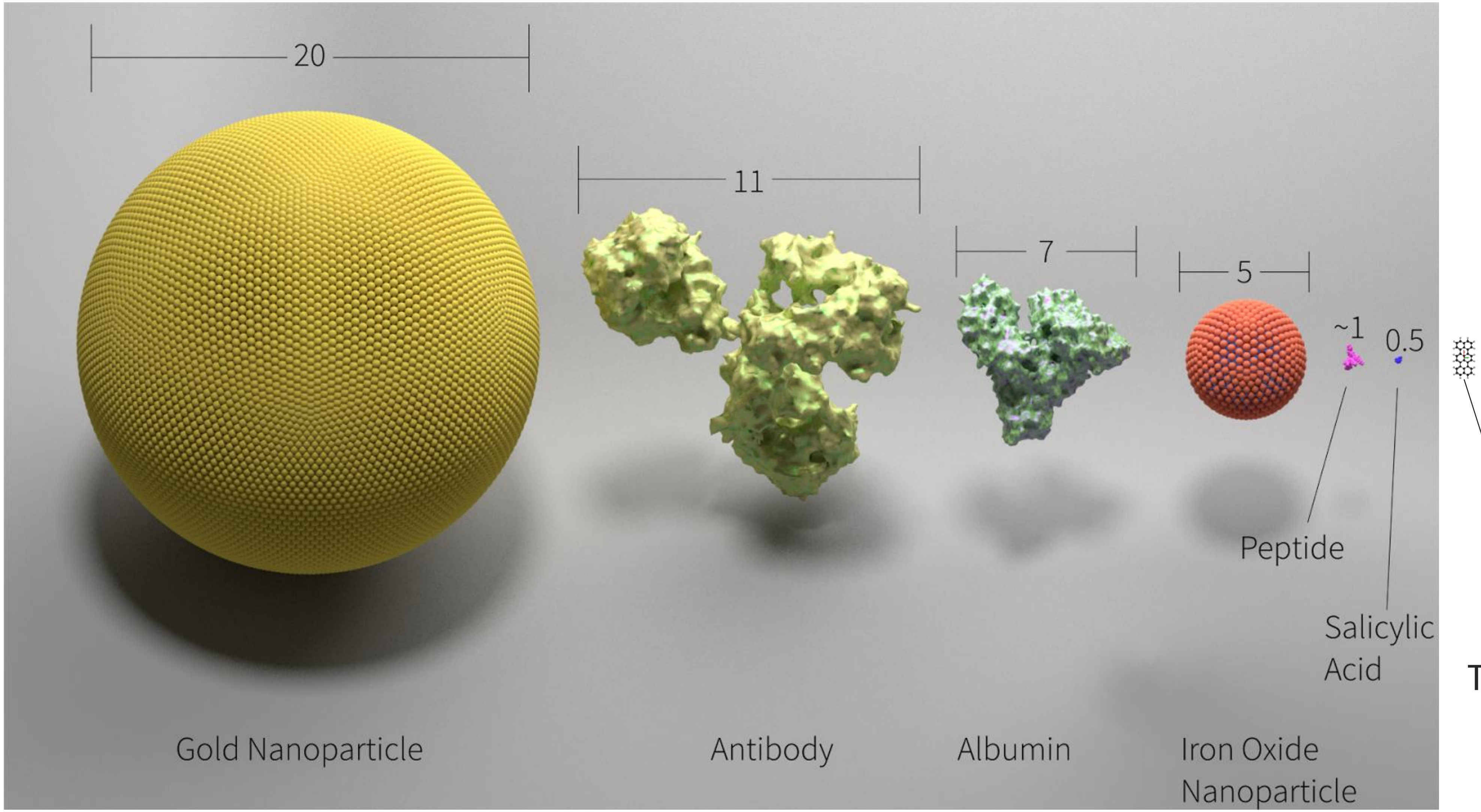
Outline

1. **Motivations**
2. **Optical Nano-antennas**
3. **Coupling to optics**
4. **Setting up a single molecule microscopy setup**
5. **Single Molecule measurements**

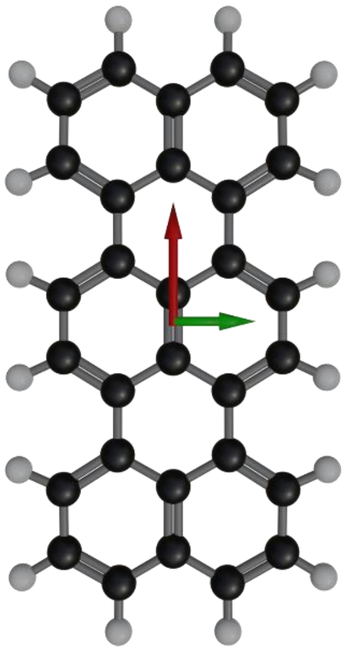


1. Motivations

Motivations



Terrylene

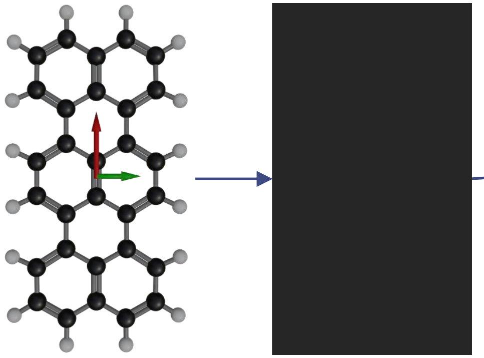


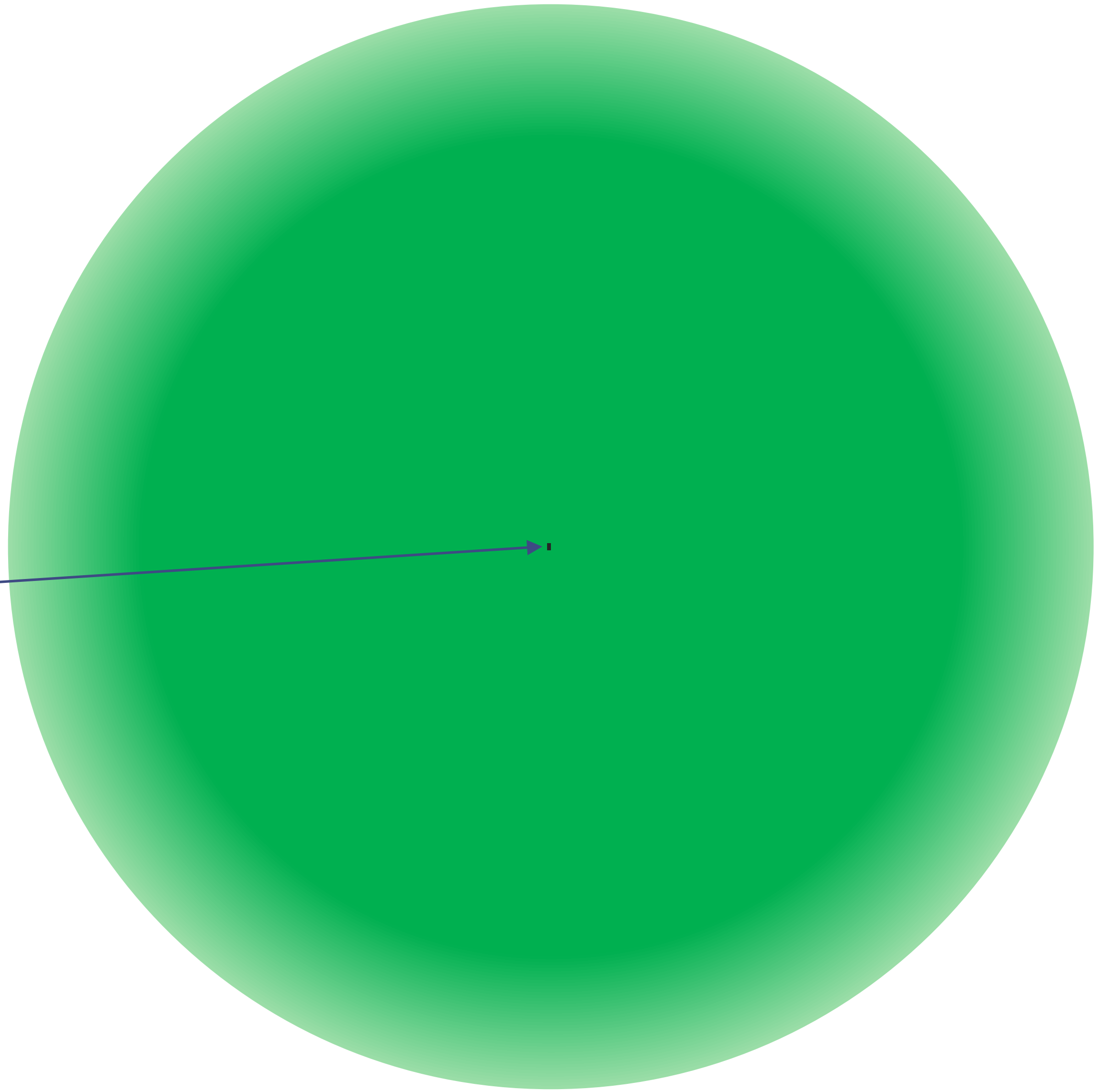
https://crowsandcats.blogspot.com/2013/04/visualizing-size-in-biochemical-systems.html

Motivations

Overcome the size mismatch

λ = 532 nm
NA = 1.4
FWHM ≈ $\lambda/2NA$ = 190nm





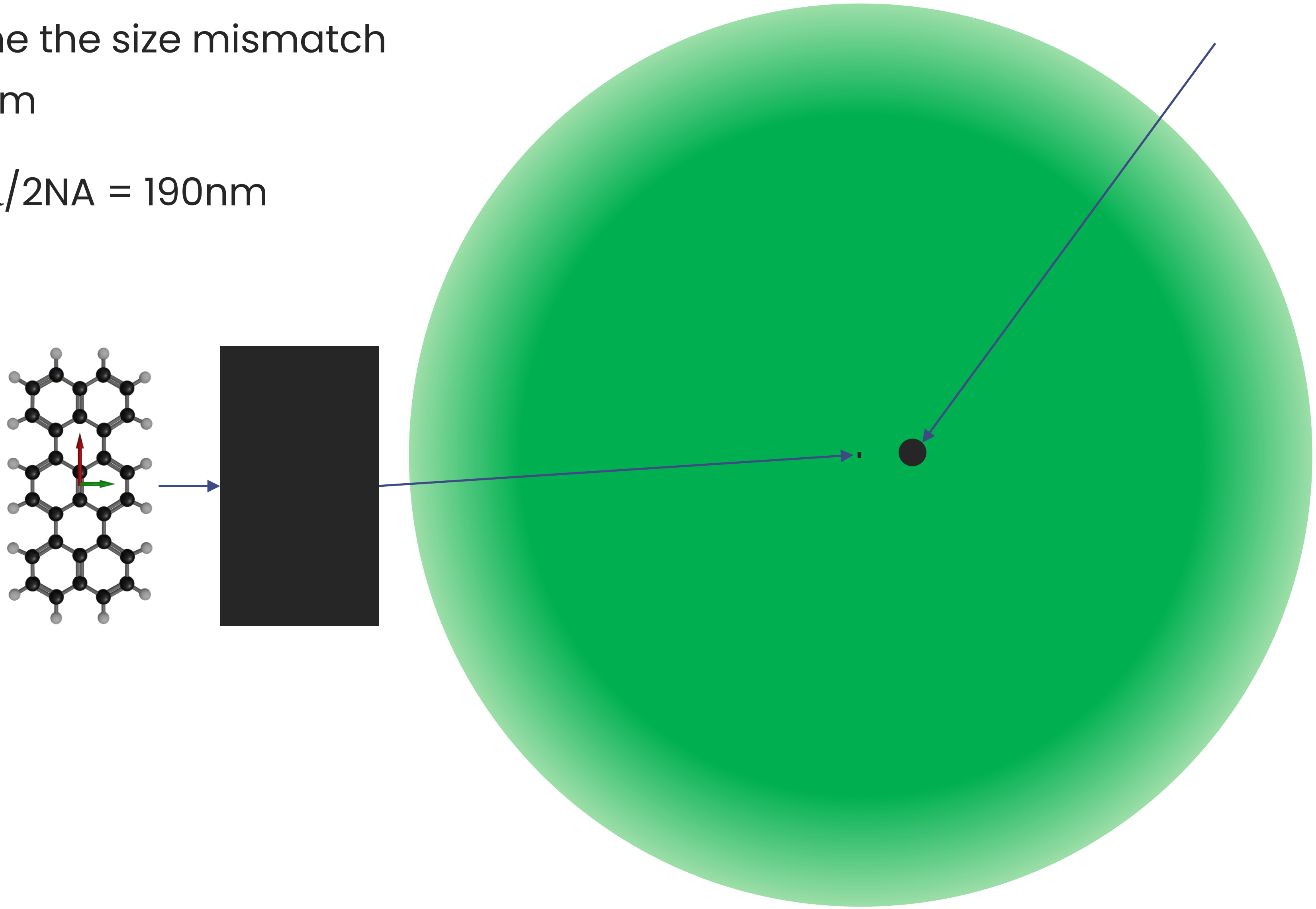
Motivations

Overcome the size mismatch

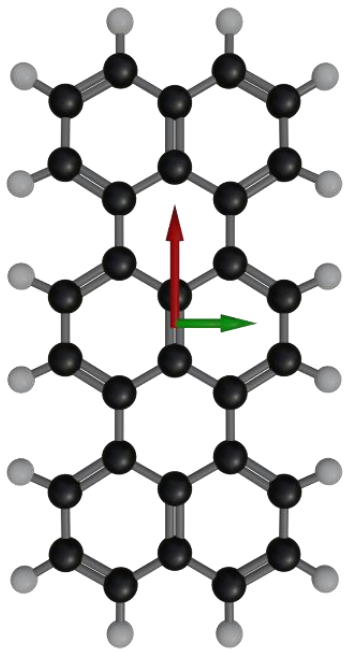
λ = 532 nm
NA = 1.4
FWHM ≈ $\lambda/2NA$ = 190nm

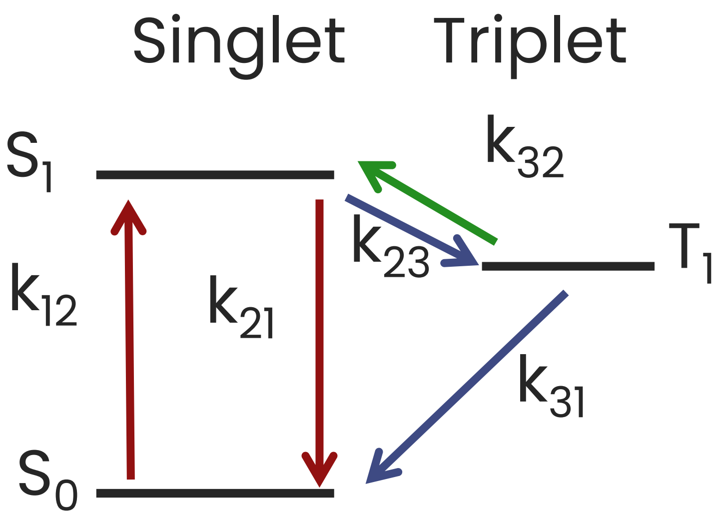
Room temperature absorption cross-section

σ_{abs}= 1.2 x 10^{-16} cm^2

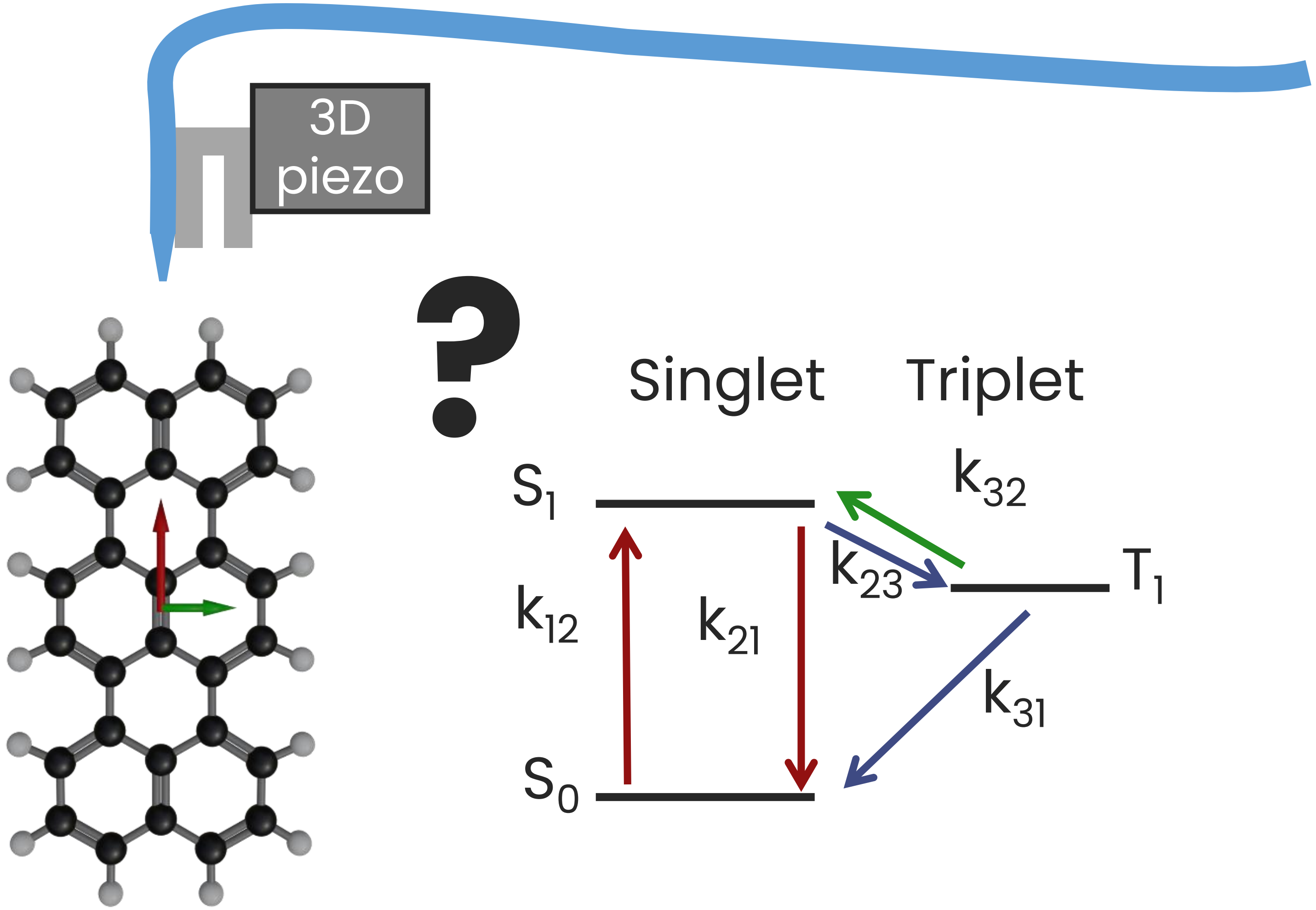


Motivations





Motivations

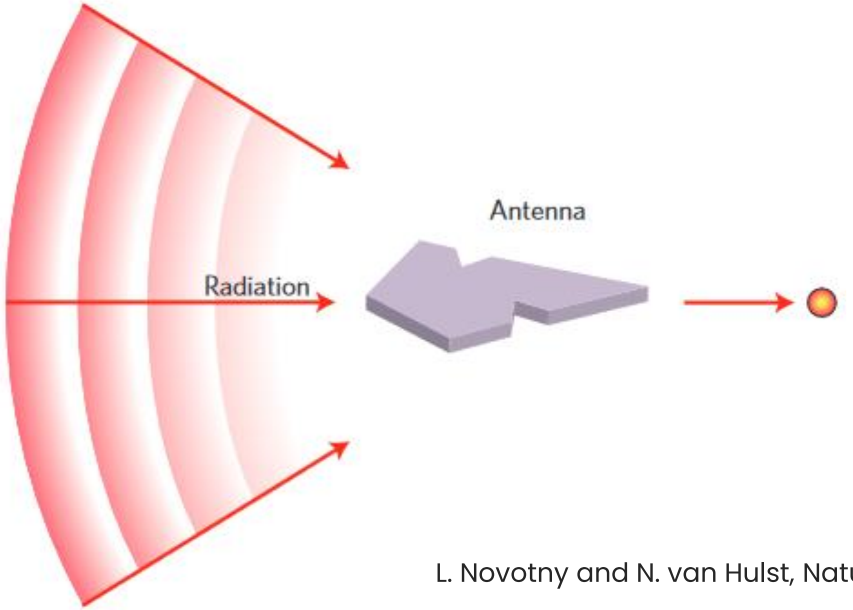


2. Optical Nano-antennas

Optical Nano-antennas

Use antennas to focus light to subwavelength dimensions :

- Dielectric antennas
- Plasmonic antennas
- Electric modes
- Magnetic modes



L. Novotny and N. van Hulst, Nature Nanophotonics 5, 83 (2011)

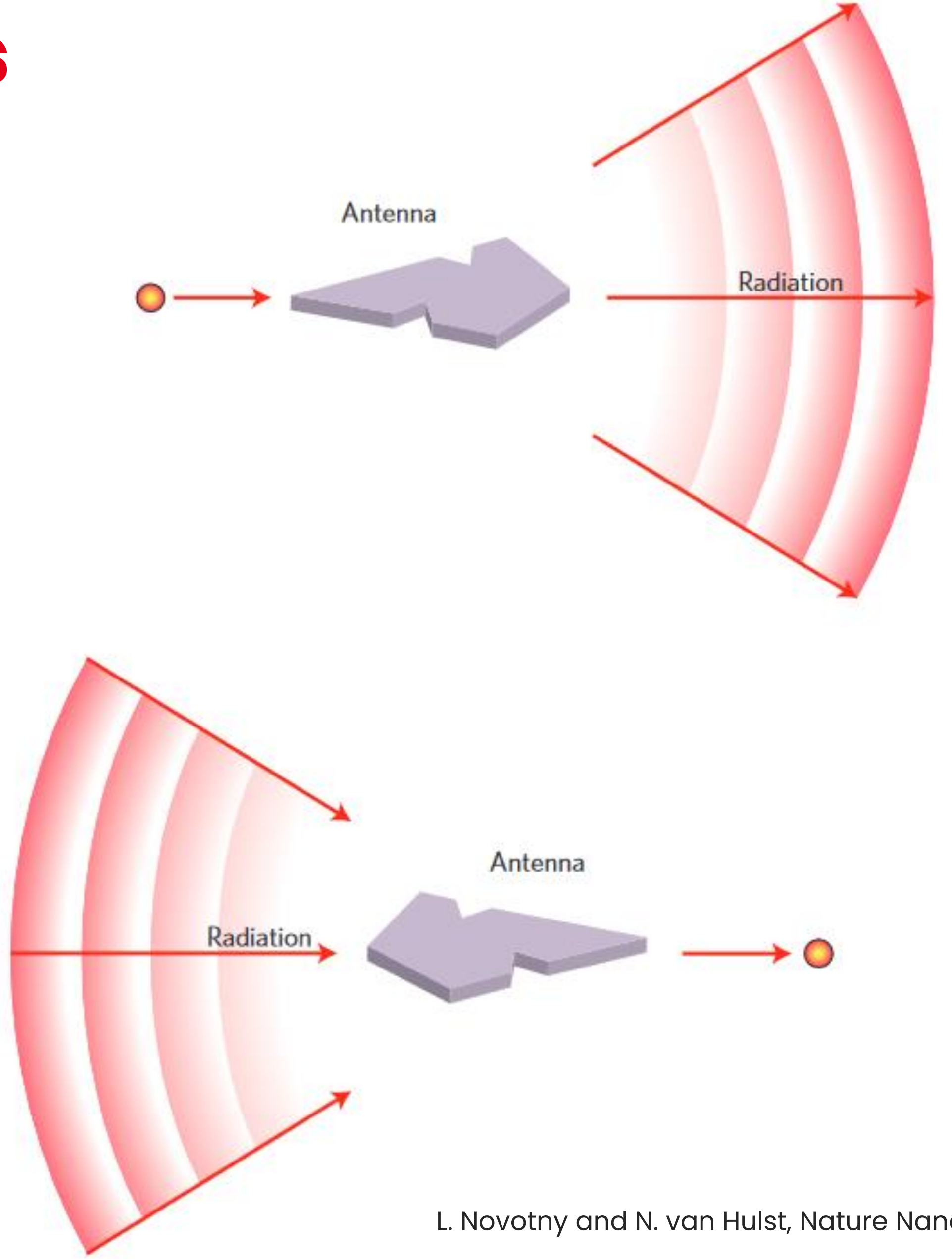
Optical Nano-antennas

Use antennas to focus light to subwavelength dimensions :

- Dielectric antennas
- Plasmonic antennas
- Electric modes
- Magnetic modes

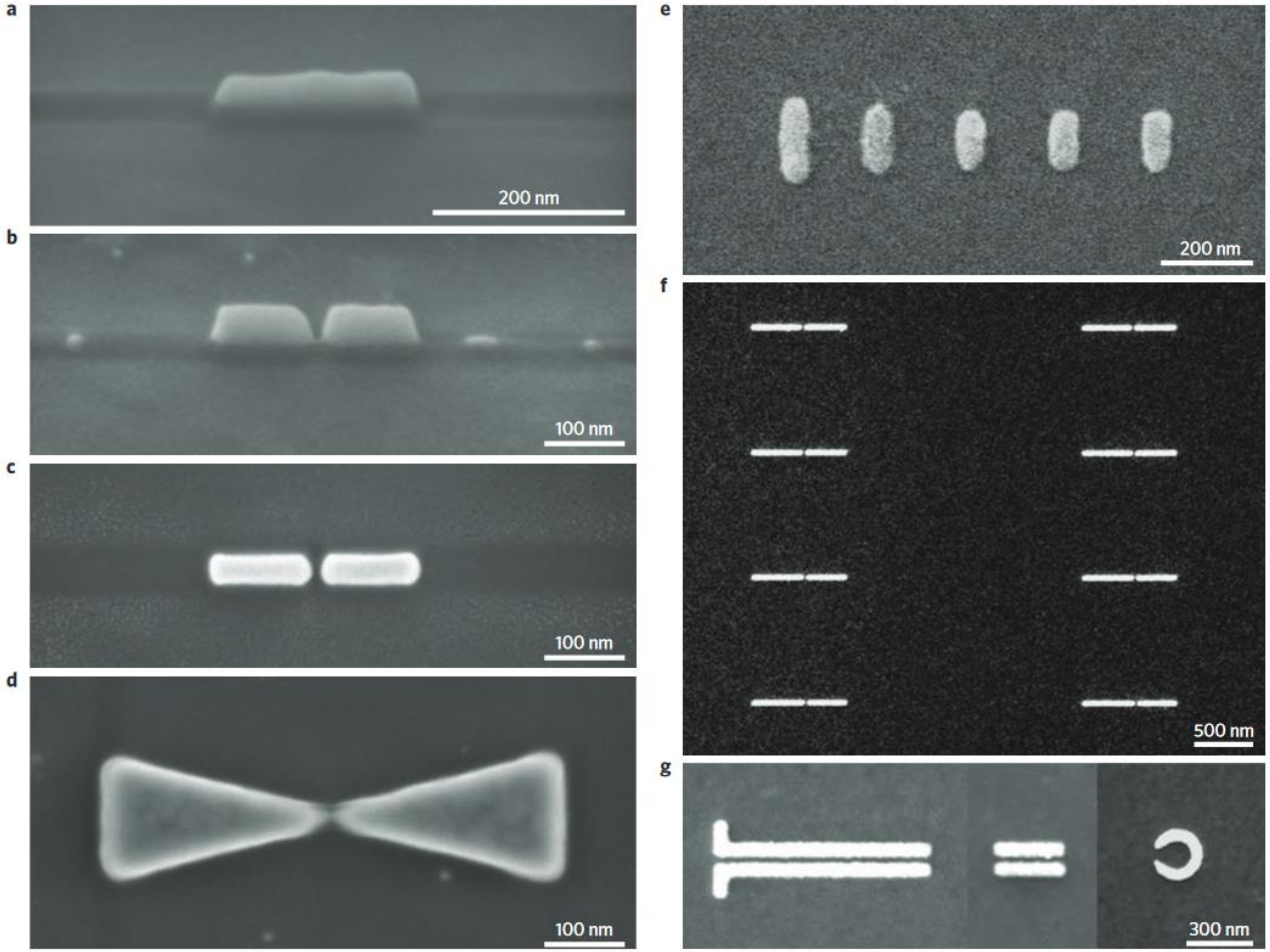
Use antennas to out-couple light

- near-field components of light
- Tailor emission direction
- Increase the local density of optical states
- Modify the photophysics of emitters



L. Novotny and N. van Hulst, Nature Nanophotonics 5, 83 (2011)

Optical Nano-antennas

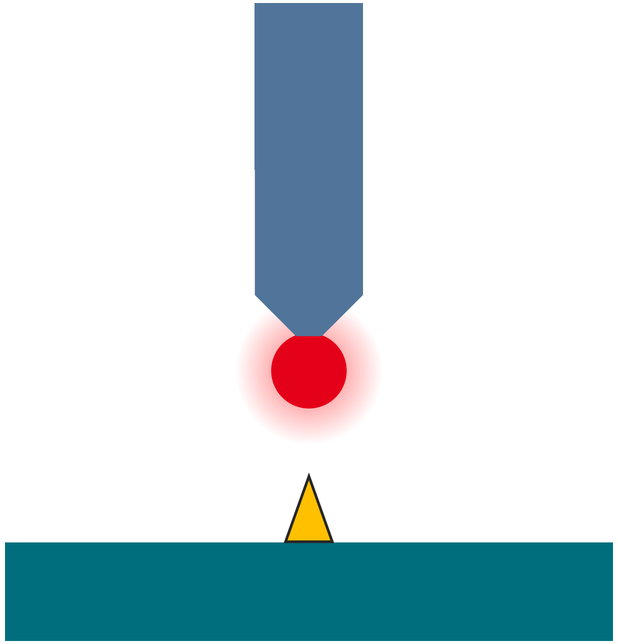


L. Novotny and N. van Hulst, Nature Nanophotonics 5, 83 (2011)

Single emitter coupled to single antenna : two approaches

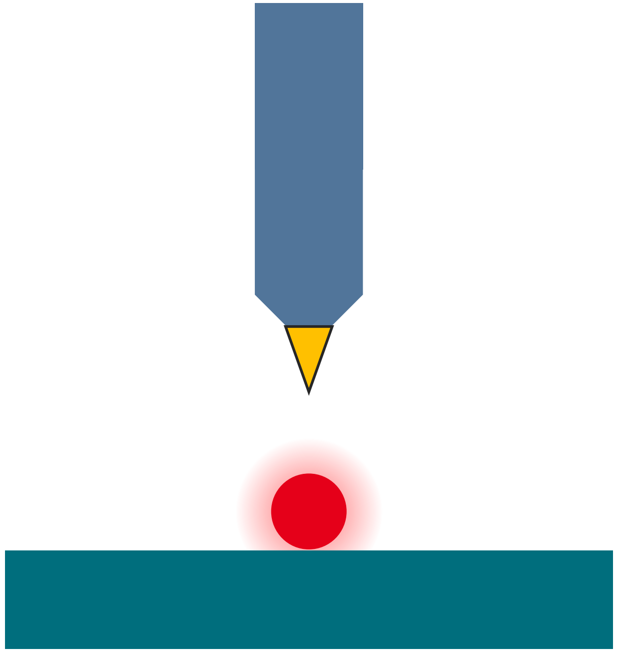
Emitter on tip

Antenna on substrate



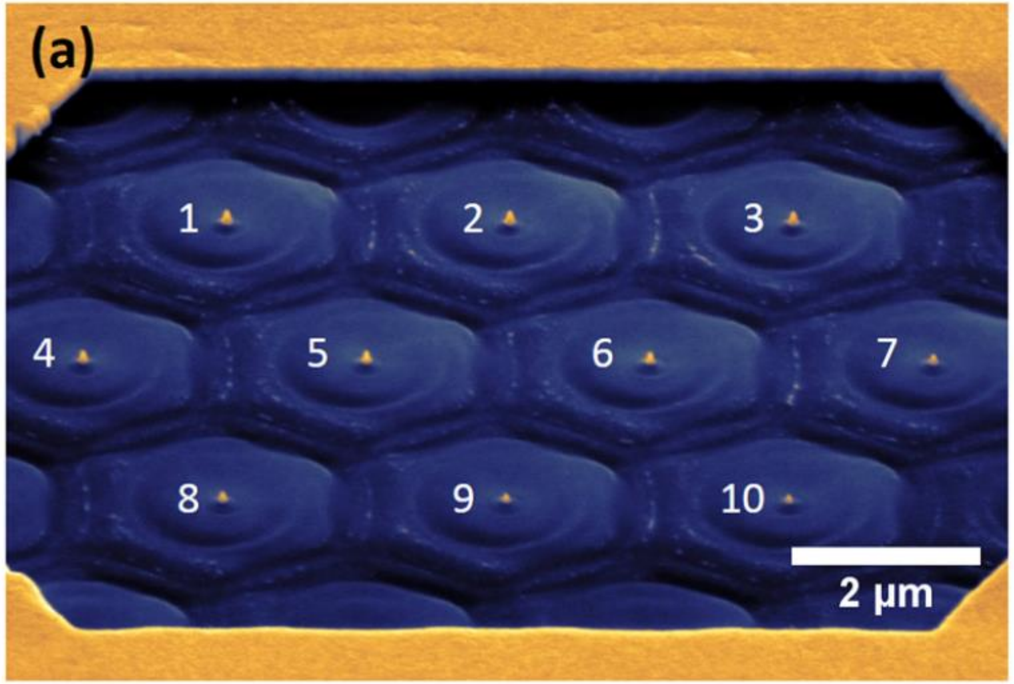
Antenna on tip

Emitter on substrate

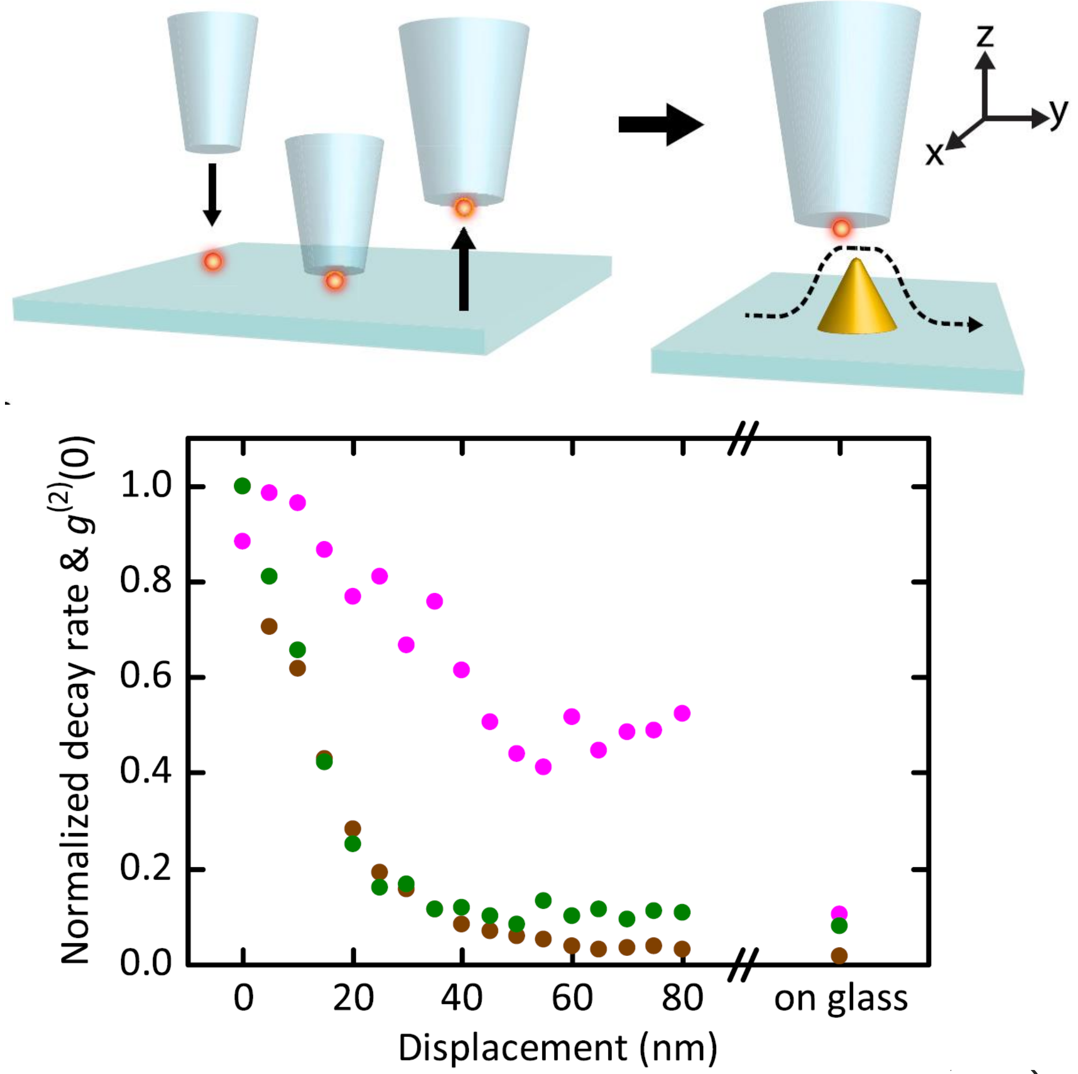


Quantitative measurements

Optical Nano-antennas (on substrates)



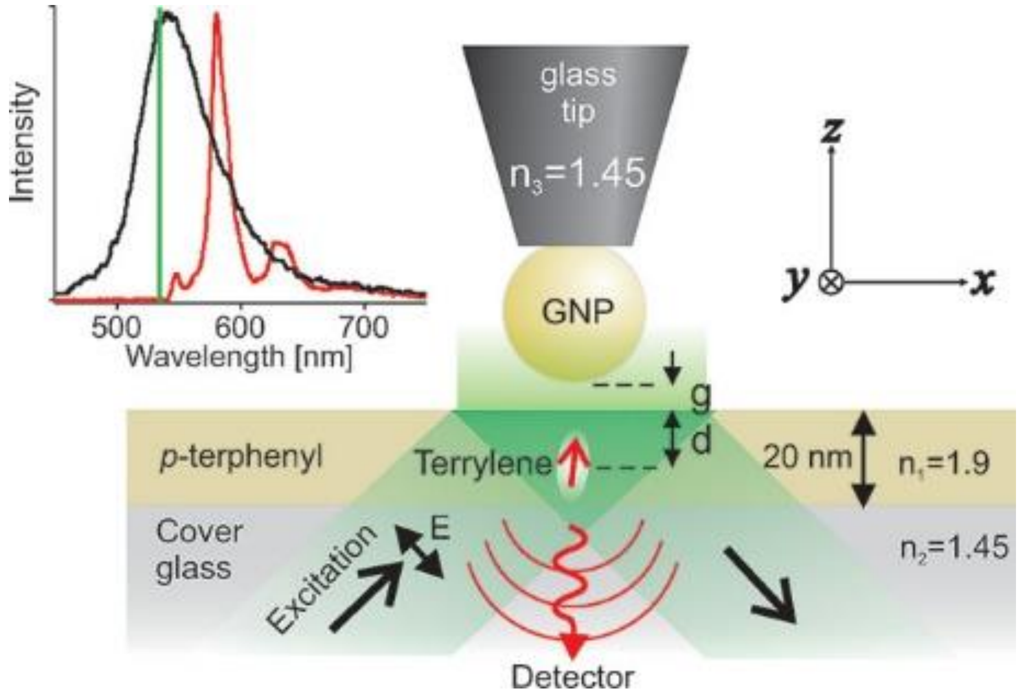
B. Hoffman, S. Vassant et al. Nanotechnology 26, 404001 (2015)

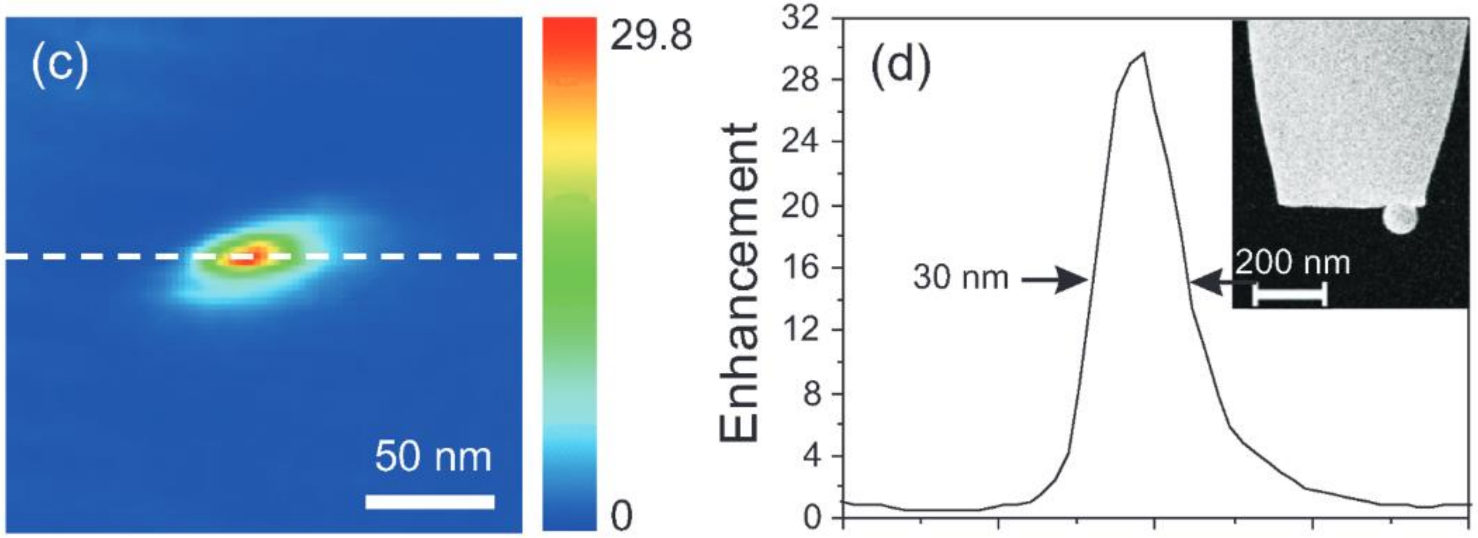


K. Matsuzaki, S. Vassant et al. Scientific Reports 7, 42307 (2017)

Optical Nano-antennas (on tip)

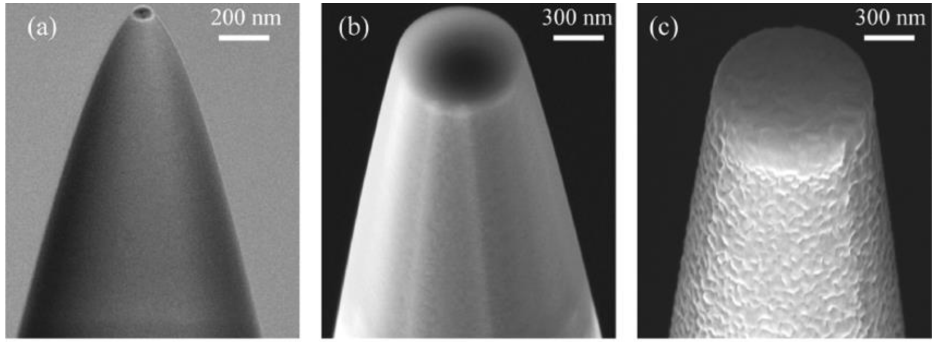
Picking-up colloïdal nanoparticles

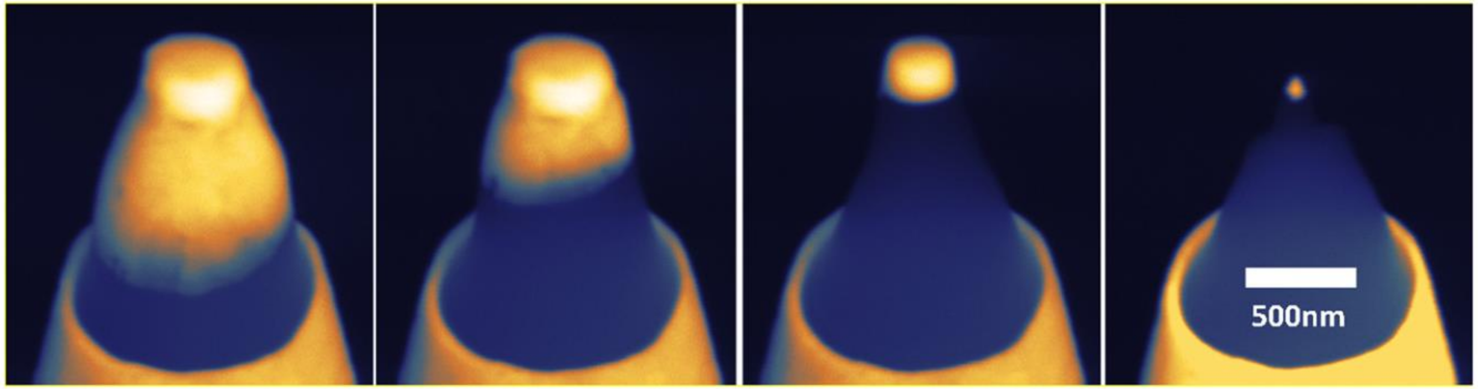




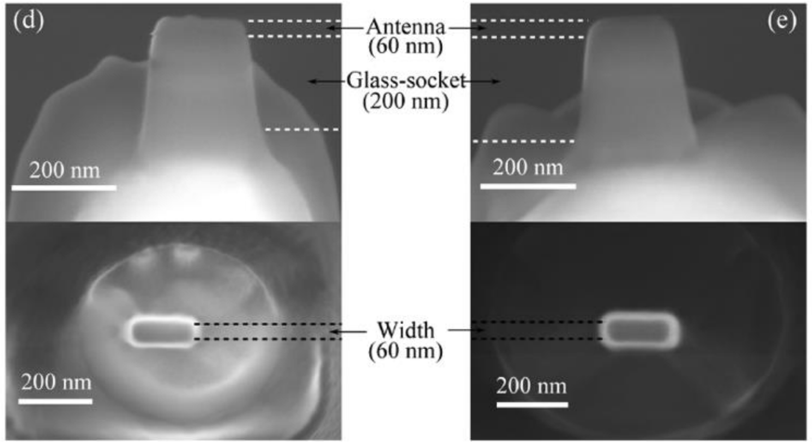
S. Kühn et al, PRL 97, 017402 (2006)
K.G. Lee et al, Nanoletters 9(12) 4007 (2009)

Optical Nano-antennas (on tip)

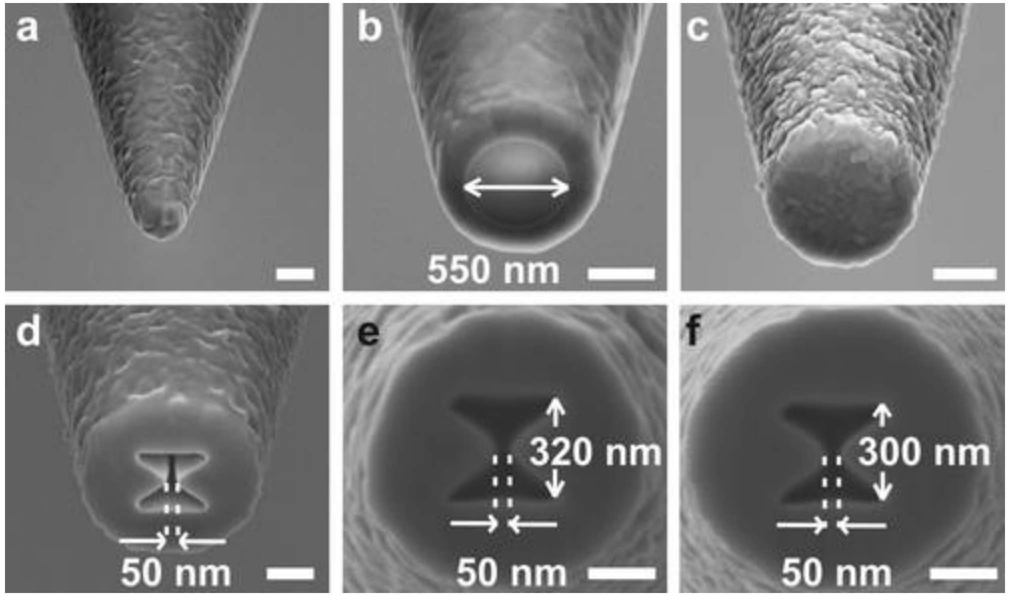




B. Hoffman, S. Vassant et al. Nanotechnology 26, 404001 (2015)

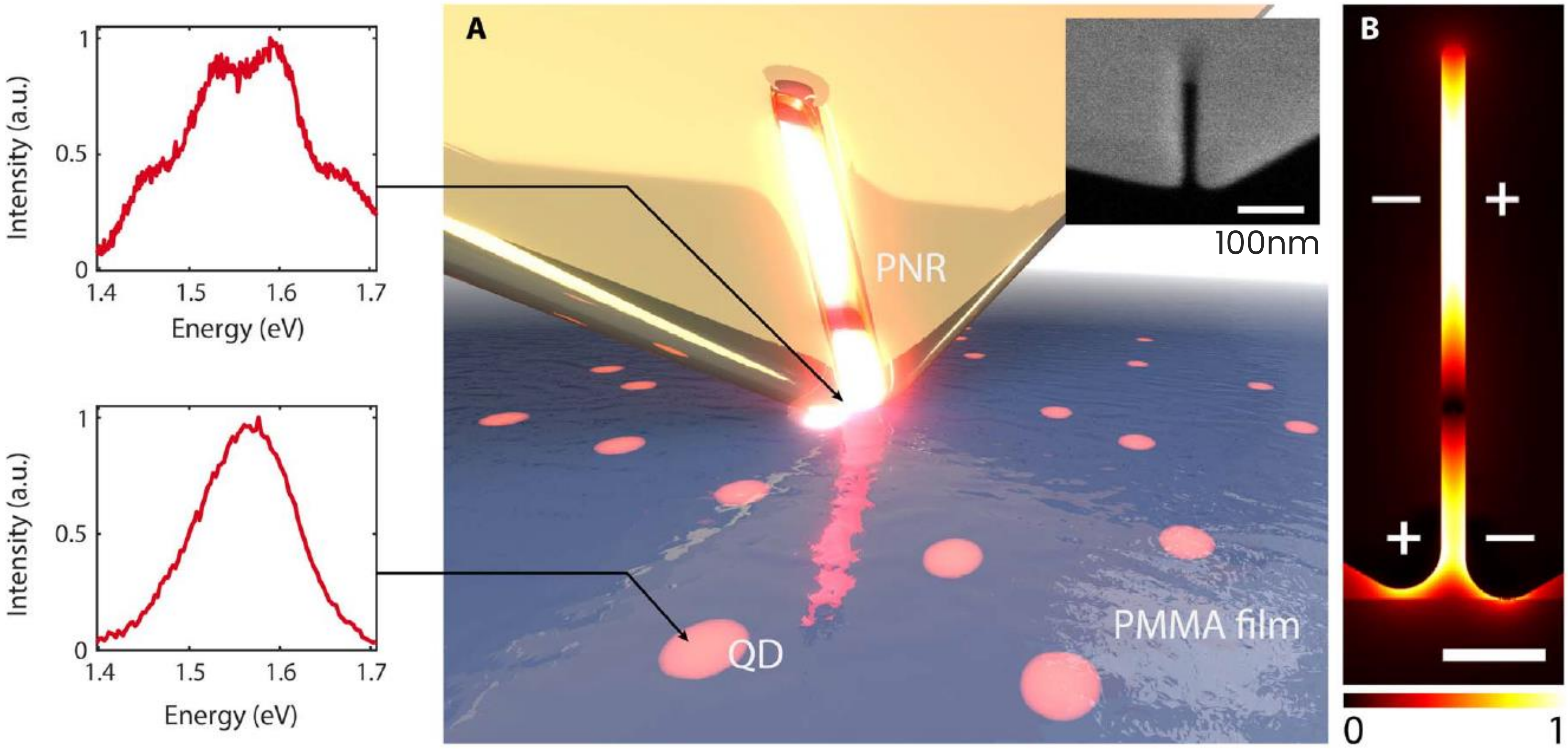


Singh et al, Nanoletters 14 (8) 4715 (2014)



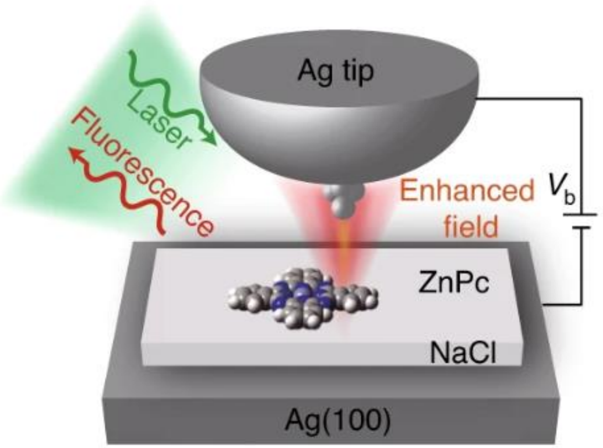
M. Mivelle et al., Nanoletters 12(11), 5972 (2012)

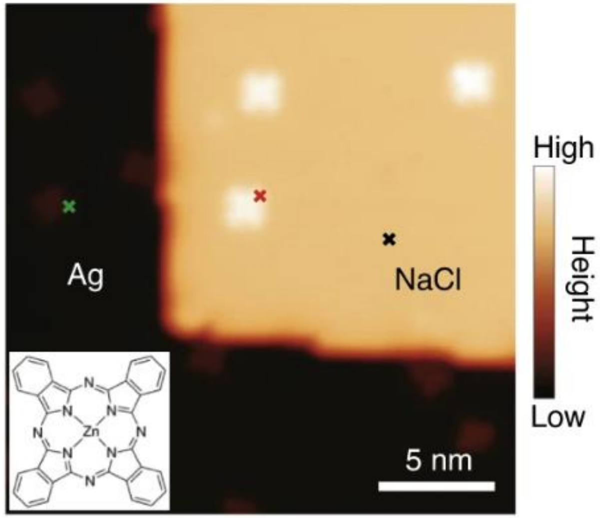
Optical Nano-antennas (on tip)

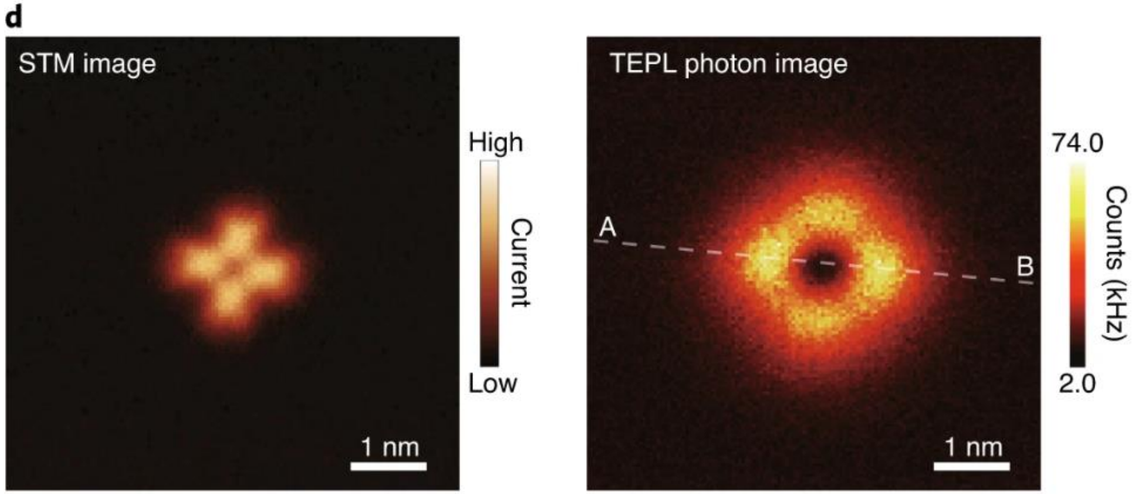


H. Gross et al. Science Advances 4, 4906 (2018)

Sub-molecular resolution with pico-cavities



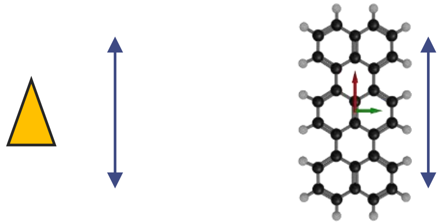




B. Yang et al. Nat. Photonics 14, 693–699 (2020)

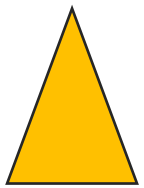
A good antenna for single photon emitters

Match the dipolar nature of the dipole



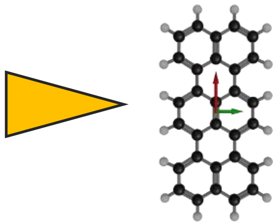
Get the proper size

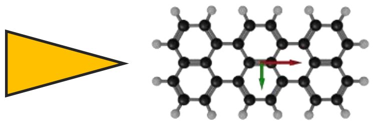
Too much absorption
Low scattering



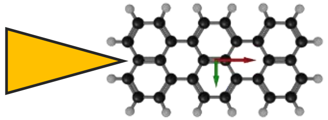
Multipolar resonances
Lower field confinement

Get the right orientation

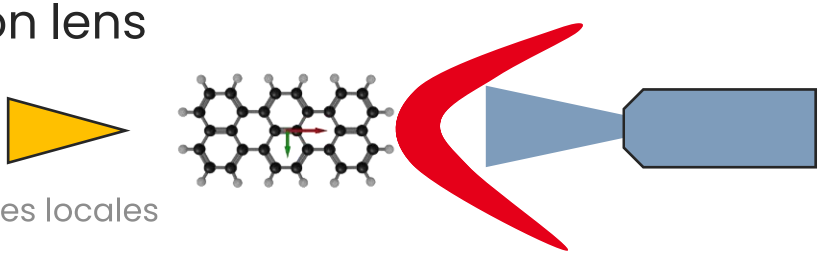




Get the right distance

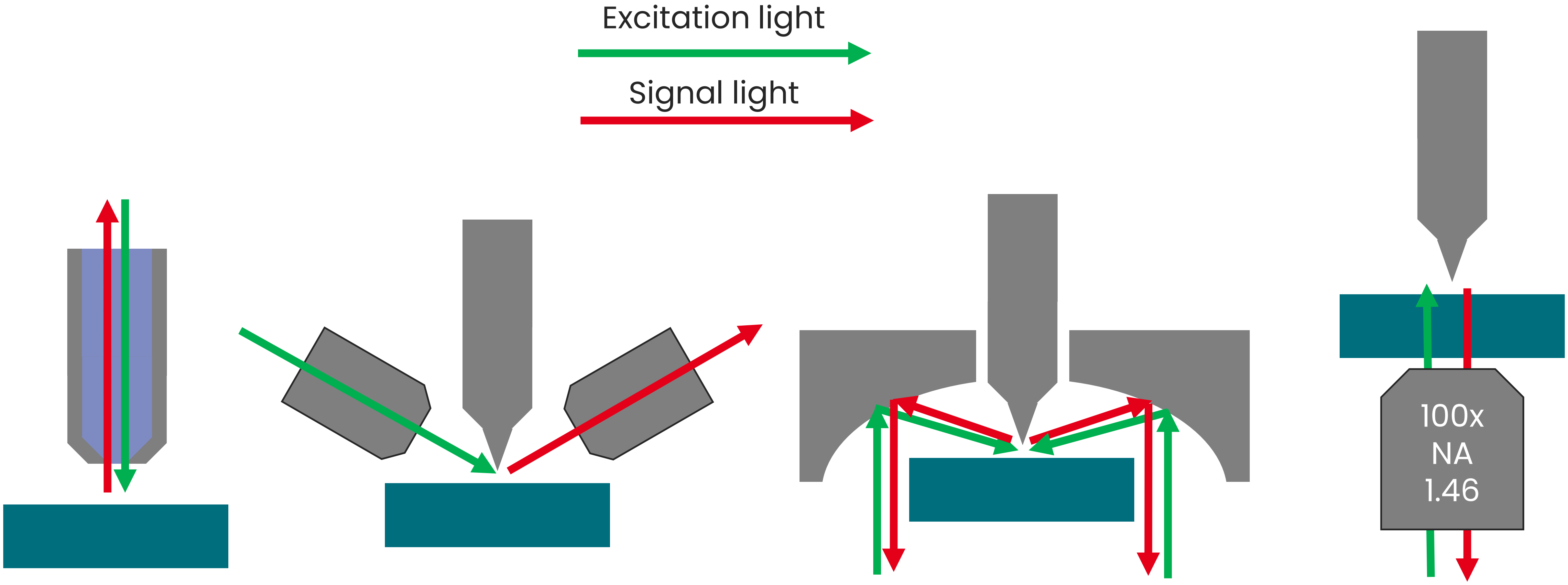


Get the radiation pattern fit the collection lens



3. Coupling to optics

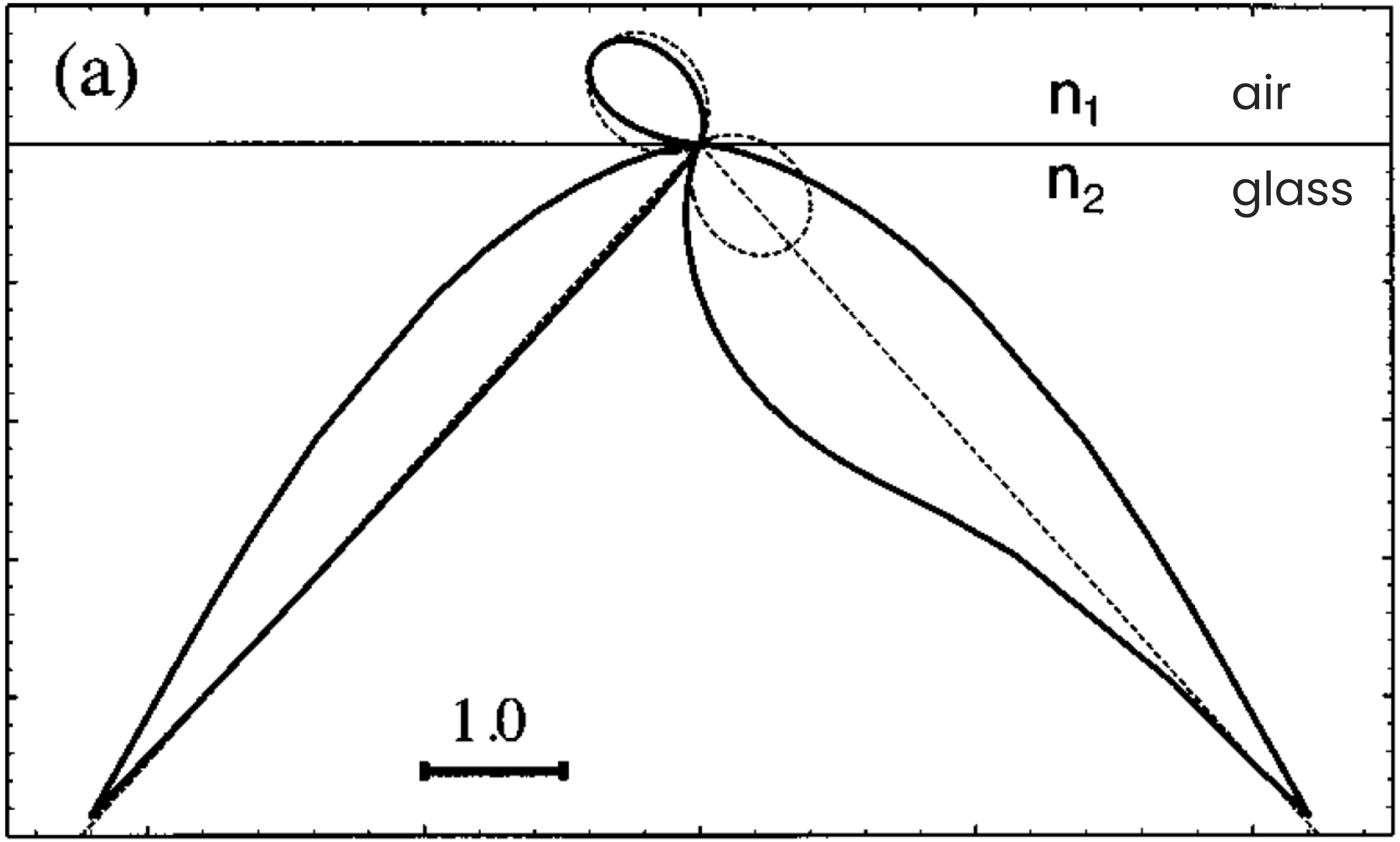
Scanning probe optical configuration



D. Pohl, Patent 1982
EP82111974A·1982-12-27

M. Lieb and A. Meixner, Optics Express 8, 458 (2001)

Dipole emission diagram at a dielectric interface



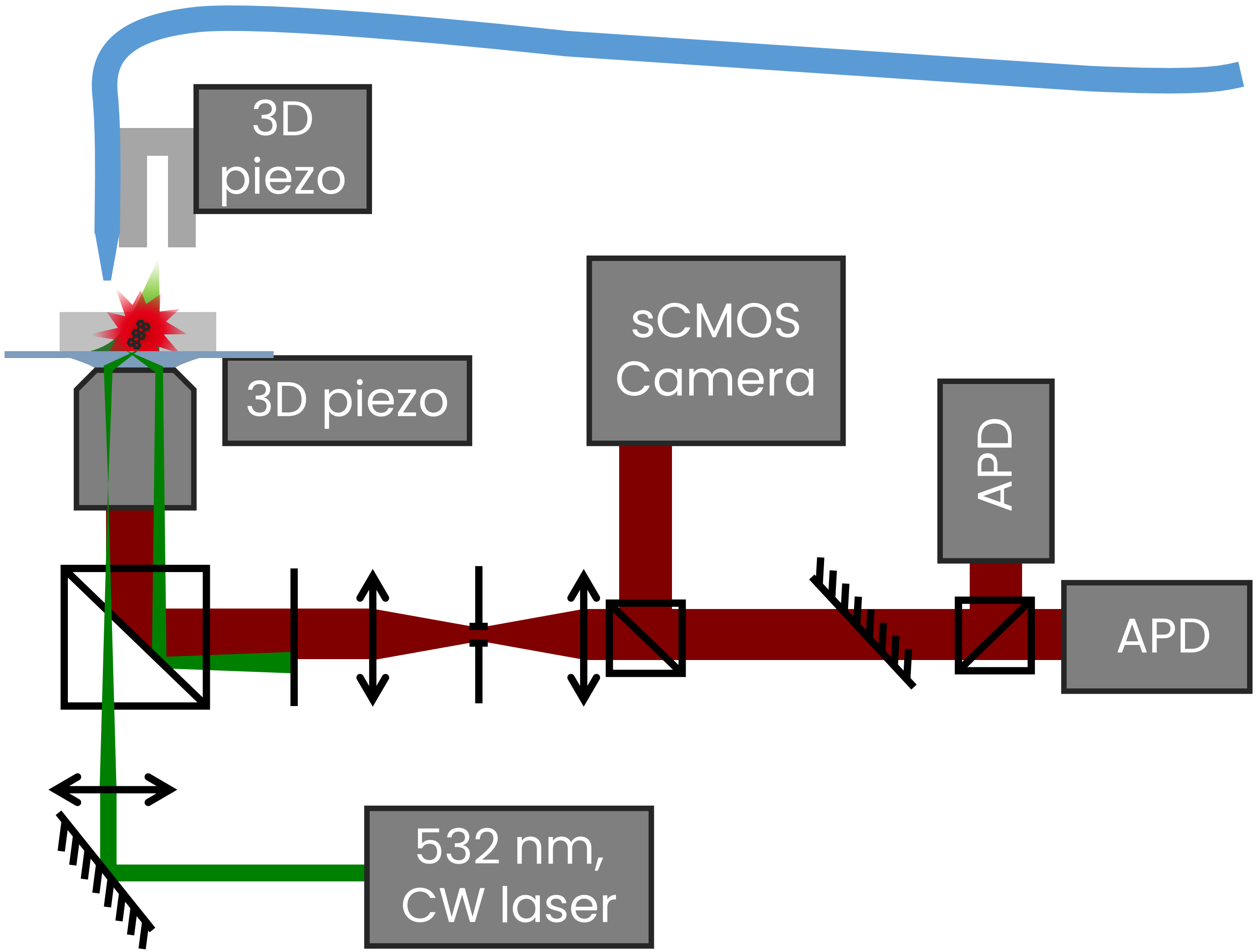
J. Mertz, J. Opt. Soc. Am. B 17 (11), 1906 (2000)

High numerical aperture
Oil immersion objectives

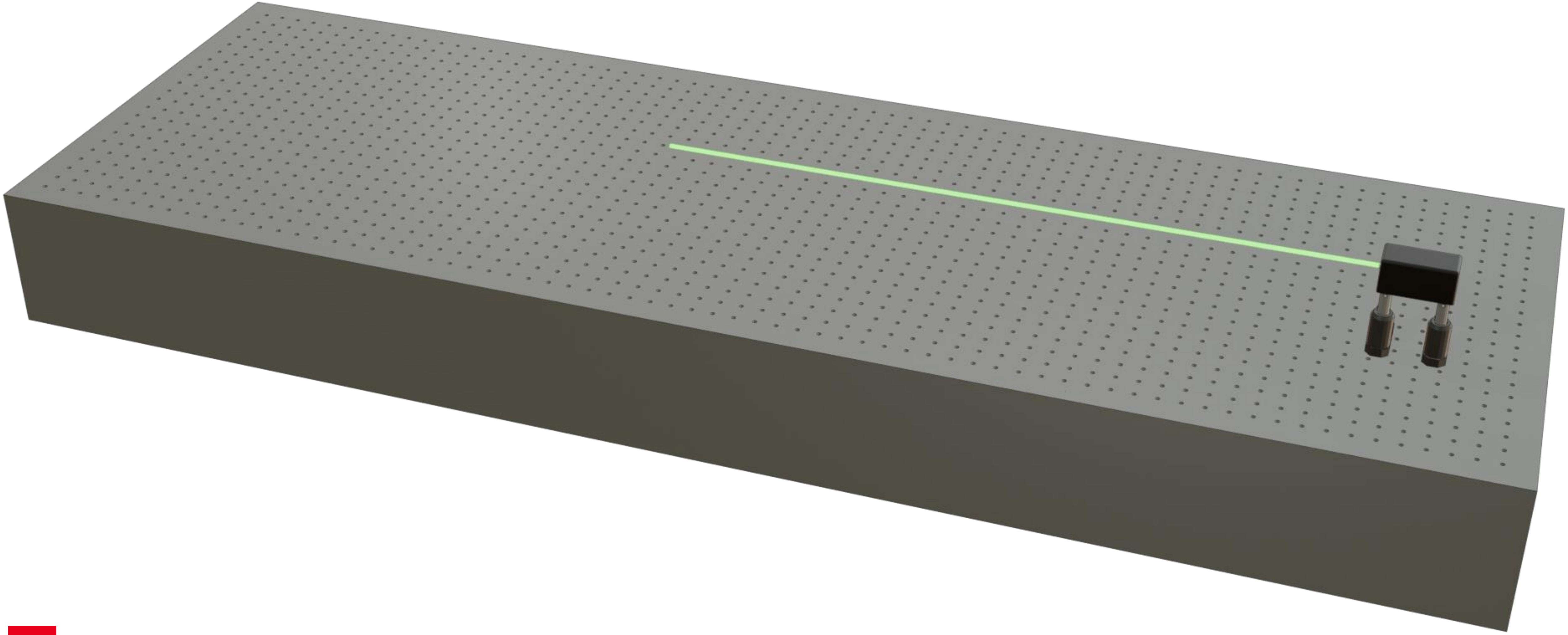
https://www.bio-equip.cn/enshow1equip_pc.asp?equipid=116603&division=405

4. Setting up a single molecule microscopy setup

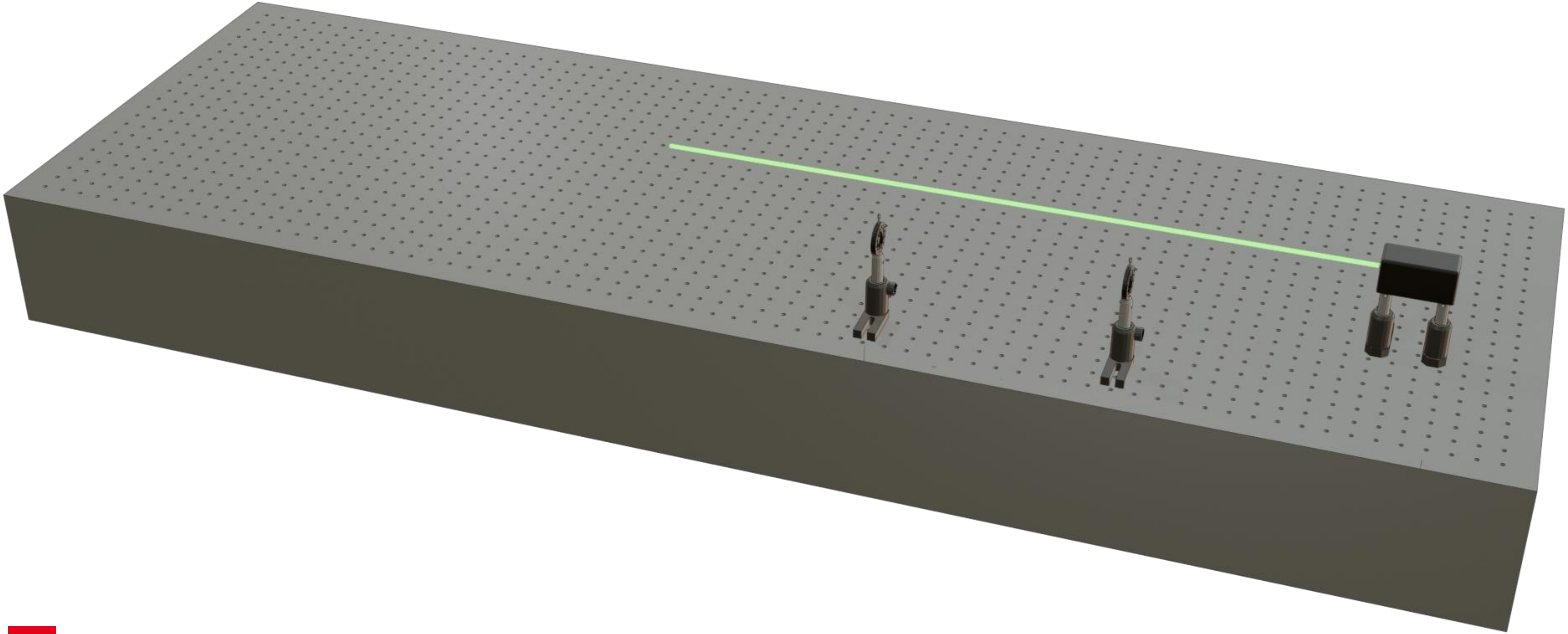
Step by step



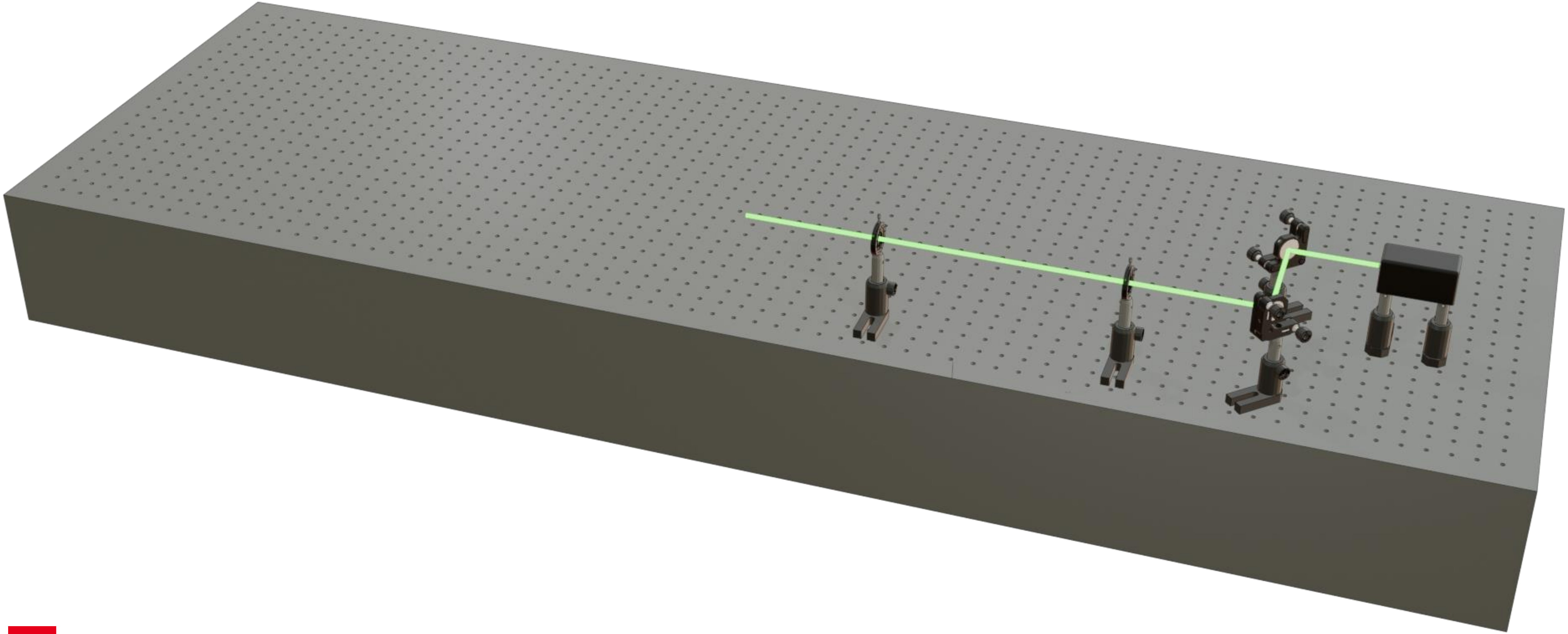
Step by step



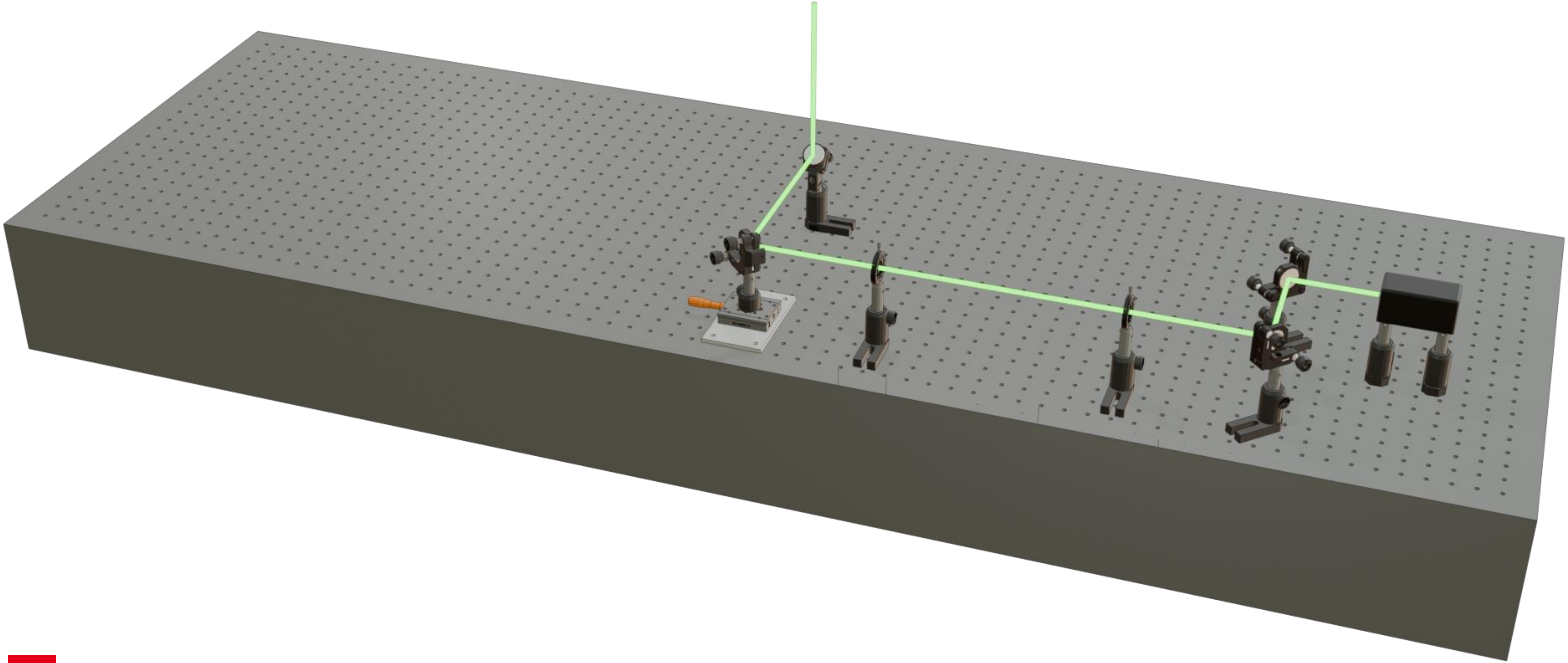
Step by step



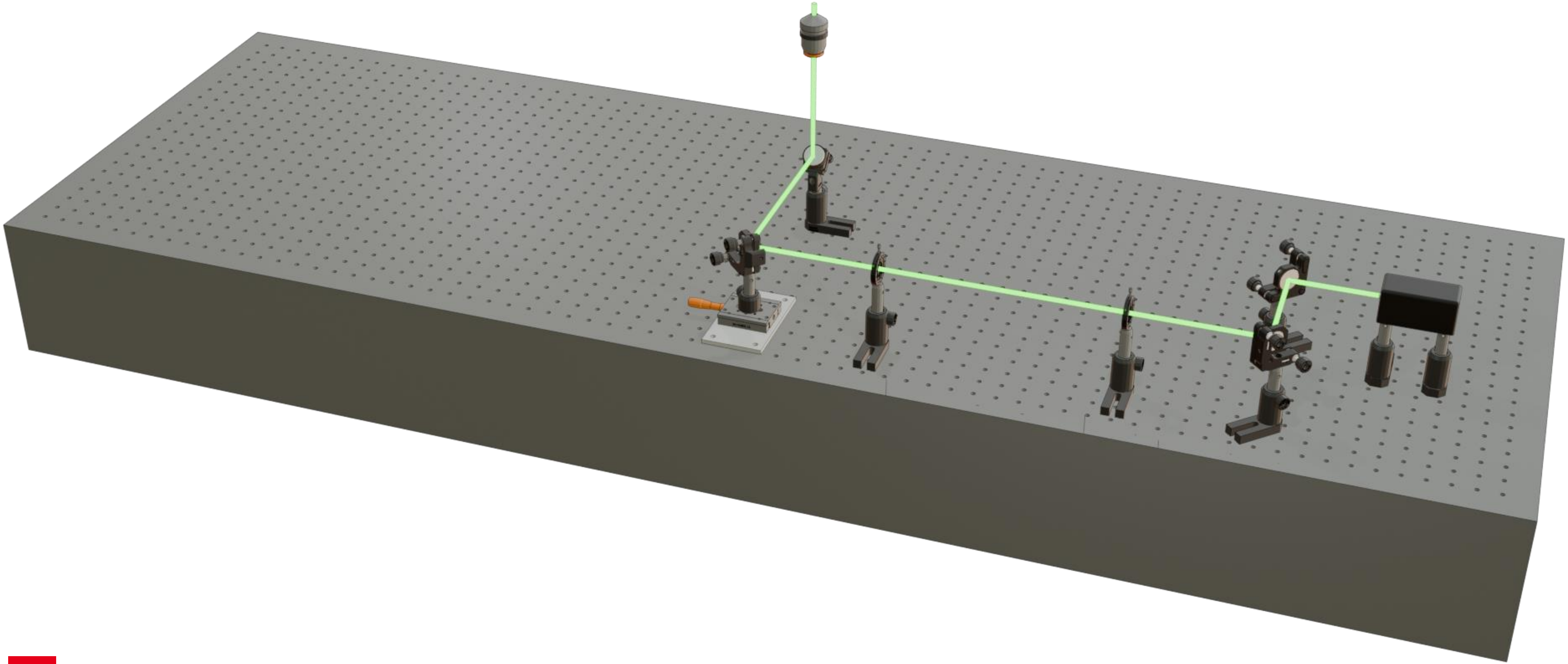
Step by step



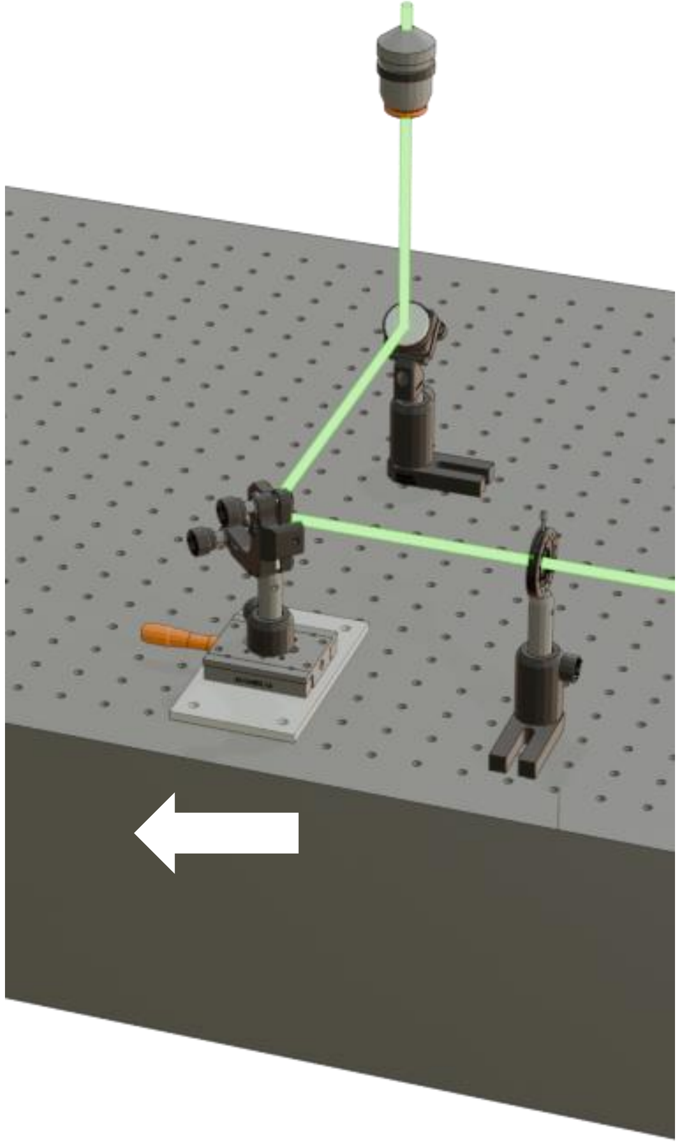
Step by step



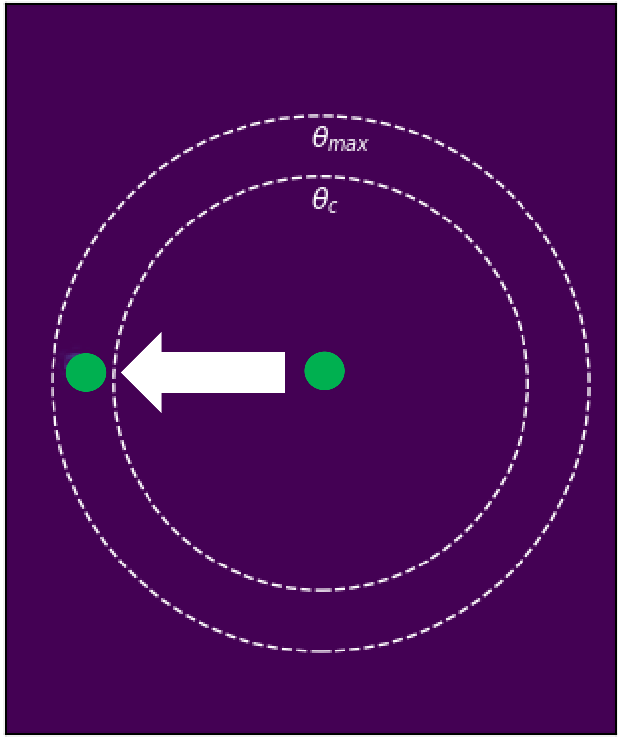
Step by step



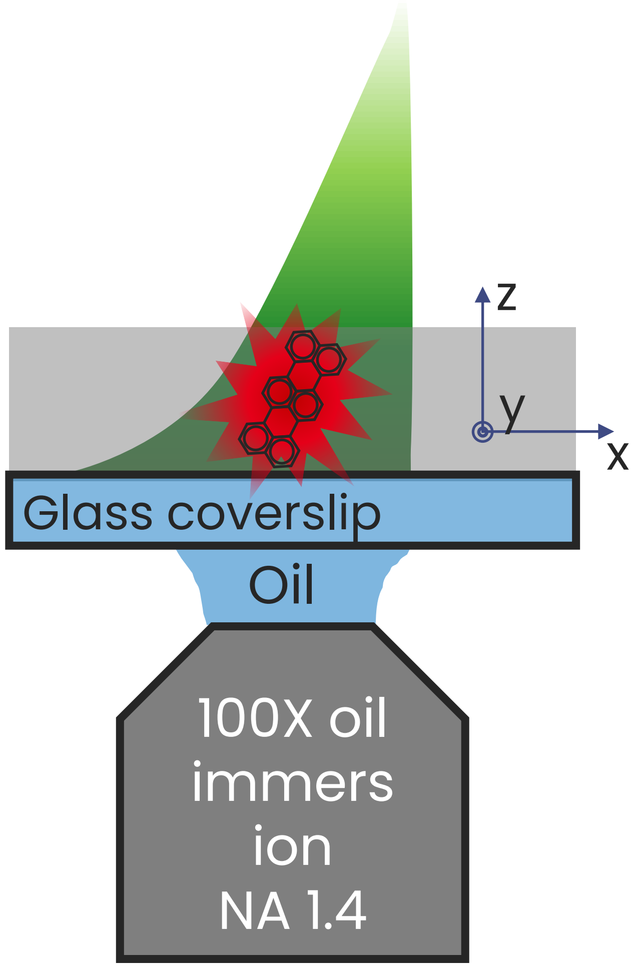
Total internal reflexion



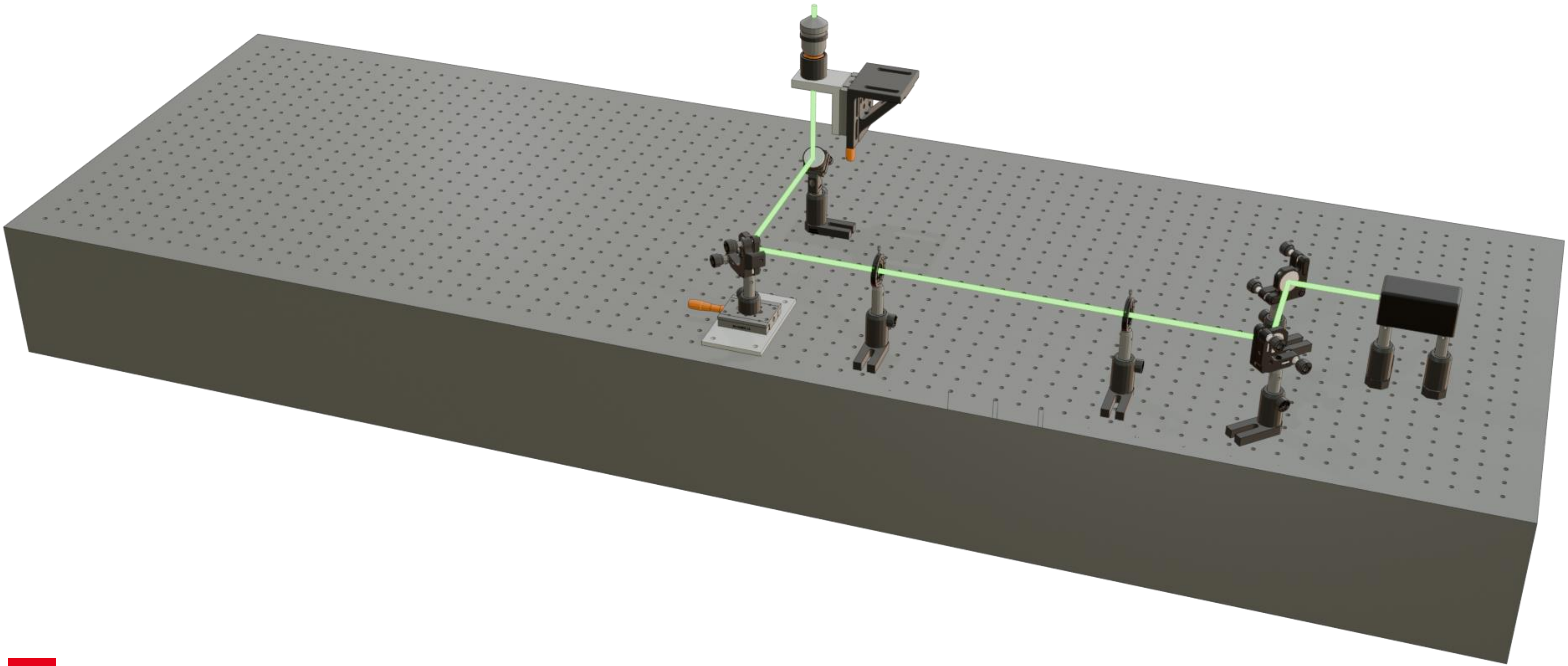
Objective entrance pupil



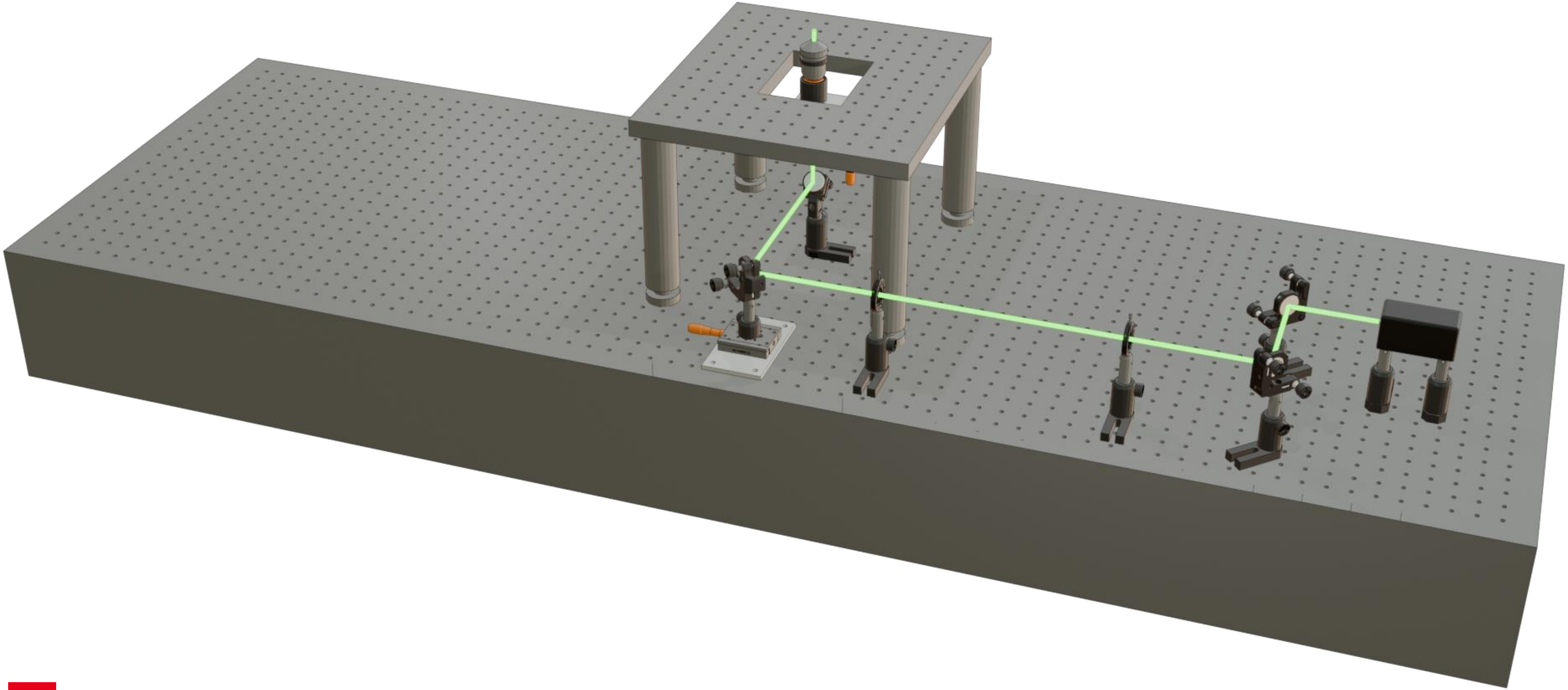
Back-focal plane image: θ_i = 51,4 deg.



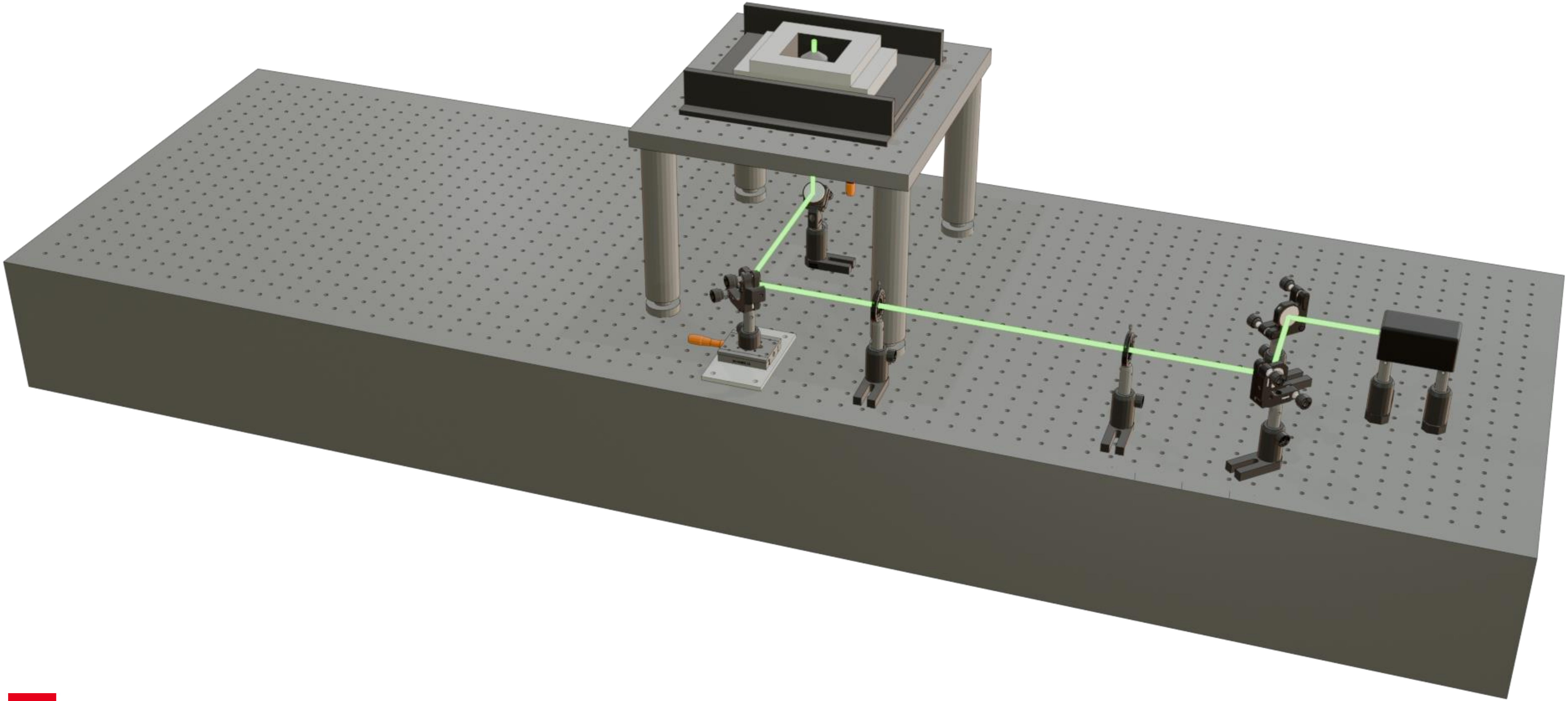
Step by step



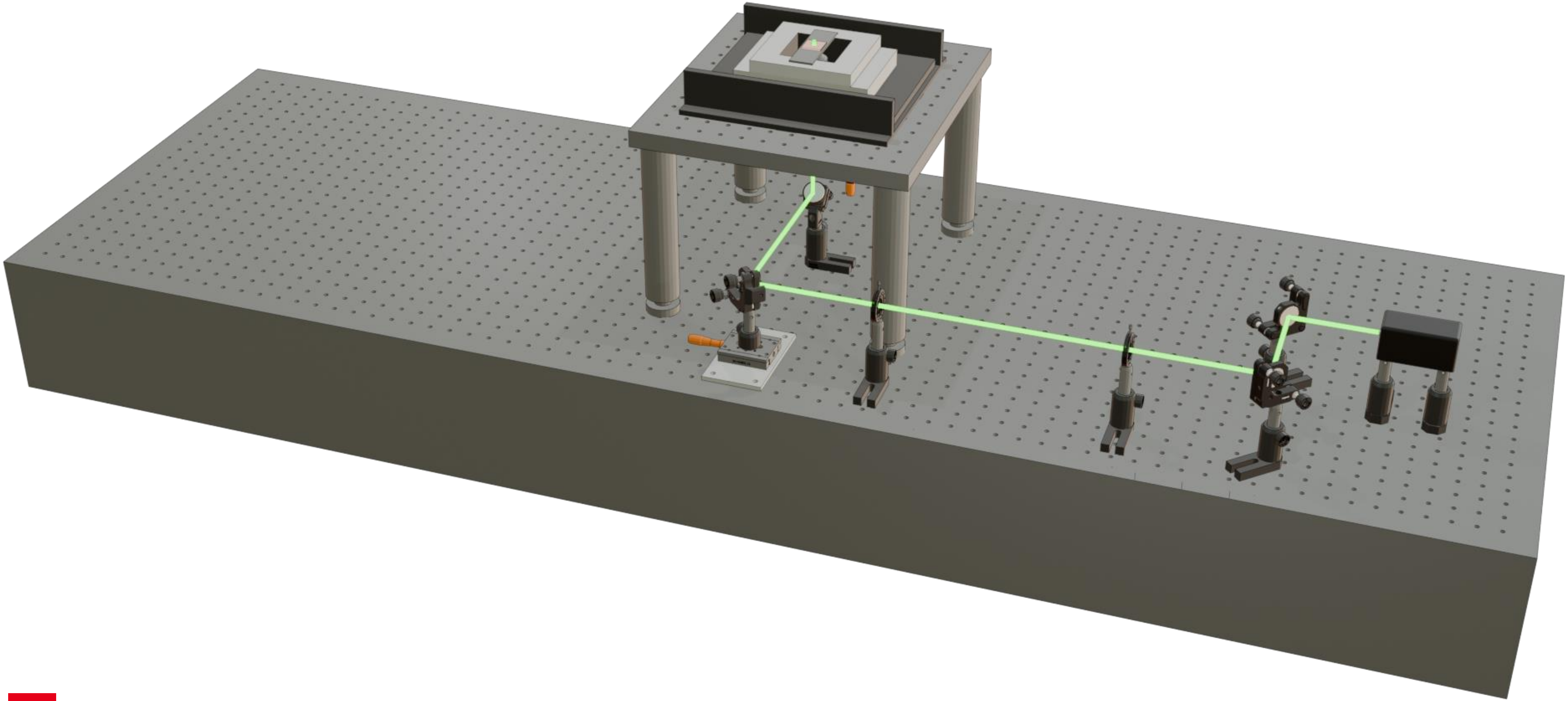
Step by step



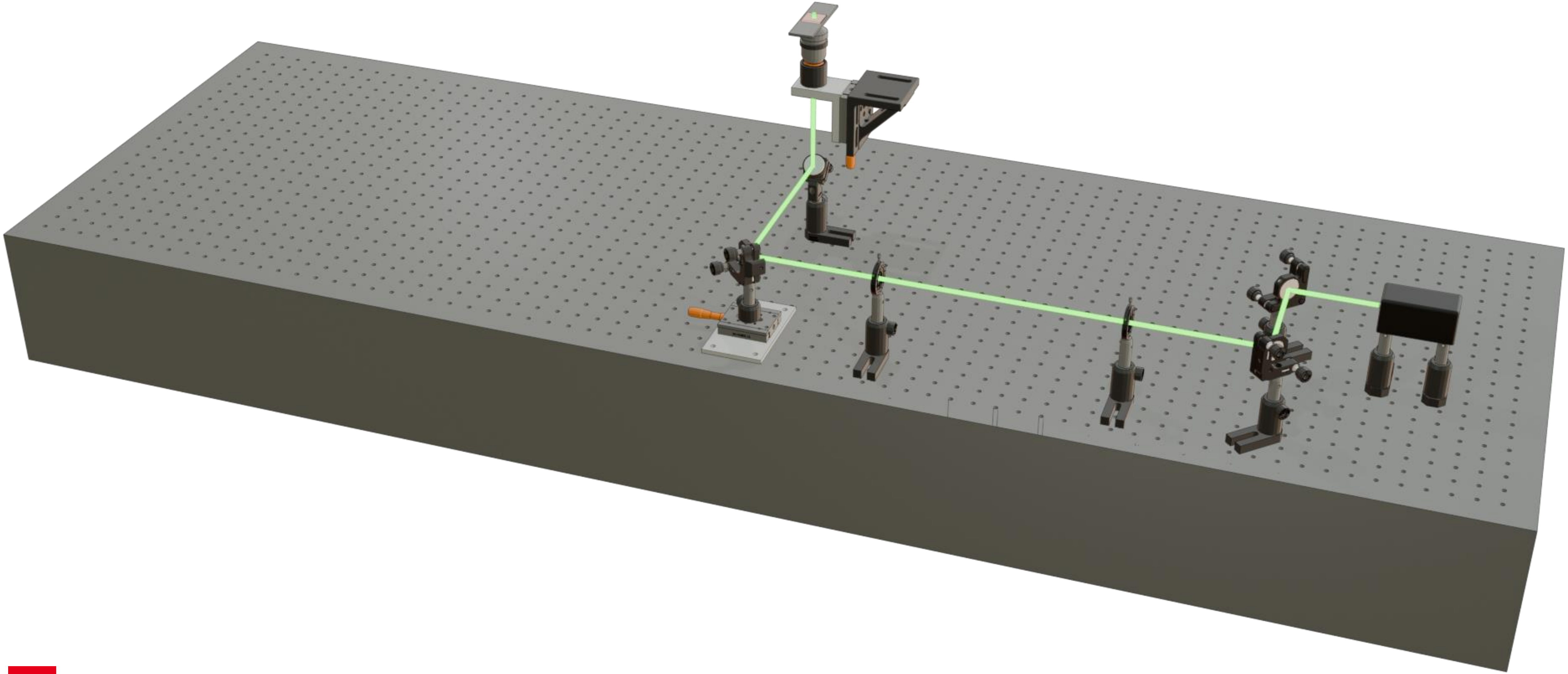
Step by step



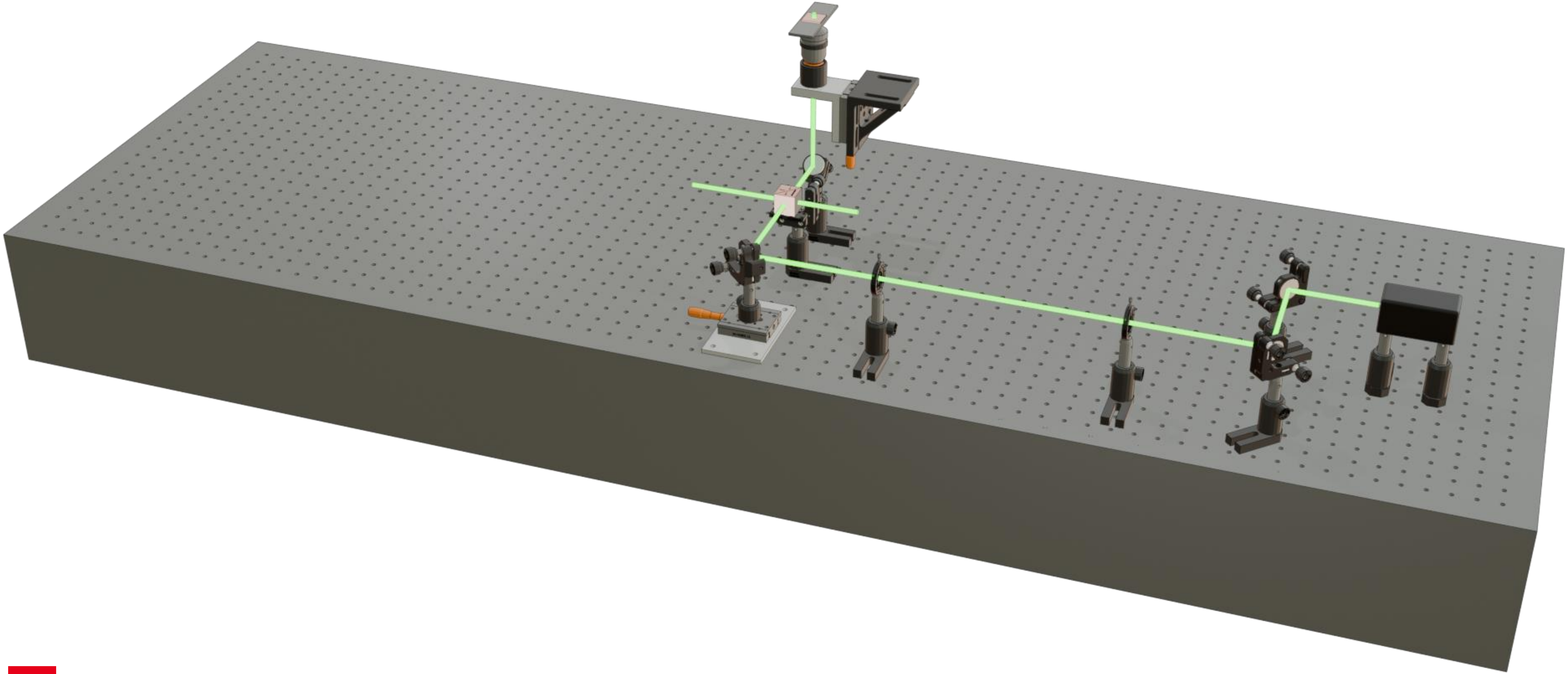
Step by step



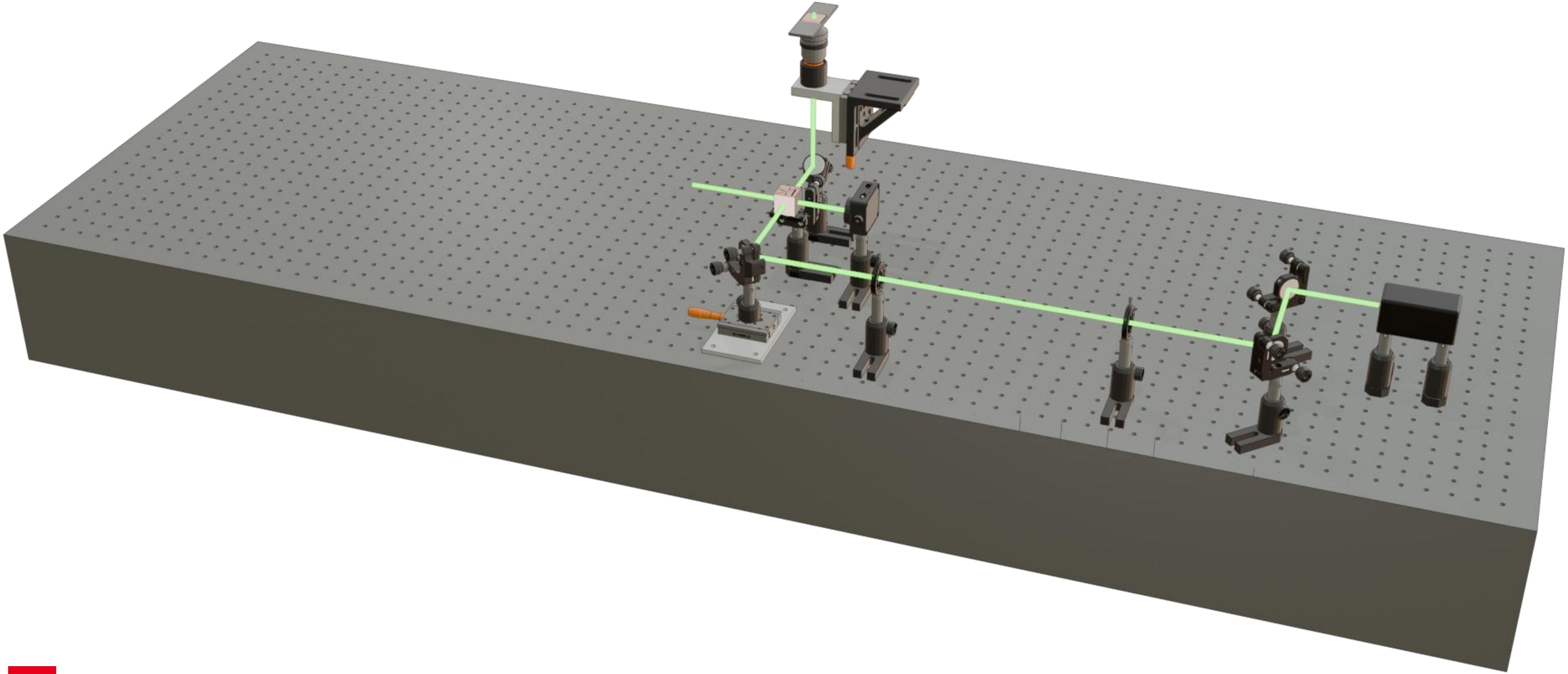
Step by step



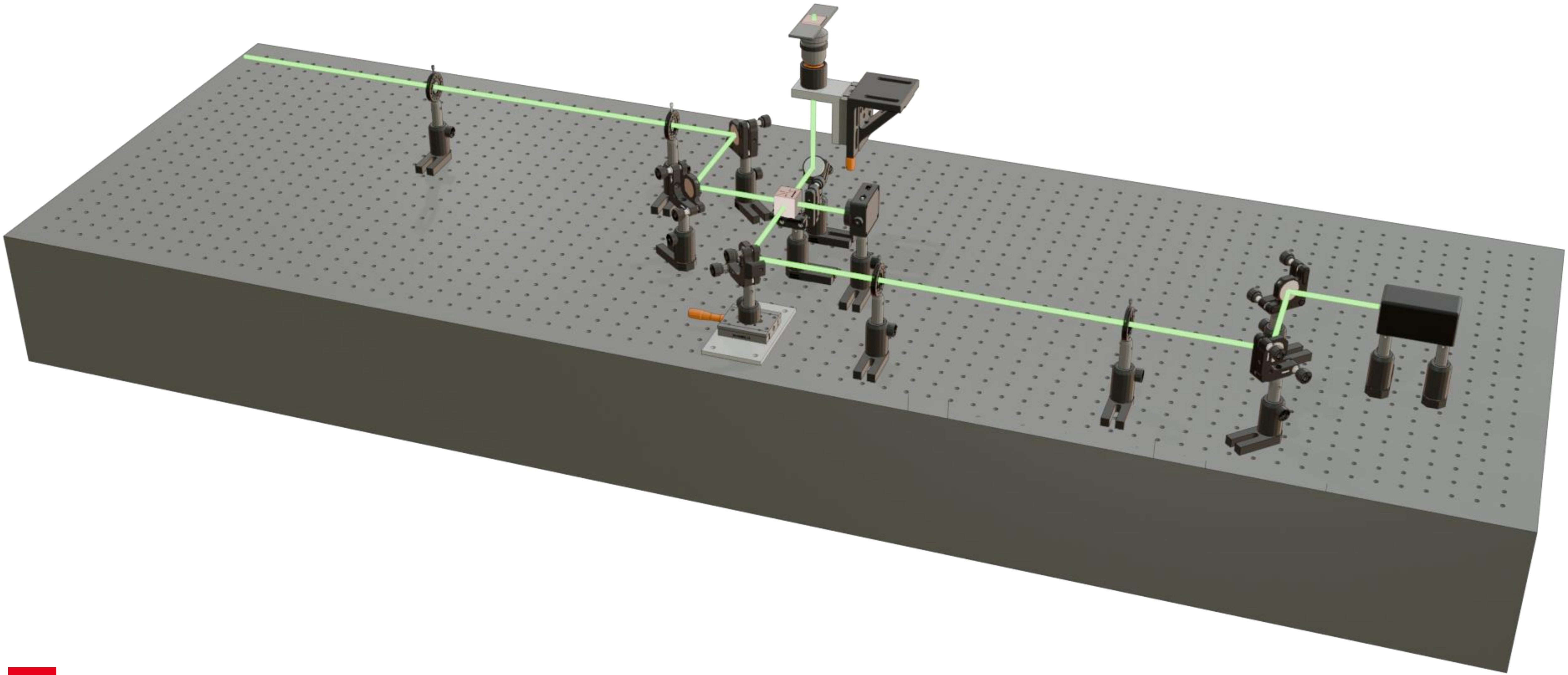
Step by step



Step by step



Step by step



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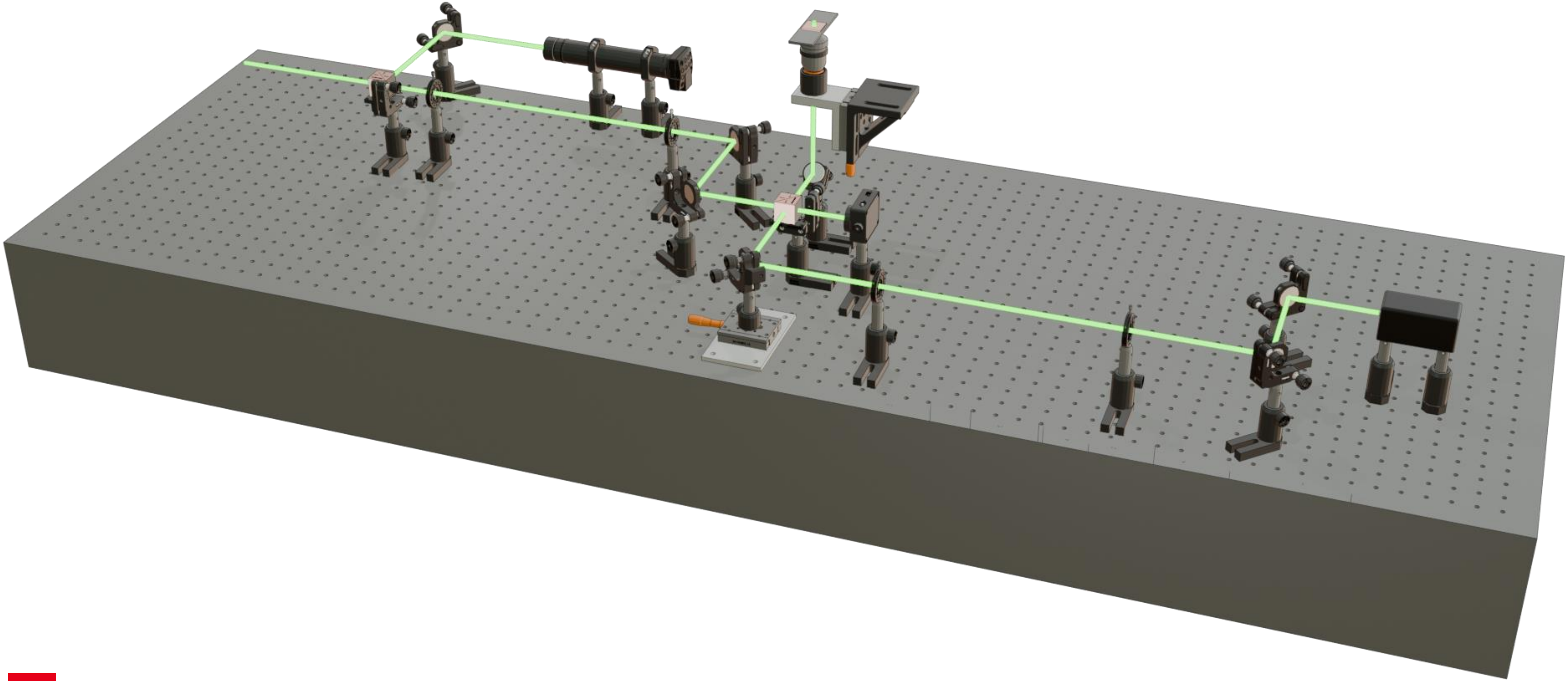
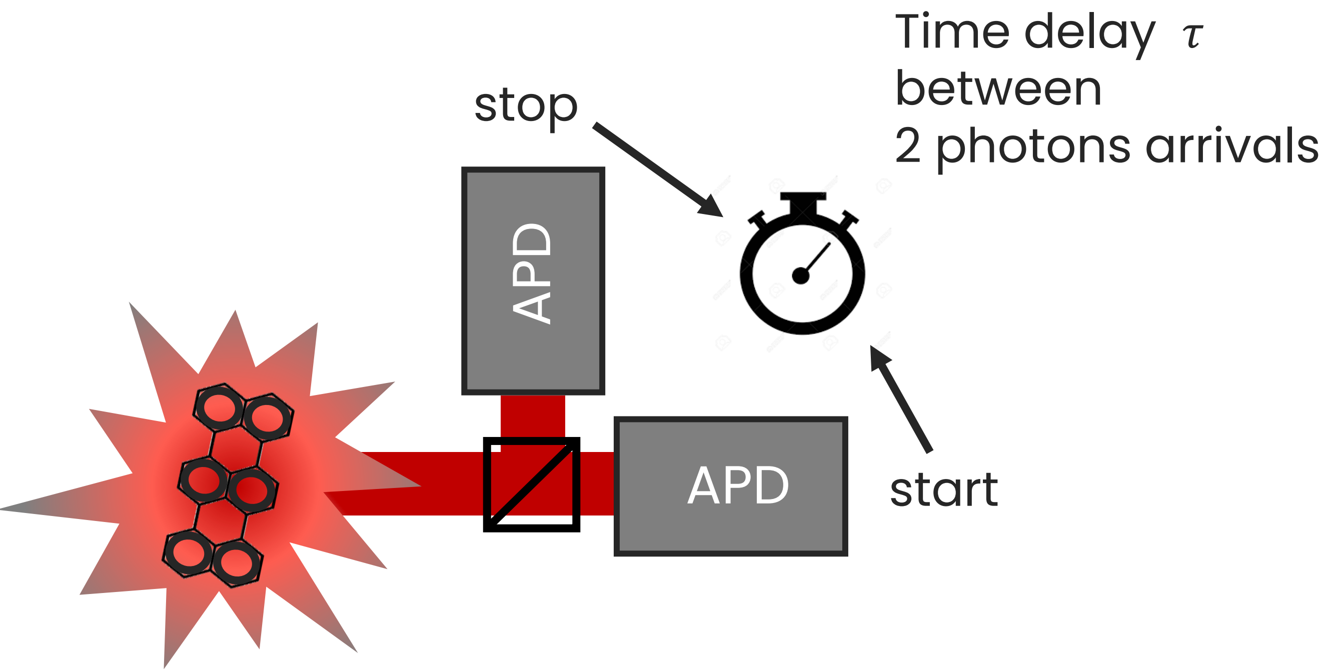
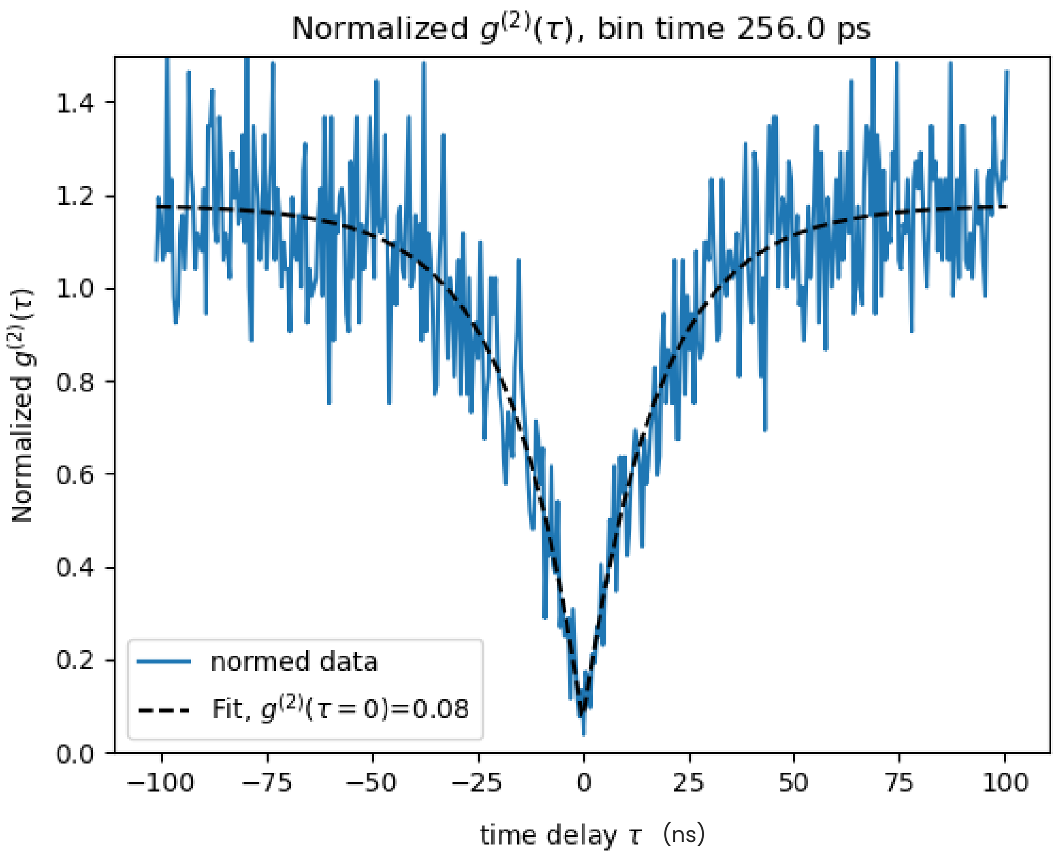


THE proof of single photon emission

Hanbury-Brown-Twiss (HBT) detection

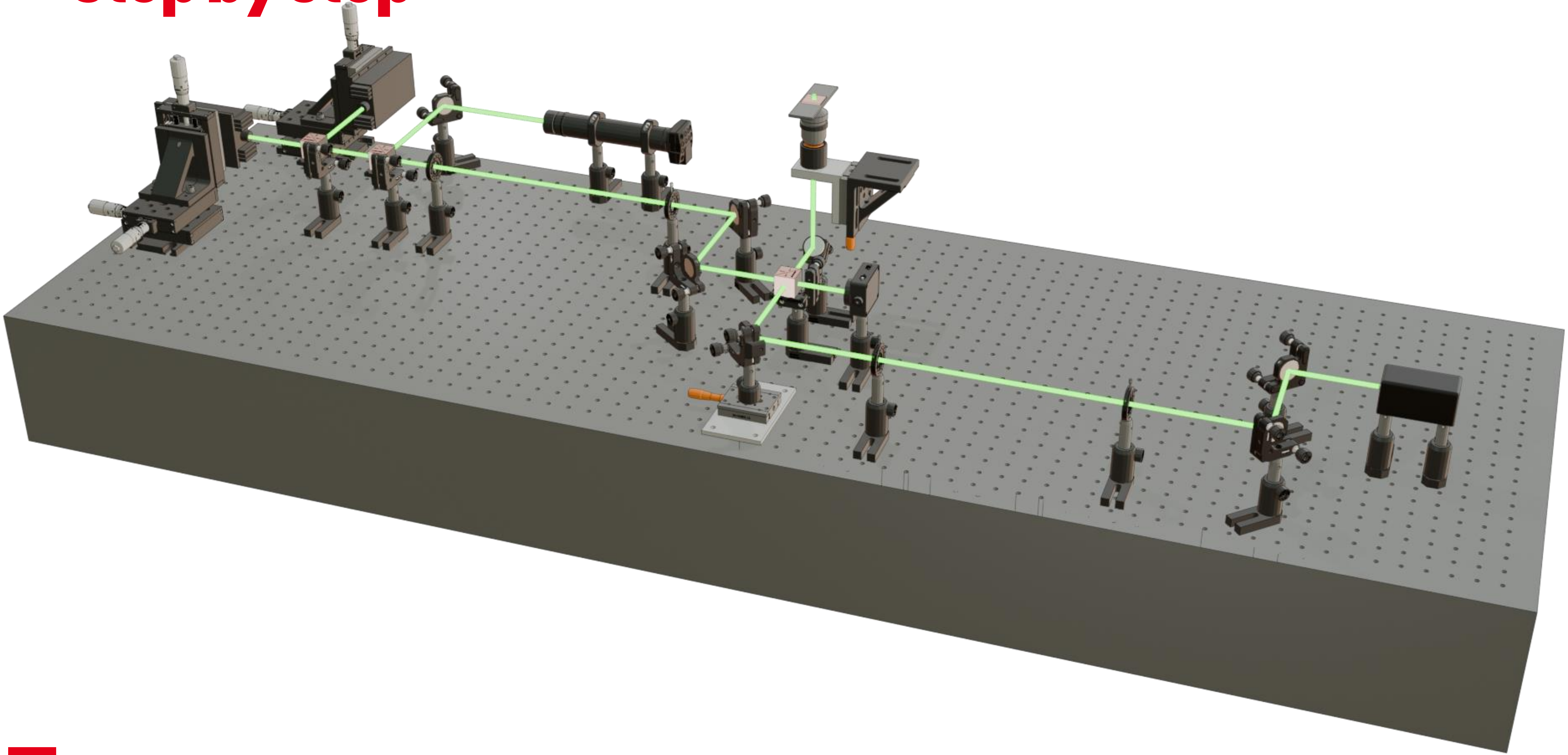




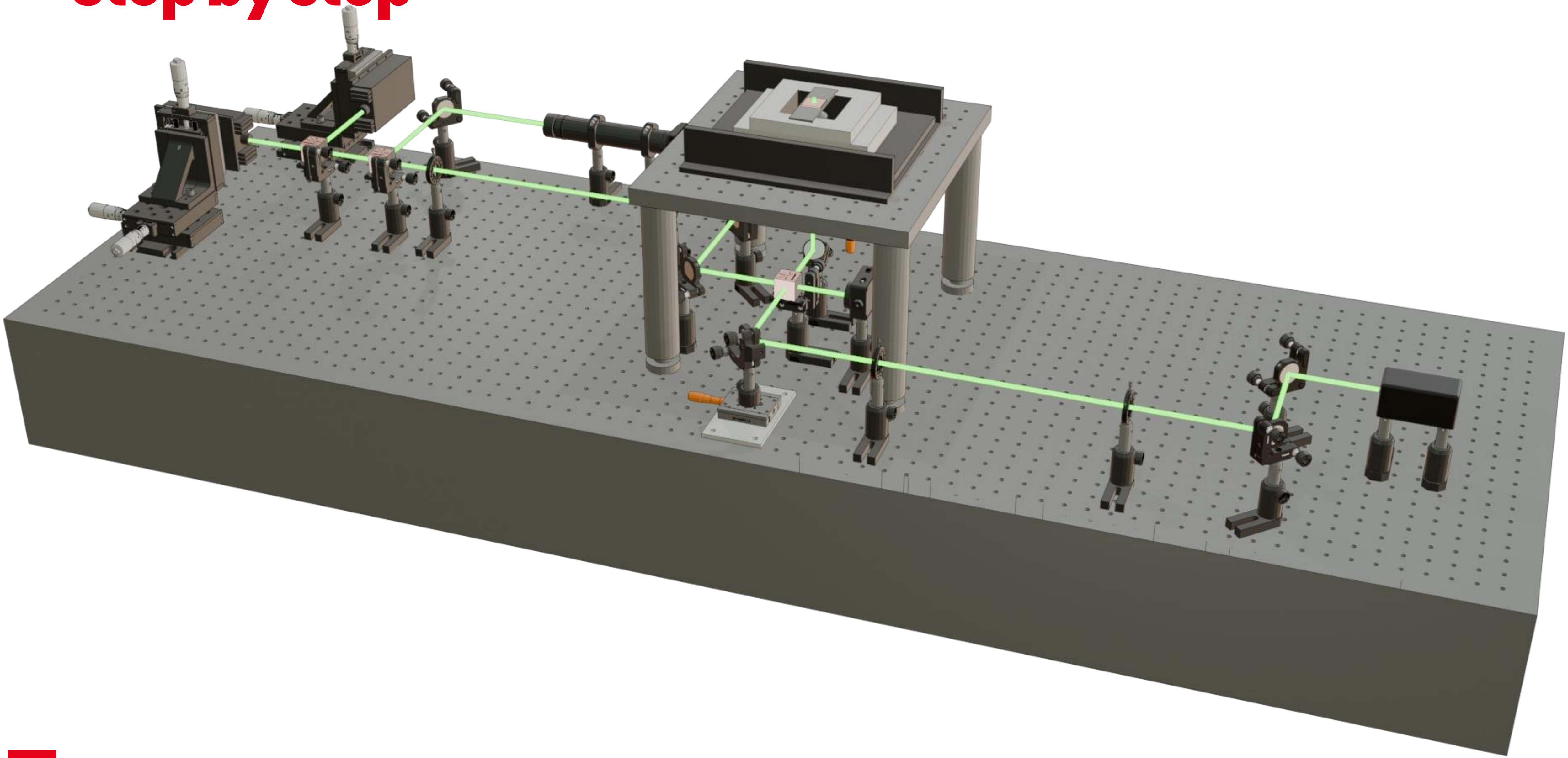
Need 1 to 2 million photons

$$g^{(2)}(\tau) = 1 - (1 + A)\exp(-\lambda_2\tau) + A\exp(-\lambda_3\tau)$$

Step by step



Step by step



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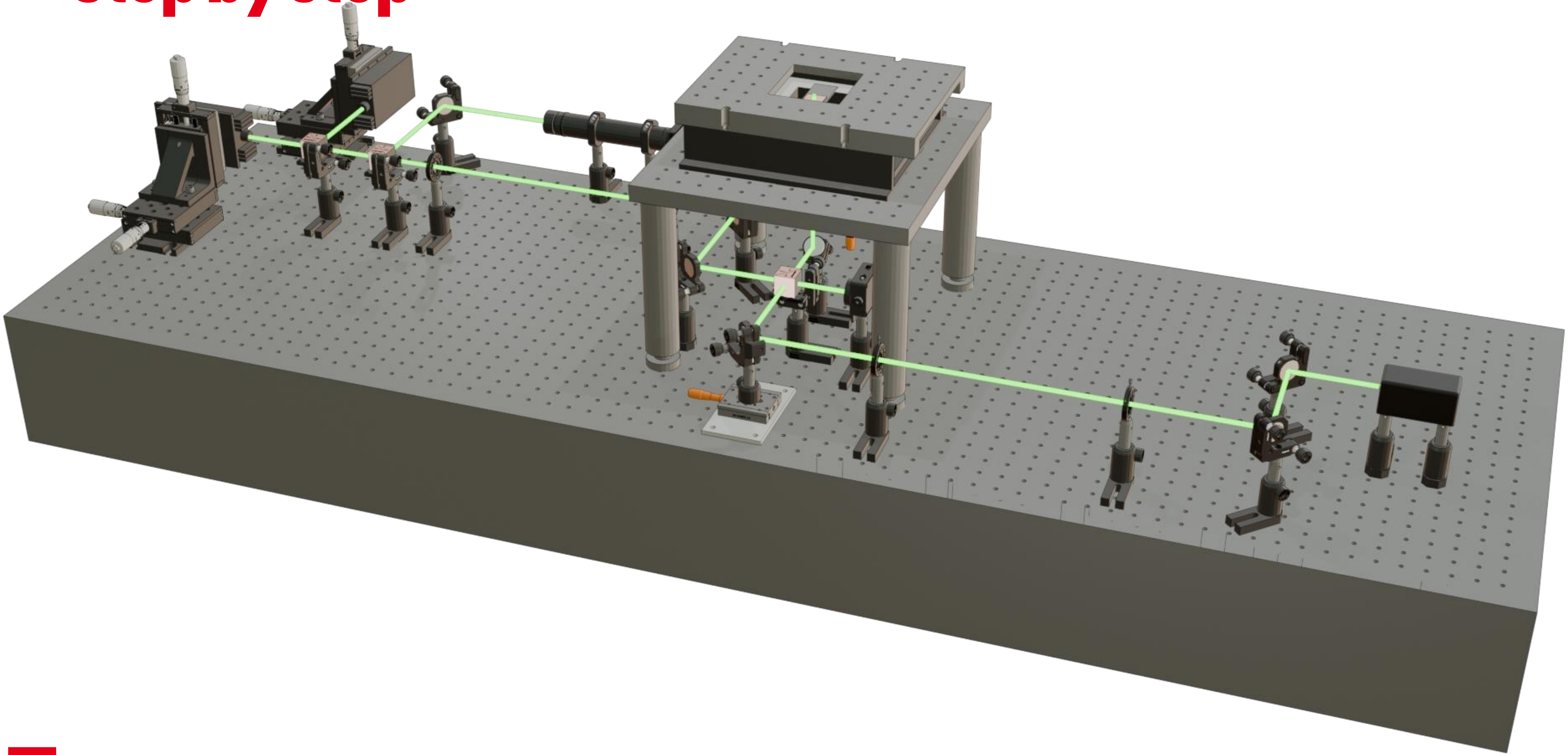
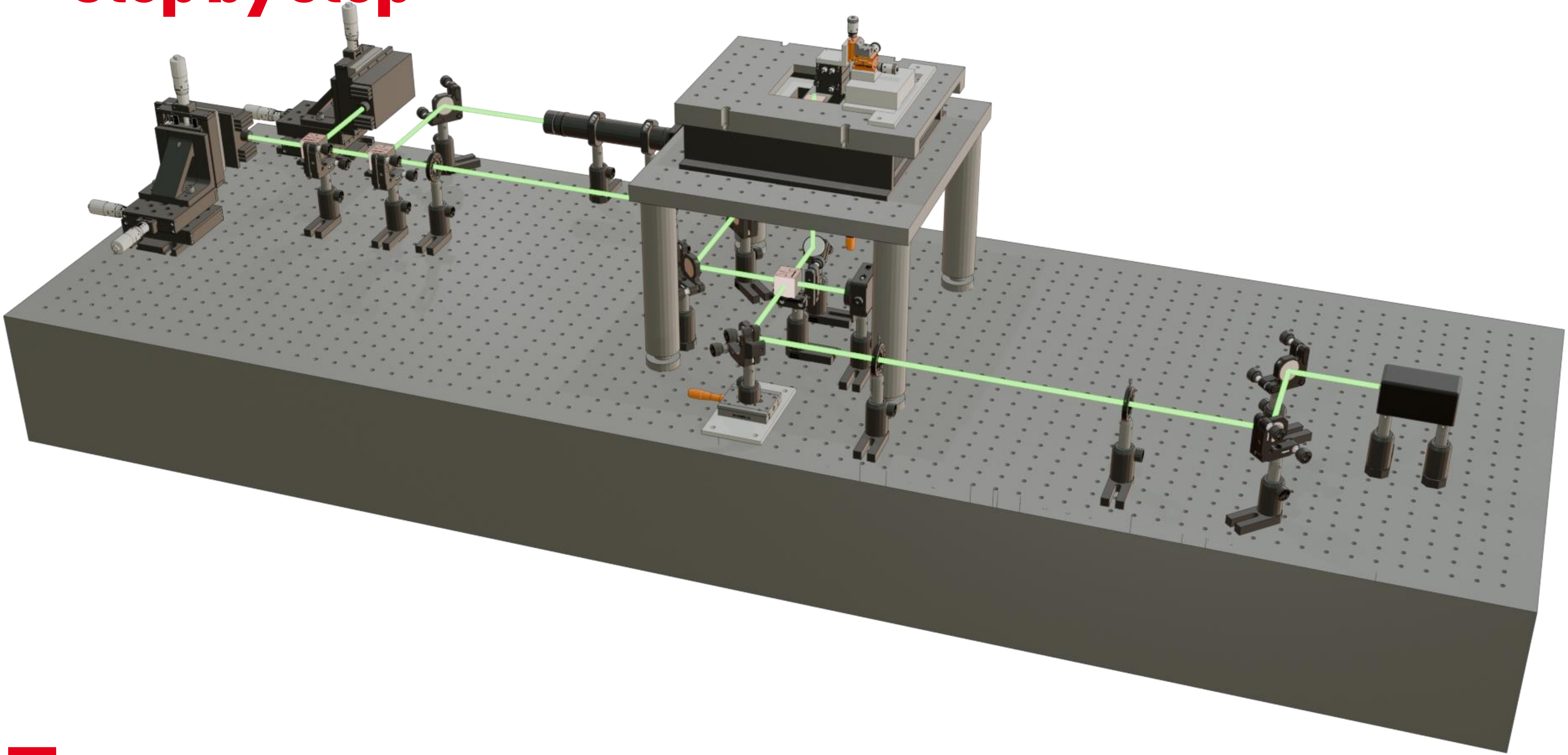


Step by step



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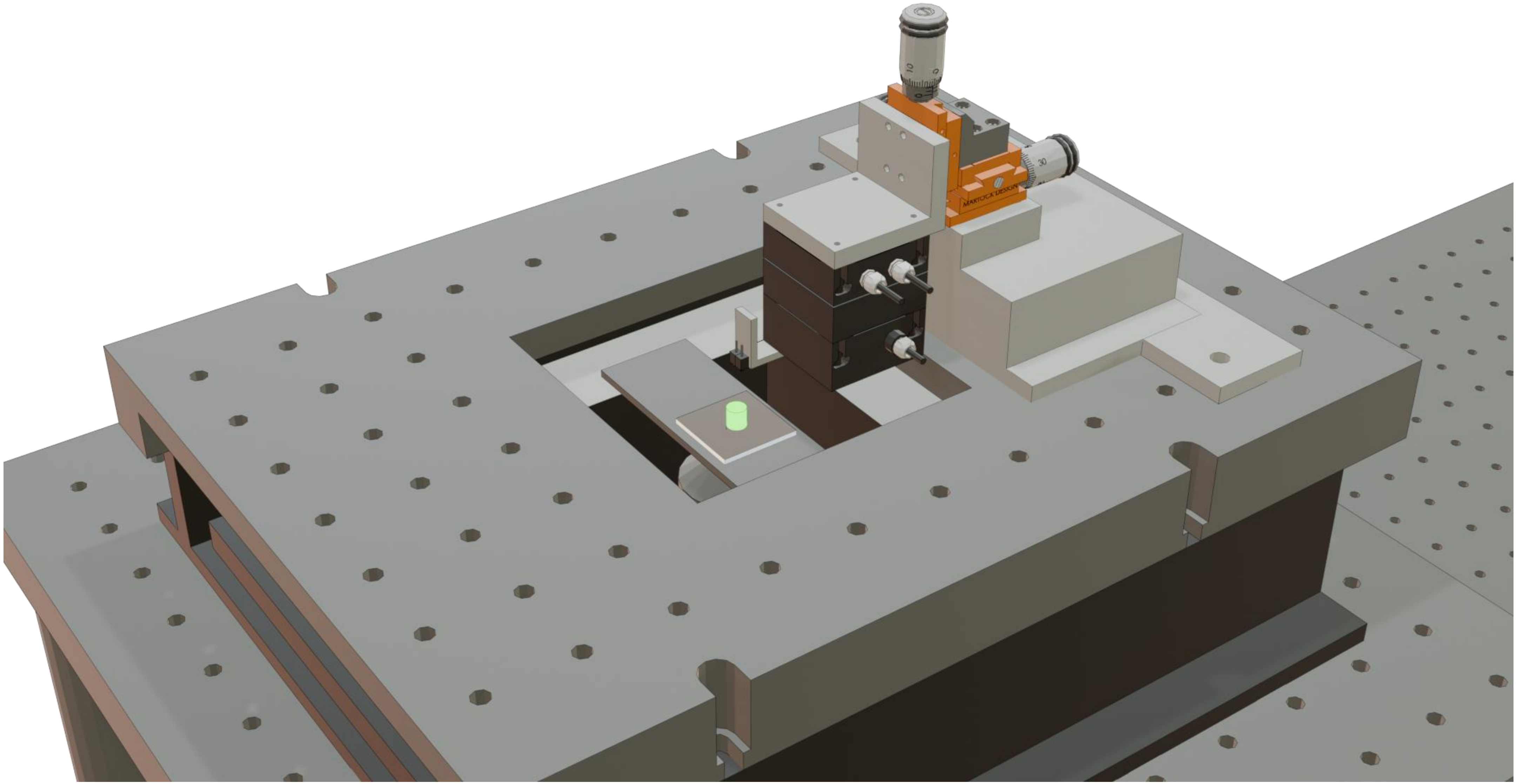
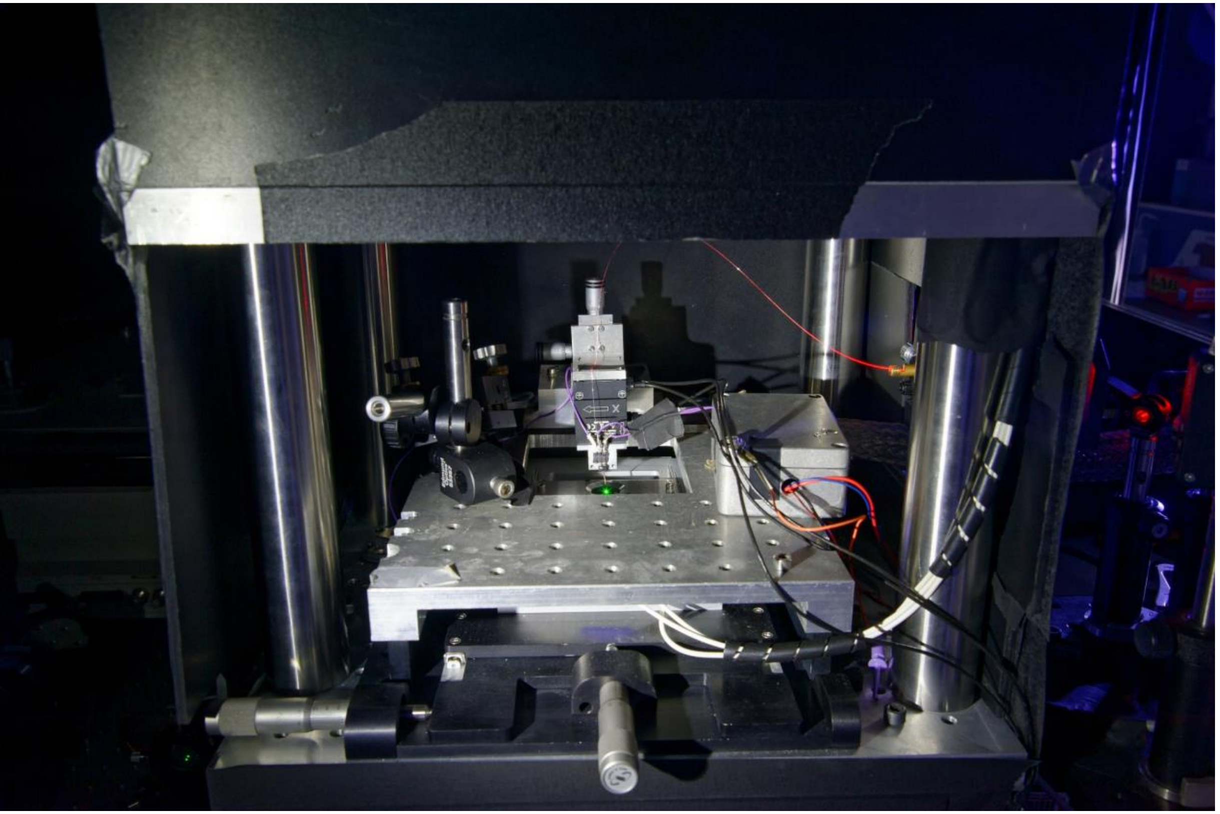
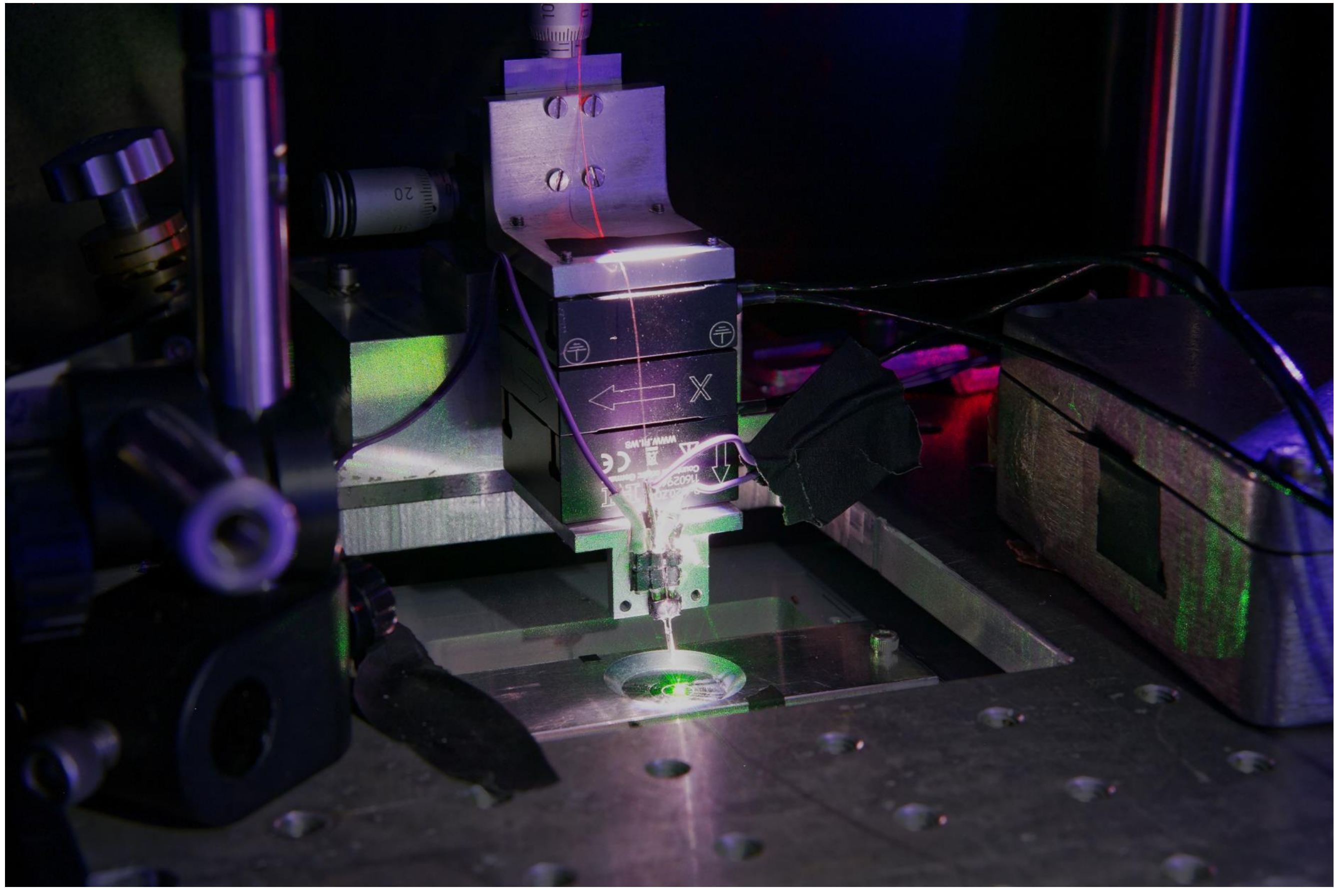


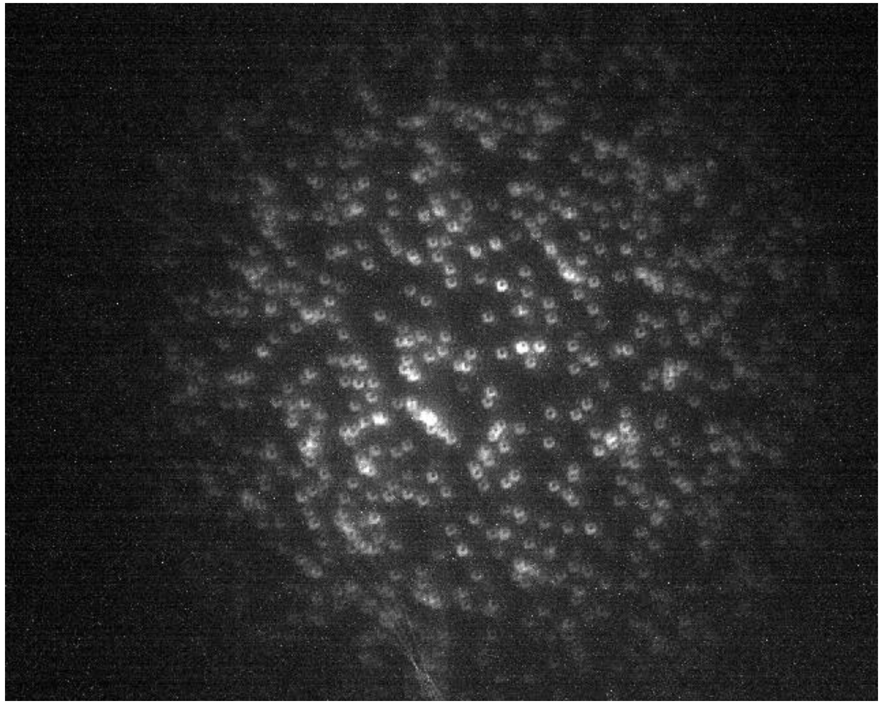
The real setup: Shear Force Head

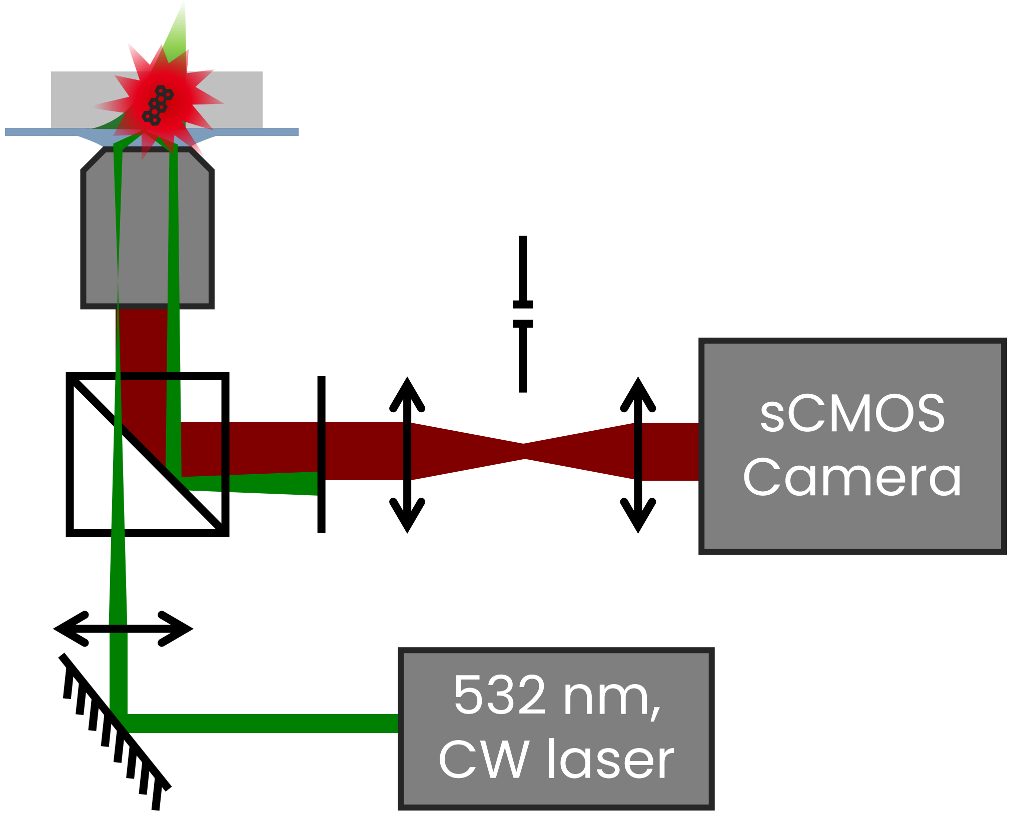




Confocal filtering

Camera image, total internal reflection wide-field illumination





Confocal filtering

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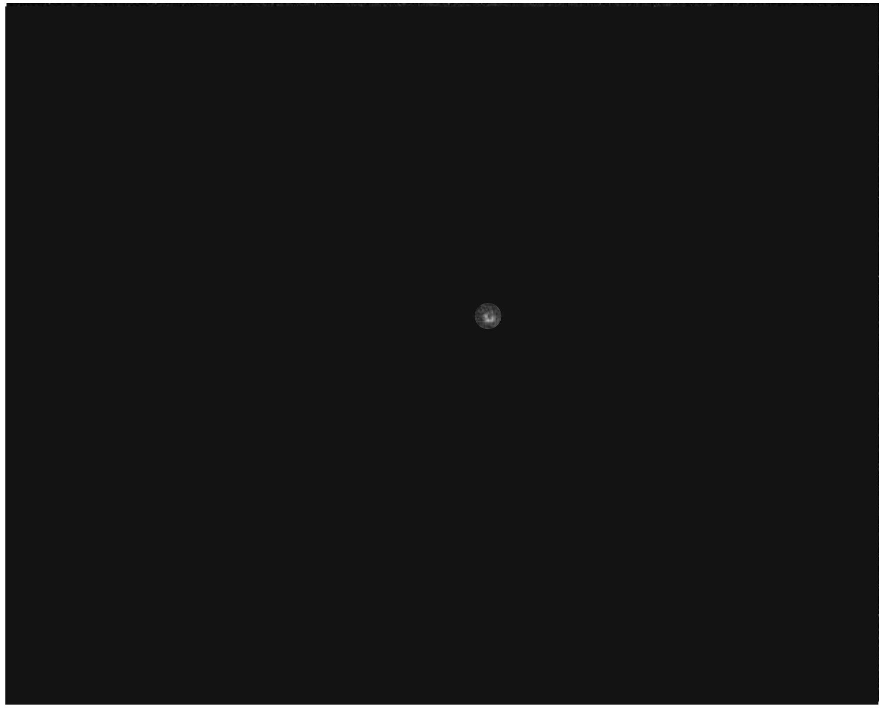
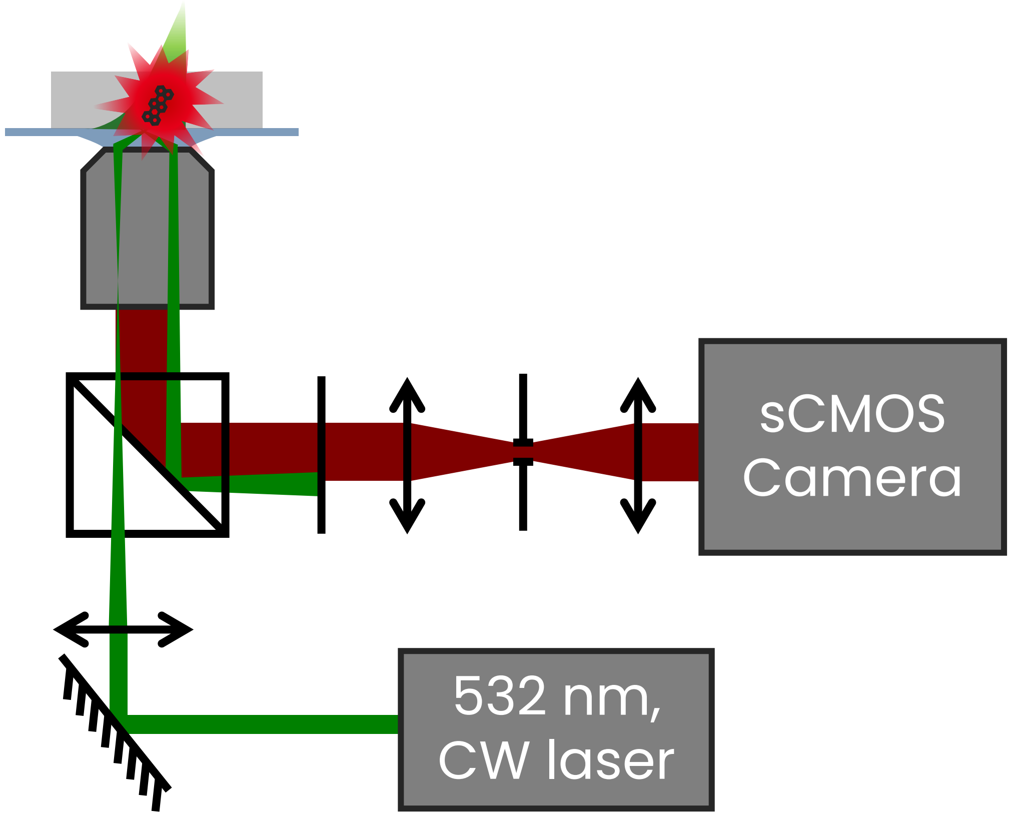


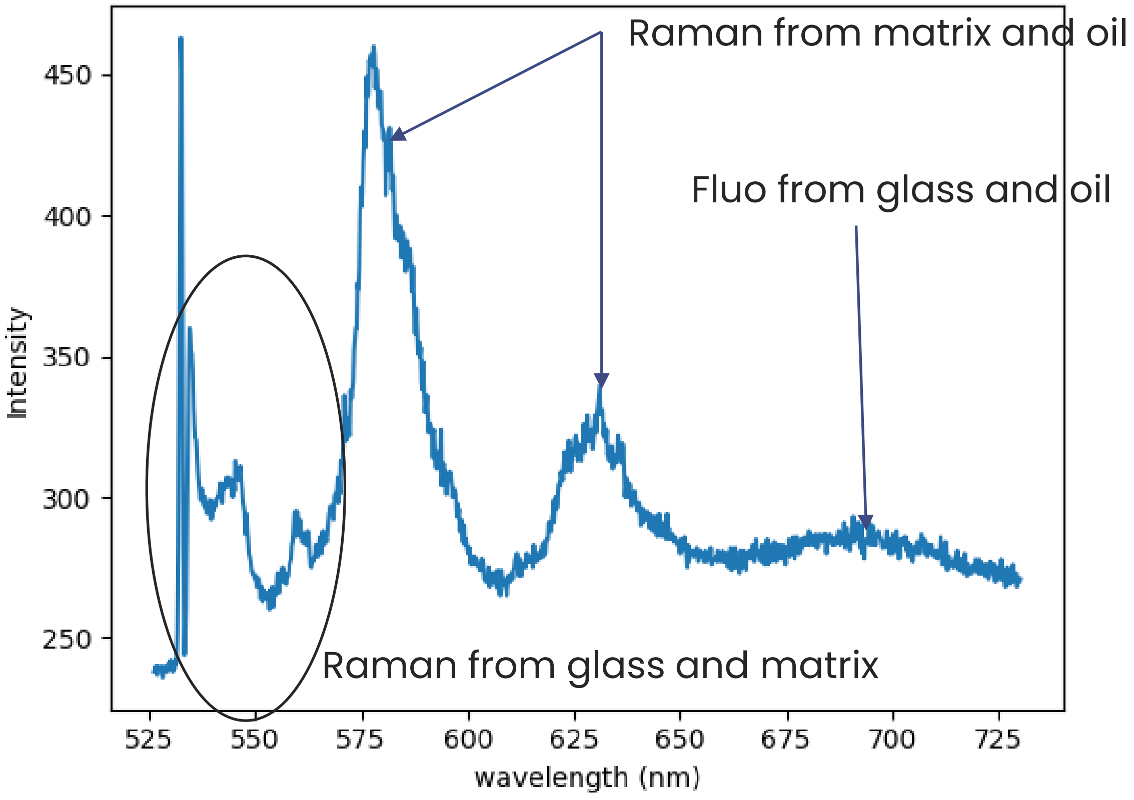


Measurement SNR > 20

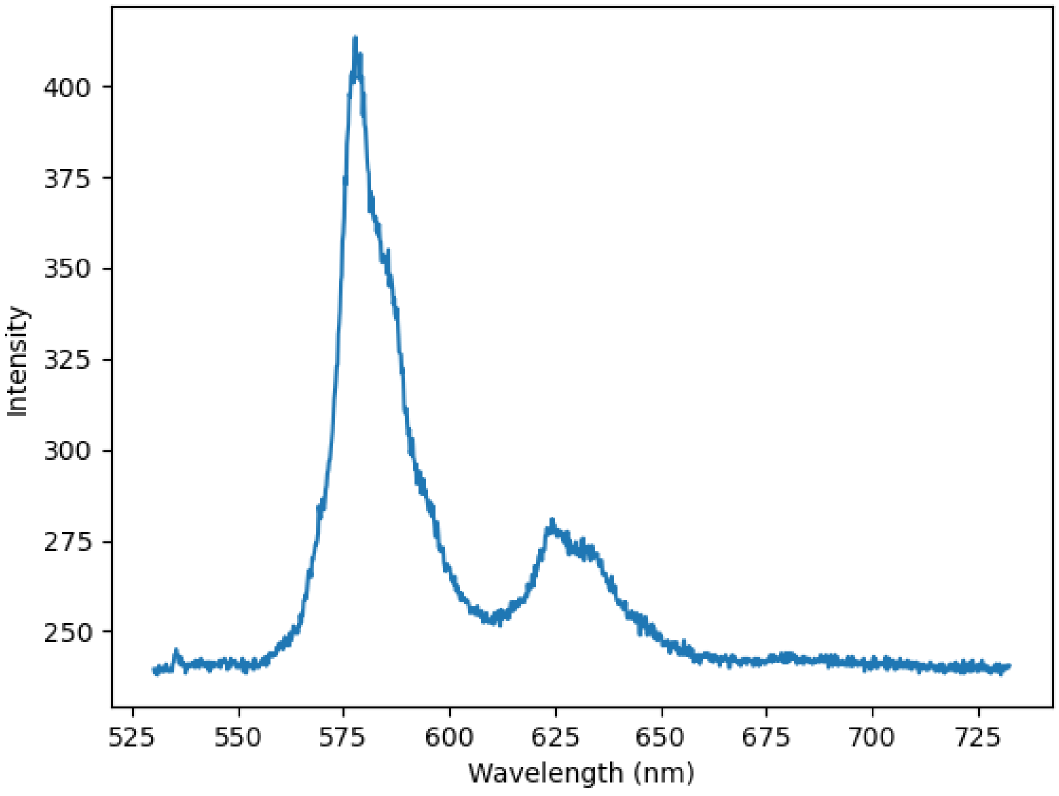
Effect of confocal filtering

Single Terrylene fluorescence spectrum

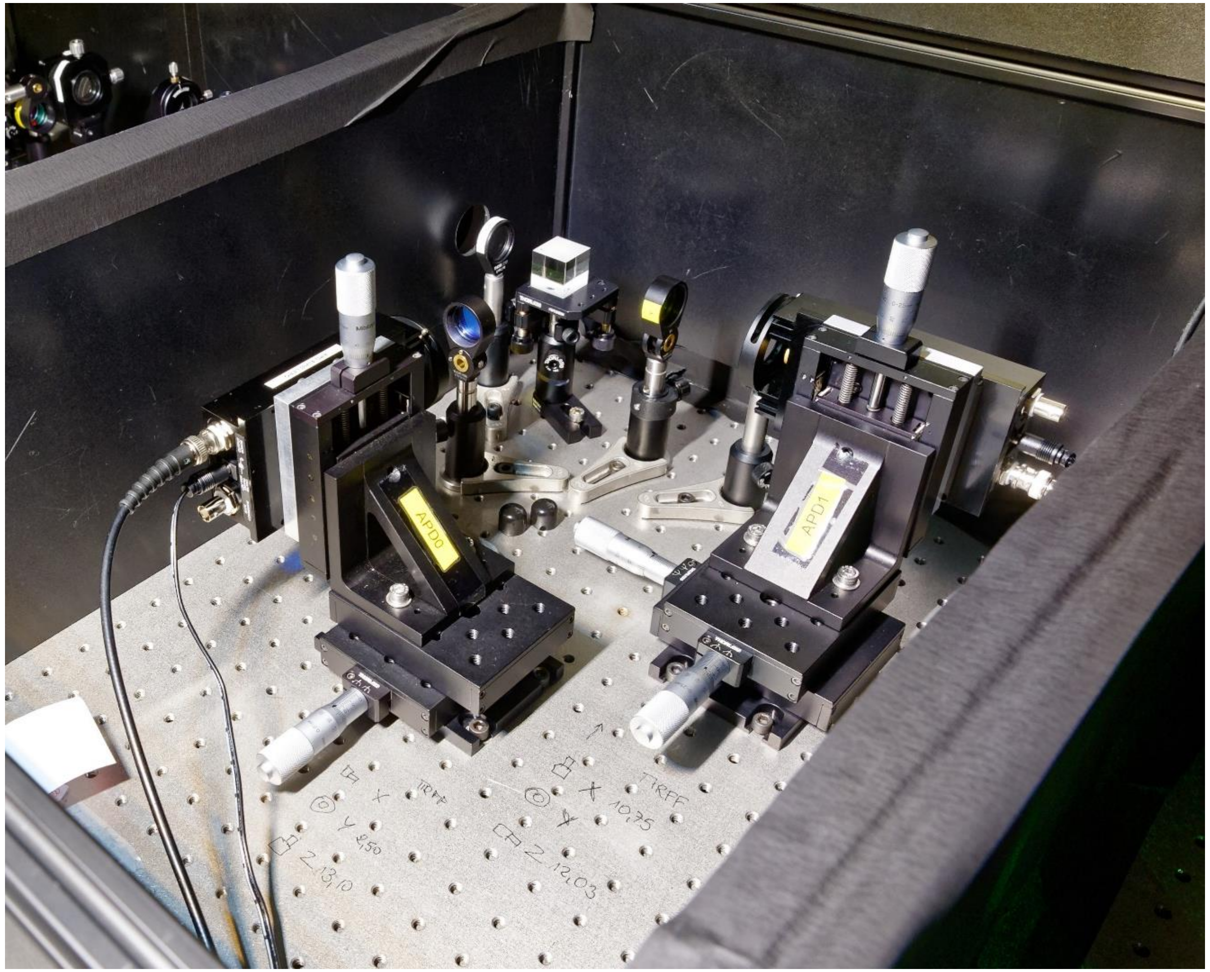
Pinhole out



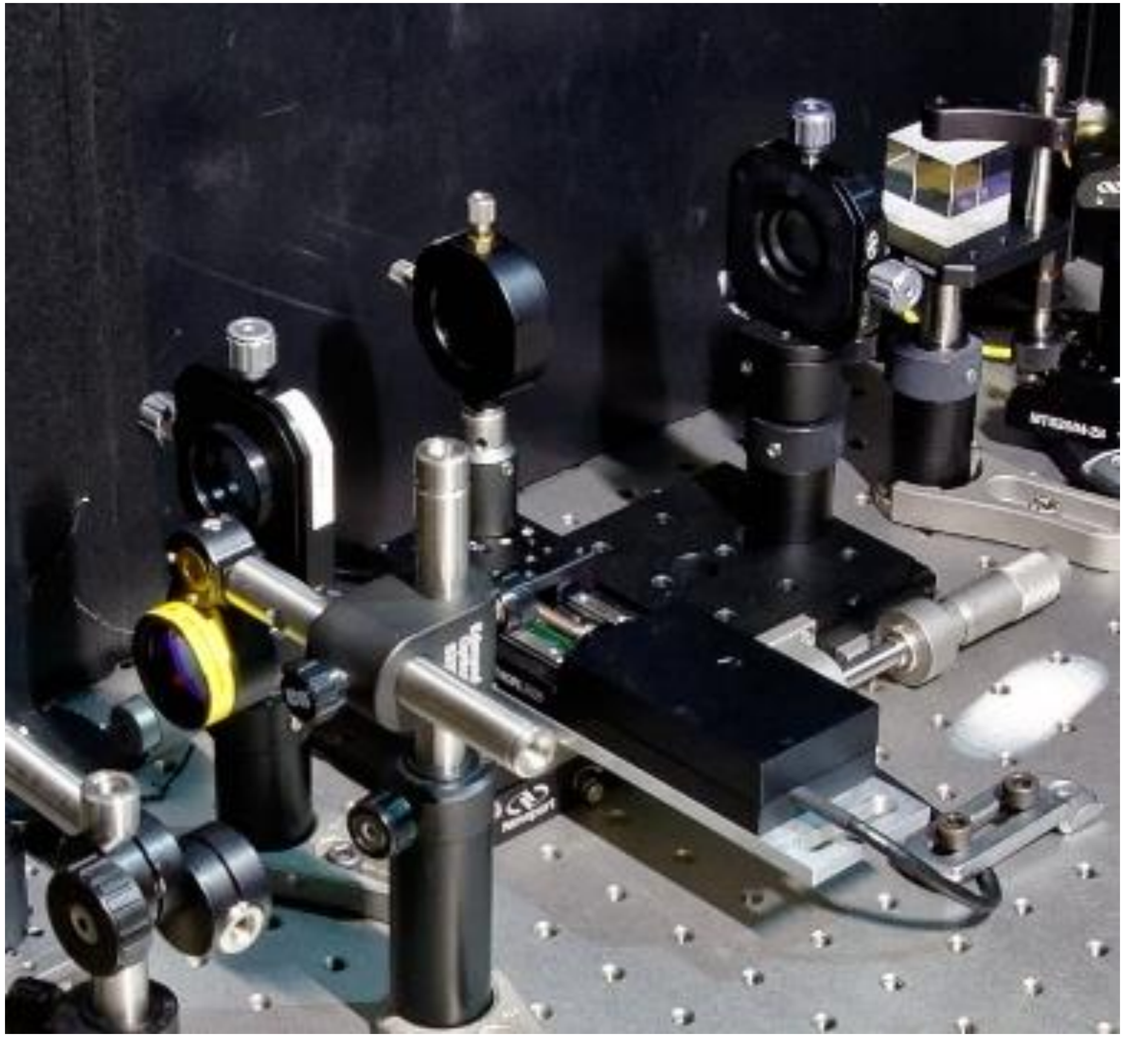
Pinhole in



The real setup: Optical detection section

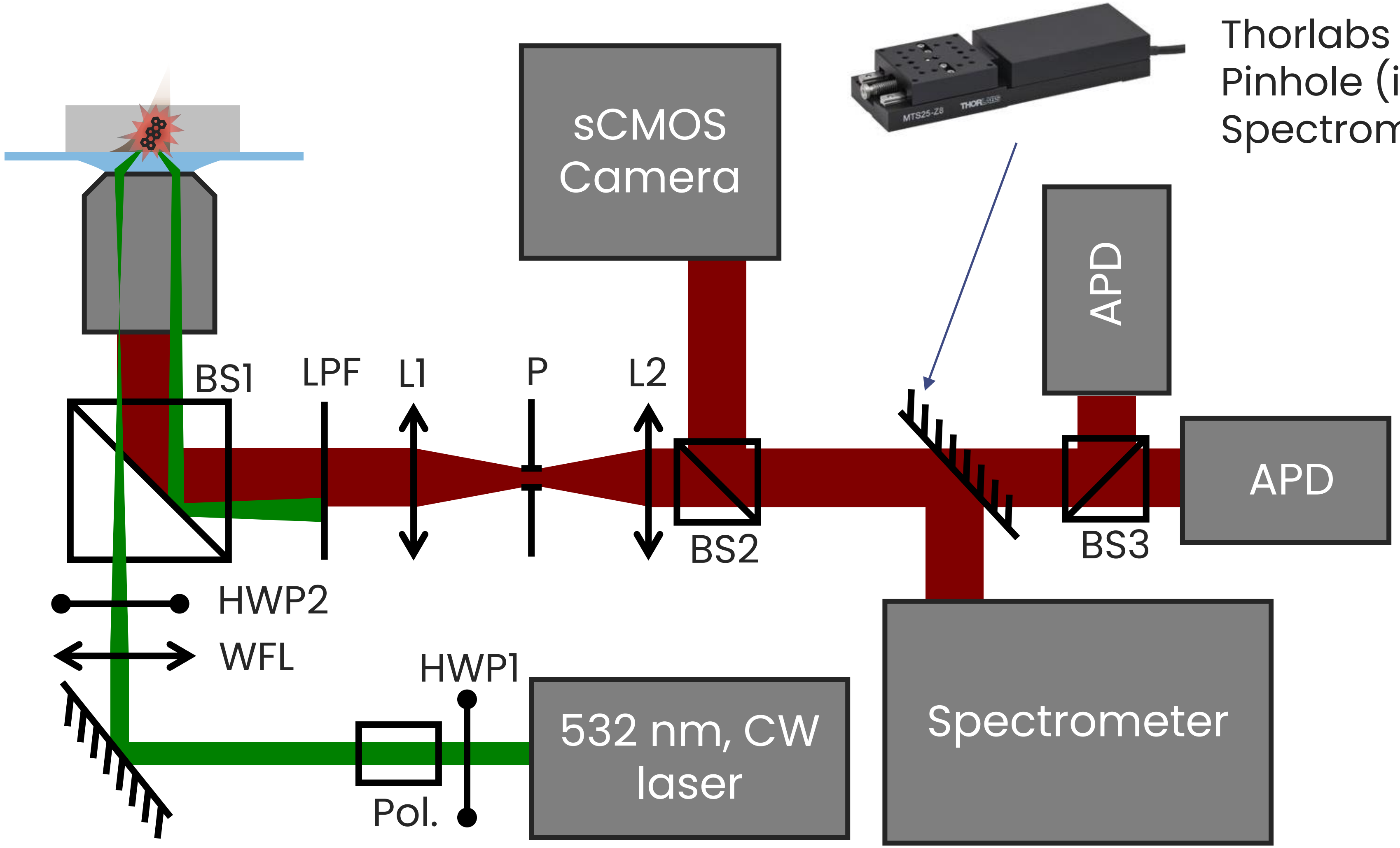


The real setup: confocal pinhole



Hamamatsu Orca Flash V4

Power and polarization control

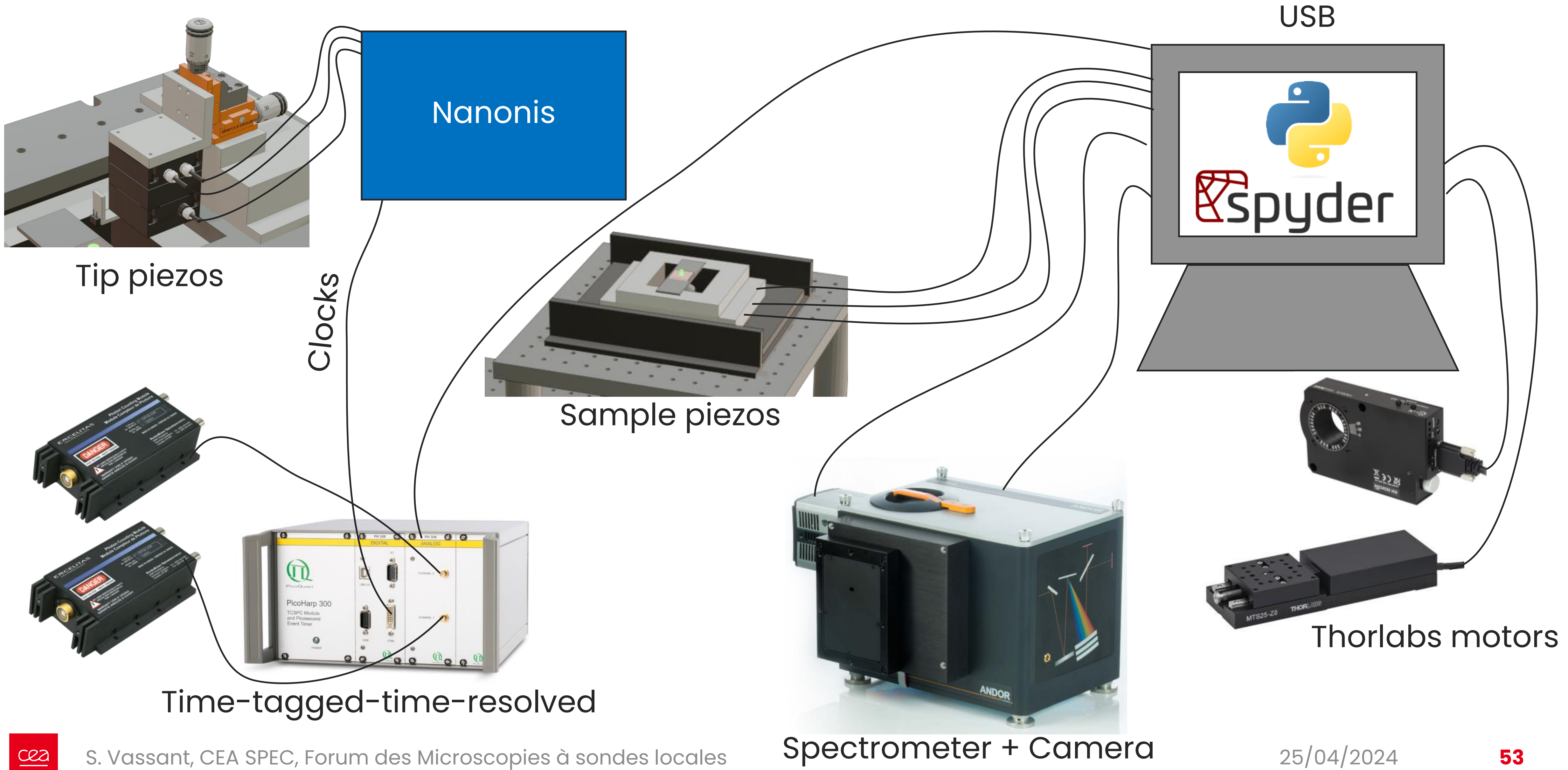


Thorlabs motorized translation
Pinhole (in and out)
Spectrometer mirror (in and out)

Thorlabs motorized rotations

Power control (with polarizer)
Input polarization control
Detection polarization control

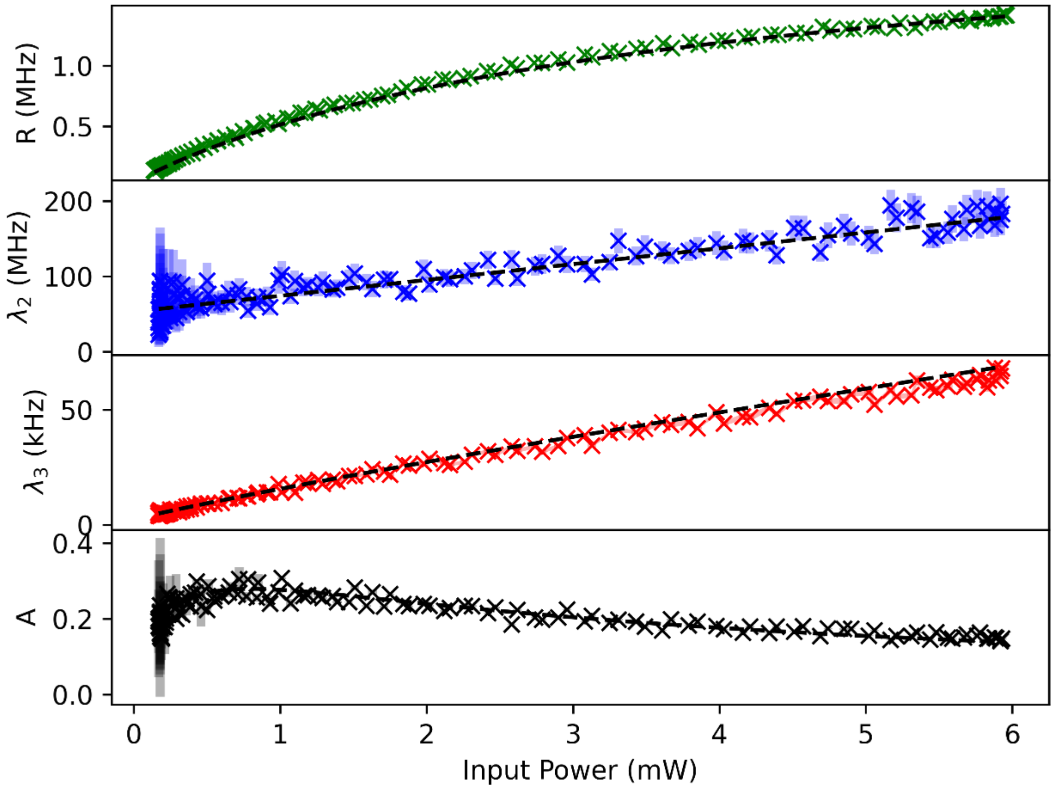
Synchronization of devices

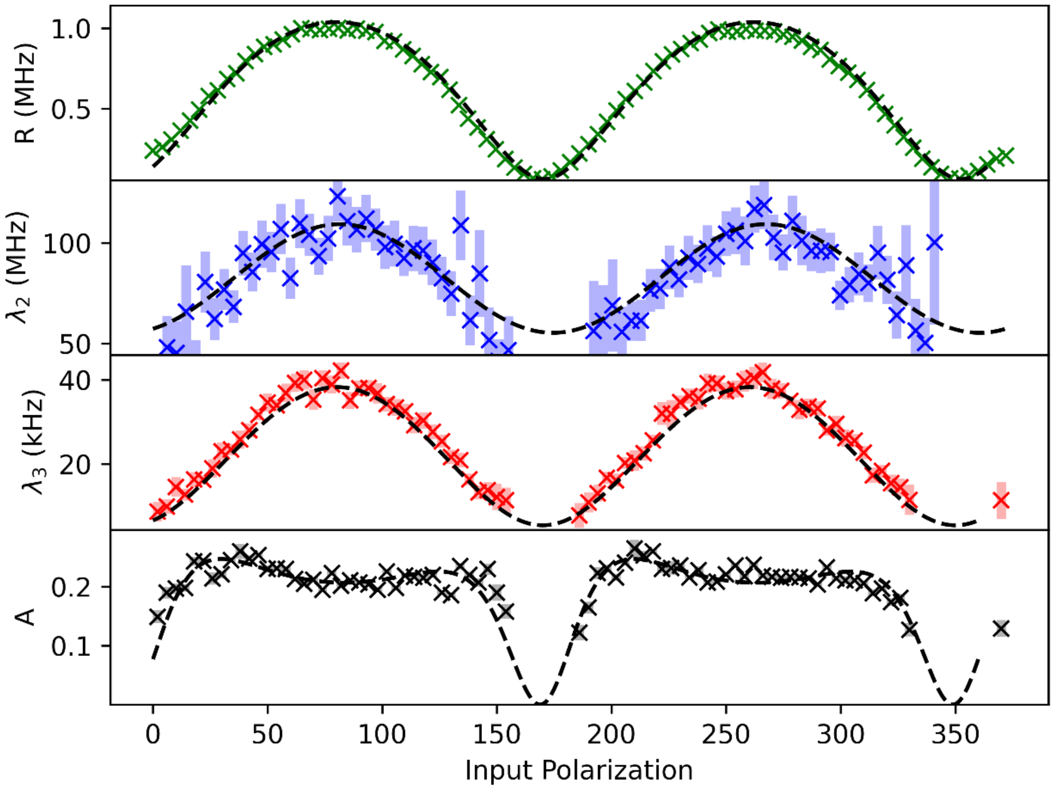


5. Single molecule measurements

=> Poster

Measurements on single molecule



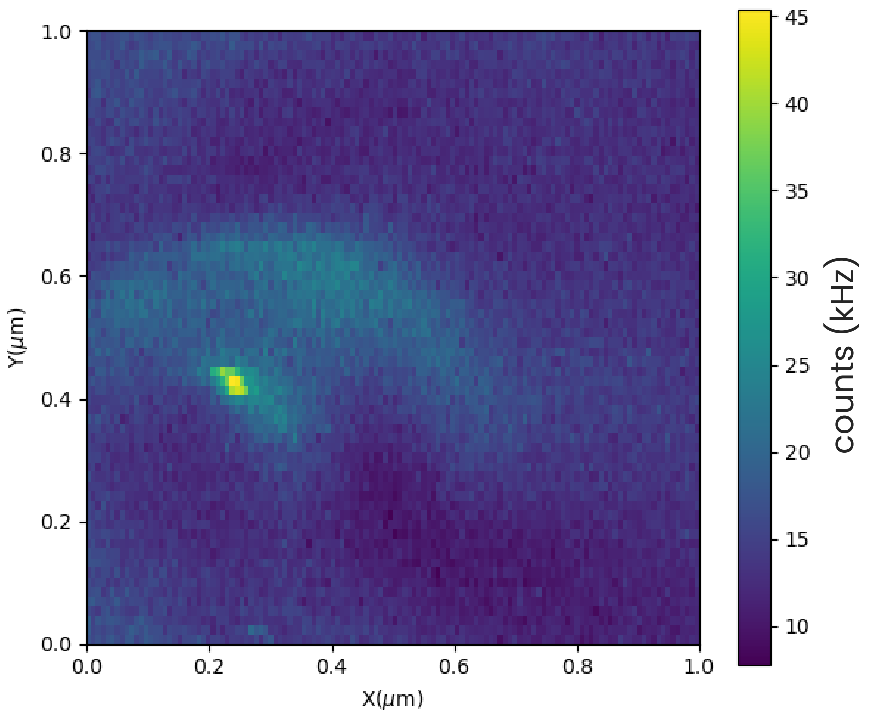


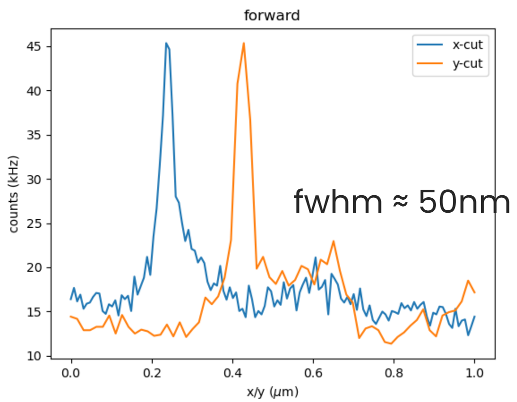
$$g^{(2)}(\tau) = 1 - (1 + A)\exp(-\lambda_2\tau) + A\exp(-\lambda_3\tau)$$

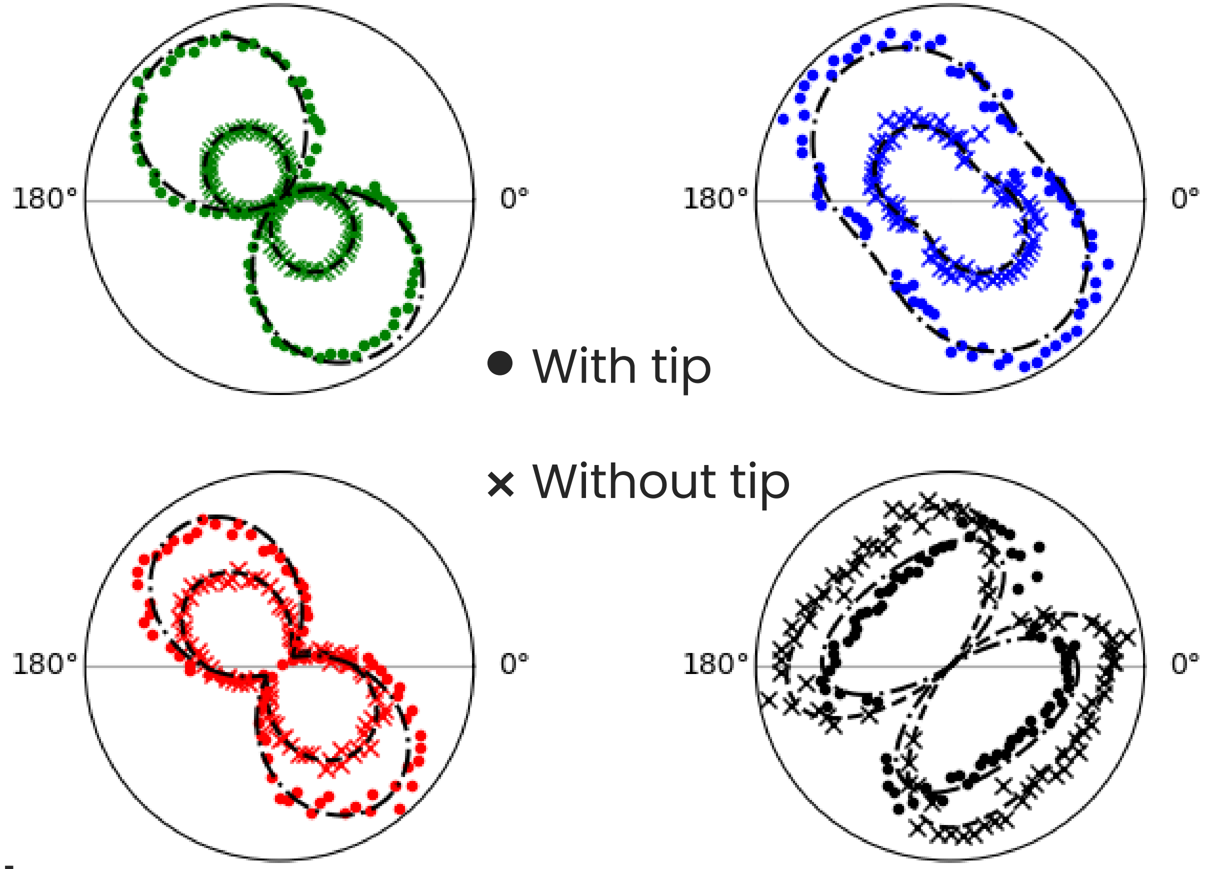
$$\begin{aligned}
\sigma_{12} &= 1.2 \times 10^{-16}\ \mathrm{cm}^2 \\
\sigma_{32a} &= 6.2 \times 10^{-20}\ \mathrm{cm}^2 \\
\sigma_{32o} &= 3.7 \times 10^{-21}\ \mathrm{cm}^2 \\
k_{21} &\approx 50\ \mathrm{MHz} \approx 20\ \mathrm{ns} \\
k_{23} &\approx 13\ \mathrm{kHz} \\
k_{31} &\approx 2.6\ \mathrm{kHz}
\end{aligned}$$

$$\begin{aligned}
\theta &= 22 \pm 9^\circ \\
\phi &= 15 \pm 8^\circ \\
\alpha &= 306 \pm 4^\circ
\end{aligned}$$

Effect of a dielectric tip







Tip effect :

Lifetime reduction (/2.3)

Increased intersystem crossin rate k_{23} (x1.5)

Increased triplet-singlet relaxation rate k_{31} (x1.4)

Enhancement of E_z (x1.2)

Collection efficiency unchanged (5.8%)

Thank you !

Région îledeFrance

Projet ANR JCJC PlasmonISC

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Lydia Sosa-Vargas

Centre de Nanosciences & de Nanotechnologies
Christophe Dupuis

How much does it cost ?

Fiber puller: 17 k€

2 APDs: 15 k€

sCmos Camera: 11 k€

High NA objective: 15 k€

Sample piezo: 27 k€

AFM control (Nanonis): 35-45 k€

TTTR electronics: 20 k€

Tip piezos: 30 k€

Spectrometer with camera: 26 k€

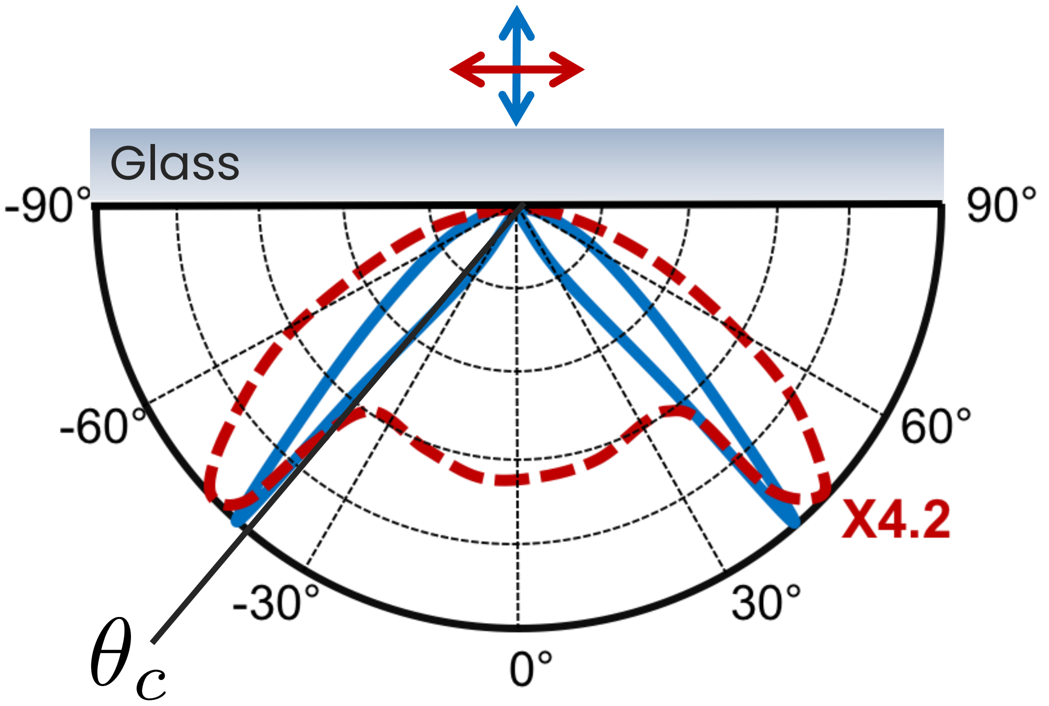
532nm CW laser: 9 k€

Thorlabs optomecanics + filters: 30-40 k€

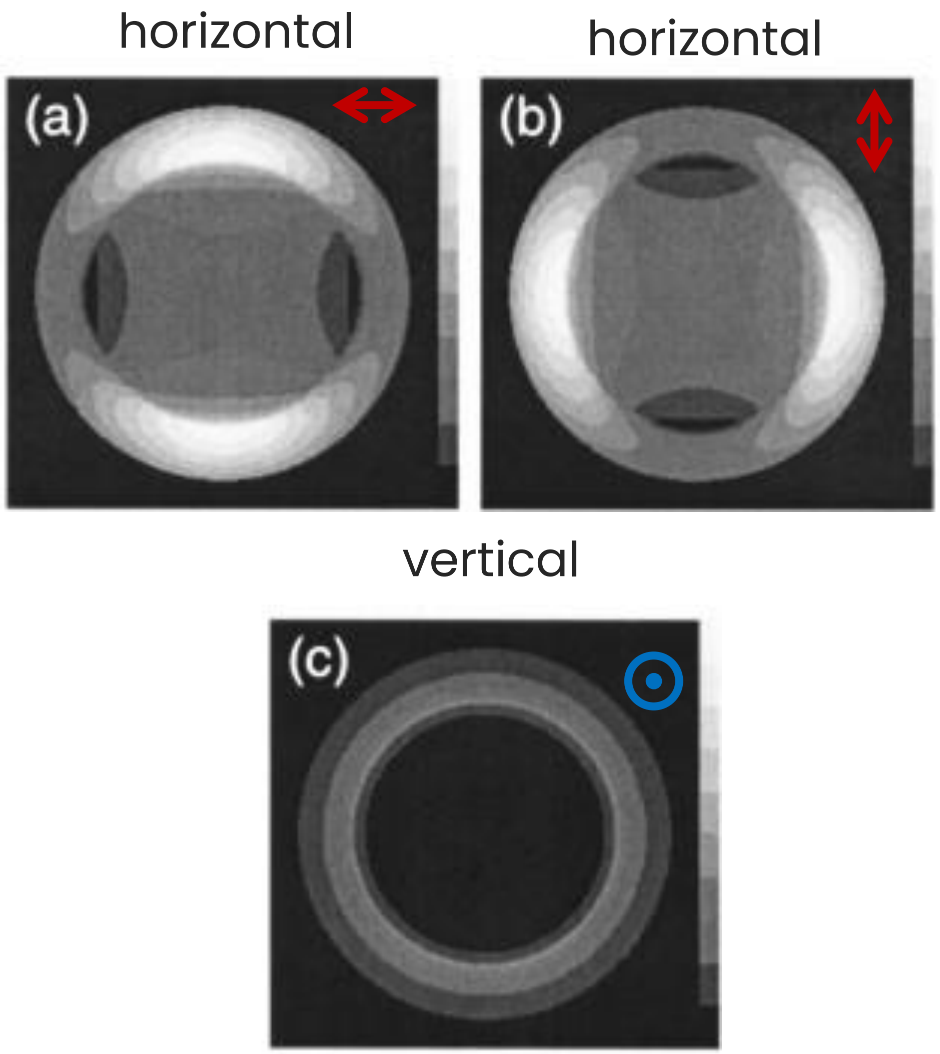
Total ≈ 235 – 255 k€

Imaging the back-focal plane

Angular emission diagram



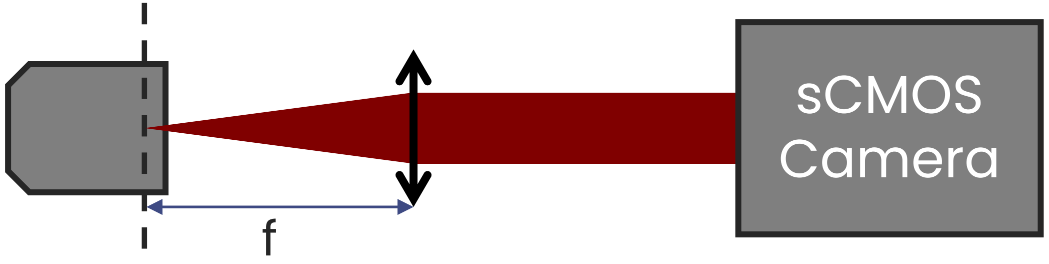
M. Trnavsky et al. J. Biomed. Opt. **13**(5), 05021 (2008)
A. Lieb et al. J. Opt. Soc. Am. B **21**, 1210 (2004)



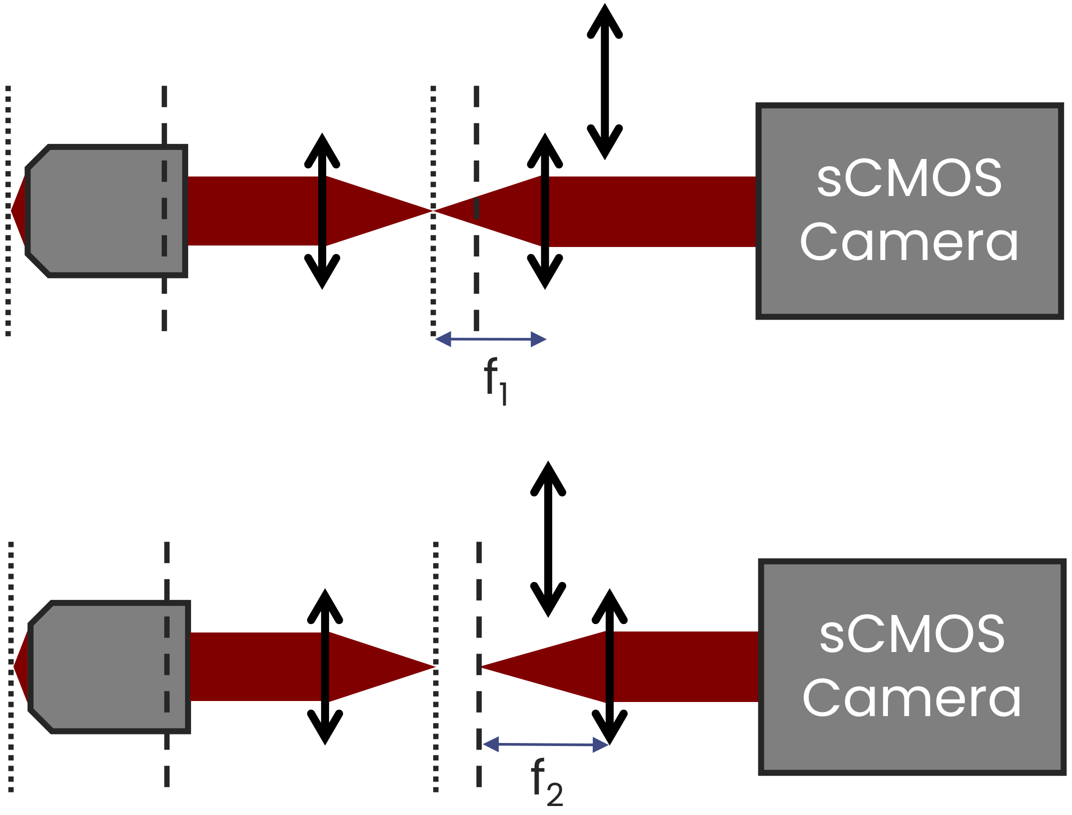
Back focal plane images

Imaging the back-focal plane

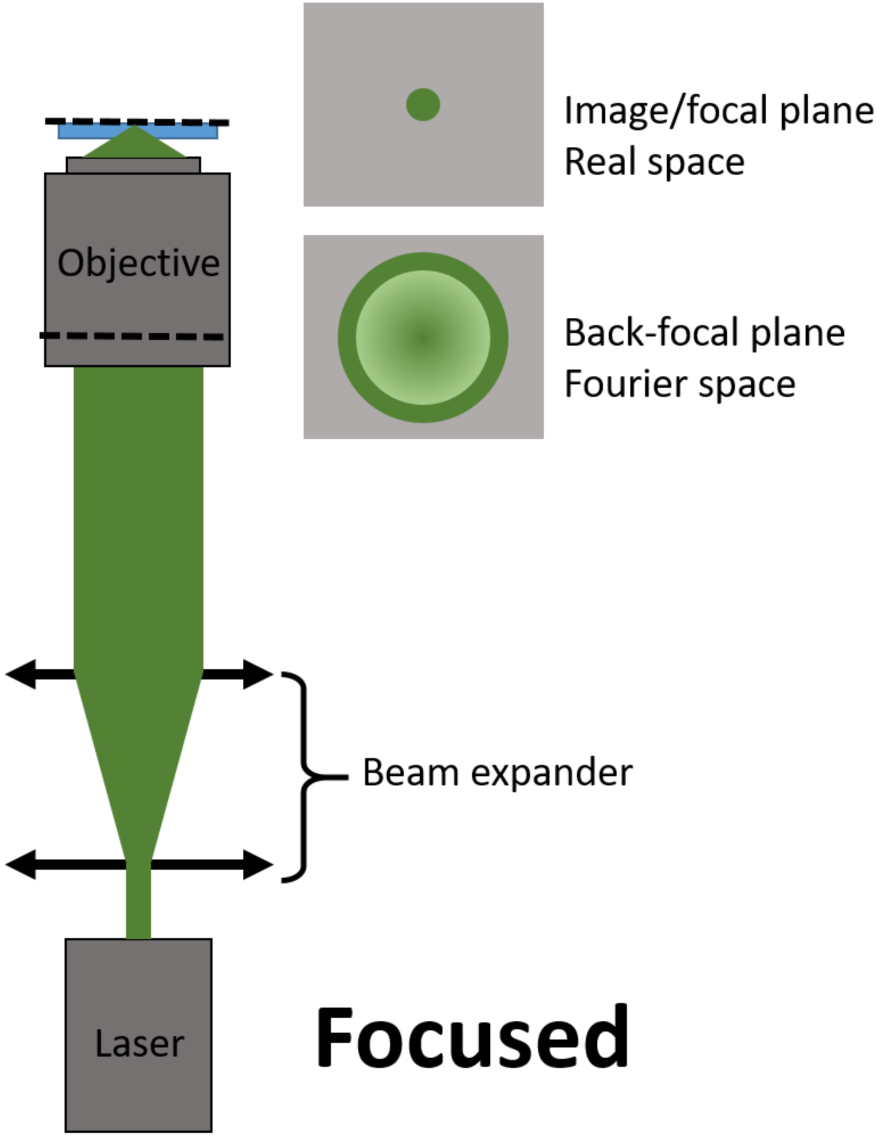
Simple BFP imaging

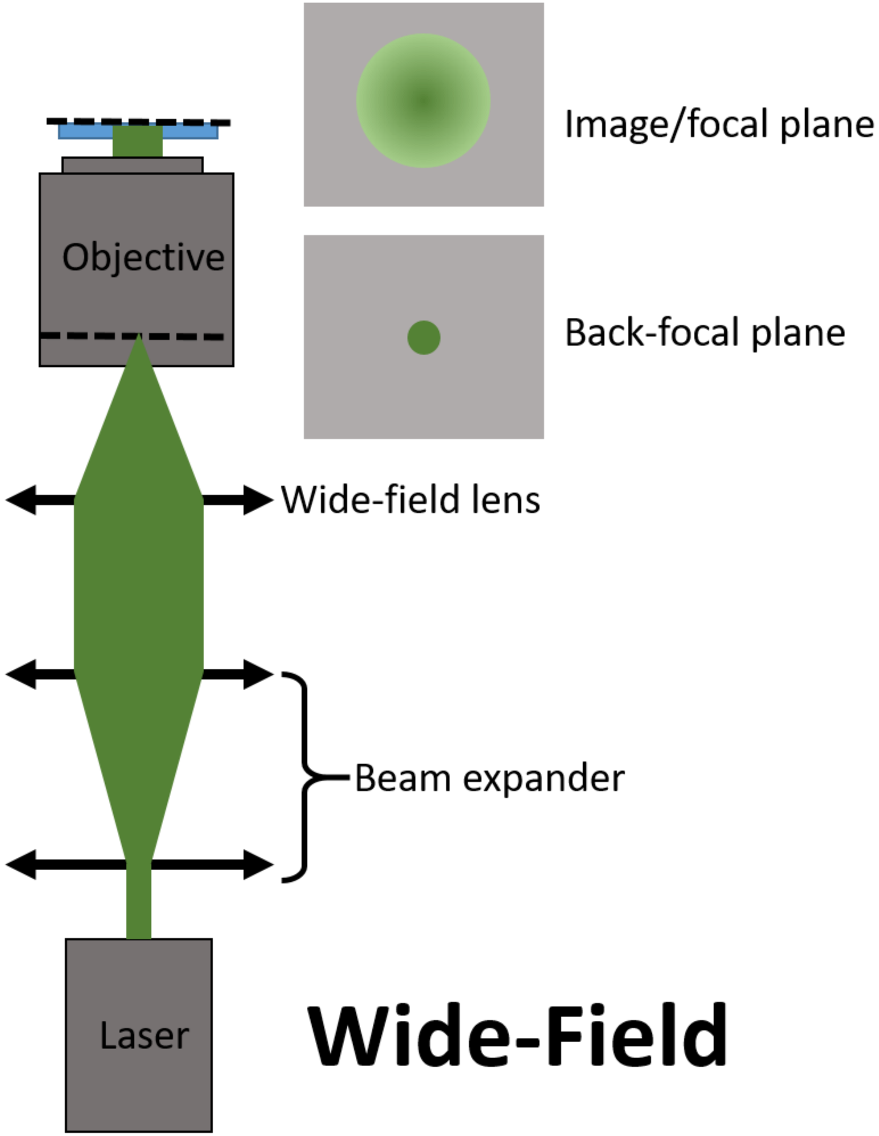


Creating a BFP conjugated plane

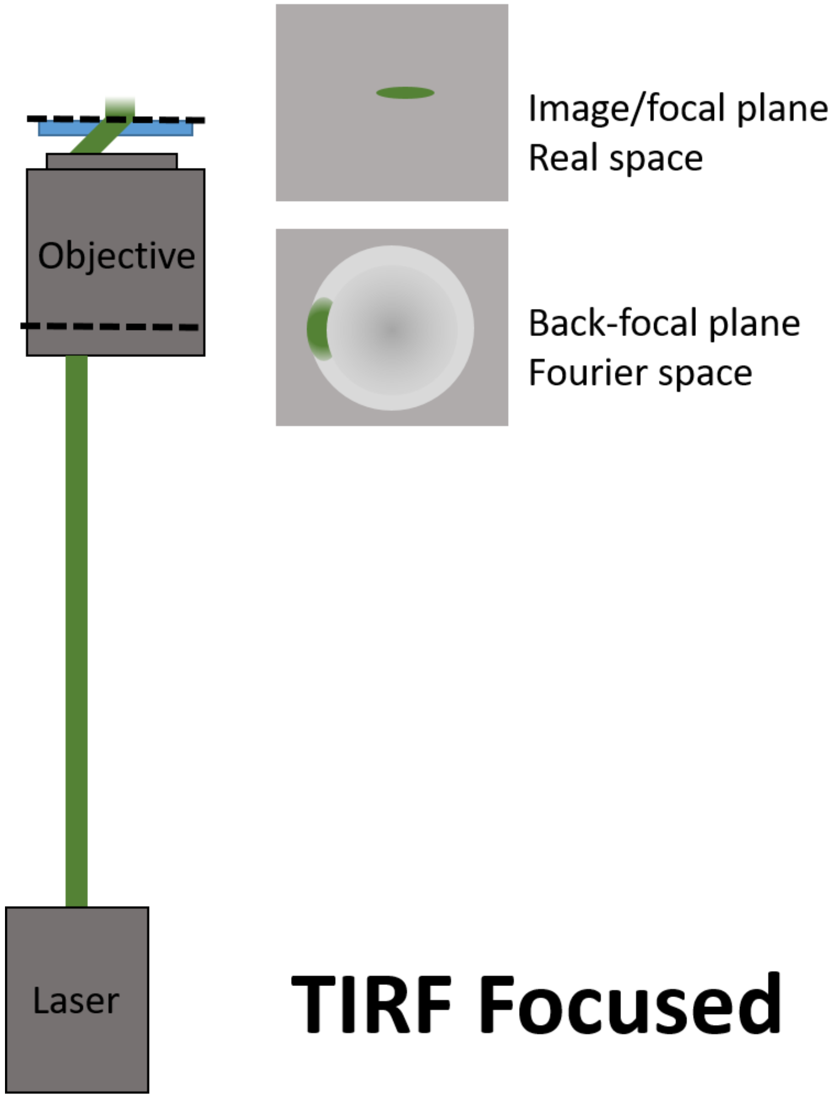


Normal incidence

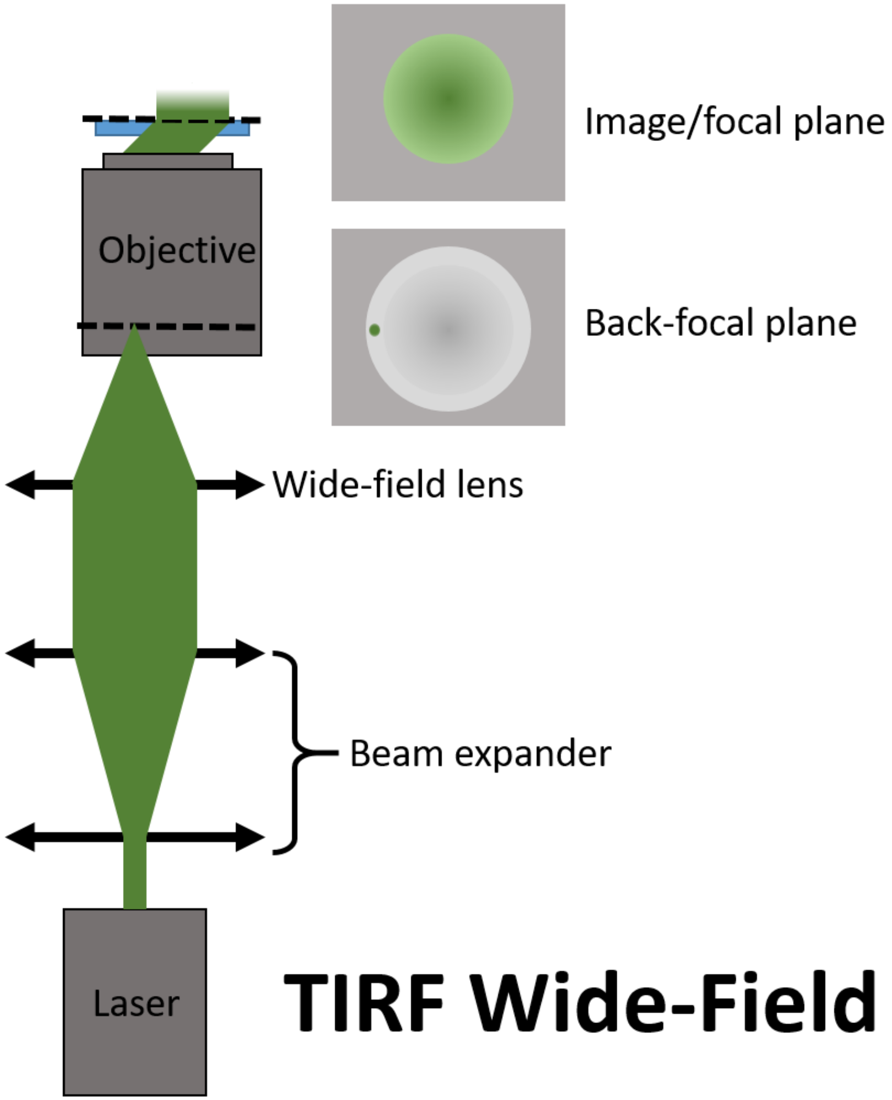




Total internal reflexion



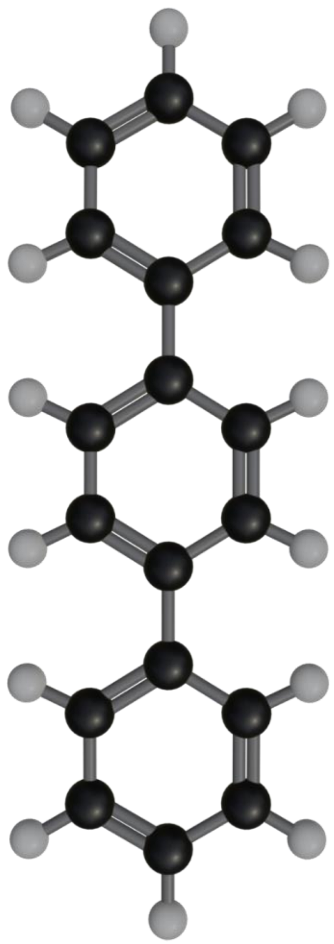
TIRF Focused



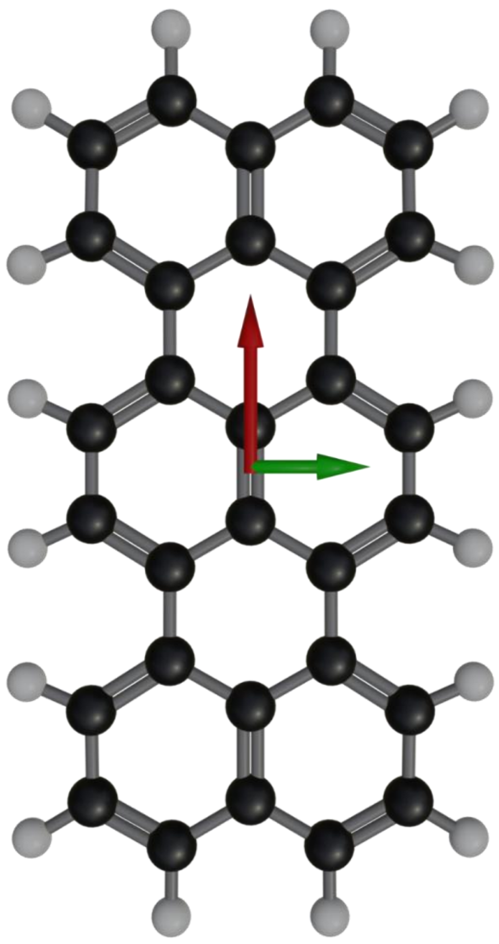
TIRF Wide-Field

Molecular System

Matrix : para-Terphenyl (pT)

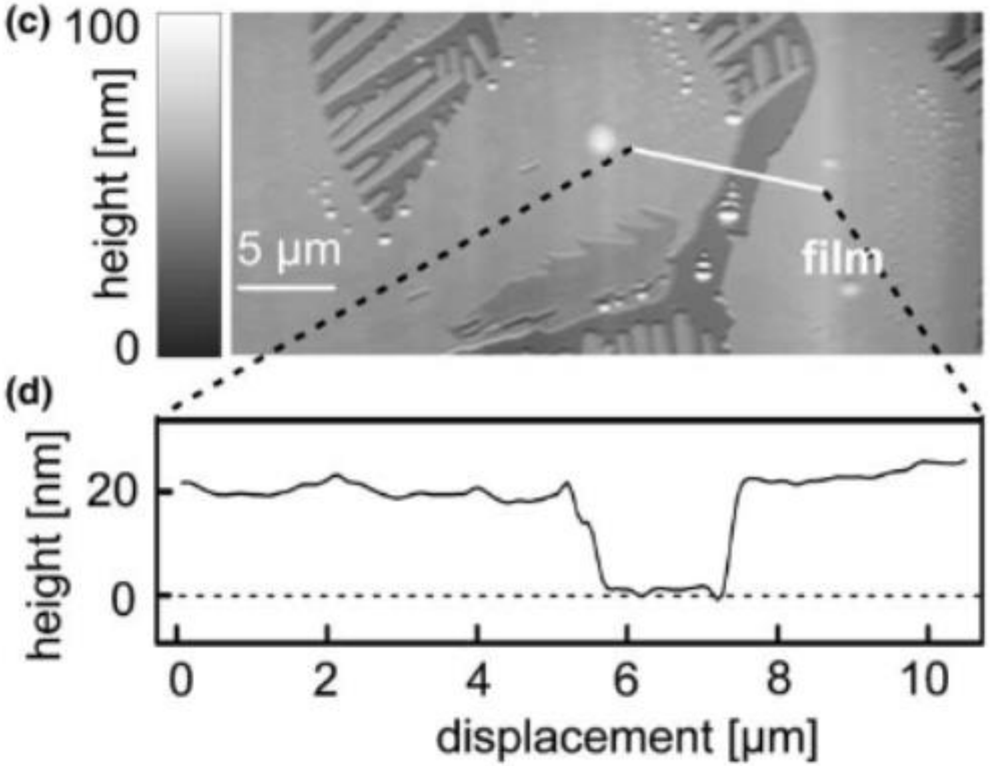


Guest molecule : Terrylene (Tr)

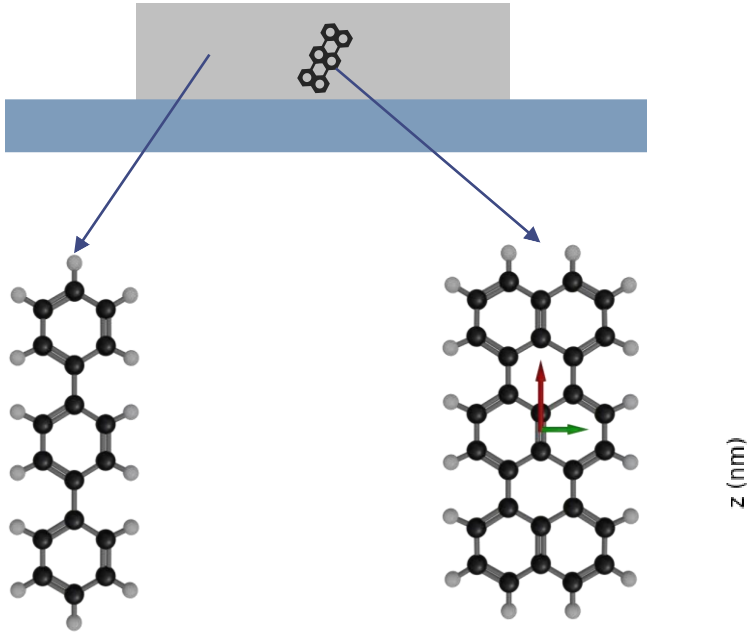


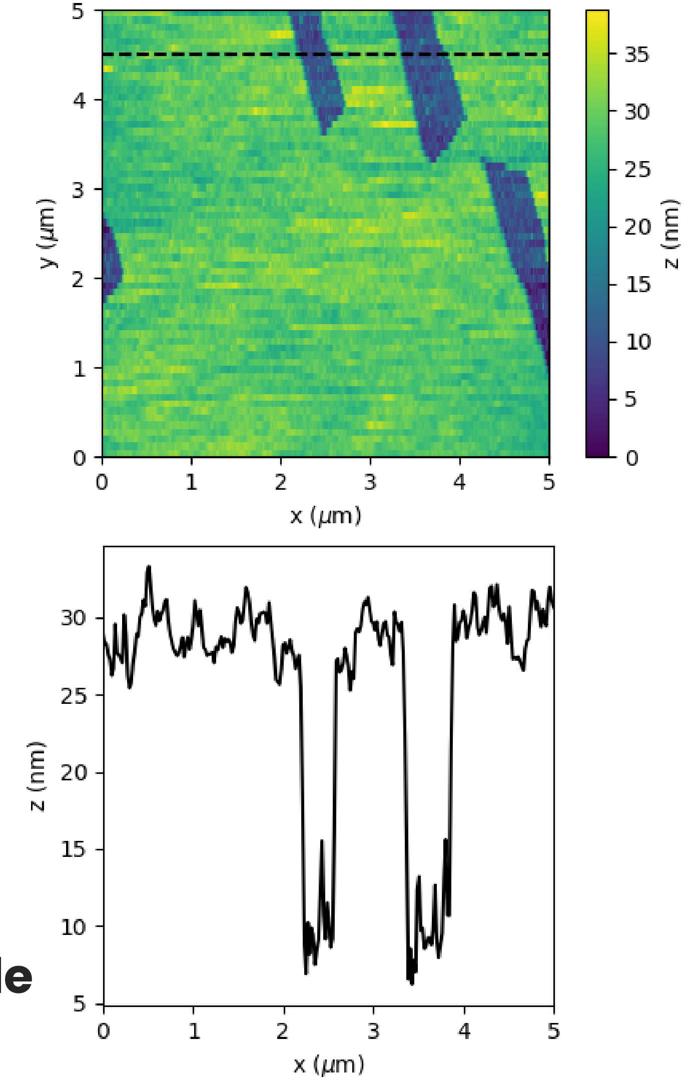
Molecular System

Sample preparation : Dilution in toluene and spin-coating

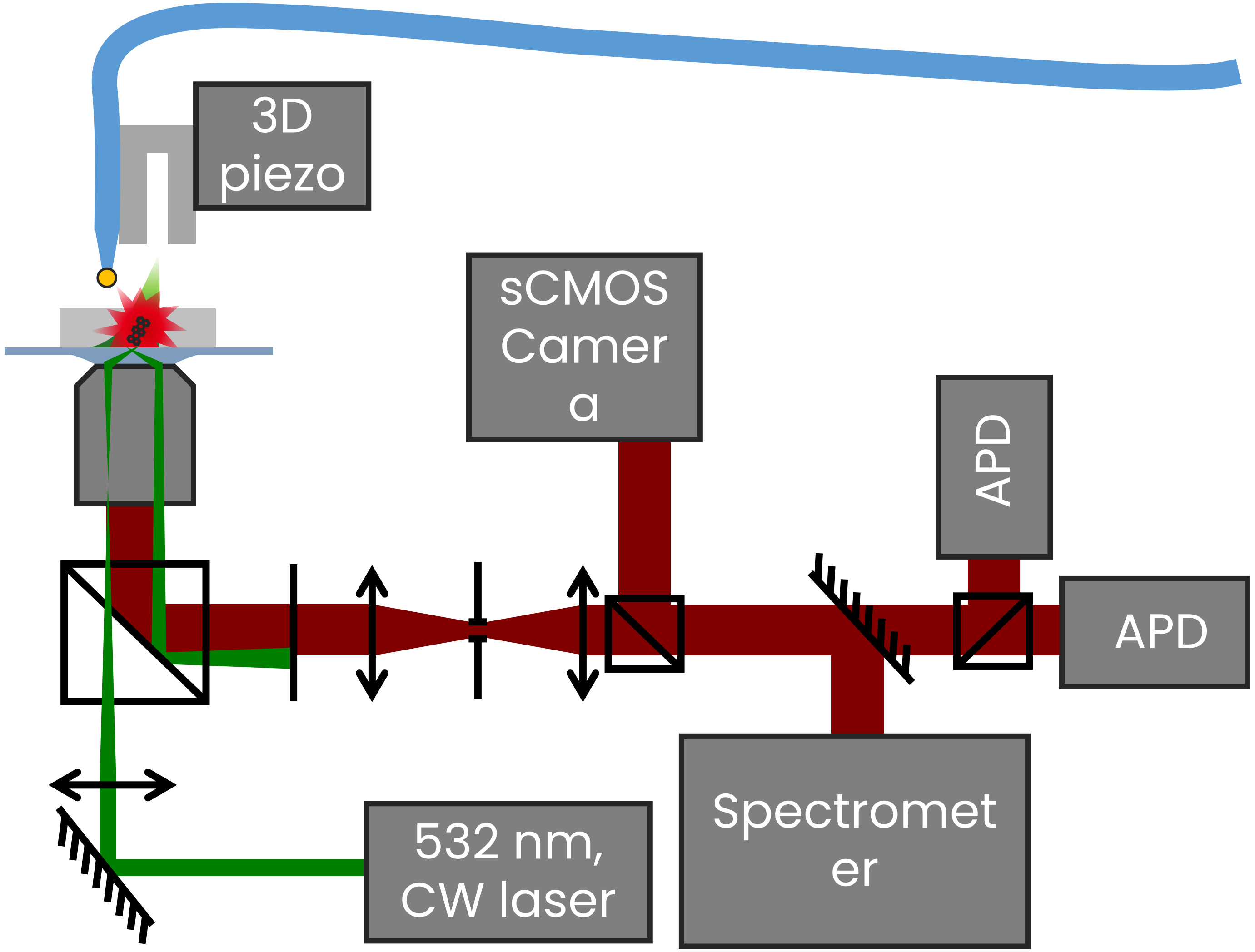


Pfab et al., Chem. Phys. Lett. **387**, 490–495 (2004)

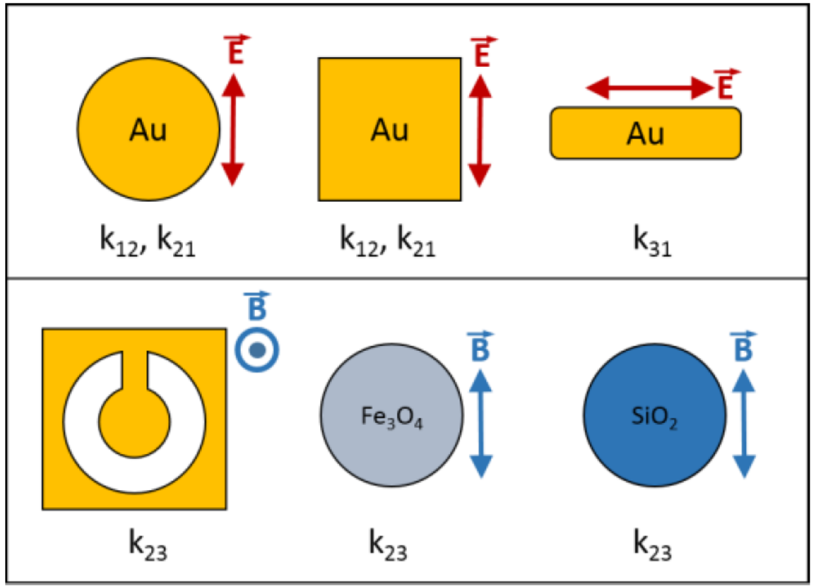




Perspectives

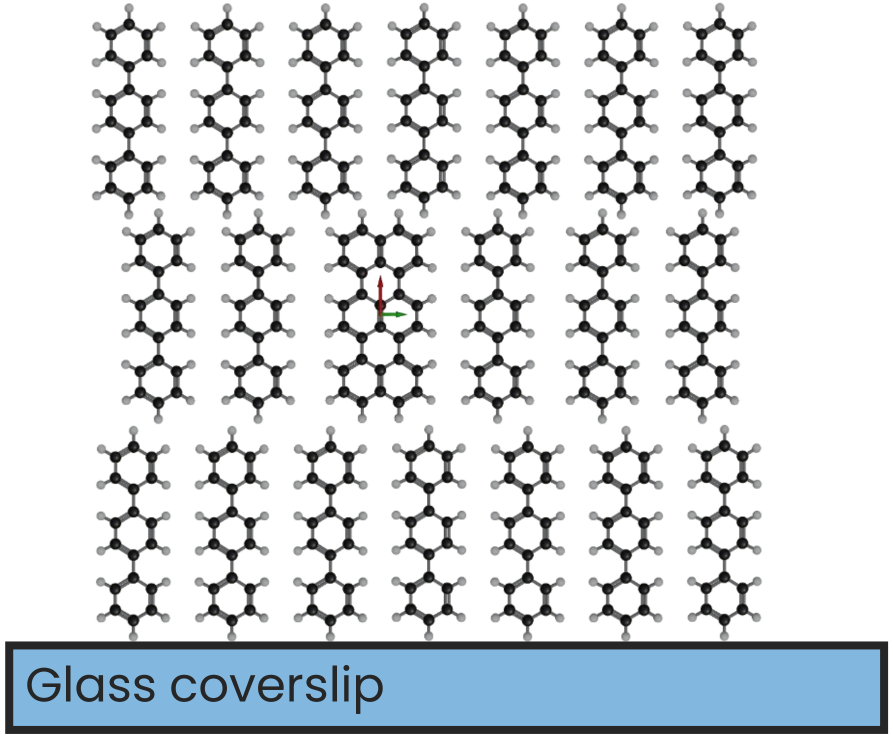


Tip functionalization

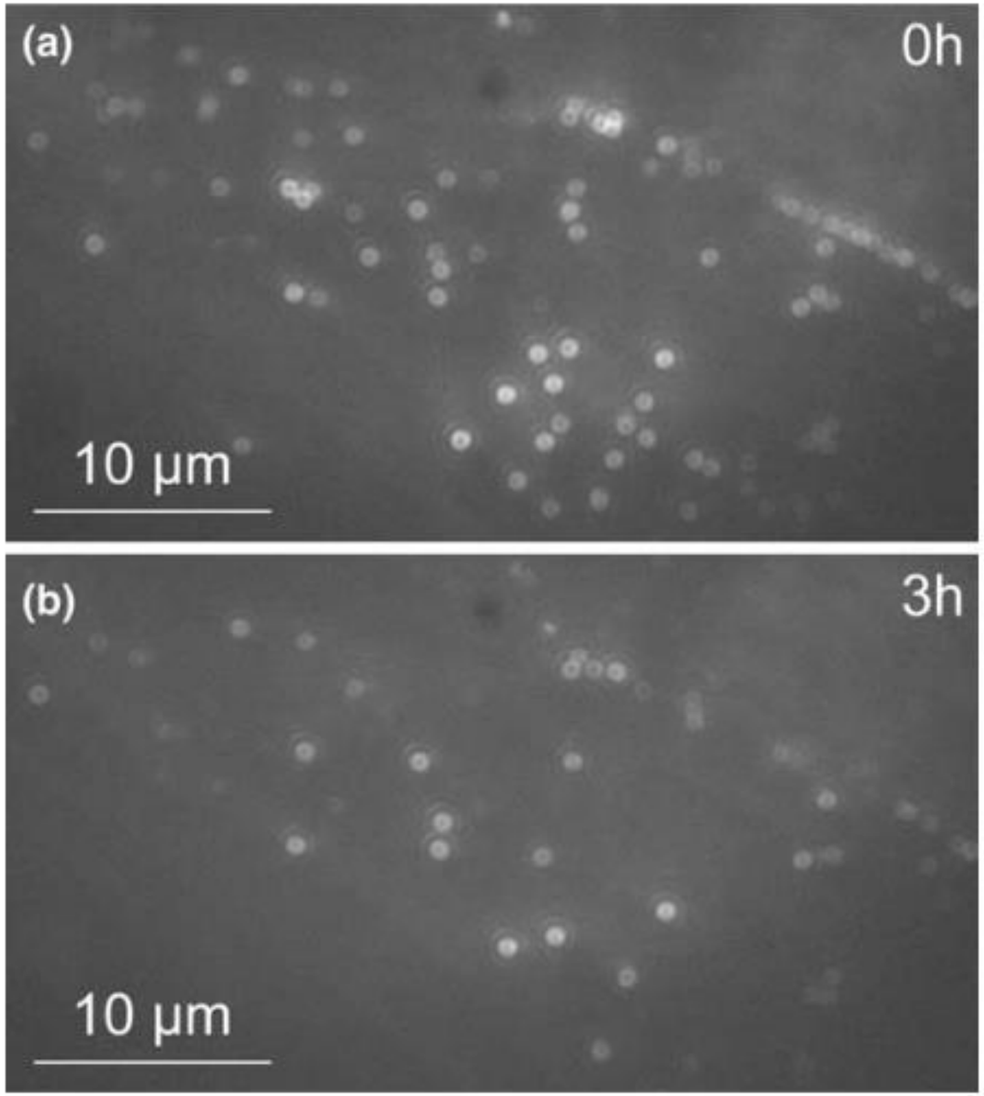


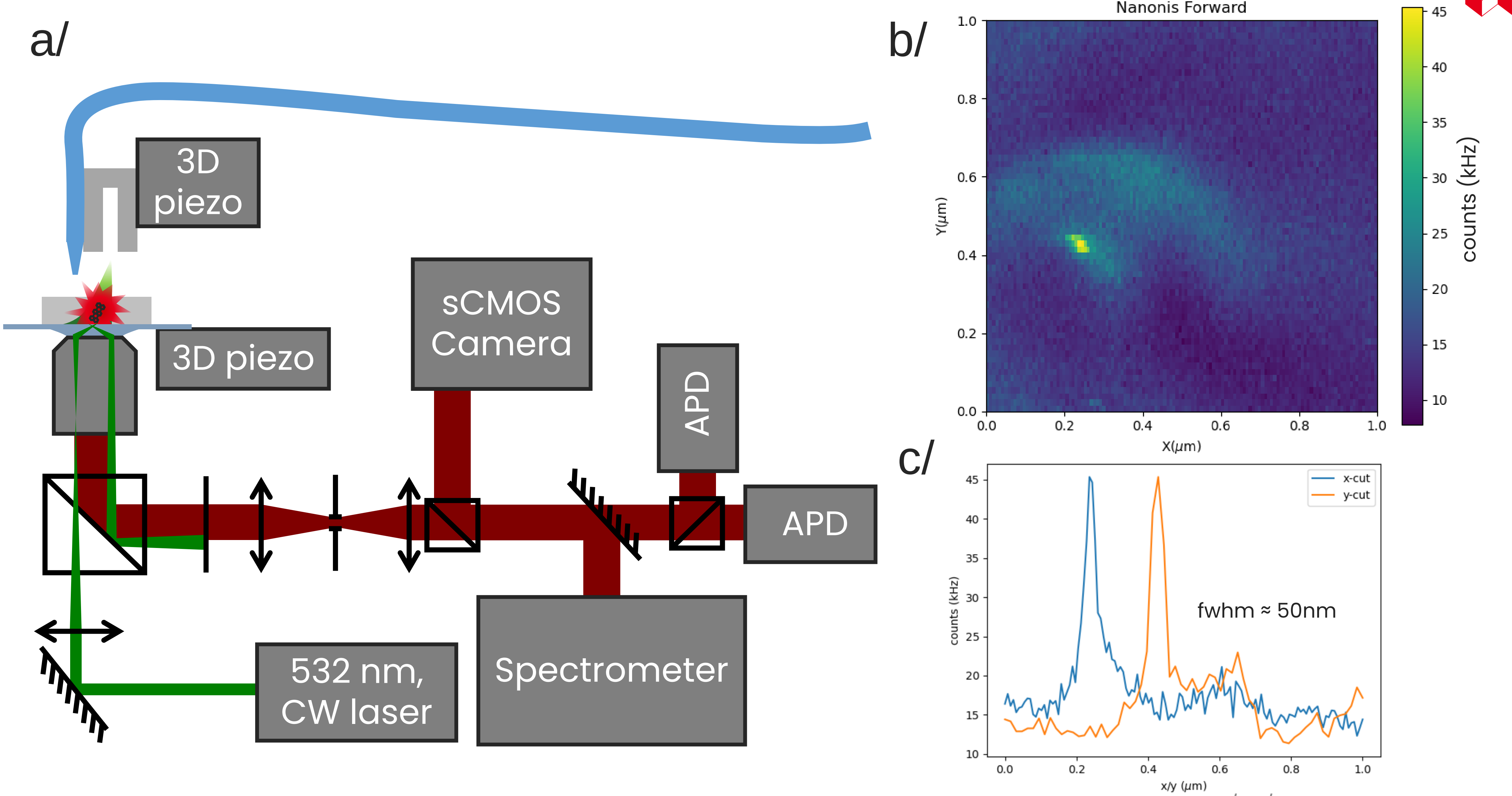
Molecular System

Highly oriented molecules, high photostability

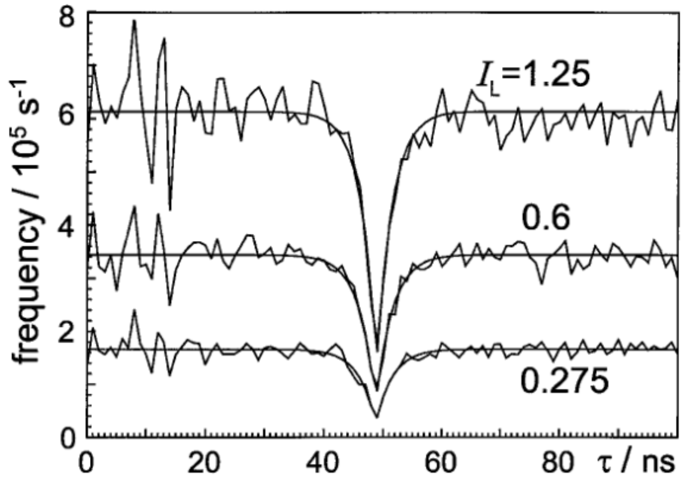


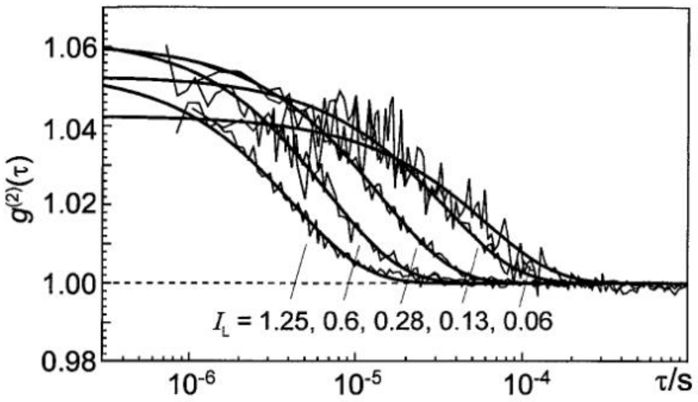
Pfab et al., Chem. Phys. Lett. **387**, 490–495 (2004)




a/
3D piezo
3D piezo
sCMOS Camera
APD
APD
532 nm, CW laser
Spectrometer
b/
Nanonis Forward
Y(µm)
X(µm)
counts (kHz)
c/
x-cut
y-cut
fwhm ≈ 50nm
counts (kHz)
x/y (µm)

Extracting rates





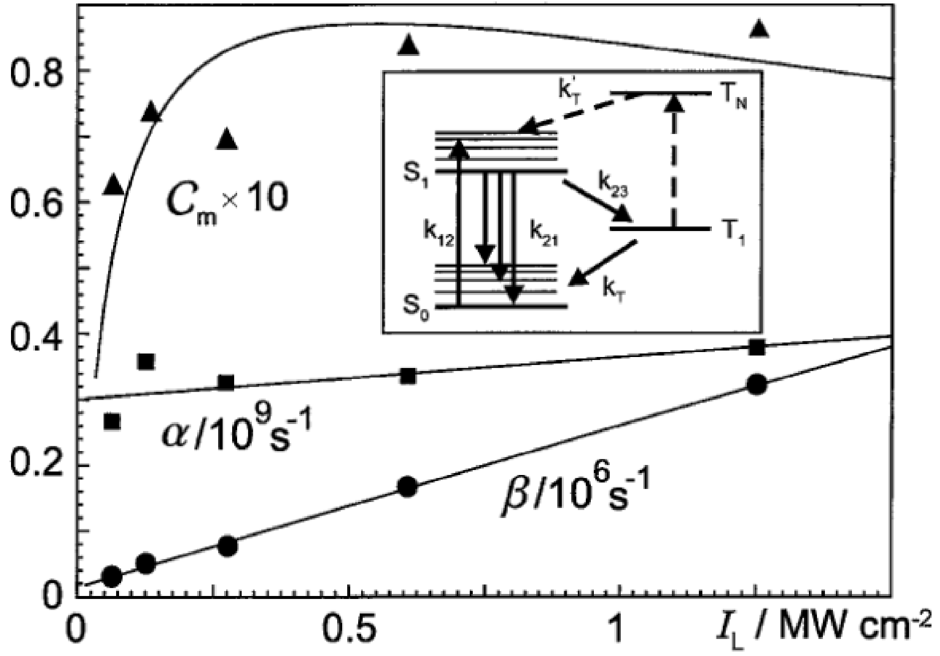
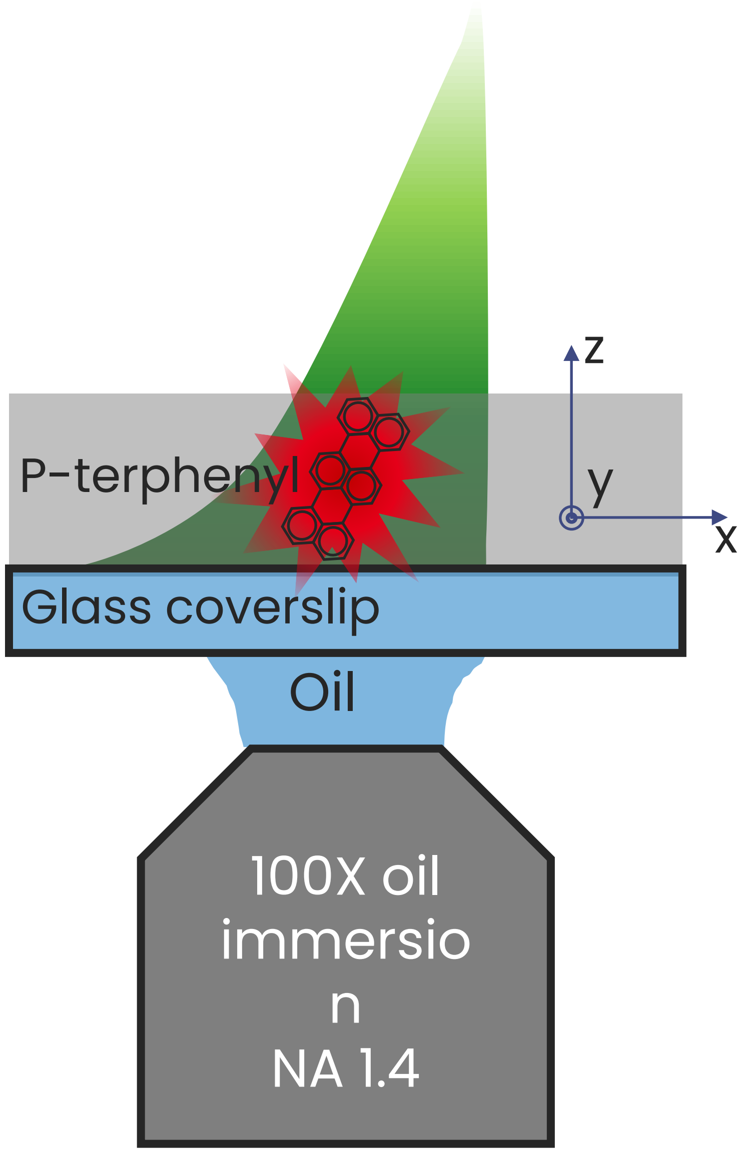


TABLE I. Transition rates of molecules M1–M3. For k_{31} the ranges of observed values are given. k_T fixed for M2.

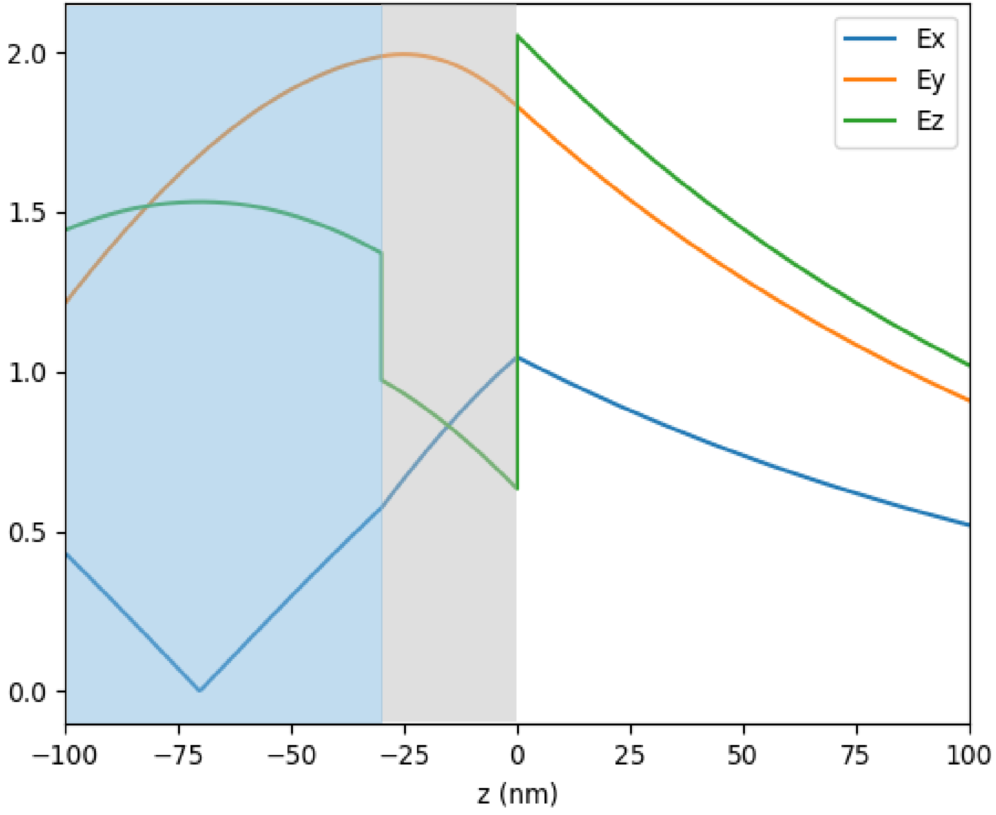
Molecule	σ 10^{-17} cm^2	k_{21} 10^8 s^{-1}	k_{23} 10^5 s^{-1}	k_T 10^3 s^{-1}	k_{31} 10^5 s^{-1}
M1	1.4	3.0	1.2	14.0	2–30
M2	7.5	1.2	23.0	3.5	2–30
M3	2.5	1.7	4.4	3.2	1–5

Fleury et al., PRL **84**, 1148 (2000), Terrylene in thick para-terphenyl crystal

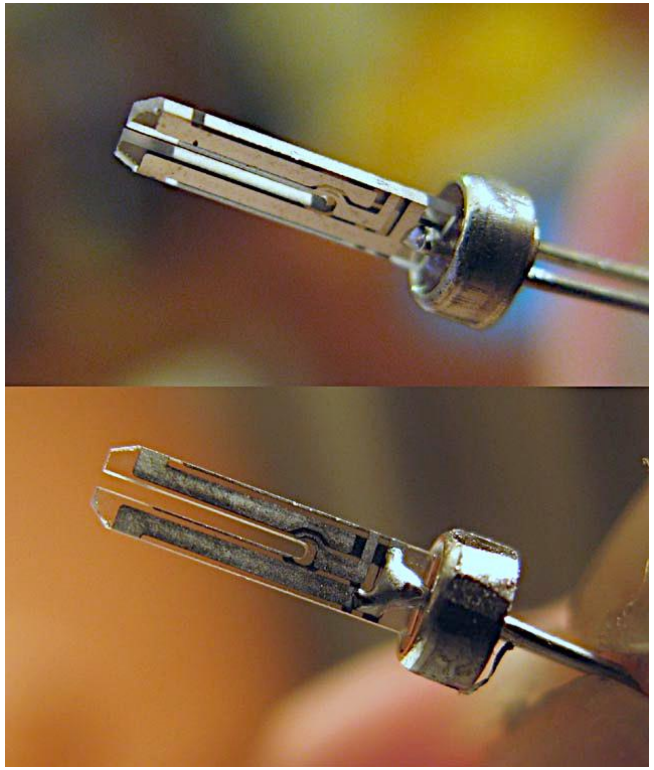
Excitation electric field distribution



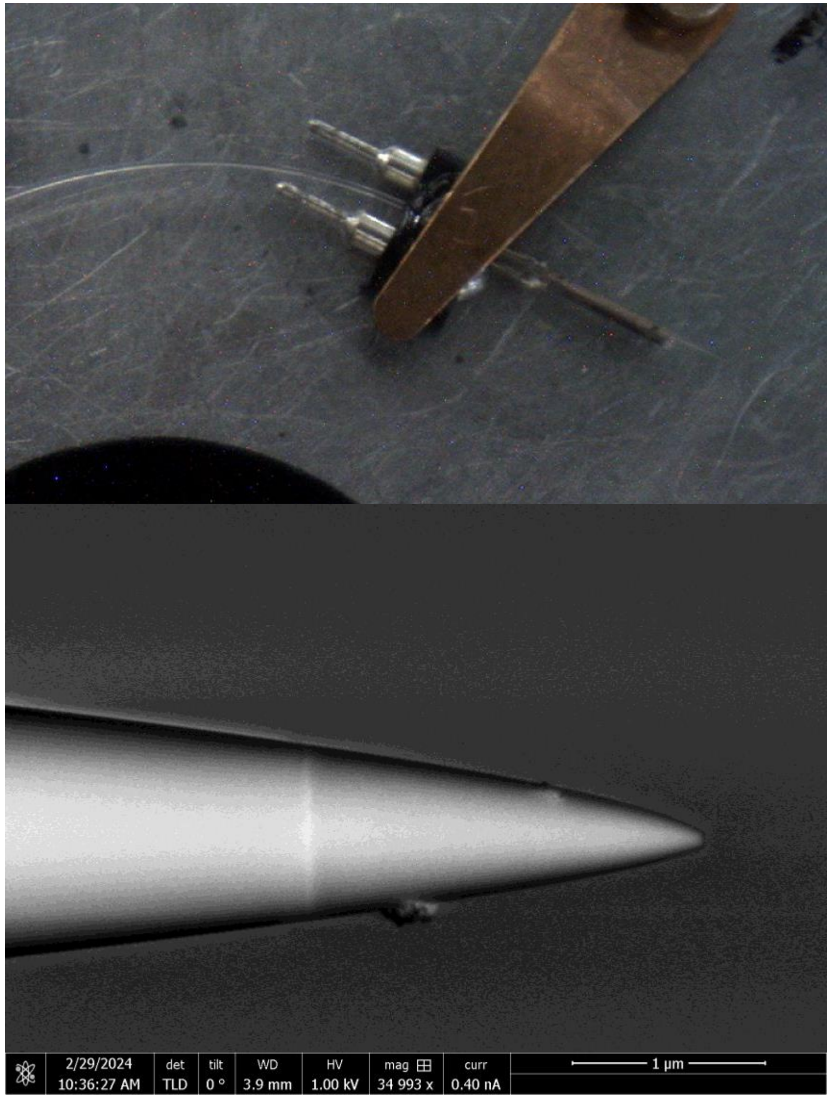
Electric Field distribution of the excitation beam

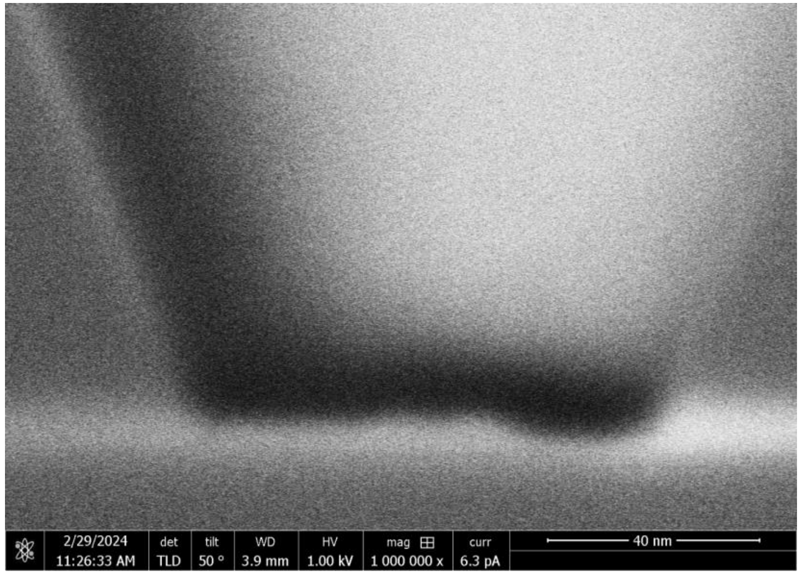


Pulled glass fiber: dielectric tip



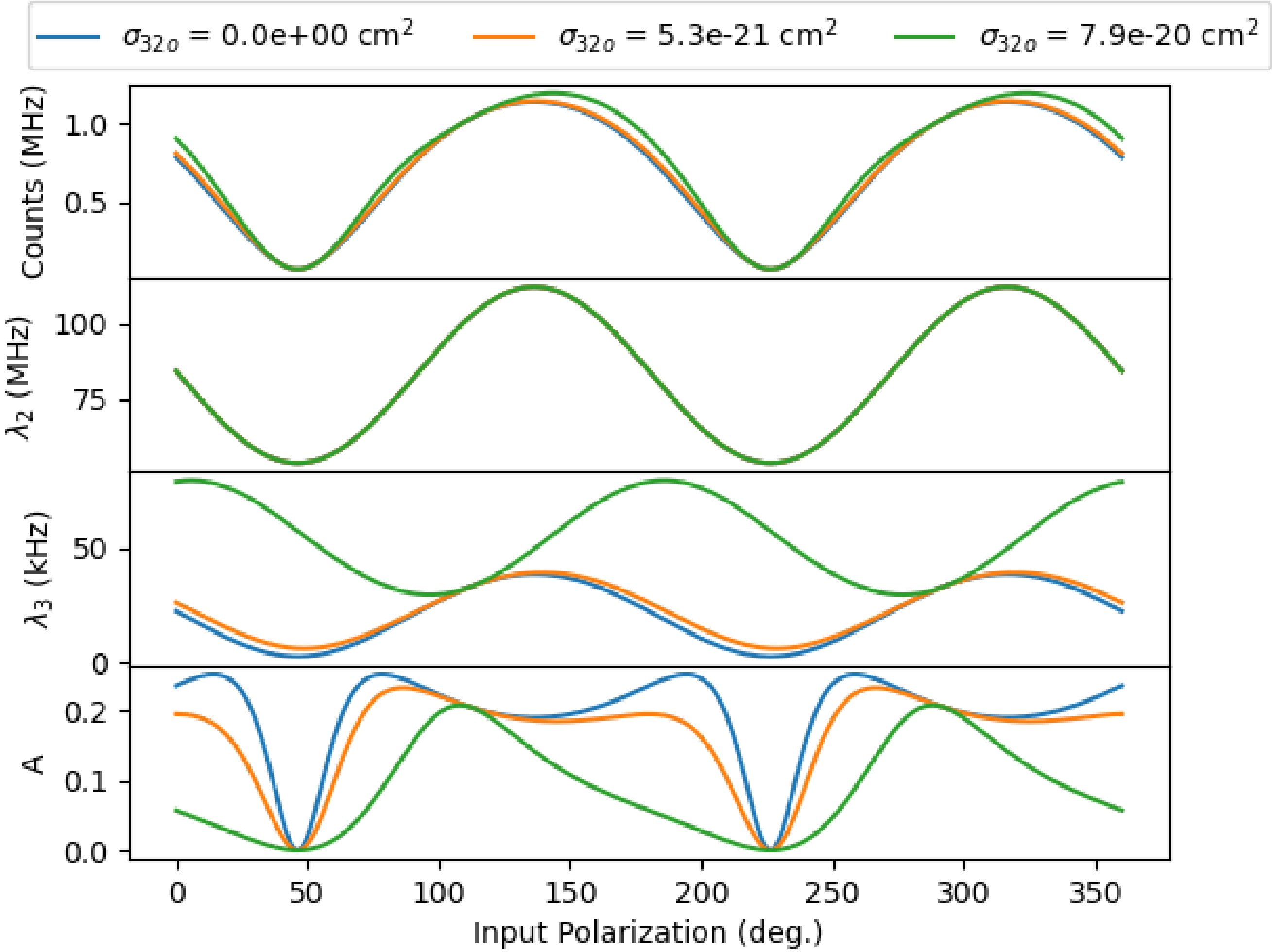
https://en.m.wikipedia.org/wiki/File:Inside_QuartzCrystal-Tuningfork.jpg

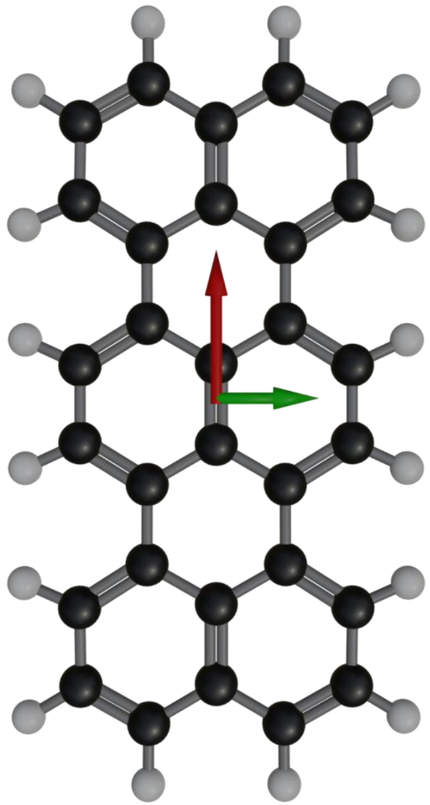




SEM : Christophe Dupuis, C2N

Calculations TT absorption





Imaging the back-focal plane

Imaging the back-focal plane of the objective:

Fourier transform of the image : light emission directions

Control of the illumination

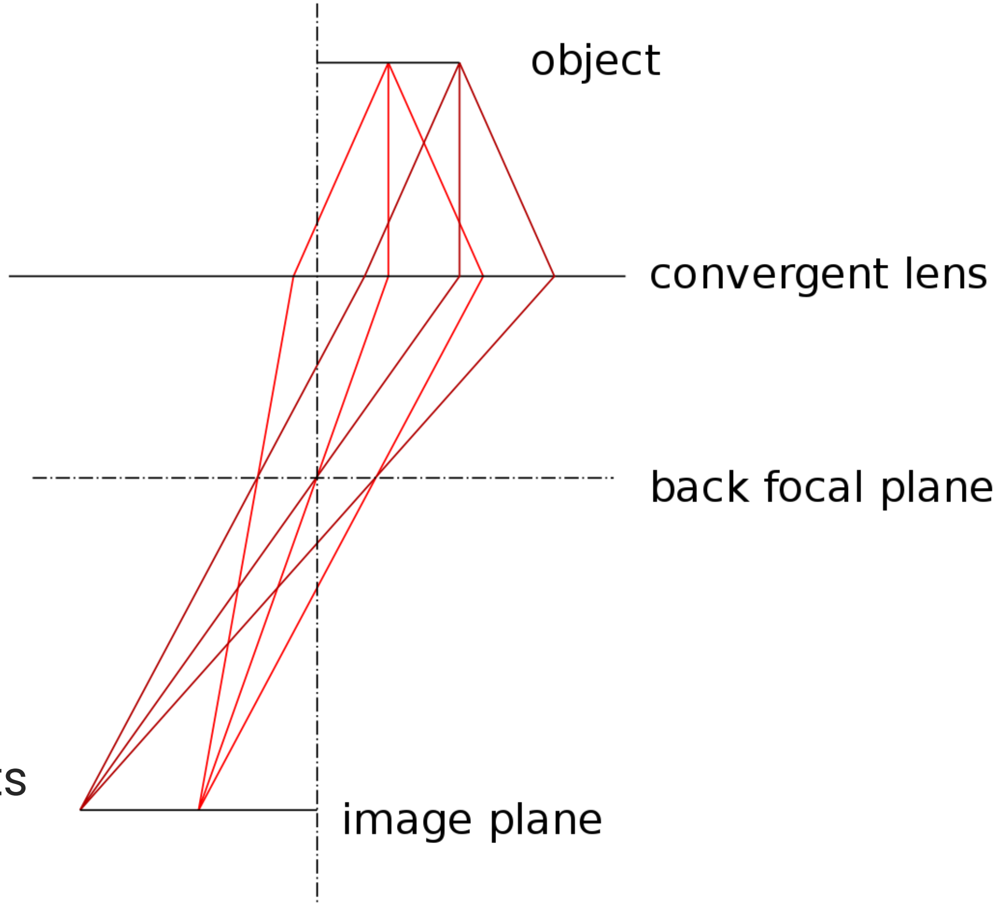
Measurement of the angle of incidence in TIRF

Determination of single emitter emission dipole

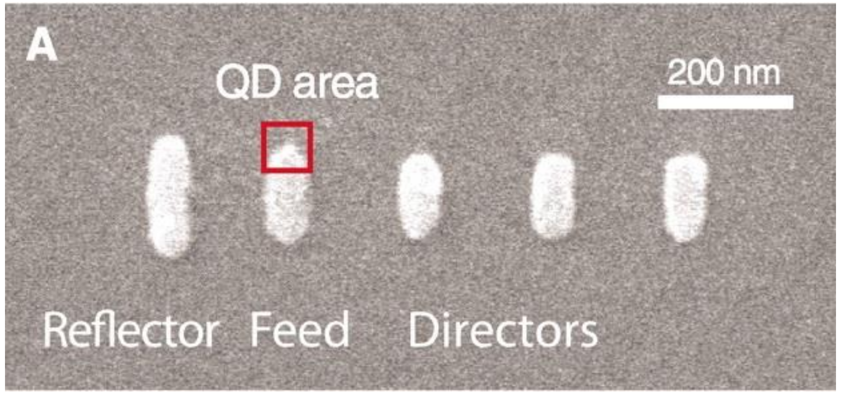
Easy way to align a fiber tip within the objective field of view

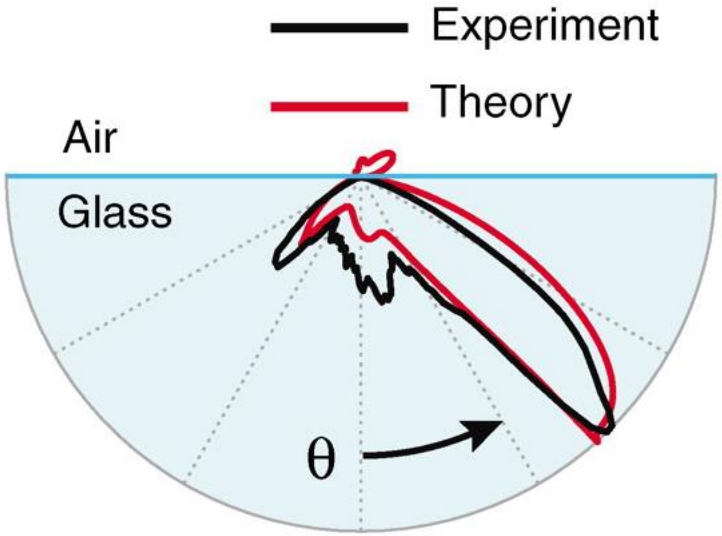
Possibility to block the incident light : dark-field measurements

Possibility to remove some angular-specific noise

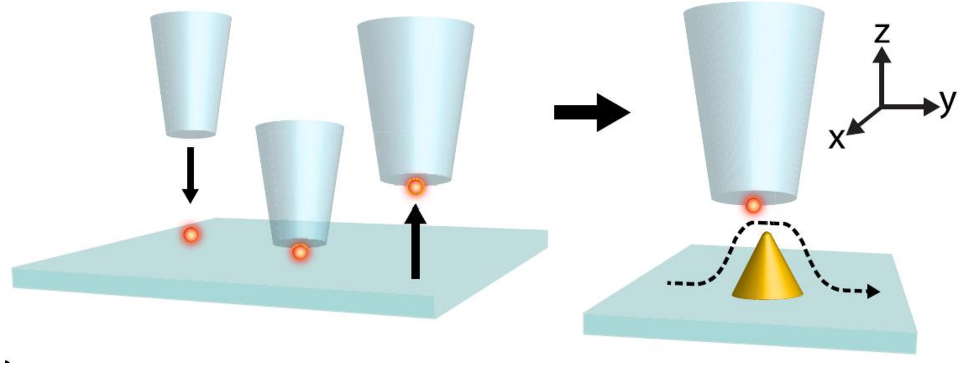


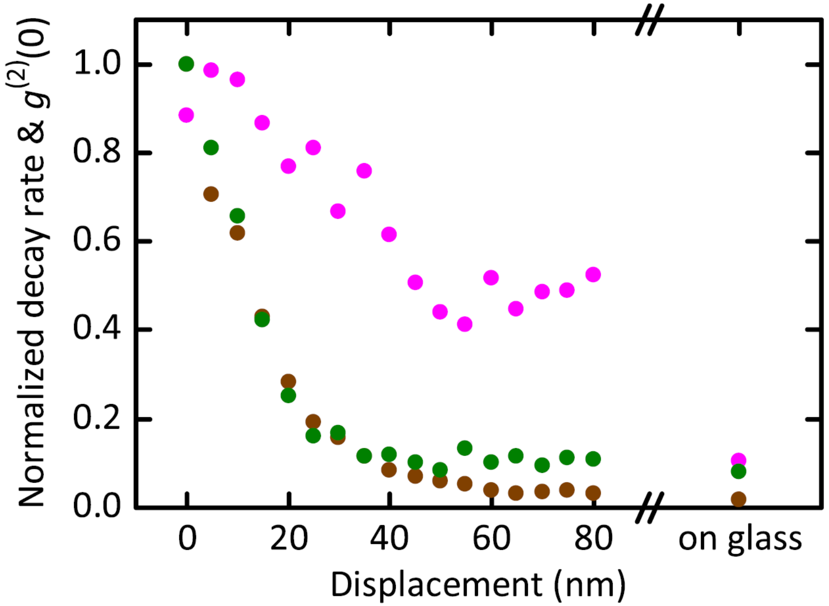
Optical Nano-antennas (on substrates)





A. Curto et al., Science 329, 5994 (2010)



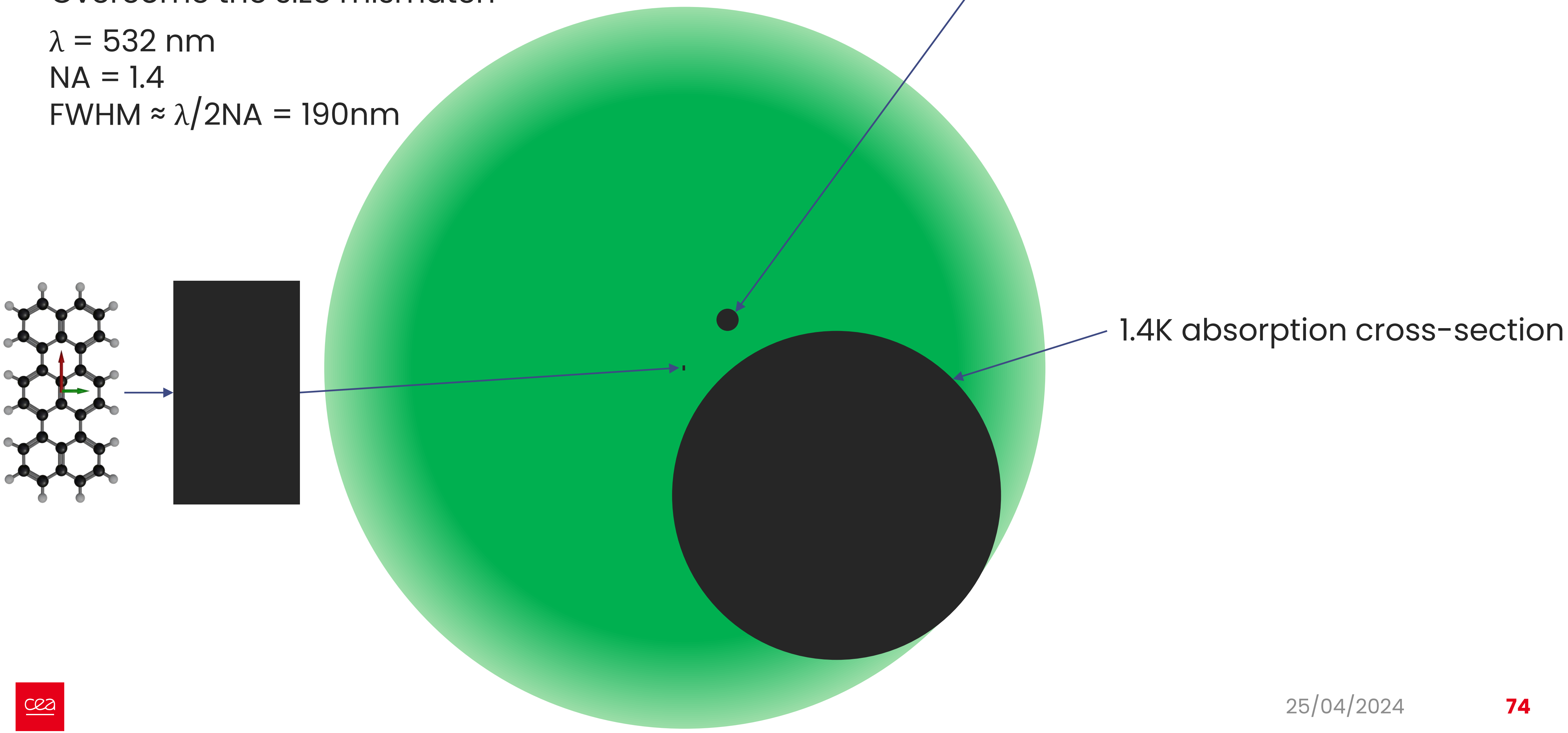


K. Matsuzaki, S. Vassant et al. Scientific Reports 7, 42307 (2017)

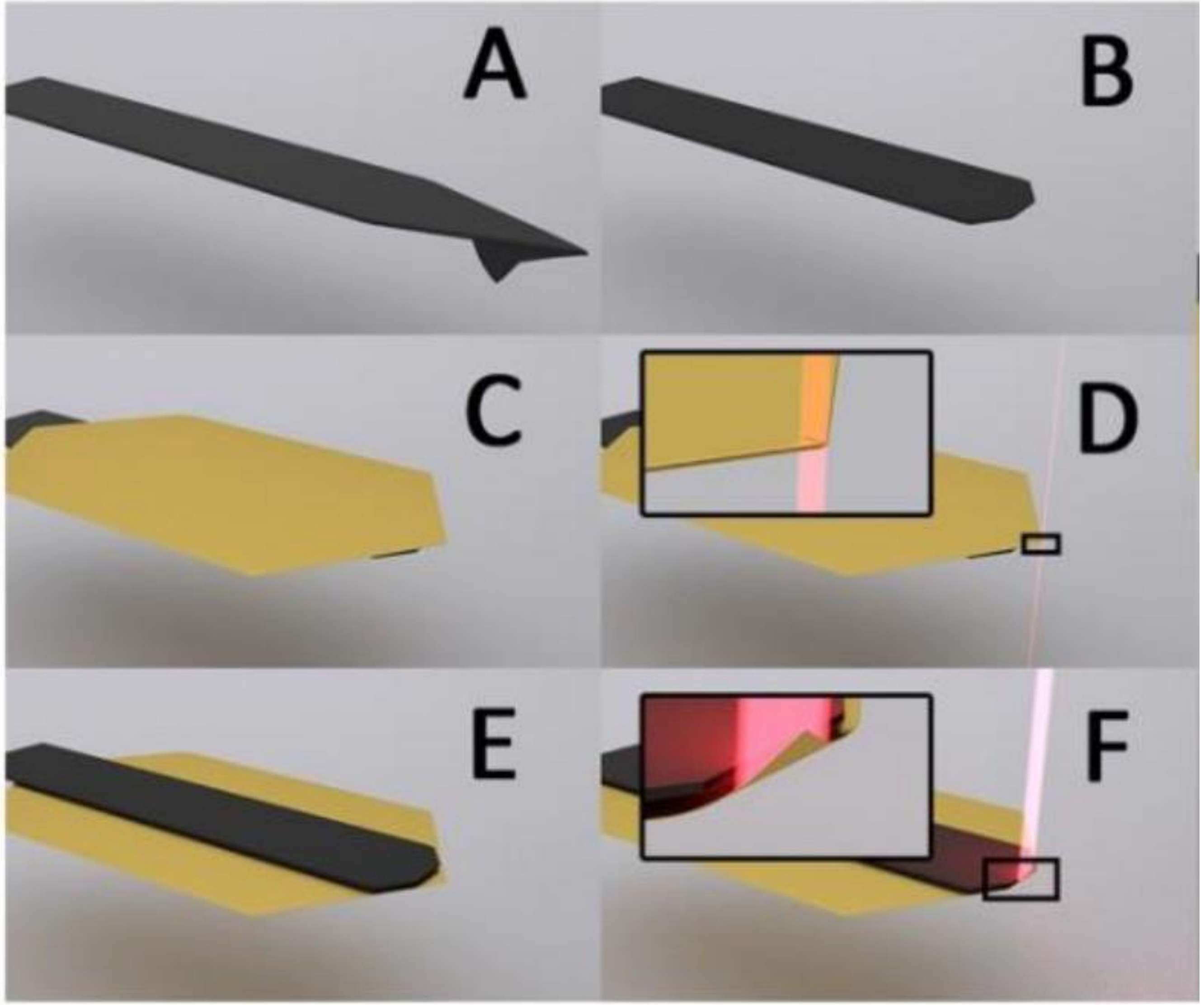
Motivations

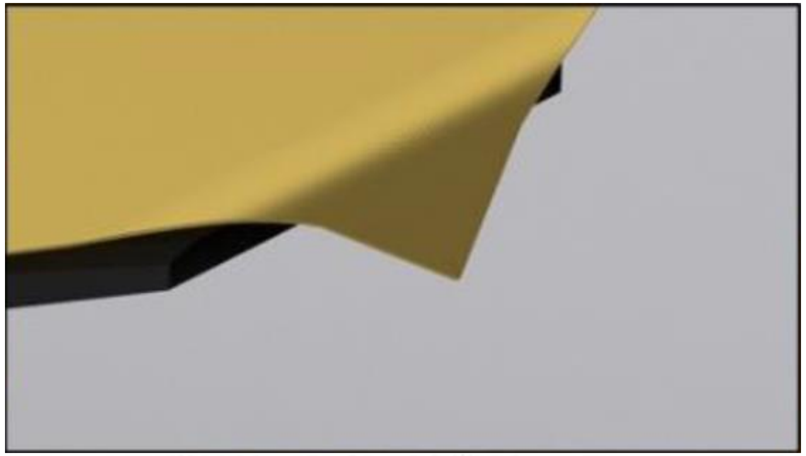
Overcome the size mismatch

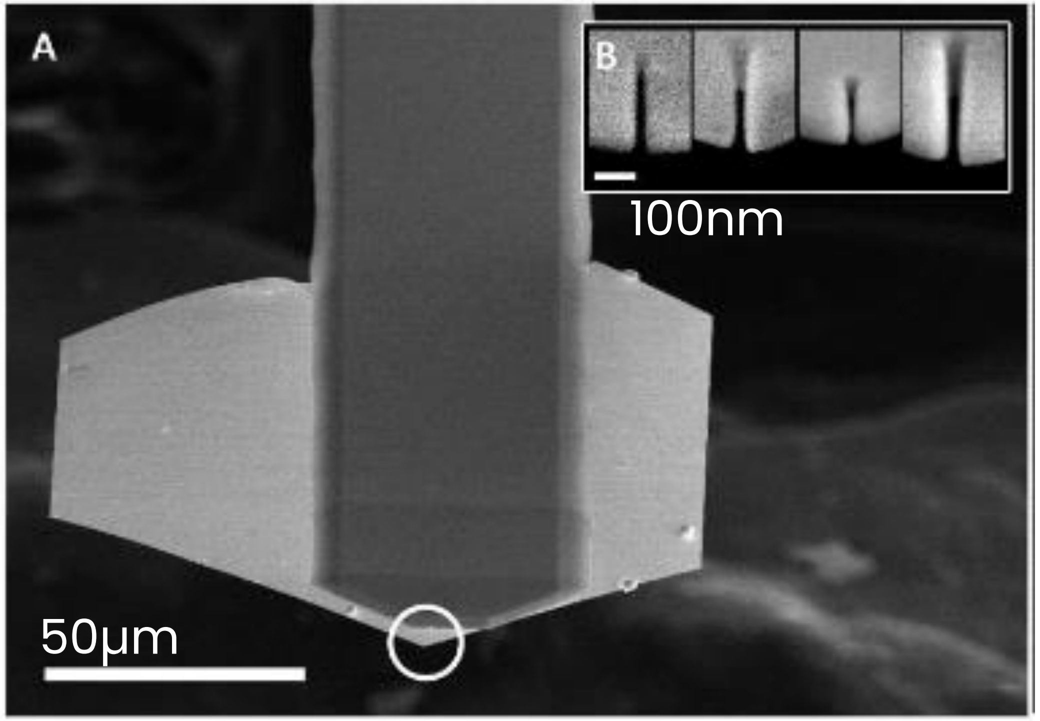
λ = 532 nm
NA = 1.4
FWHM ≈ $\lambda/2NA$ = 190nm



Optical Nano-antennas (on tip)

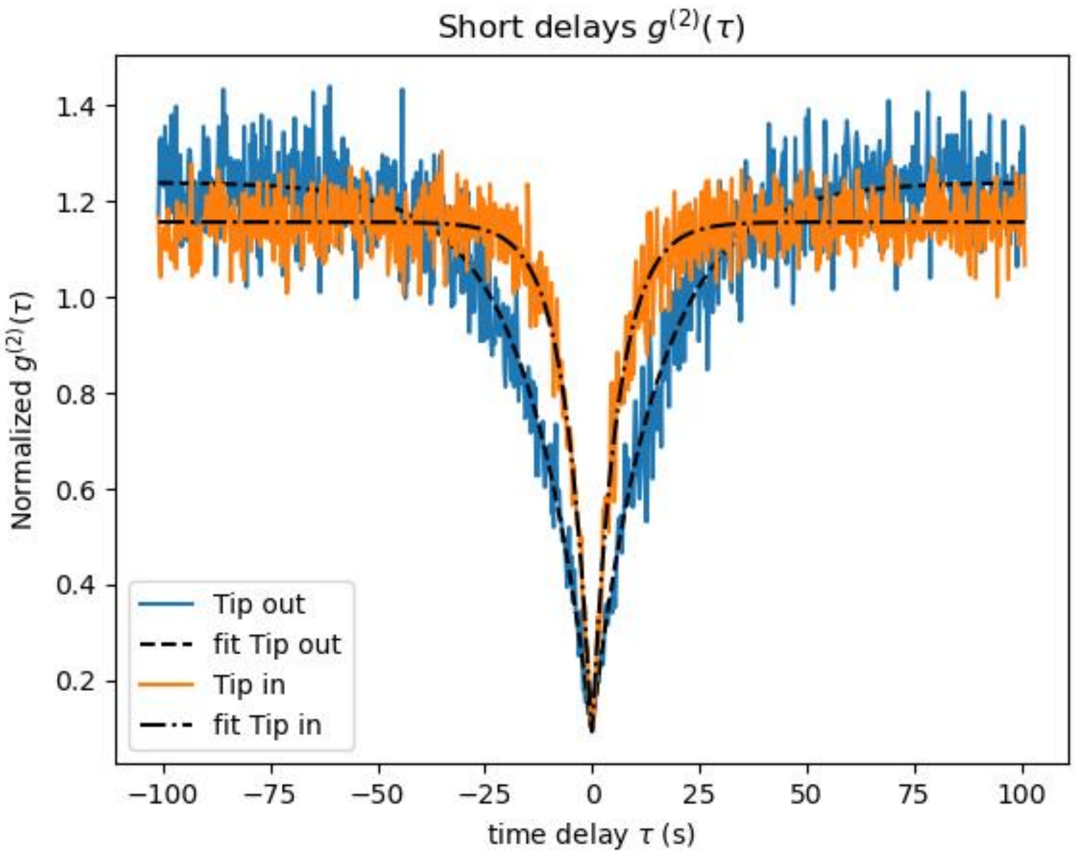


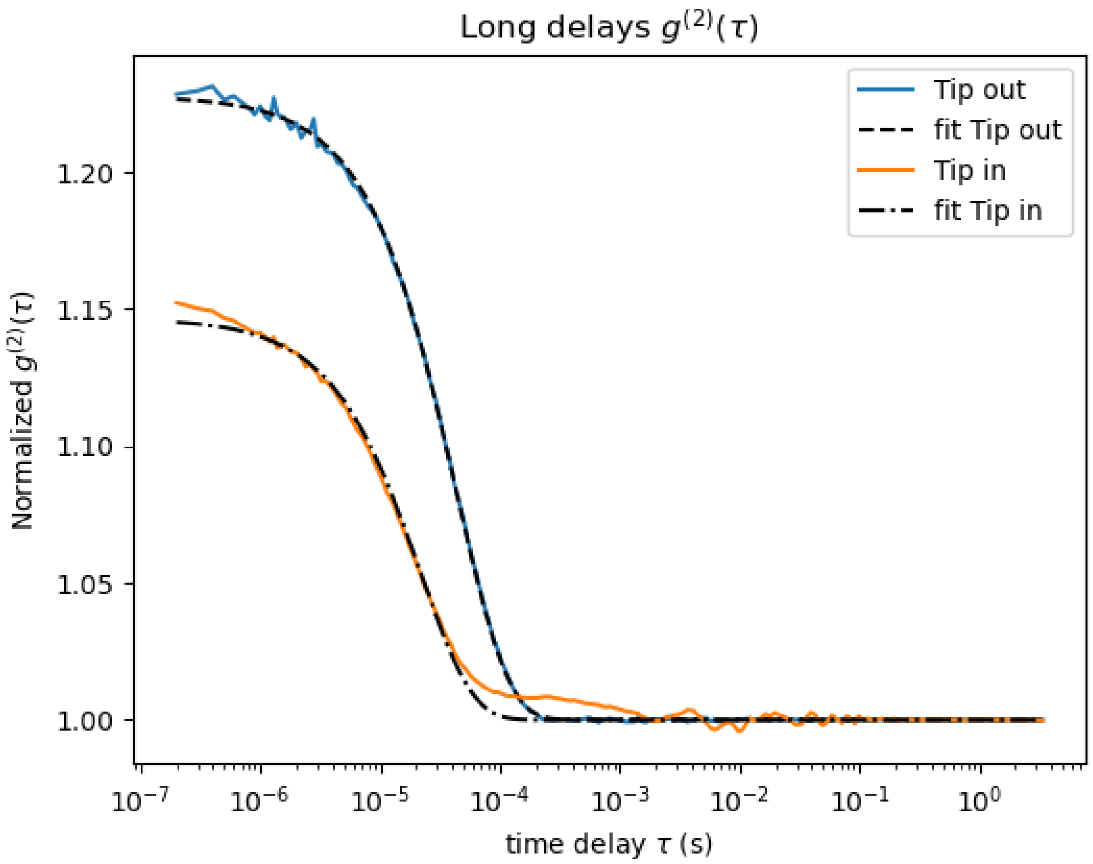




H. Gross et al. Science Advances 4, 4906 (2018)

Tip effect on $g^{(2)}$





Single photon emitter : what informations ?

Fluorescence spectrum

Fluorescence lifetime

Excitation rate

Intersystem crossing rates

Orientation of the emitter in the laboratory frame