Nanolight 2022

Centro de Ciencias de Benasque Pedro Pascual – Benasque, Spain

March 6 – 12 2022

Single molecule localization through sequential structured illumination

Fernando D. Stefani

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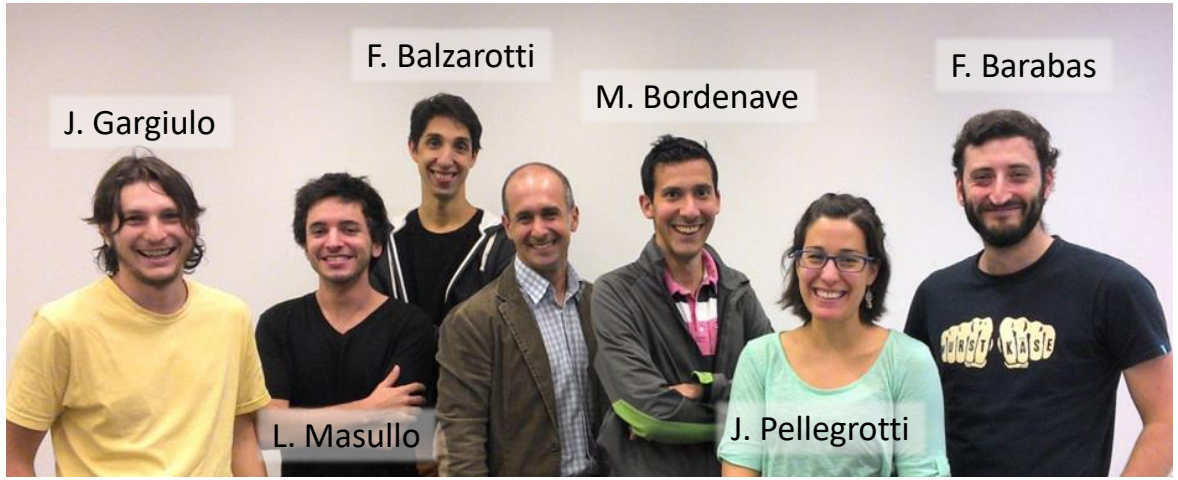
Center for Bionanoscience Research (CIBION)
Buenos Aires, Argentina

Applied nanoPhysics @ CIBION

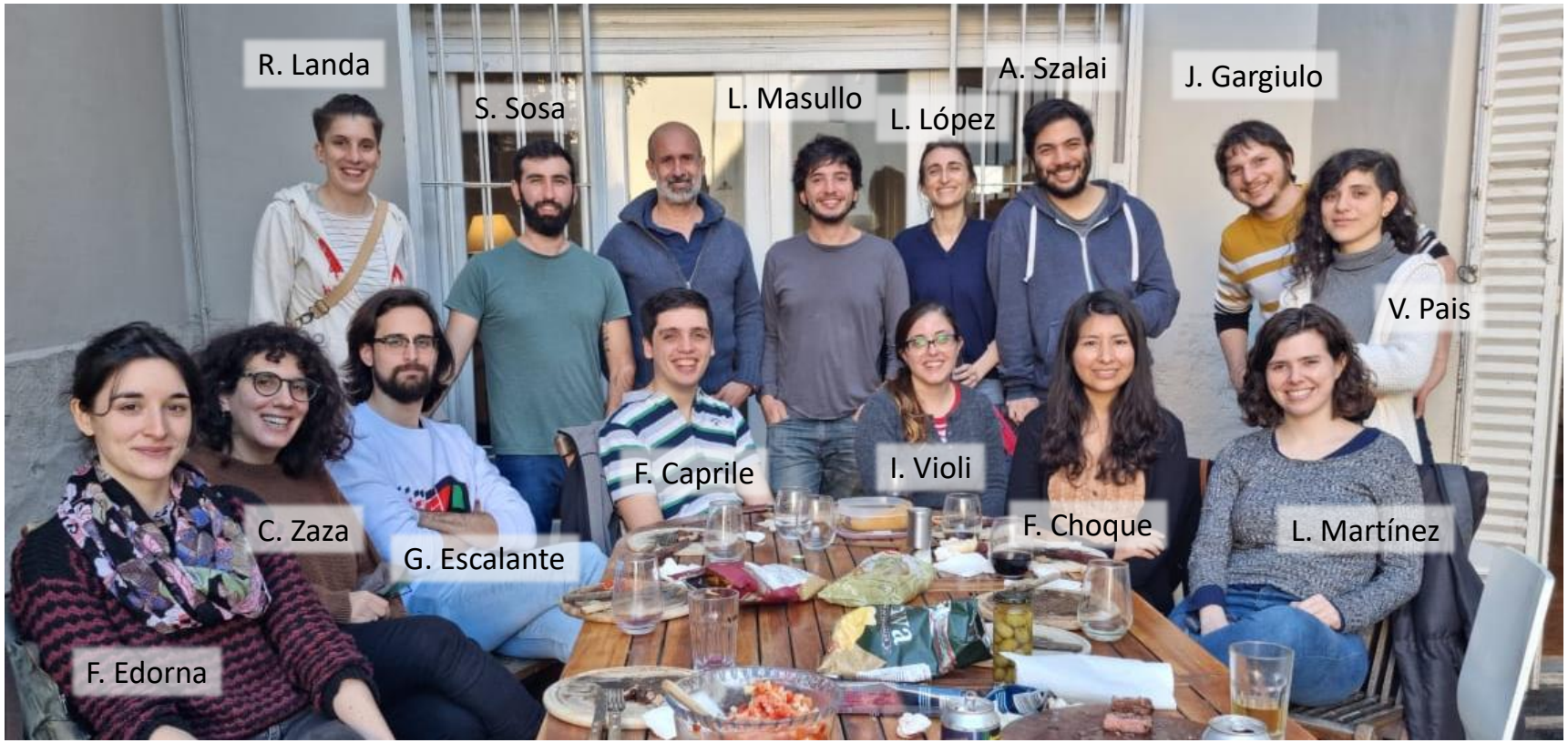
2015



2018

https://stefani-lab.ar/

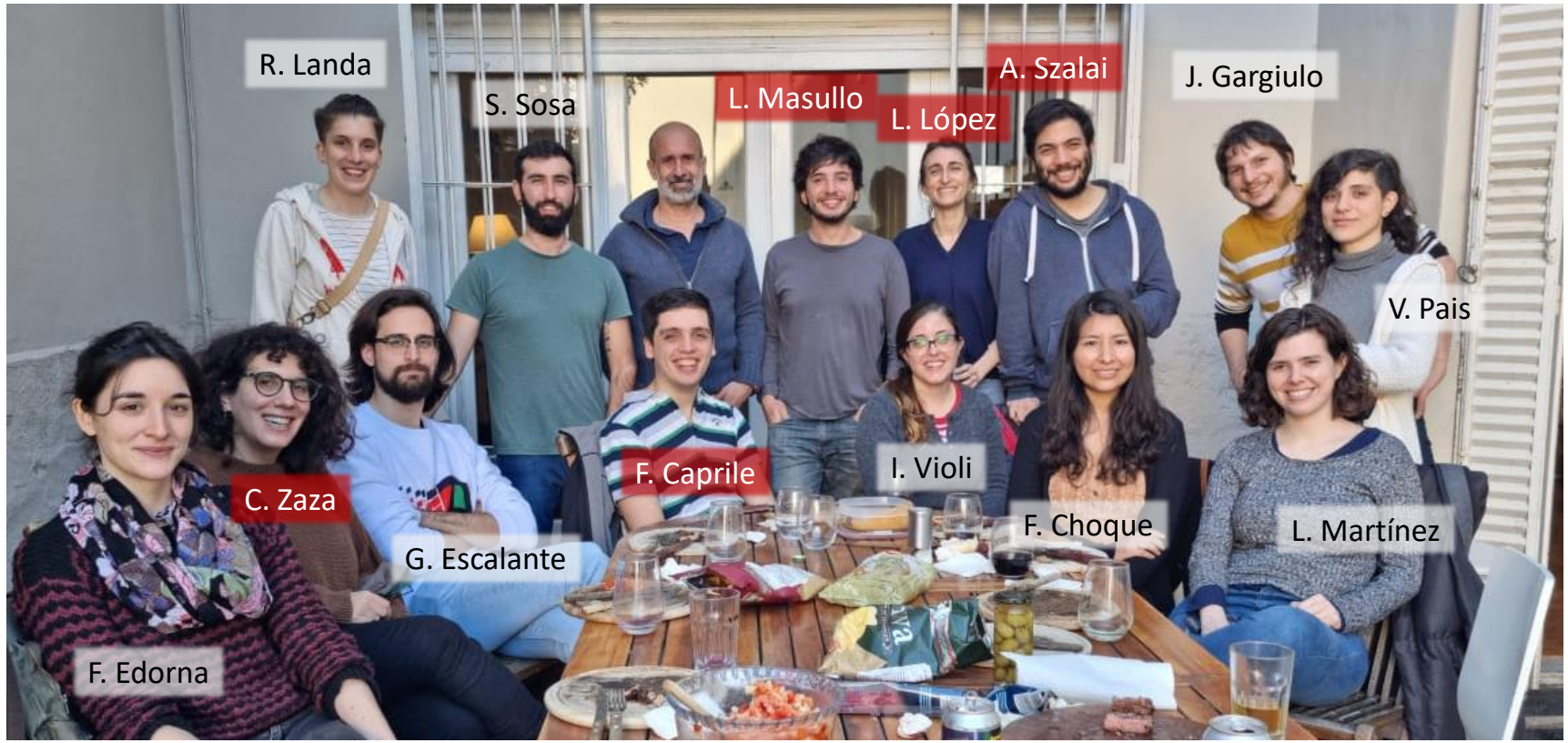
Applied nanoPhysics @ CIBION



2021

https://stefani-lab.ar/

Applied nanoPhysics @ CIBION



2021

https://stefani-lab.ar/

Current Research

- Optical manipulation (printing) of colloidal nanoparticles
- Self-assembled nanophotonic devices – DNA-origami
- Fluorescence nanoscopy
  - New methods
  - Applications to neurobiology
  - 3D chromatin organization

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Acknowledgements

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Damián Refojo

LMU Munich
Philip Tinnefeld
Jochen Feldmann
Stefan Maier

UNSAM Buenos Aires
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Juan Mucci
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Dante Chialvo

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FIL Buenos Aires
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UBA Buenos Aires
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Lía Pietrasanta

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Juan José Sáenz

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Sabrina Simoncelli

Adios Juanjo

ACS Photonics

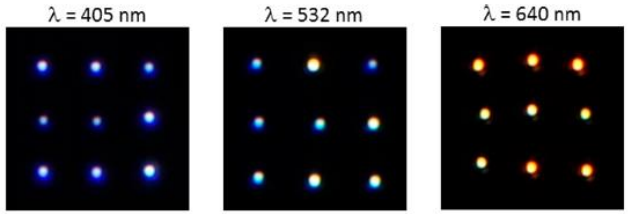
Letter

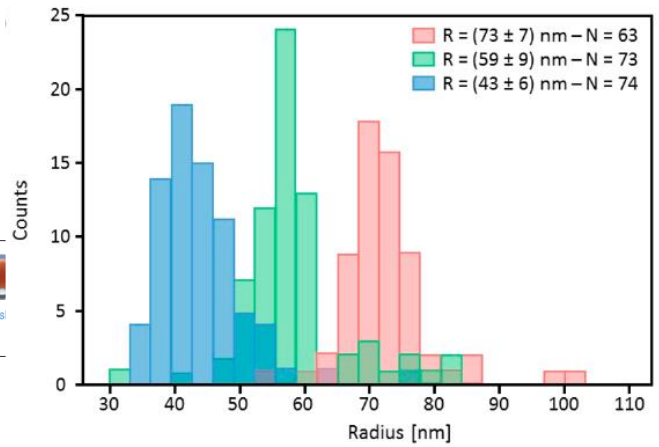
Cite This: *ACS Photonics* 2019, 6, 815–822

pubs.acs.org/journal/apchd5

Size-Selective Optical Printing of Silicon Nanoparticles through Their Dipolar Magnetic Resonance

Cecilia Zaza,[†,‡] Ianina L. Violi,[*,†] Julián Gargiulo,[§] Germán Chiarelli,[†,‡] Ludmilla Schumacher,[∥] Jurij Jakobi,[⊥] Jorge Olmos-Trigo,[#] Emiliano Cortes,[§,▽] Matthias König,[∥] Stephan Barcikowski,[⊥] Sebastian Schlücker,[∥] Juan José Sáenz,[#,○] Stefan A. Maier,[§,▽] and Fernando D. Stefani[*,†,‡]





Challenges on optical printing of colloidal nanoparticles

Cite as: J. Chem. Phys. 156, 034201 (2022); doi: 10.1063/5.0078454
Submitted: 12 November 2021 • Accepted: 27 December 2021 •
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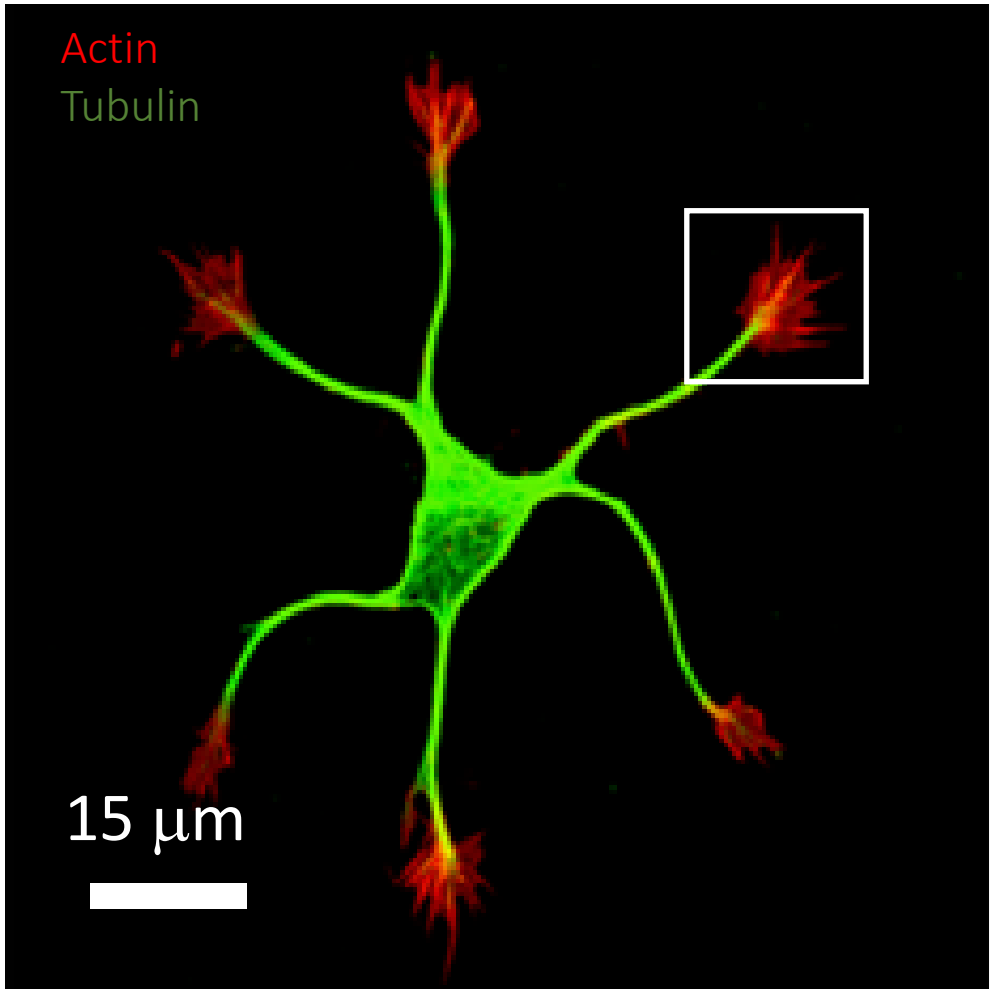
Ianina L. Violi,[1,2,a)] Luciana P. Martinez,[1] Mariano Barella,[1] Cecilia Zaza,[1,3] Lukáš Chvátal,[4] Pavel Zemánek,[4] Marina V. Gutiérrez,[5] María Y. Paredes,[5] Alberto F. Scarpettini,[5] Jorge Olmos-Trigo,[6] Valeria R. Pais,[3] Iván Díaz Nóblega,[3] Emiliano Cortes,[7] Juan José Sáenz,[6] Andrea V. Bragas,[3] Julian Gargiulo,[1,7,a)] and Fernando D. Stefani[1,3,a)]

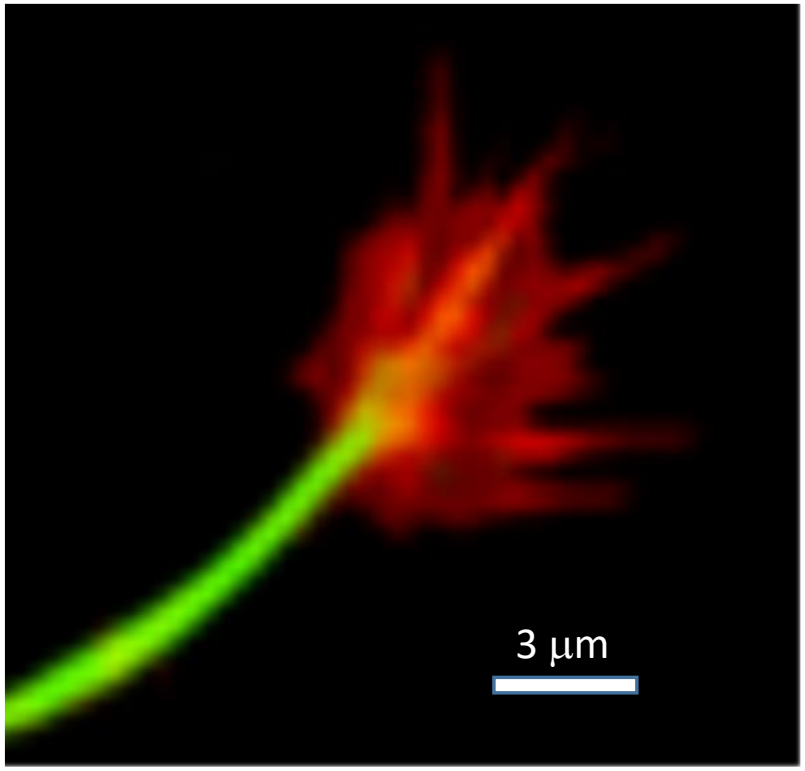
Cecilia Zaza wins OSA Emil Wolf Student Paper Competition

September 19th, 2019

Single molecule localization through sequential structured illumination

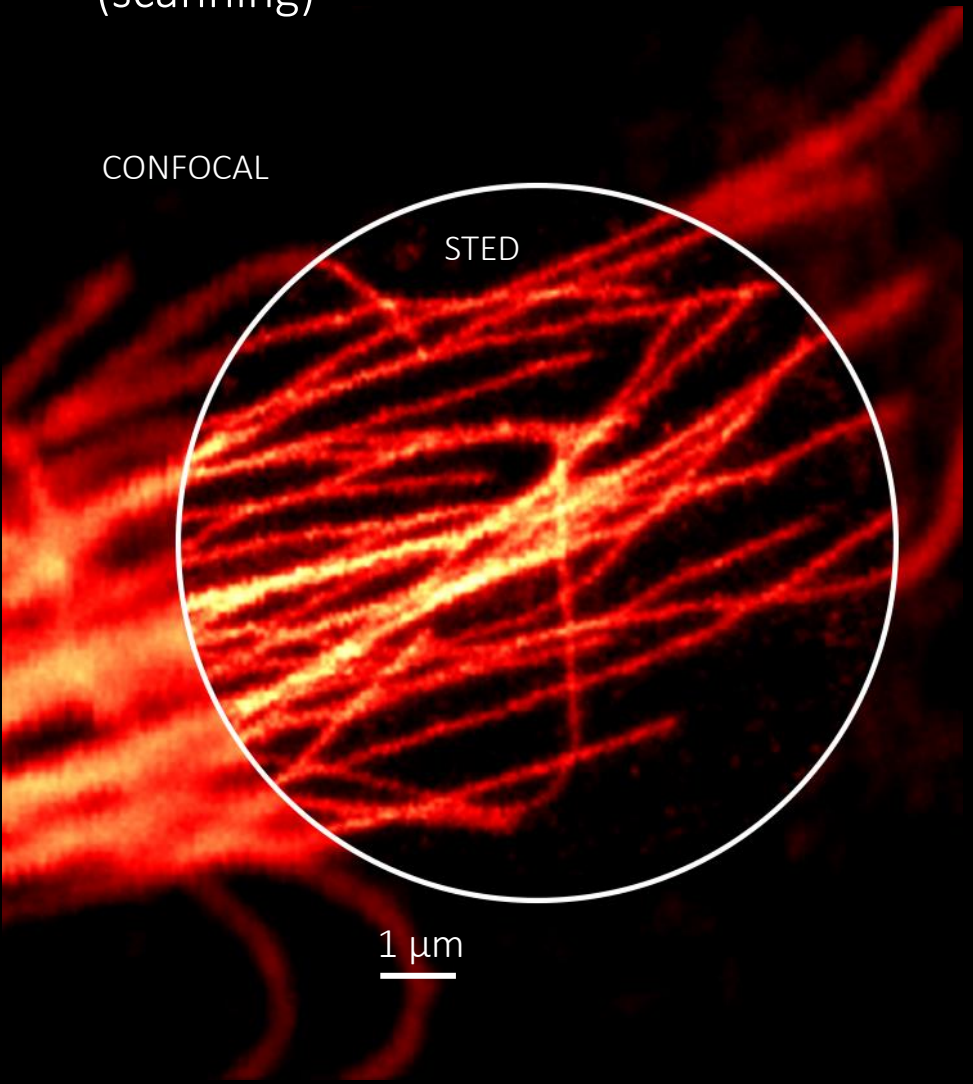
Fluorescence microscopy





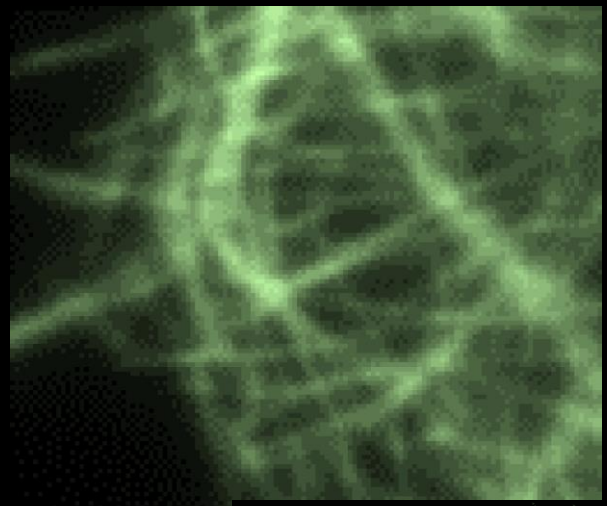
Fluorescence Nanoscopy - 1st Generation

Coordinate-targeted nanoscopy (scanning)

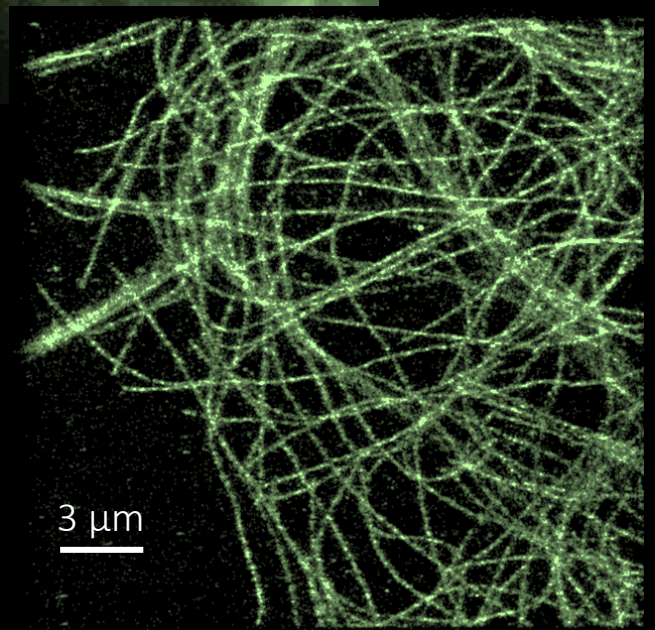


Coordinate-stochastic nanoscopy (wide-field)

Wide-field

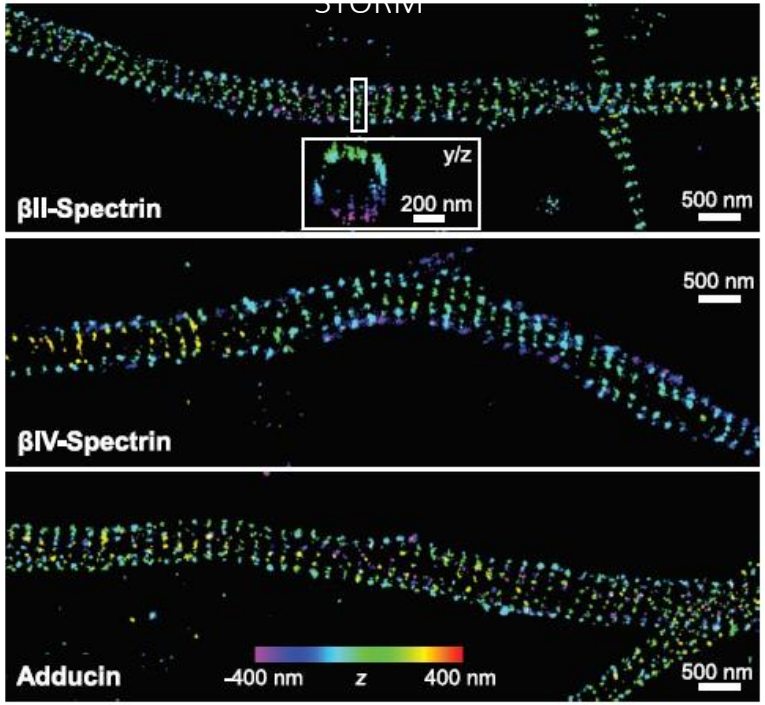


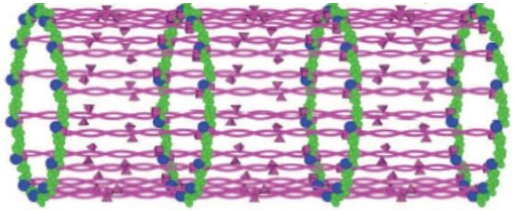
STORM

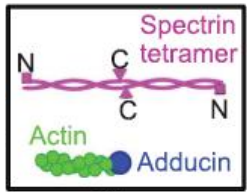


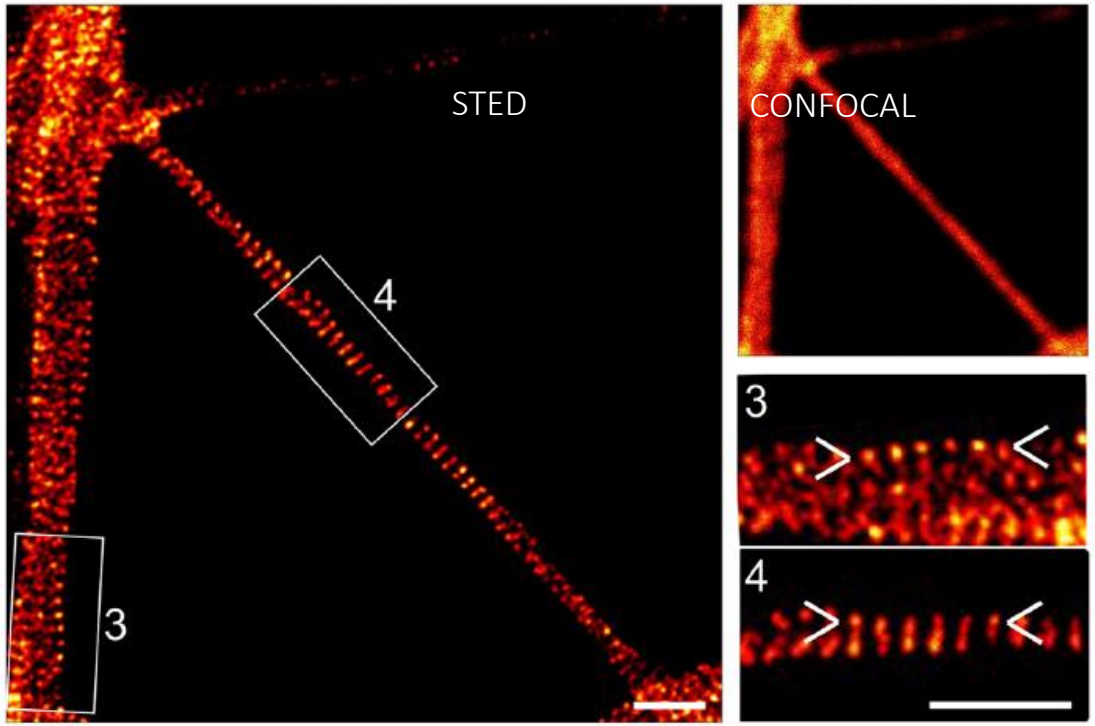
Supramolecular protein nanostructures

Membrane associated periodic skeleton (MPS) of neurons









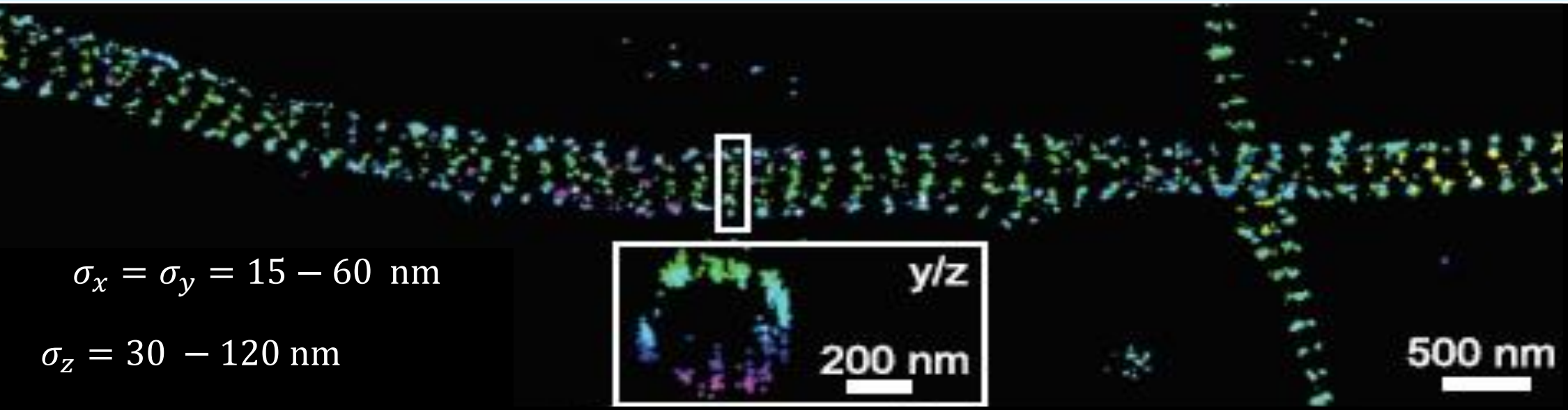
Unsain et al. *Scientific Reports* **8** (2018) 3007

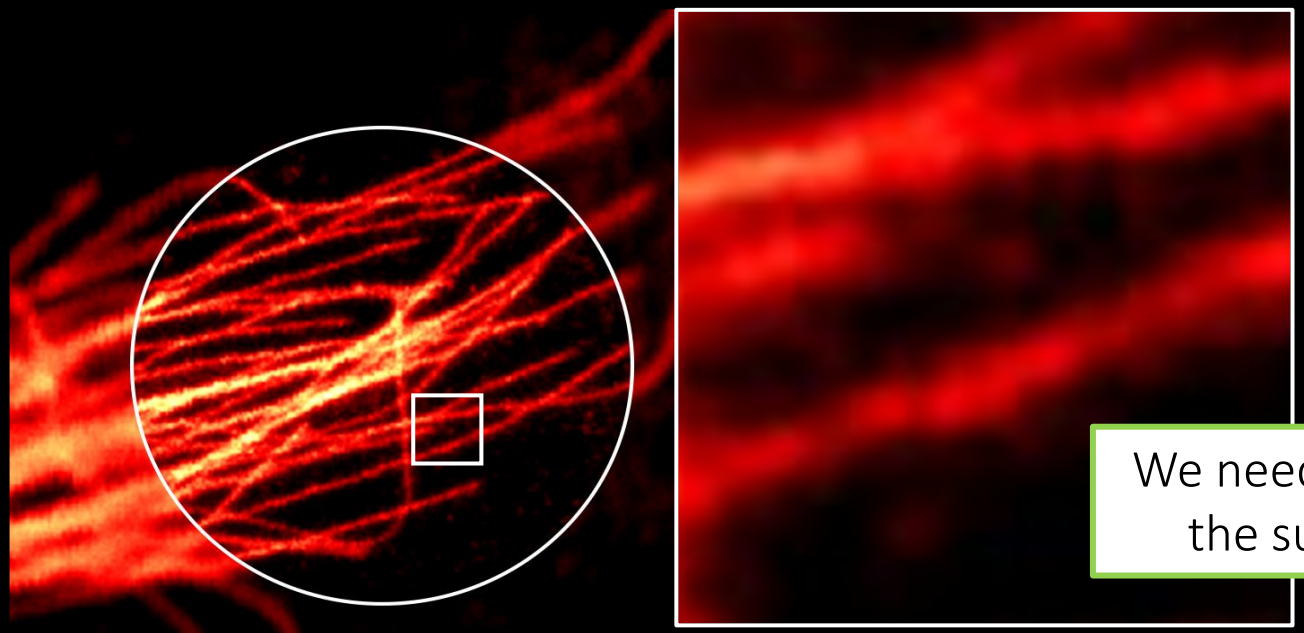
Xiaowei Zhuang Lab
Xu et al. *Science* **2013**, *339*, 452–456.

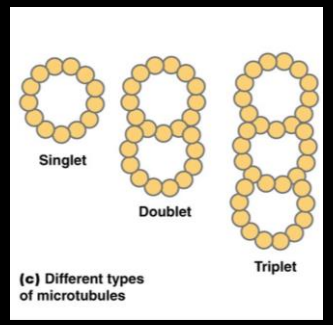
Typical nanoscopy resolution in biological systems

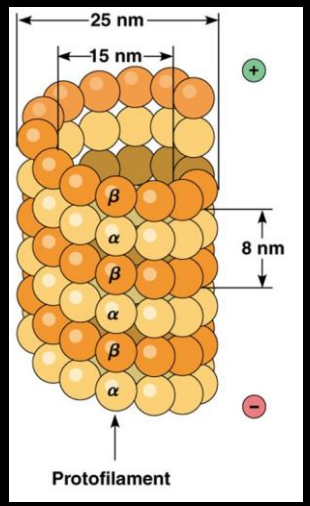
$\sigma_x = \sigma_y = 15 - 60$ nm

$\sigma_z = 30 - 120$ nm









We need another push into the sub-10 nm regime

Sub-10 nm resolution

SML-SSI Single-Molecule Localization with Sequential Structured Illumination

Science 355 (2017) 606-612

Nano Letters 21 (2021) 840-846

Biophysical Reports 2 (2022) 100036

LSA (2022) News & Views accepted

New data

STED-FRET Super-resolved energy transfer imaging

Nano Letters 21 (2021) 2296–2303

Nanoscale 13 (2021) 18421-18433

SIMPLER Supercritical Illumination Microscopy Photometric z-Localization with Enhanced Resolution

Nature Communications 12 (2021) 517

Nanometer resolution with fluorescence… what´s the problem?

3 basic obstacles:

1. SBR
2. Label size
3. Sample drift

Masullo et al. "Fluorescence nanoscopy at the sub-10 nm scale" ***Biophysical Reviews*** 13 (2021) 1101–1112

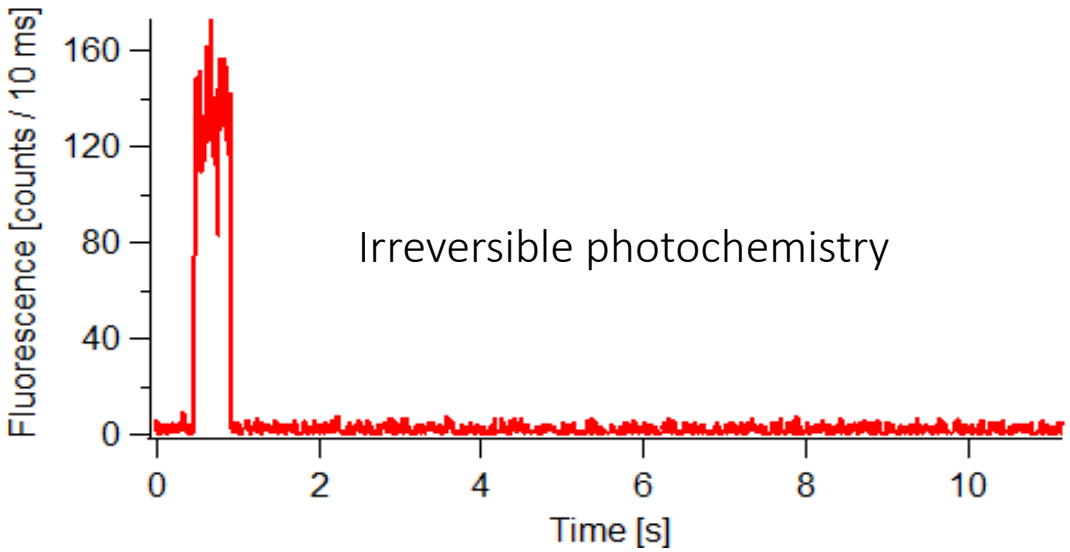
Nanometer resolution with fluorescence, why not?

3 basic obstacles:

1. SBR — Fluorescence photon budget, detectors, methods
2. Sample drift — Active stabilization, post-processing corrections
3. Label size — Fluorophores, nanobodies, aptamers,…

Masullo et al. "Fluorescence nanoscopy at the sub-10 nm scale" ***Biophysical Reviews*** 13 (2021) 1101–1112

Limited fluorescence photon budget



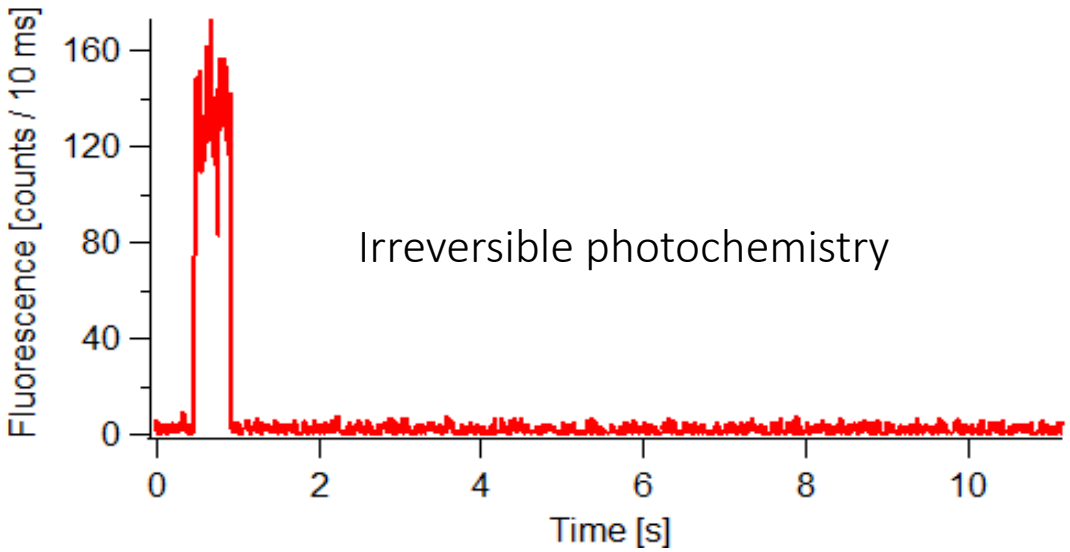
SBR limits:

localization precision

imaging resolution

tracking length/temporal resolution

Limited fluorescence photon budget



Irreversible photochemistry

Solutions

Get more photons:

DNA-PAINT

Get more information:

SML-SSI

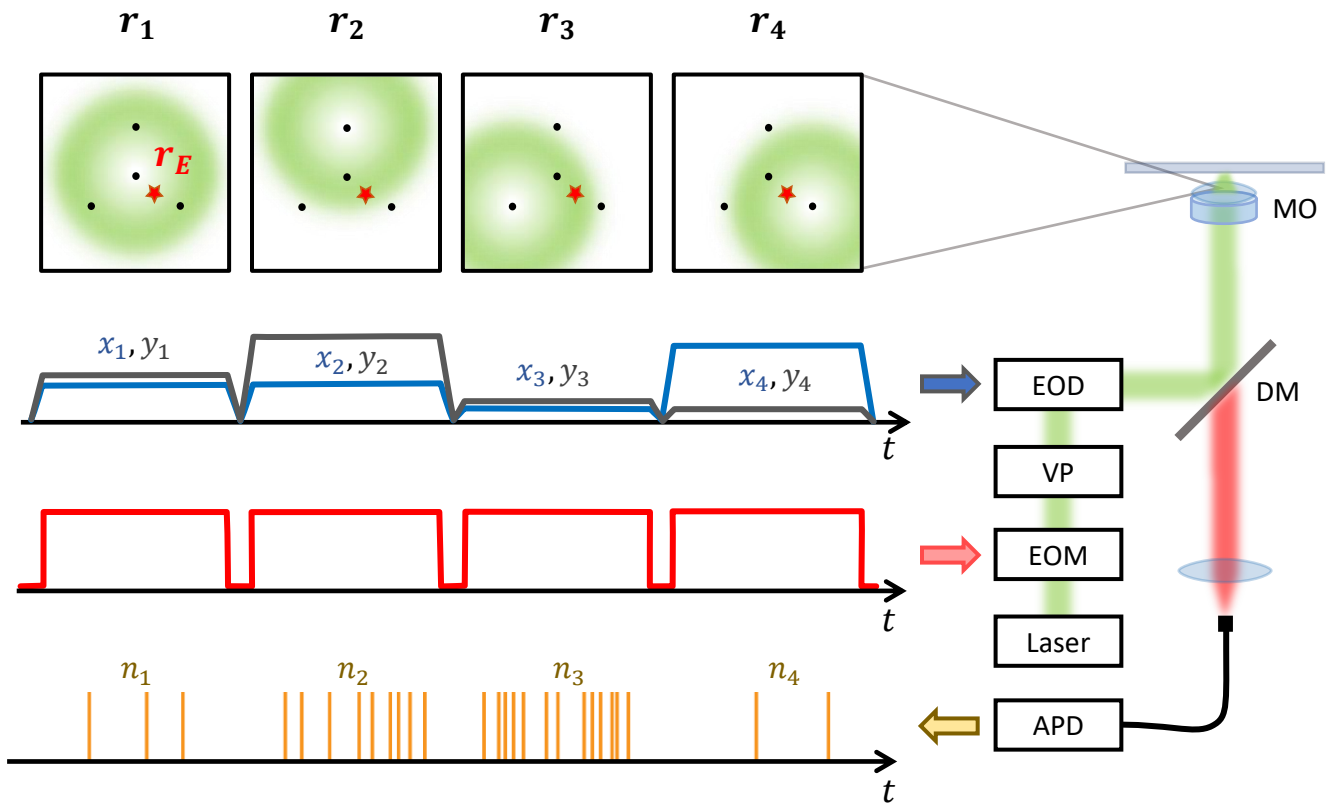
SBR limits:

localization precision

imaging resolution

tracking length/temporal resolution

MINFLUX



$$\bar{n} = [n_1, n_2, n_3, n_4]$$

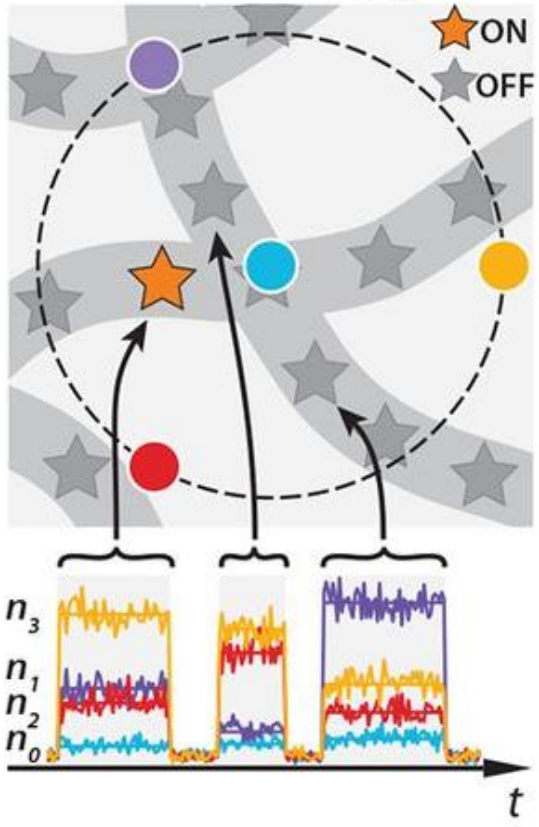
$$\mathcal{L}(\boldsymbol{r_E}|\bar{n}) = \frac{N!}{\prod_{i=1}^{K} n_i!} \prod_{i=1}^{K} p_i(\boldsymbol{r_E})^{n_i}$$

$$p_i(\boldsymbol{r_E}) = \frac{I(\boldsymbol{r_E} - \boldsymbol{r_i})}{\sum_{j=1}^{K} I(\boldsymbol{r_E} - \boldsymbol{r_j})}$$

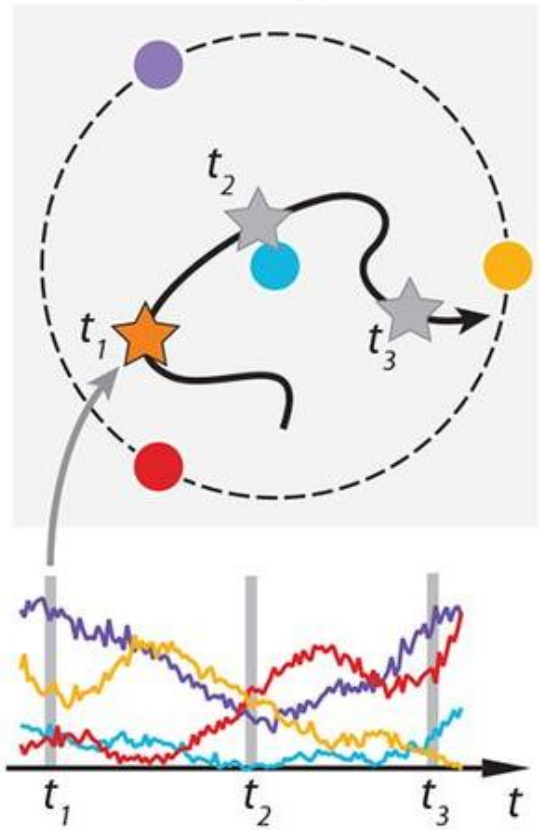
Balzarotti et al. *Science* 355 (2017) 606-612

MINFLUX

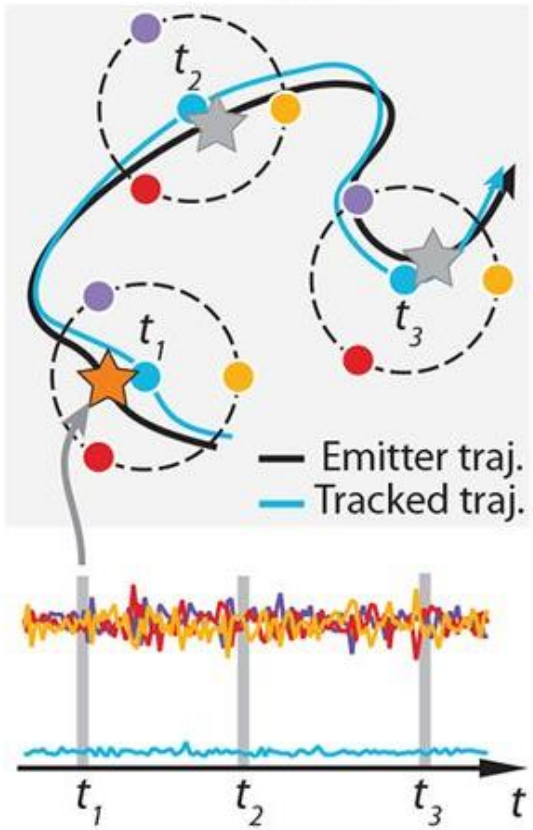
Nanoscopy



nm - Tracking



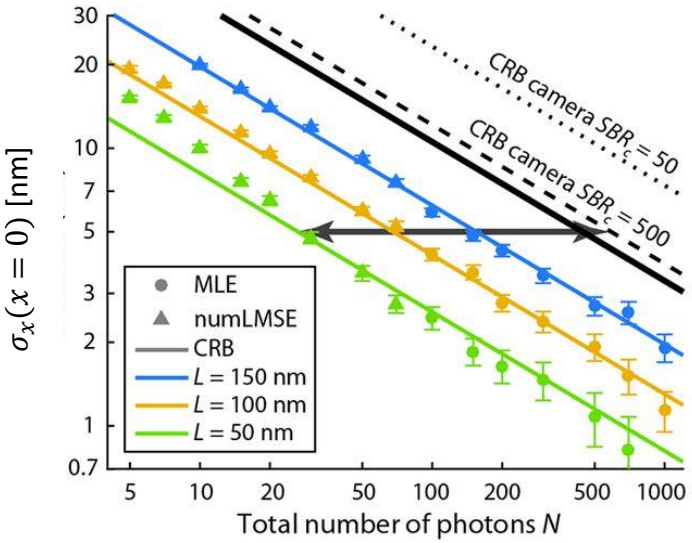
μm - Tracking



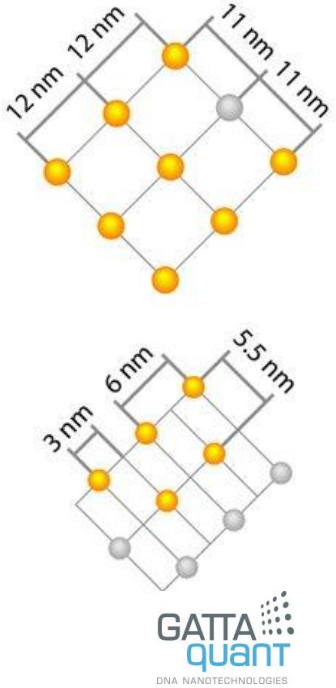
Balzarotti et al. ***Science*** 355 (2017) 606-612

MINFLUX

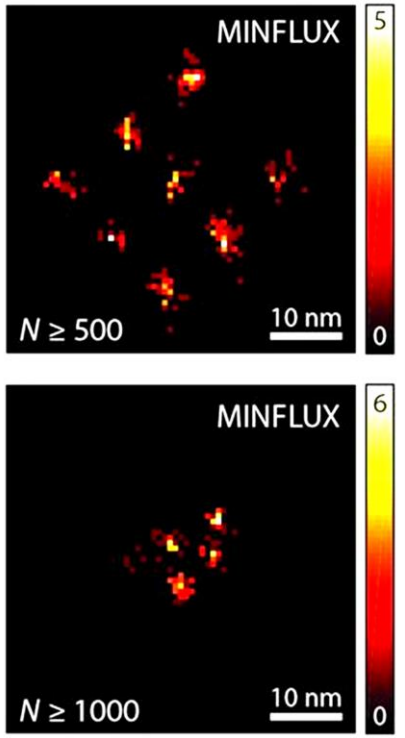
Tunable nanometer resolution



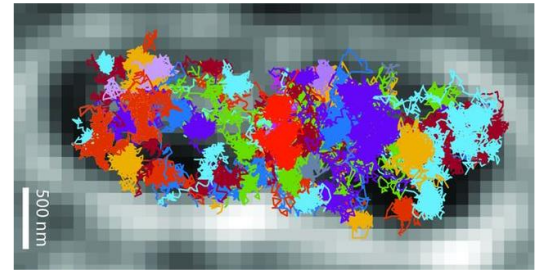
DNA origami

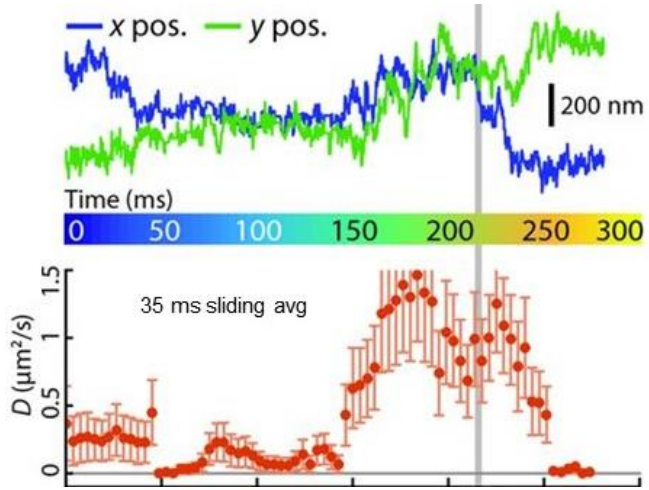


Nano imaging



Superfast tracking



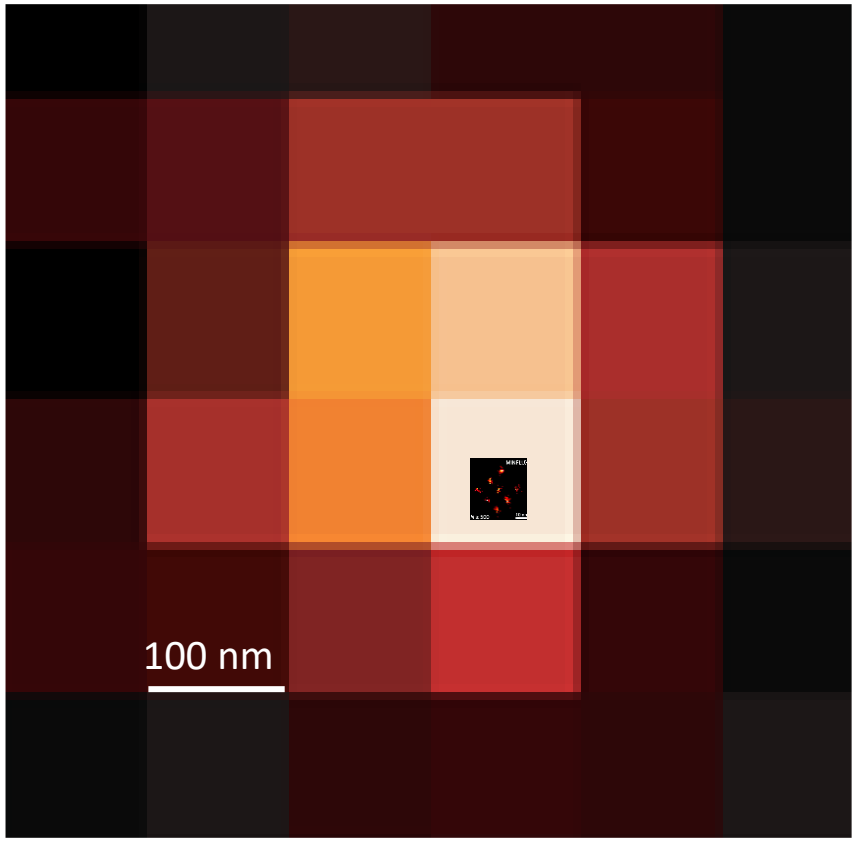


Balzarotti et al. ***Science*** 355 (2017) 606-612

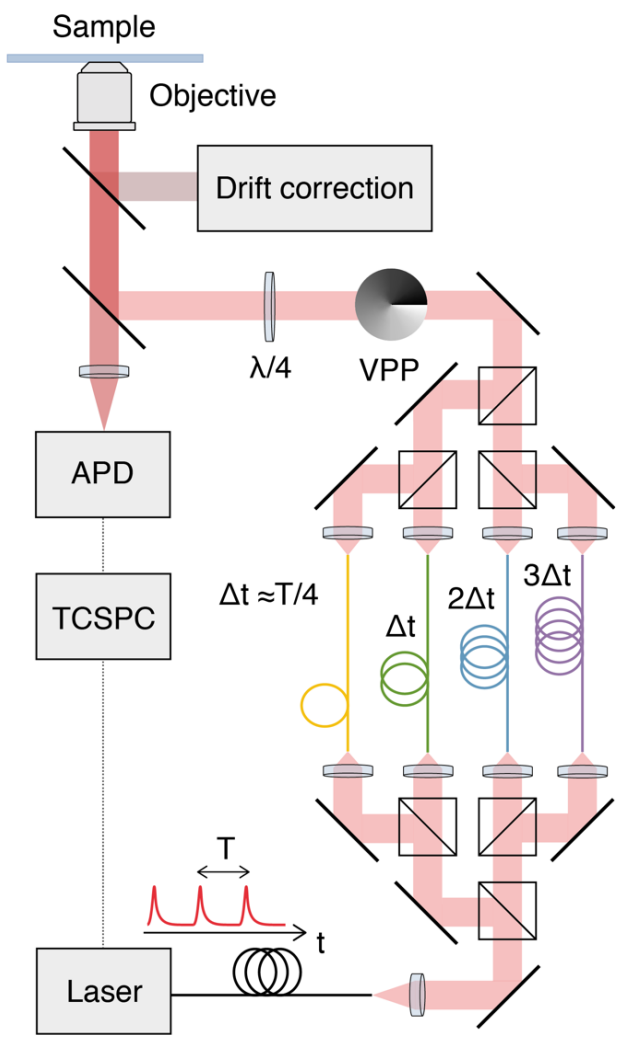
MINFLUX pros and cons

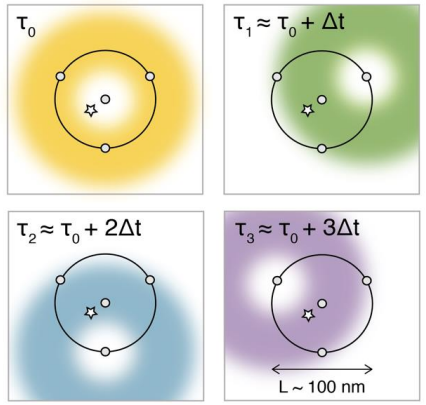
- SM localization using minima of light is more photon-efficient
- Routinely delivers 1-2 nm precision/resolution

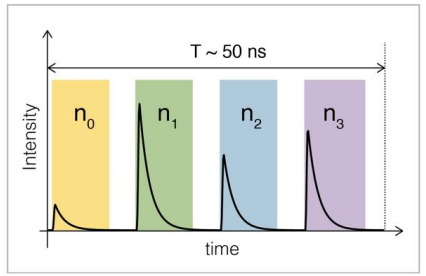
- Instrumentally very complex / costly
- Conceptually difficult to grasp



p-MINFLUX



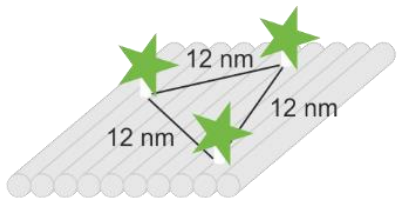


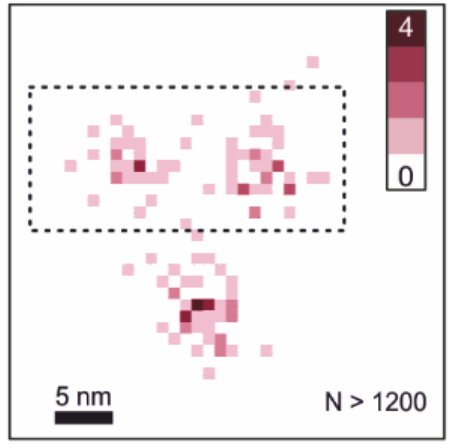


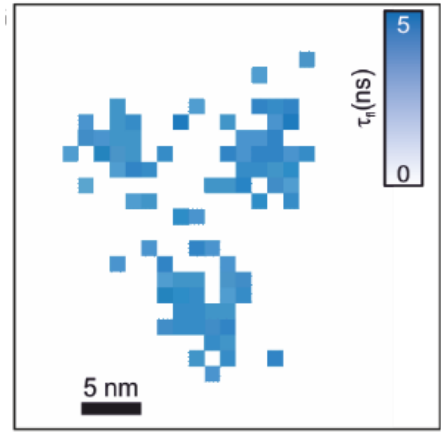
$$[n_0, n_1, n_2, n_3]$$

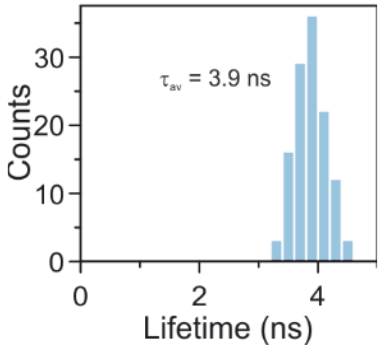
$$\downarrow$$

$$\boldsymbol{r_E} = (x, y)$$



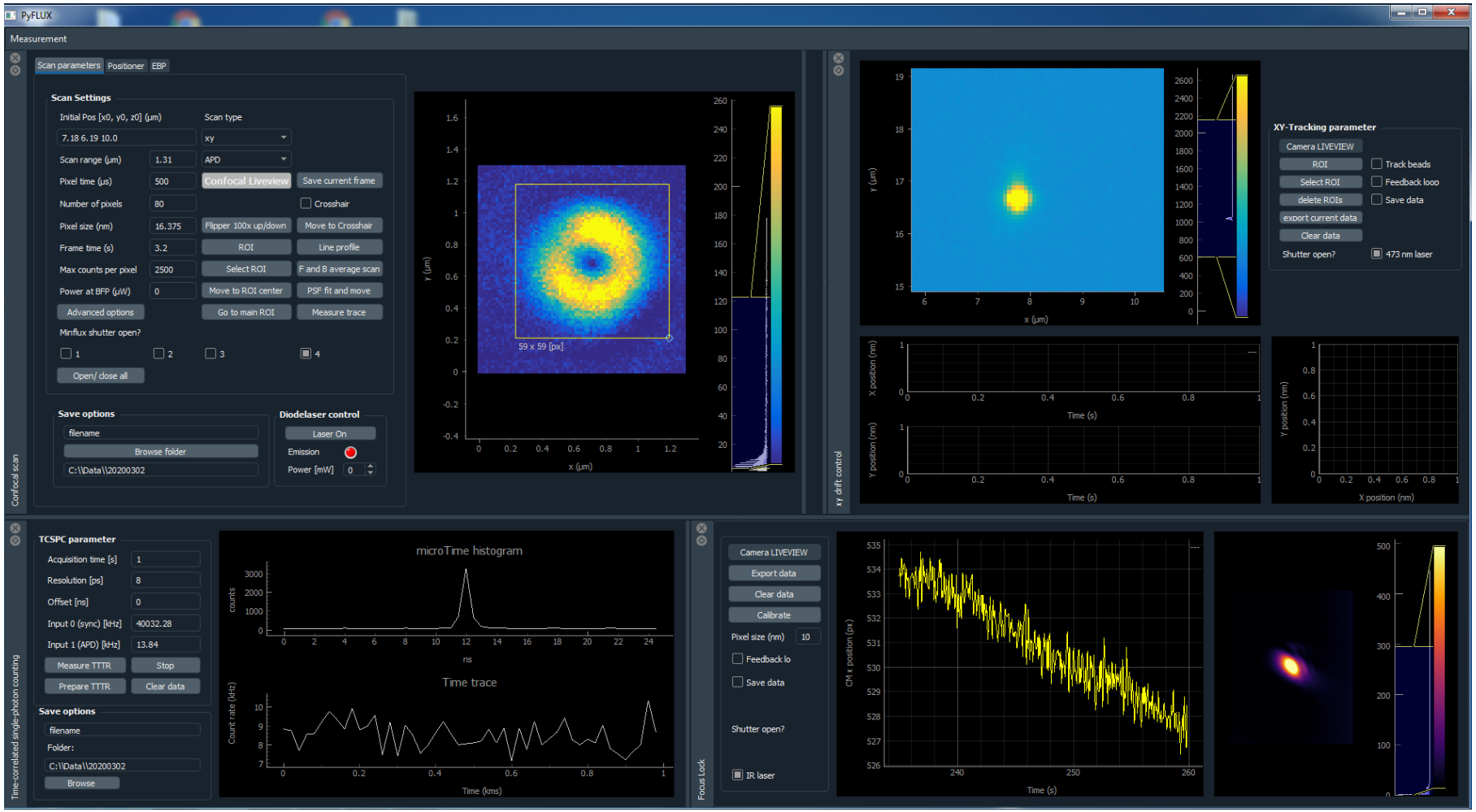






Masullo et al. ***Nano Letters*** 21 (2021) 840-846

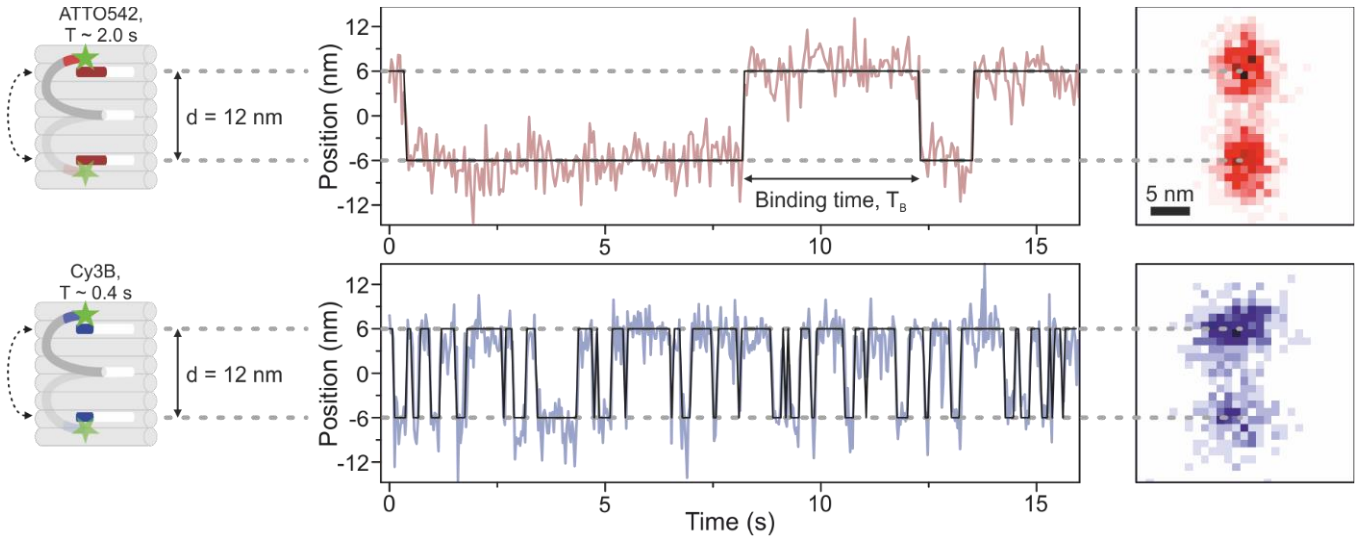
PyFLUX: open-source Python package for MINFLUX

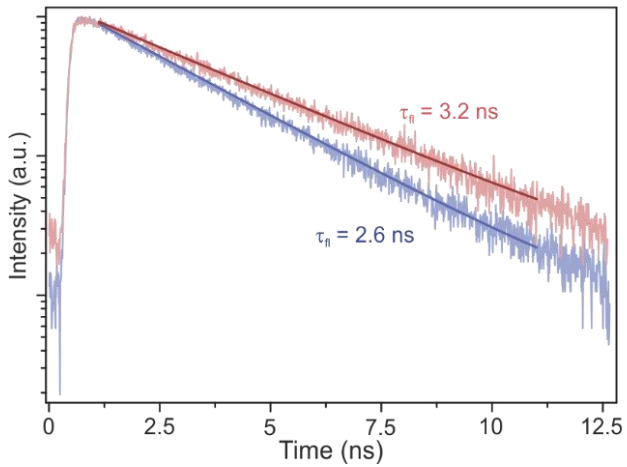


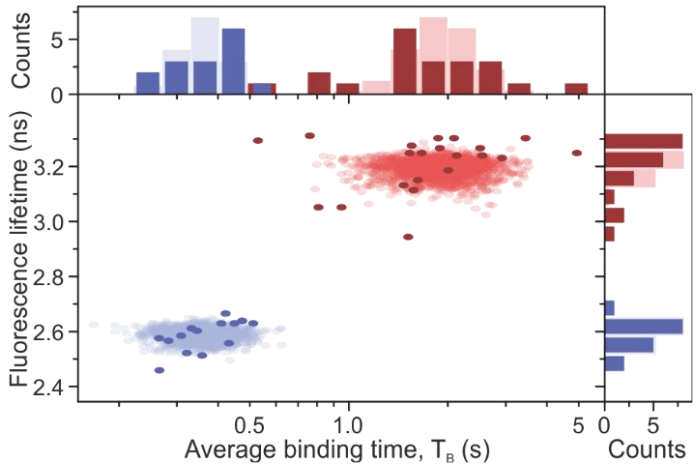
https://github.com/Stefani-Lab

Luciano Masullo – PhD thesis

p-MINFLUX tracking and lifetime multiplexing







p-MINFLUX

- Instrumentally simpler
- Maximum speed

Masullo et al. ***Nano Letters*** 21 (2021) 840-846

SML-SSI Single-Molecule Localization with Sequential Structured Illumination

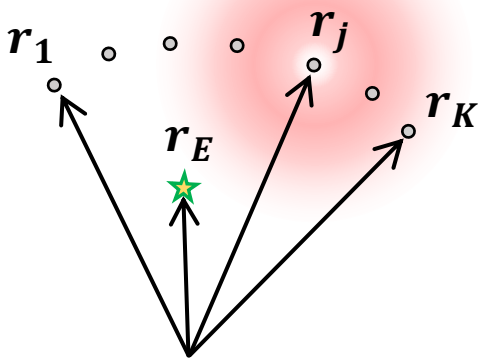
Spatially structured illumination $I(\boldsymbol{r})$

I_{gauss}

I_{donut}

Sequential single-molecule fluorescence measurement

$I(\boldsymbol{r}-\boldsymbol{r_j})$



$$\bar{n} = [n_1, n_2, \ldots, n_K]$$

Molecular position estimation

$$\mathcal{L}(\boldsymbol{r_E}|\bar{n}) = \frac{N!}{\prod_{i=1}^{K} n_i!} \prod_{i=1}^{K} p_i(\boldsymbol{r_E})^{n_i}$$

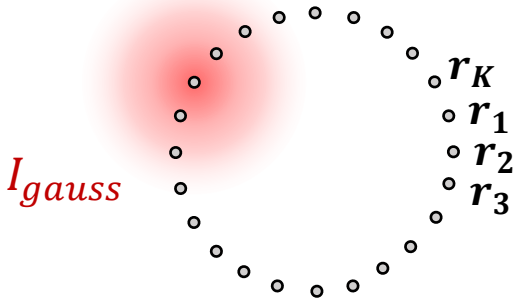
$$p_i(\boldsymbol{r_E}) = \frac{I(\boldsymbol{r_E}-\boldsymbol{r_i})}{\sum_{j=1}^{K} I(\boldsymbol{r_E}-\boldsymbol{r_j})}$$

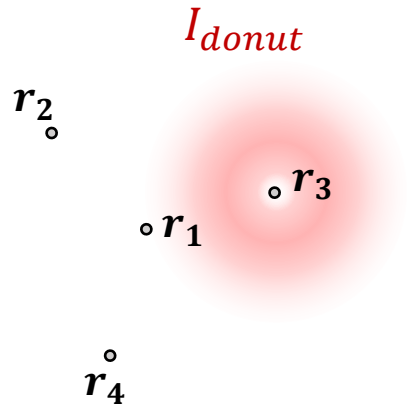
Masullo et al. "A common framework for single-molecule localization using sequential structured illumination" ***Biophysical Reports*** 2 (2022) 100036

SML-SSI

ORBITAL TRACKING (2001)

Enderlein et al. 2001
Gratton et al. 2003
…





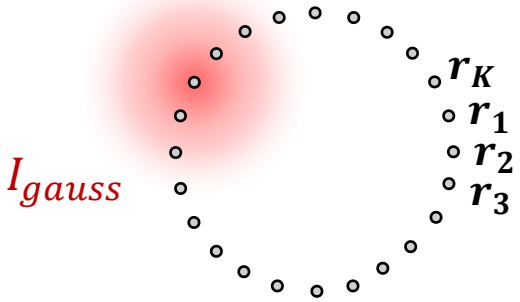
MINFLUX (2017)

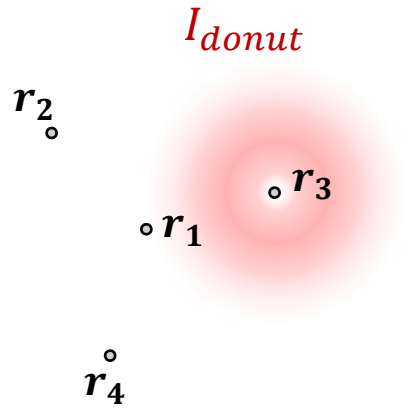
Hell et al.
Stefani et al.
Tinnefeld et al.

SML-SSI

ORBITAL TRACKING (2001)
Enderlein et al. 2001
Gratton et al. 2003
…

MINSTED (2021)
Hell et al.





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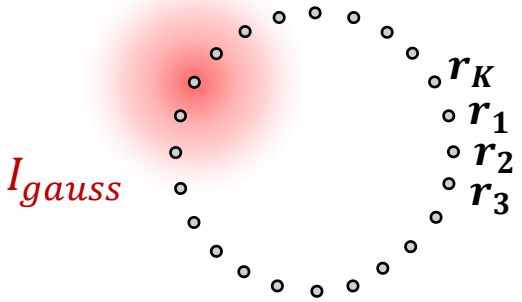

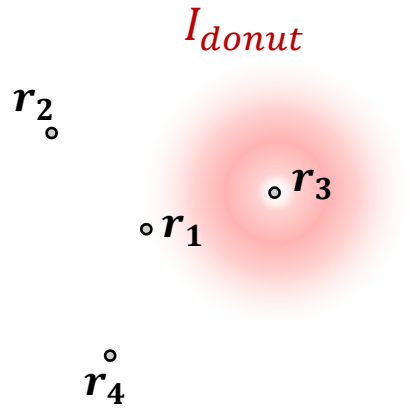

MINFLUX (2017)

Hell et al.
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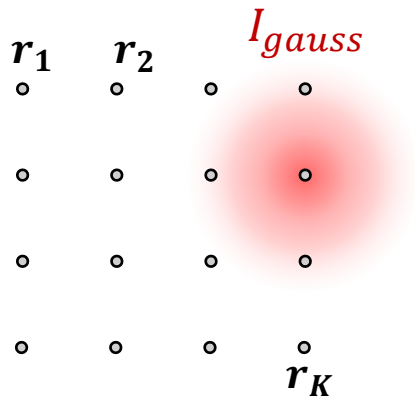
Tinnefeld et al.

SINGLE-MOLECULE CONFOCAL (2020)

Enderlein et al.



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Biophysical Reports 2 (2022) 100036

ORBITAL TRACKING (2001)

Enderlein et al. 2001
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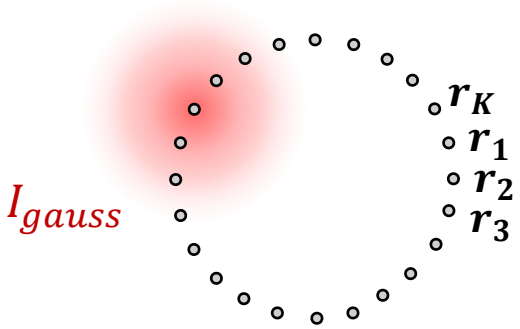
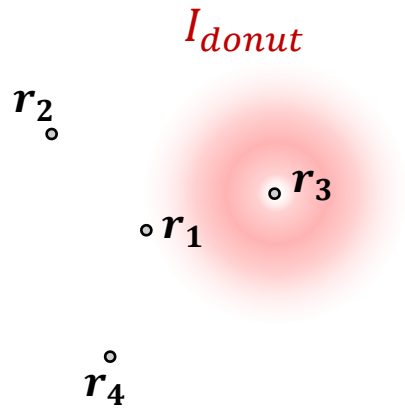
…

MINSTED (2021)

Hell et al.





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SINGLE-MOLECULE CONFOCAL (2020)

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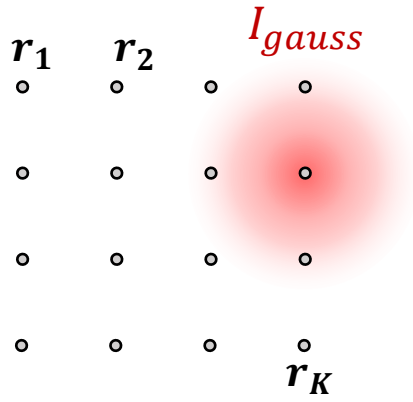

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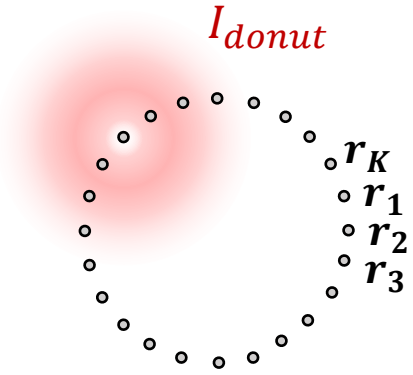
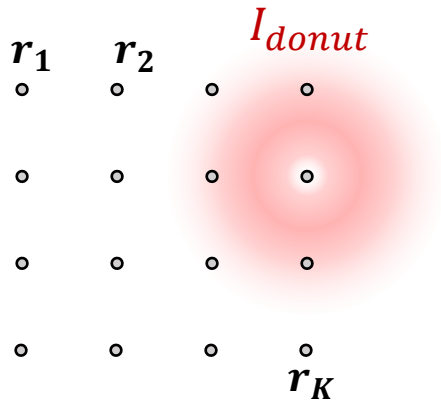


RASTMIN (2021)

Stefani et al.

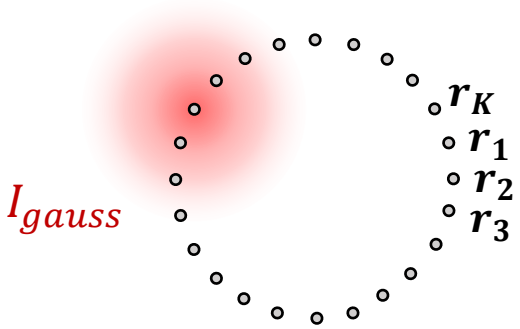


SML-SSI

Biophysical Reports 2 (2022) 100036

ORBITAL TRACKING (2001)

Enderlein et al. 2001
Gratton et al. 2003
…

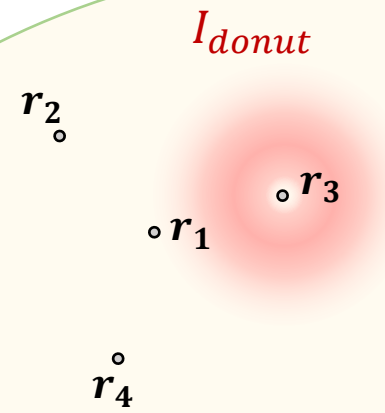


MINSTED (2021)

Hell et al.

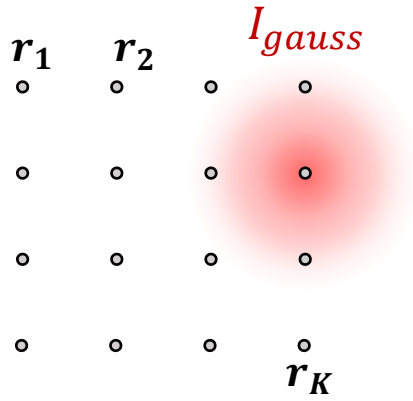
MINFLUX (2017)

Hell et al.
Stefani et al.
Tinnefeld et al.



SINGLE-MOLECULE CONFOCAL (2020)

Enderlein et al.



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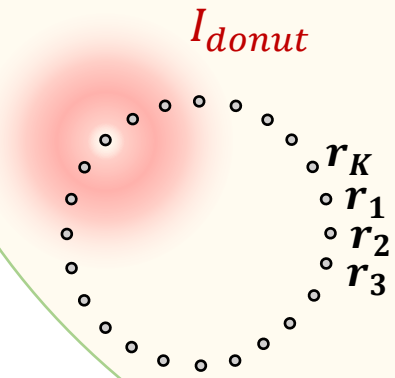
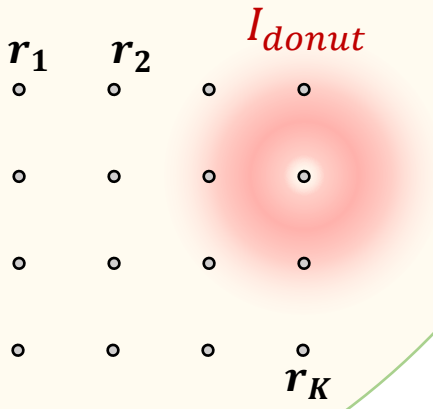


RASTMIN (2021)

Stefani et al.



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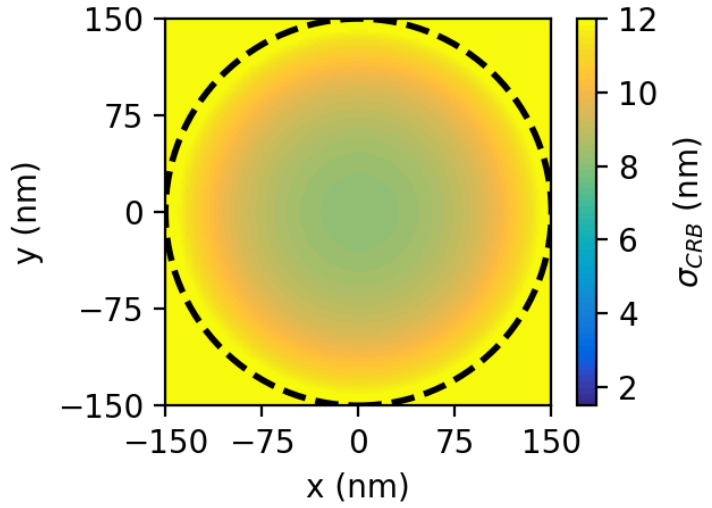
ORBITAL TRACKING



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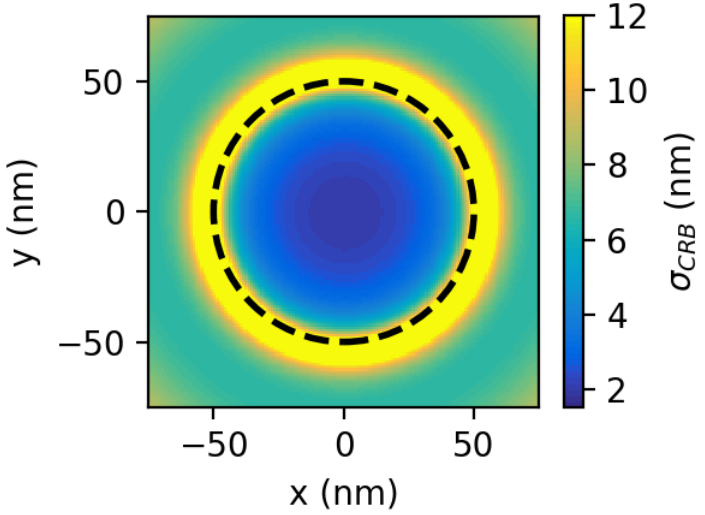
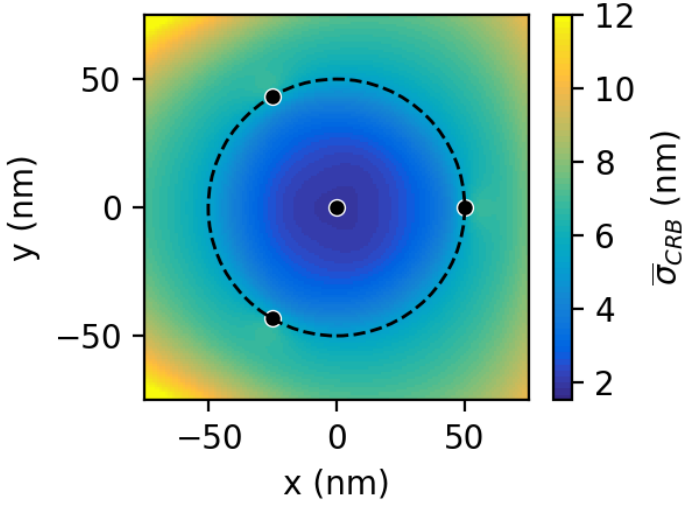


MINFLUX



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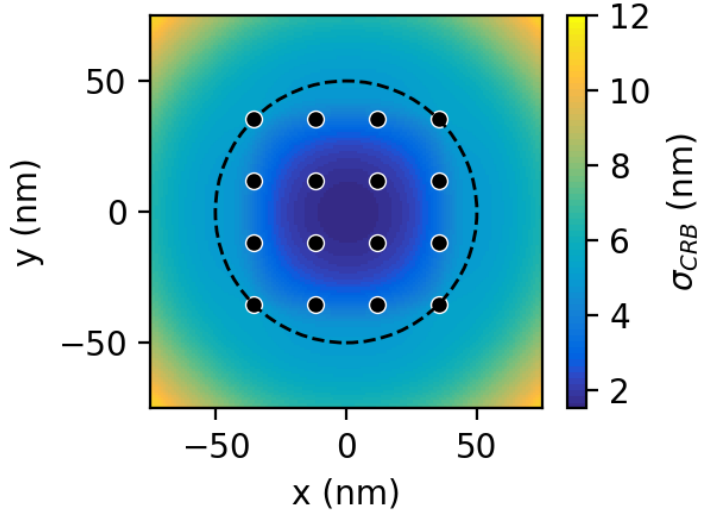
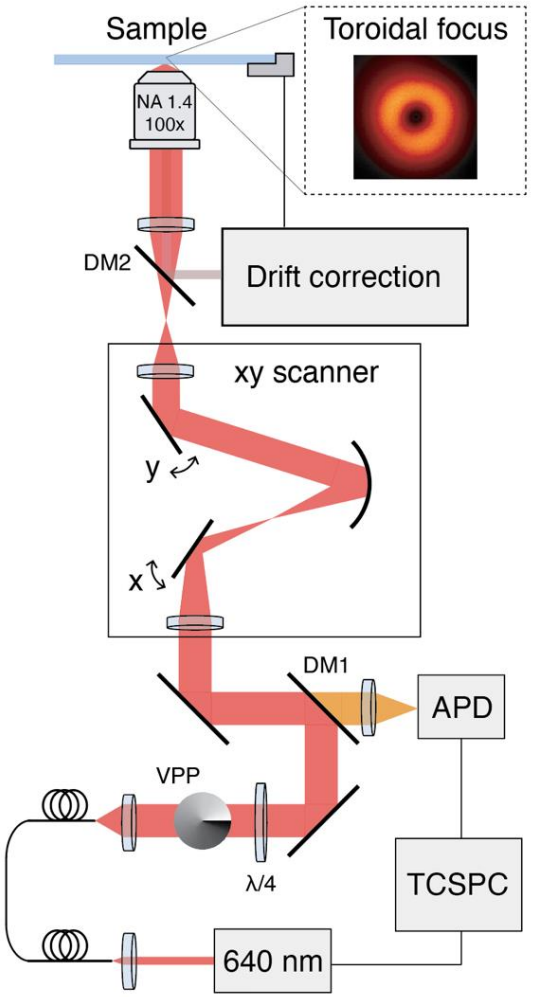


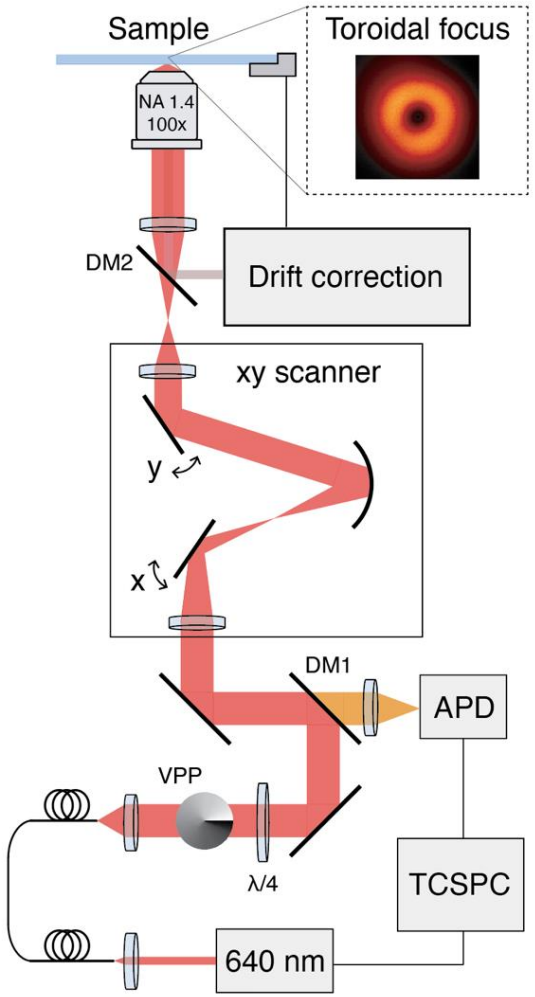
RASTMIN an alternative to MINFLUX implementable in a confocal

SETUP

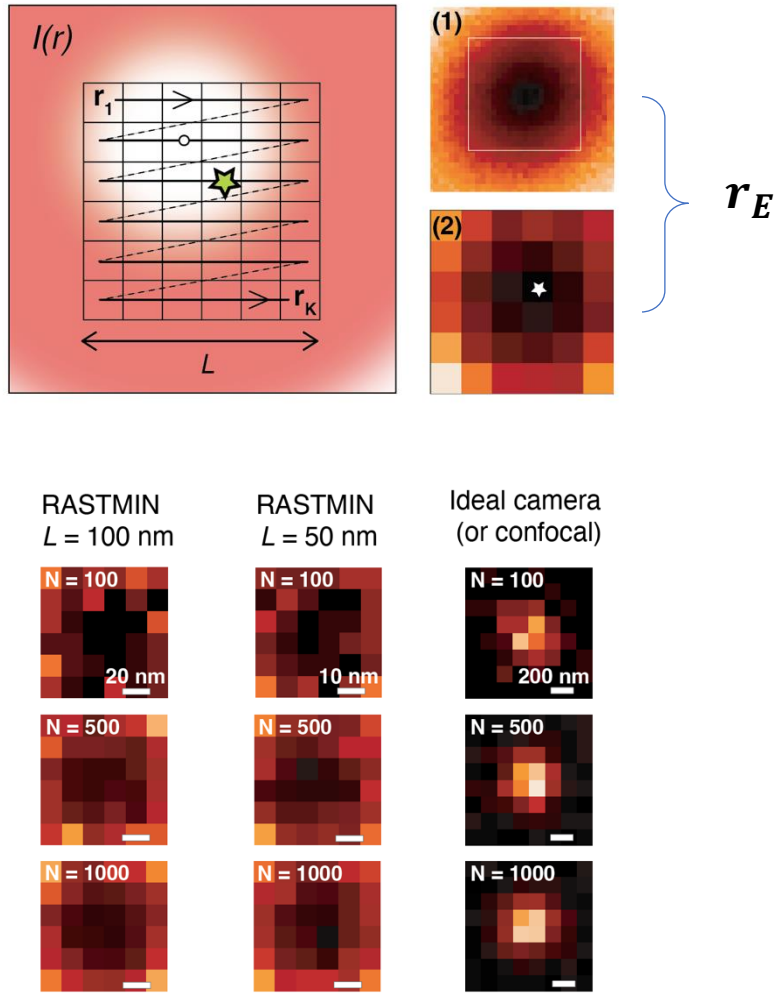


RASTMIN an alternative to MINFLUX implementable in a confocal

SETUP



MEASRUREMENT PIPELINE



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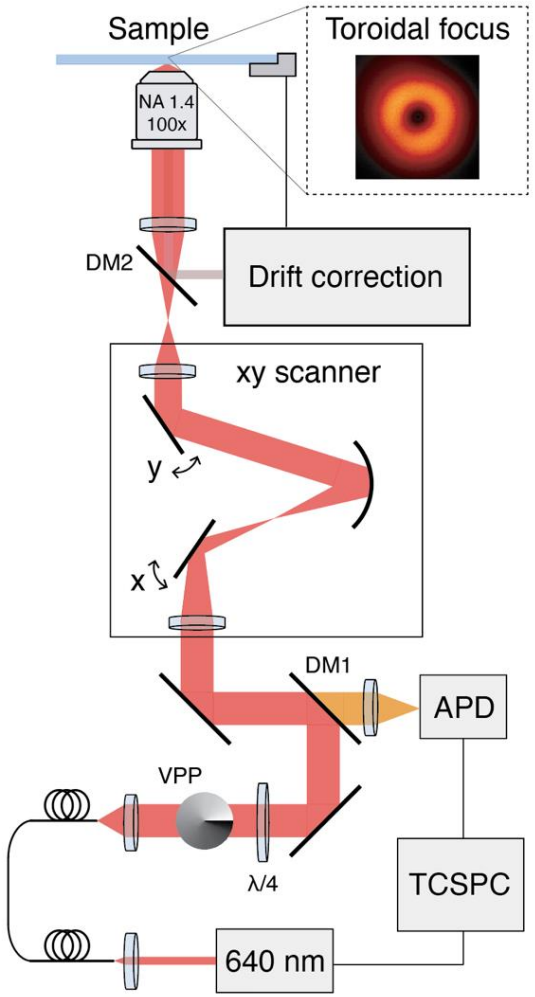
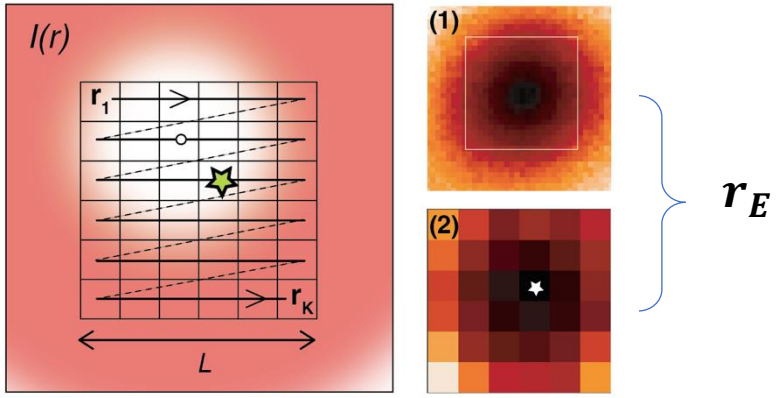
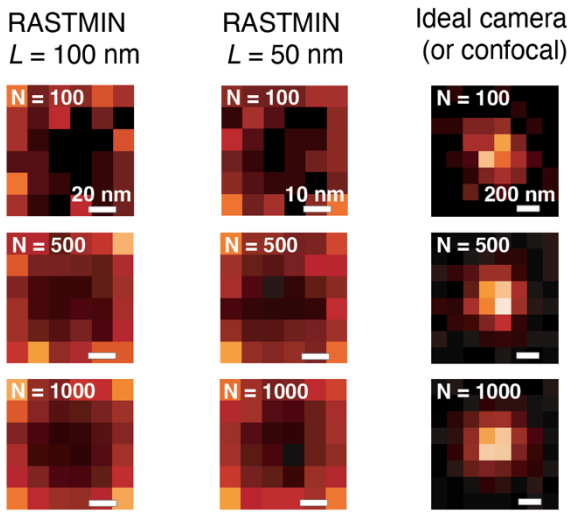
SETUP

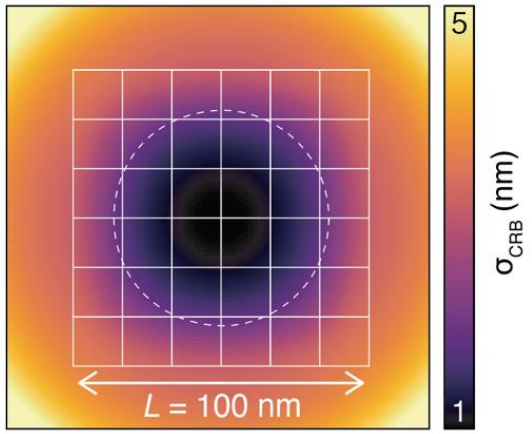


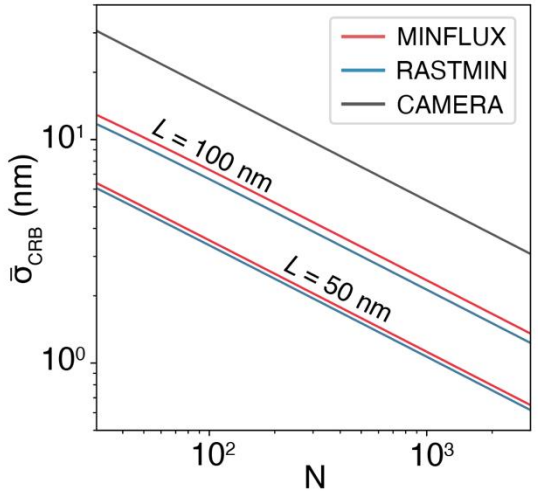
MEASUREMENT PIPELINE





THEORETICAL LOCALIZATION PRECISION





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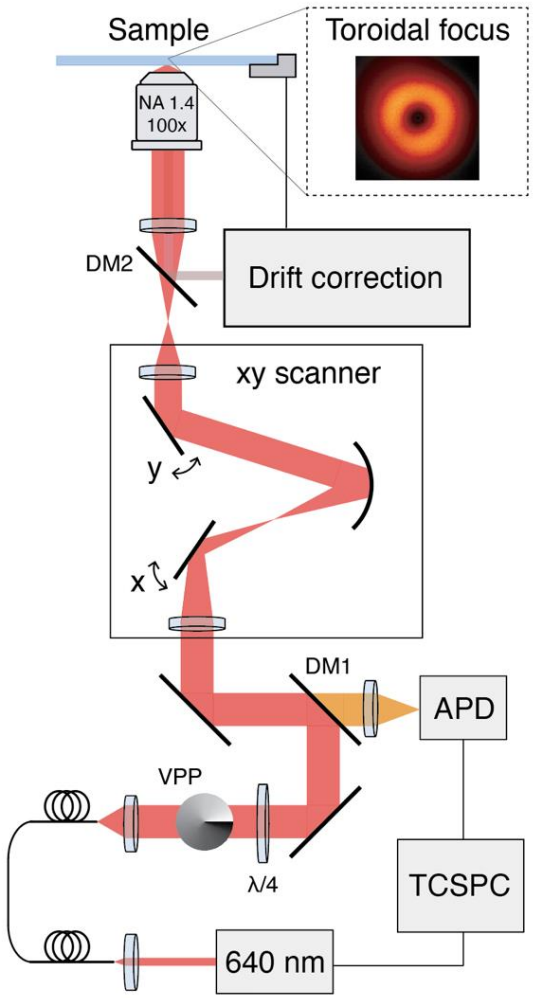
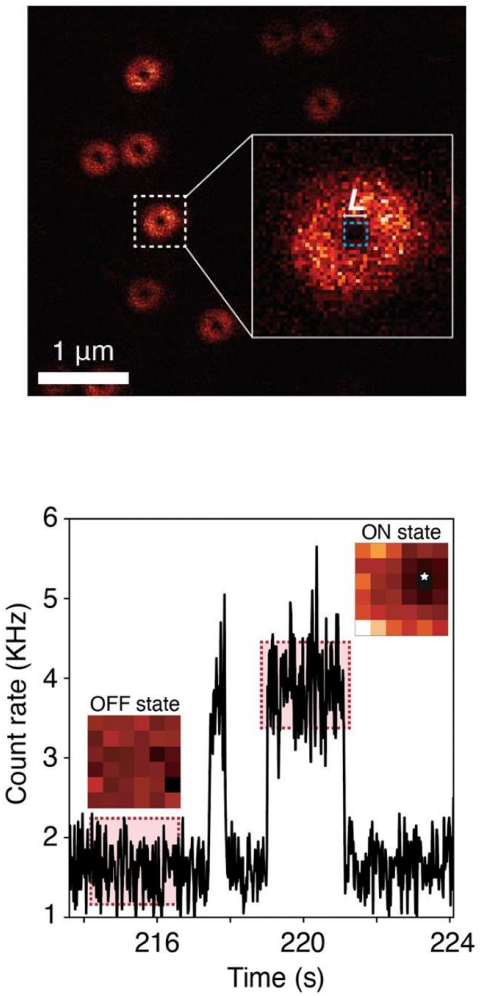
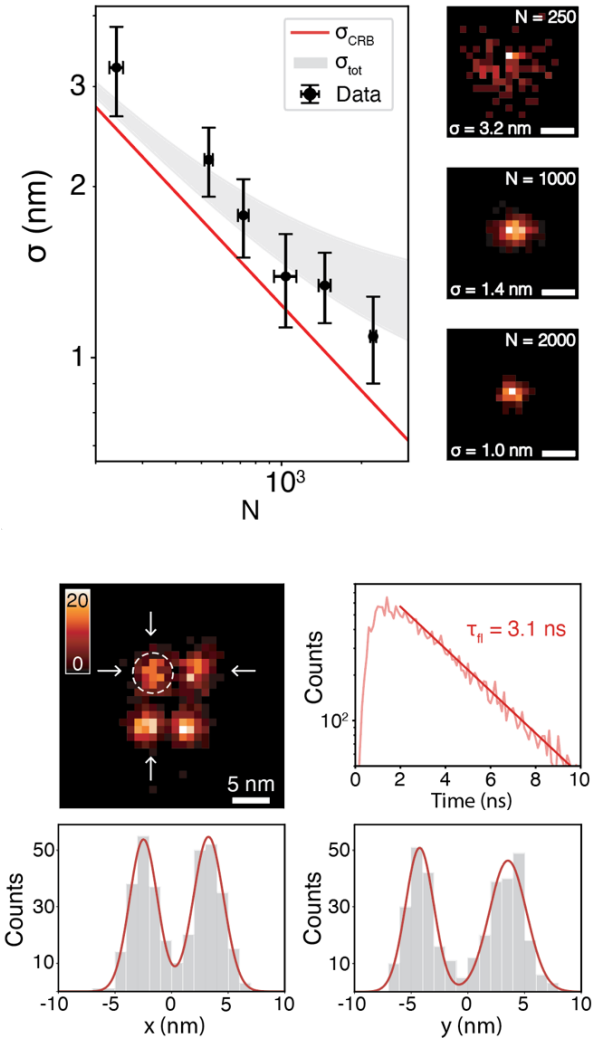


MEASUREMENT

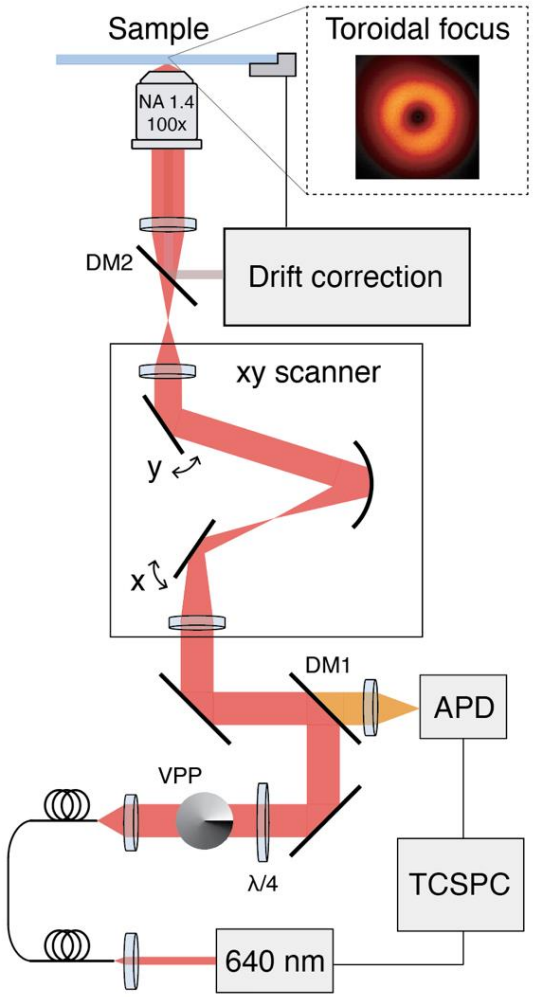


EXPERIMENTAL LOCALIZATION PRECISION



RASTMIN an alternative to MINFLUX implementable in a confocal

SETUP



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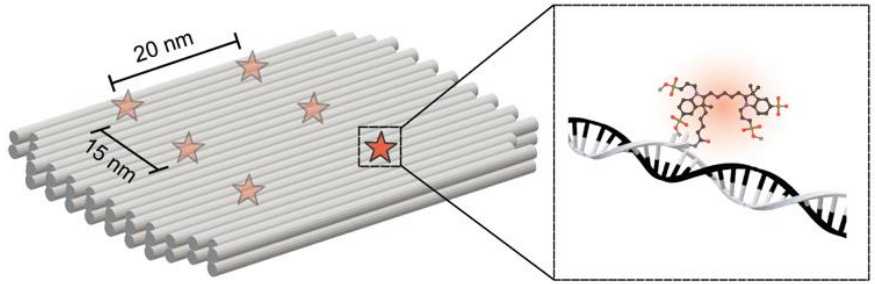
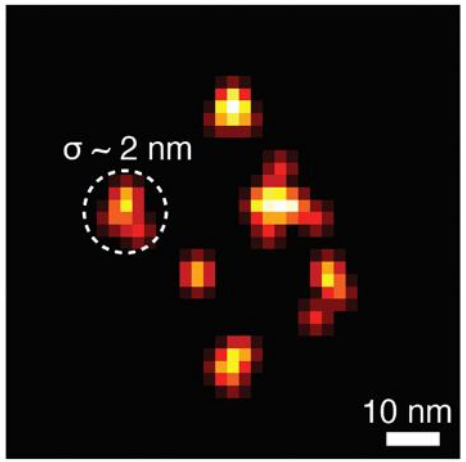




https://stefani-lab.ar/

fernando.stefani@df.uba.ar

Luciano Masullo (now at MPI biochem)

Alan Szalai (now at LMU)

Lucía Lopez

Santiago Sosa

Julián Gargiulo

Ianina Violi

Florencia Edorna

Florencia Choque

Gonzalo Escalante

Luciana Martínez

Guillermo Acuna & co (Fribourg)

Philip Tinnefeld & co (LMU)

Sub-10 nm resolution

Nature Communications 12 (2021) 517

Nano Letters 21 (2021) 2296–2303

Nanoscale 13 (2021) 18421-18433

Biophysical Reviews 13 (2021) 1101–1112

SML-SSI

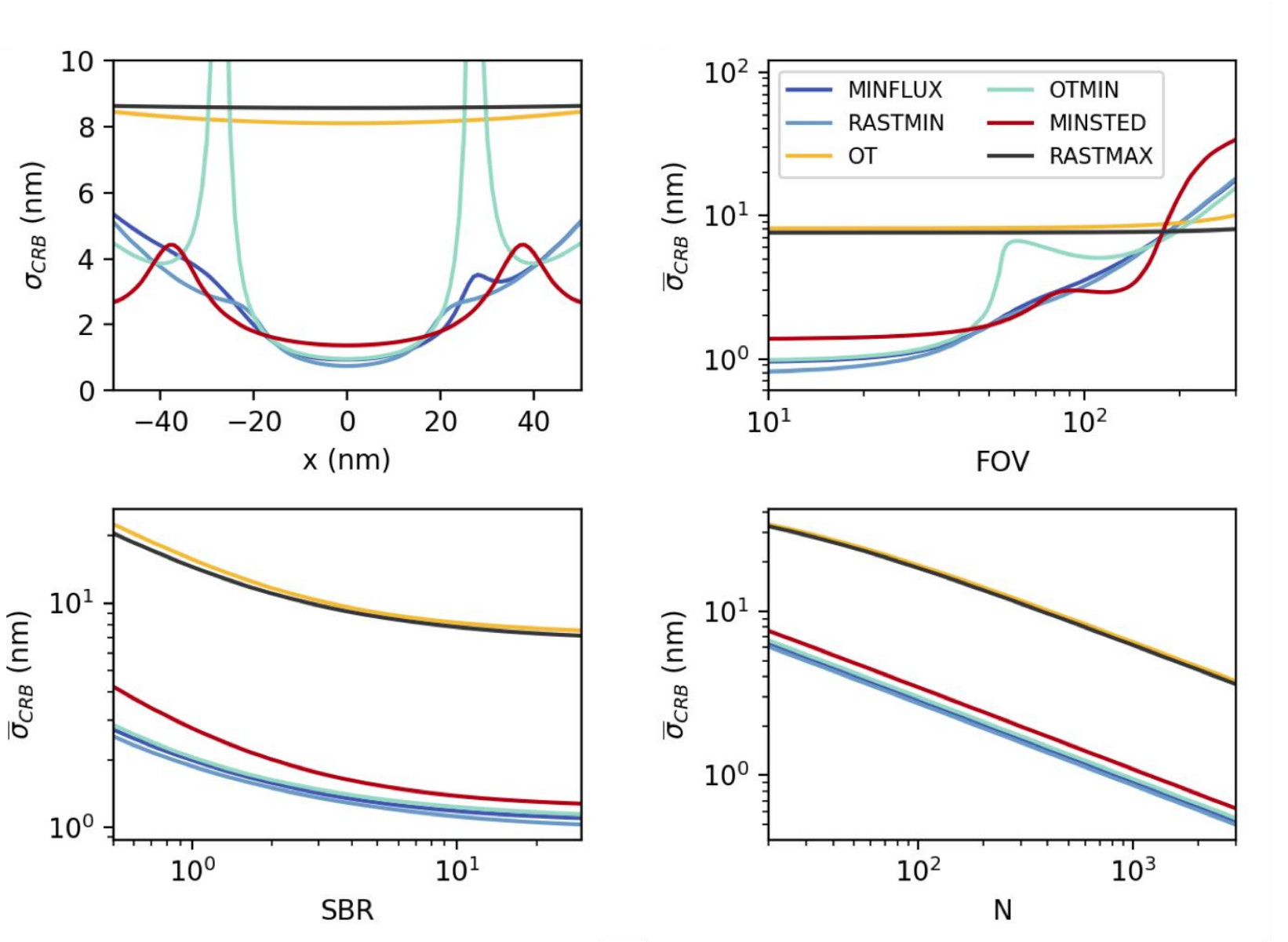
Science 355 (2017) 606-612

Nano Letters 21 (2021) 840-846

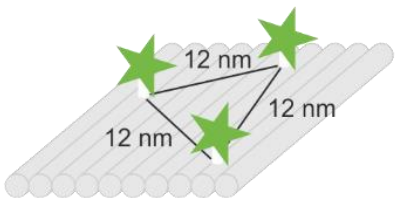
Biophysical Reports 2 (2022) 100036

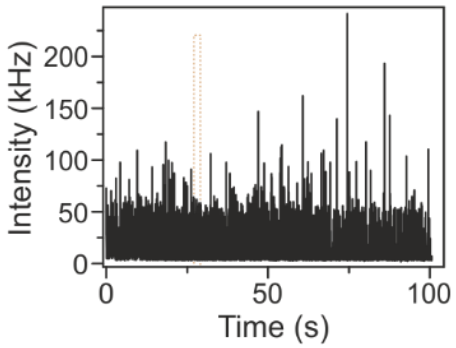
LSA (2022) News & Views to appear soon

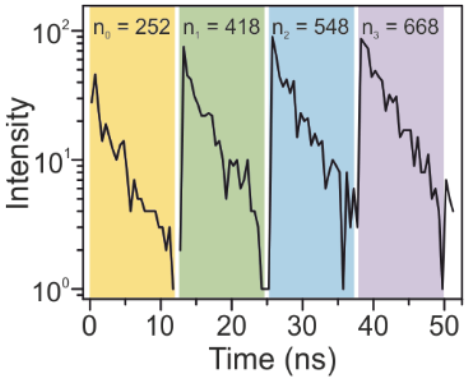
Benchmarking SML-SSI methods

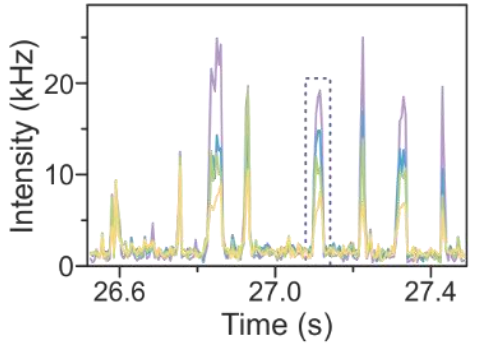


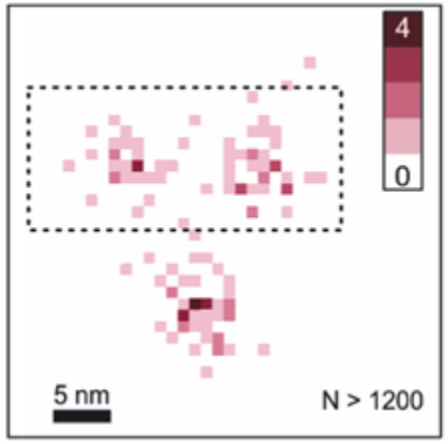
p-MINFLUX (lifetime) nanoscopy

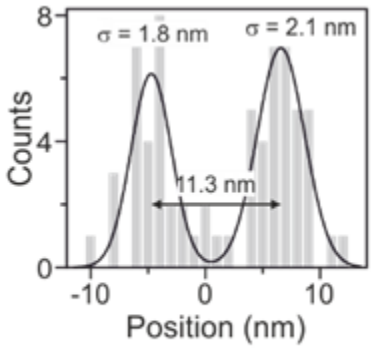


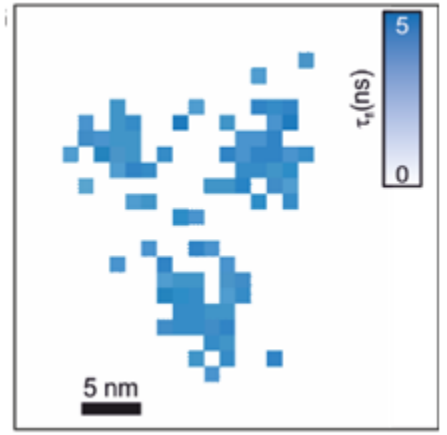


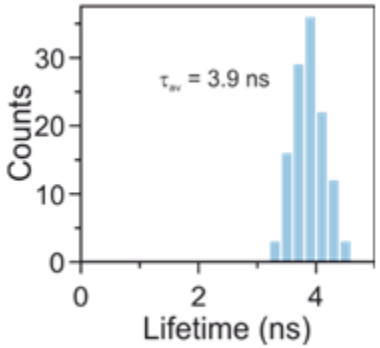








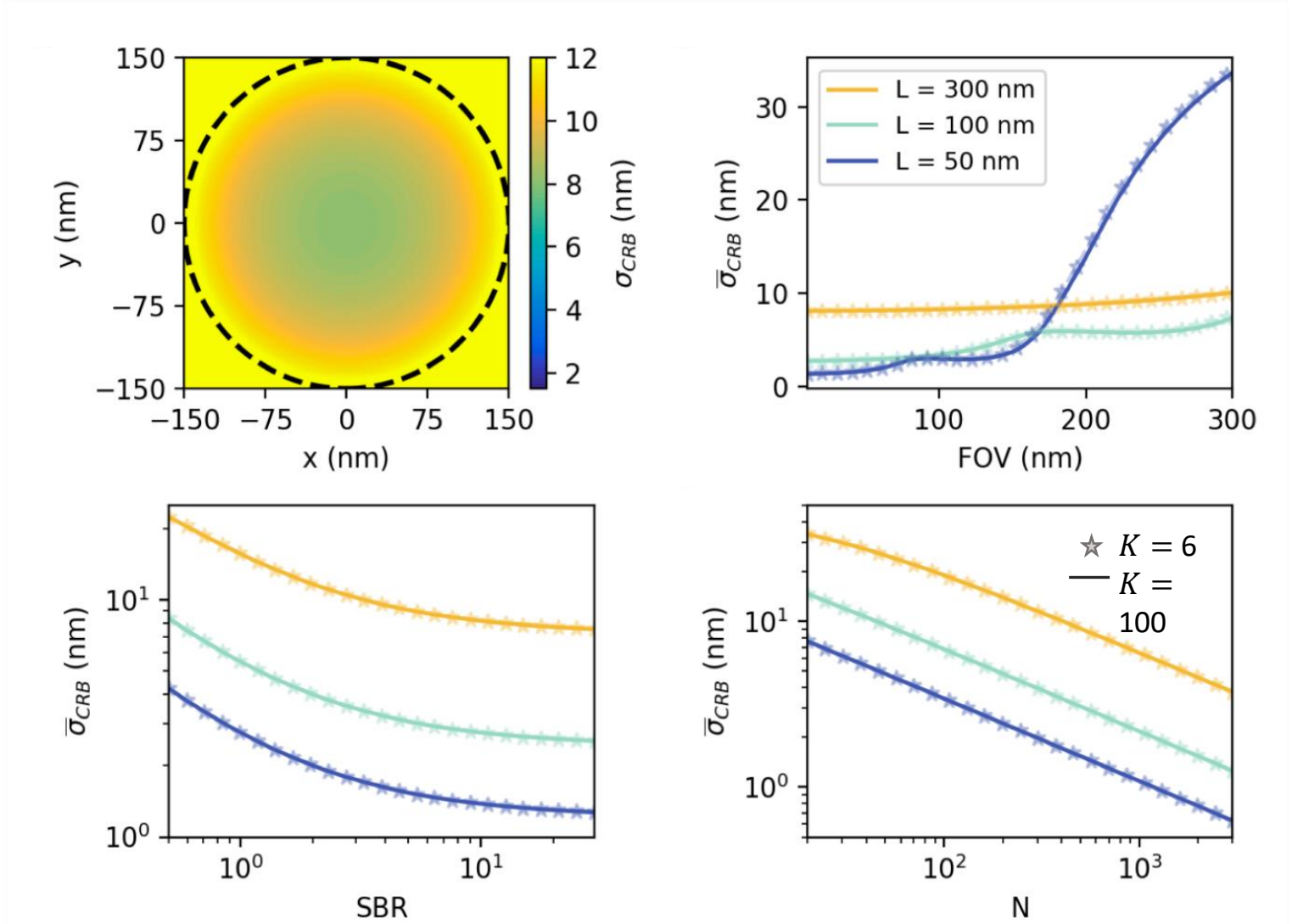




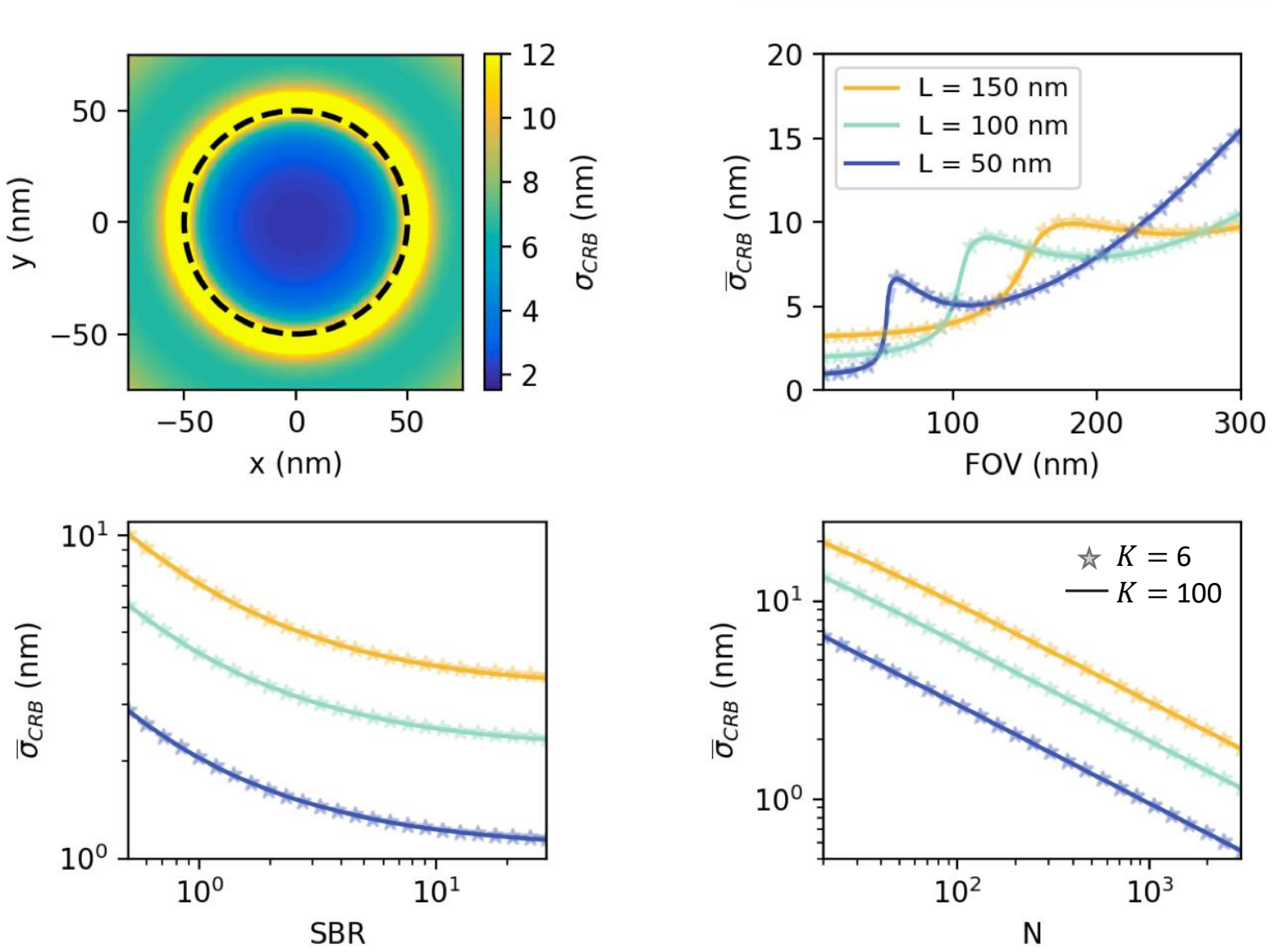
Masullo et al. ***Nano Letters*** 21 (2021) 840-846

Orbital tracking

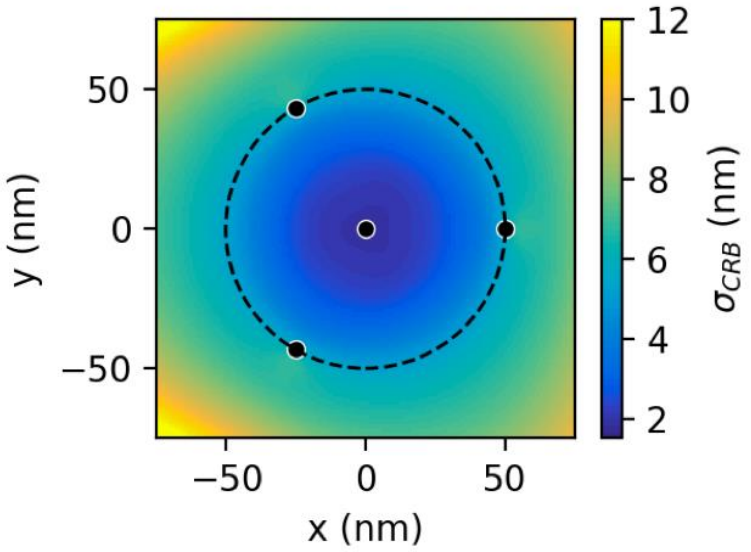
Optimal orbit radius ~ ½ FWHM of the excitation

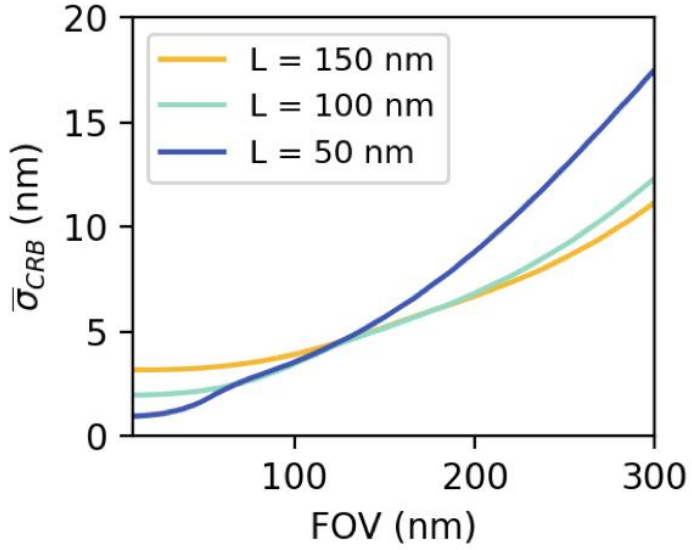


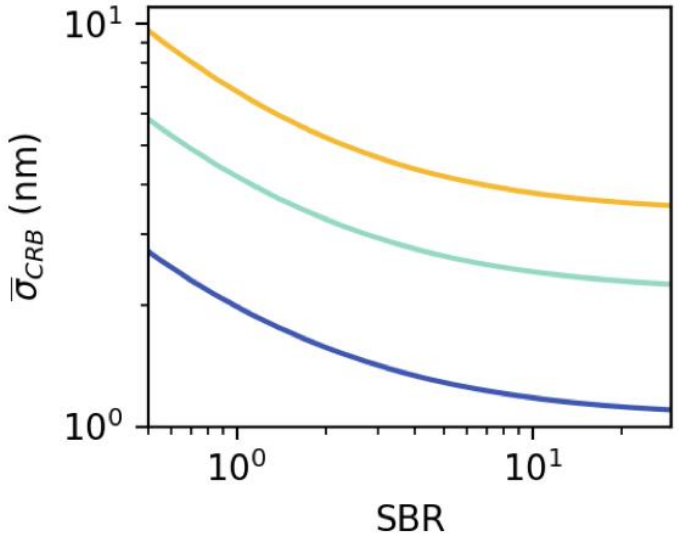
OTMIN

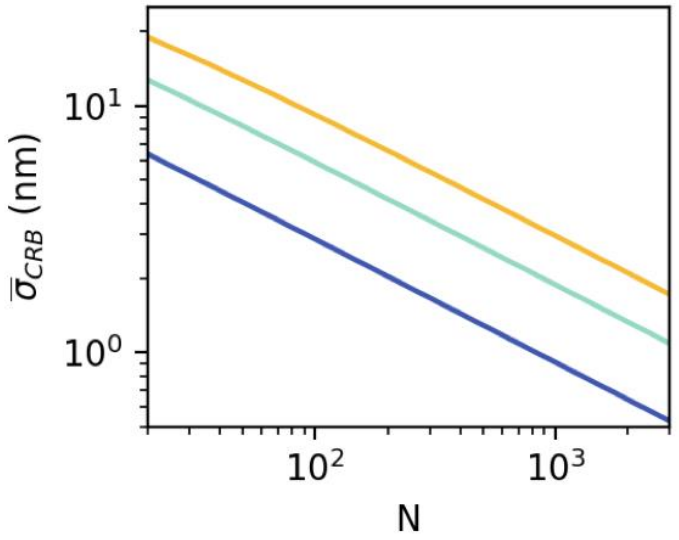

50
0
−50
y (nm)
−50
0
50
x (nm)
12
10
8
6
4
2
σ_{CRB} (nm)
20
15
10
5
0
$\overline{\sigma}_{CRB}$ (nm)
L = 150 nm
L = 100 nm
L = 50 nm
100
200
300
FOV (nm)
10^1
10^0
$\overline{\sigma}_{CRB}$ (nm)
10^0
10^1
SBR
$K = 6$
$K = 100$
10^1
10^0
$\overline{\sigma}_{CRB}$ (nm)
10^2
10^3
N

MINFLUX

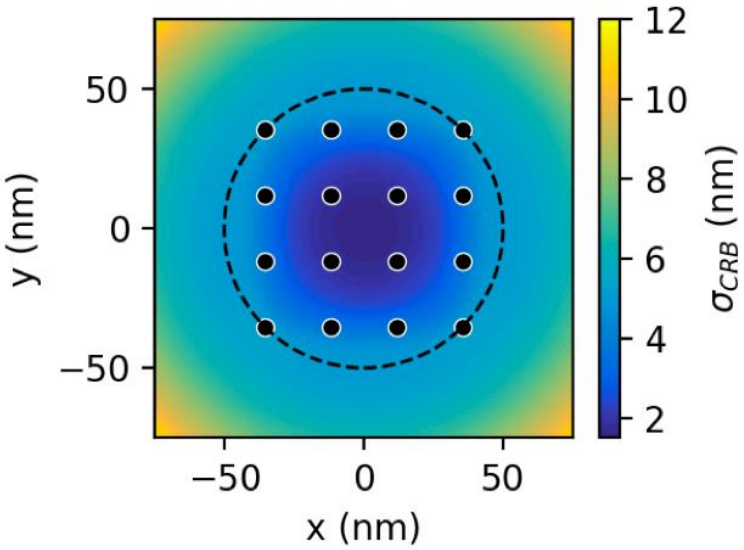

50
0
−50
y (nm)
−50
0
50
x (nm)
12
10
8
6
4
2
σ_{CRB} (nm)

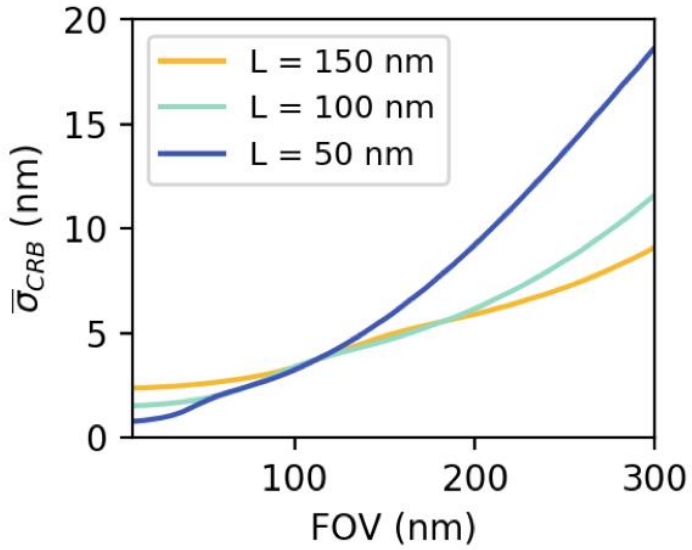

20
15
10
5
0
$\overline{\sigma}_{CRB}$ (nm)
L = 150 nm
L = 100 nm
L = 50 nm
100
200
300
FOV (nm)

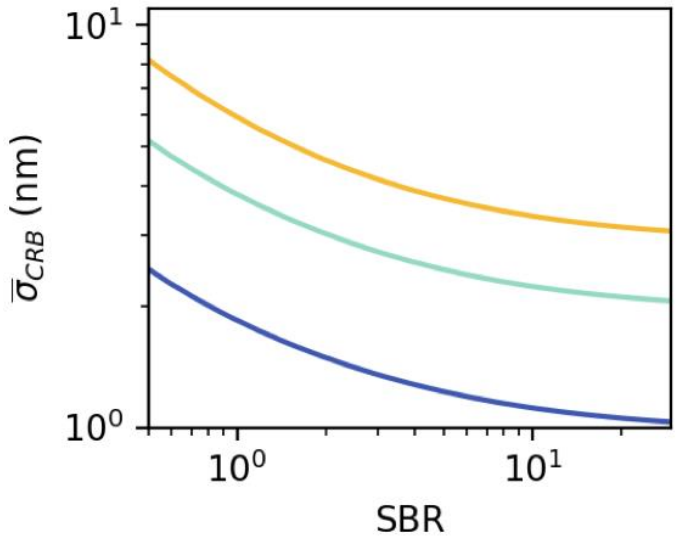

10^1
10^0
$\overline{\sigma}_{CRB}$ (nm)
10^0
10^1
SBR

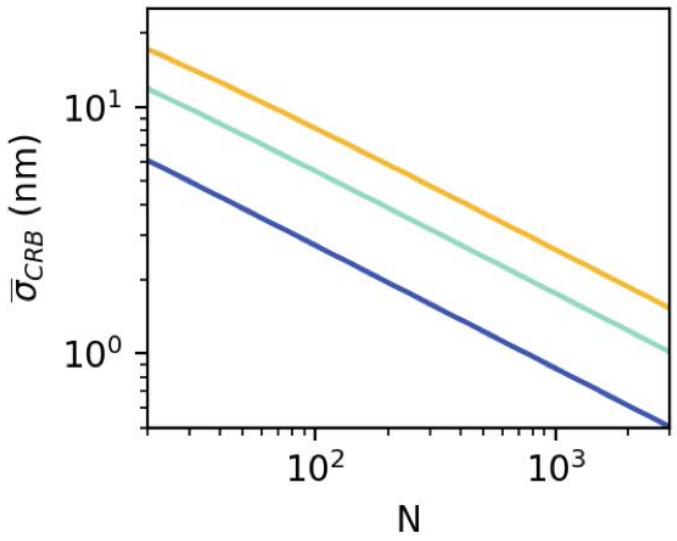

10^1
10^0
$\overline{\sigma}_{CRB}$ (nm)
10^2
10^3
N

RASTMIN


50
0
−50
y (nm)
−50
0
50
x (nm)
12
10
8
6
4
2
σ_{CRB} (nm)


20
15
10
5
0
$\overline{\sigma}_{CRB}$ (nm)
L = 150 nm
L = 100 nm
L = 50 nm
100
200
300
FOV (nm)


10^1
10^0
$\overline{\sigma}_{CRB}$ (nm)
10^0
10^1
SBR


10^1
10^0
$\overline{\sigma}_{CRB}$ (nm)
10^2
10^3
N

RASTMAX (a.k.a. single-molecule confocal localization)