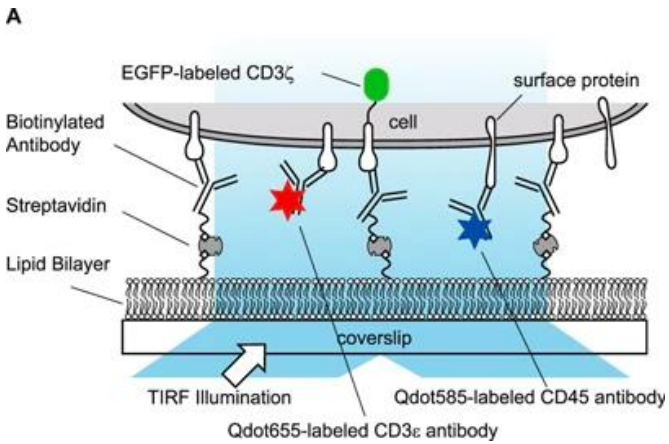
Multi-modal Single-molecule Imaging with Continuously Controlled Spectral-resolution (CoCoS🌴) Microscopy

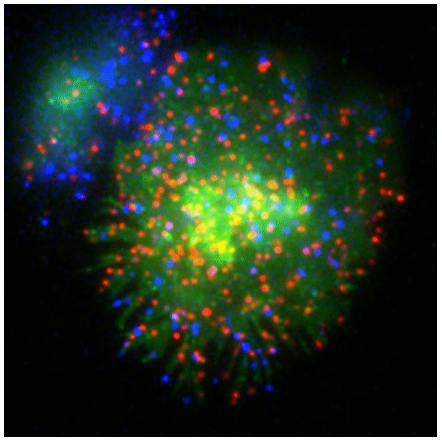
Jonathan Jeffet
School of Physics and Astronomy
Supervisor: Prof. Yuval Ebenstein

NanoBioPhotonixLab

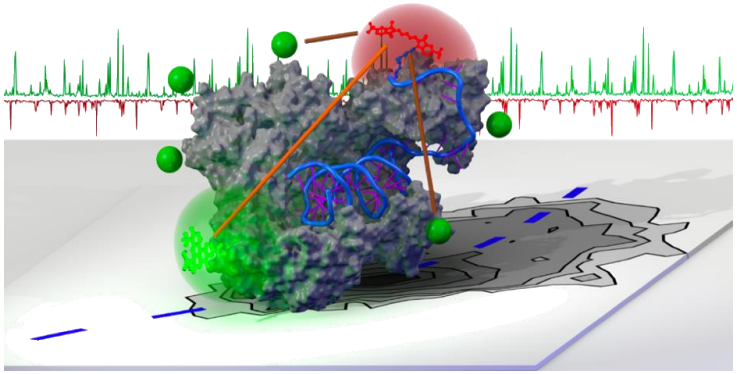
Fluorescence microscopy: Color as a contrast mechanism

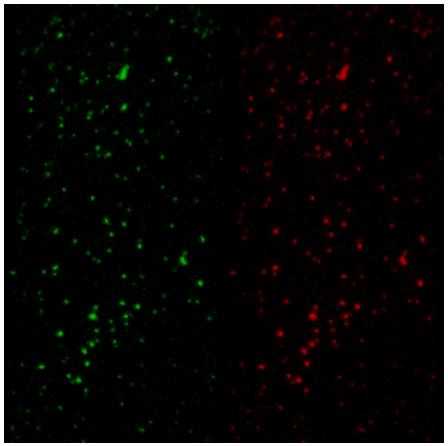
Single molecules interactions





Single molecule Förster Resonance Energy Transfer (smFRET)



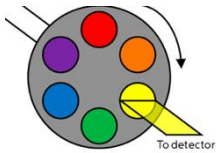


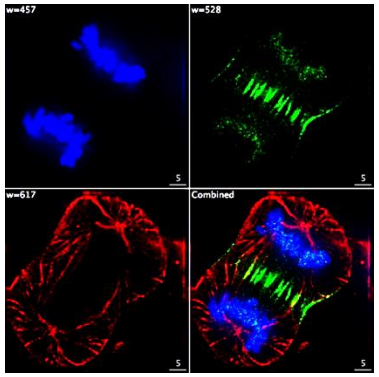
Ito, Y. et al., *Sci Rep* **7,** 6994 (2017)

Kalinin, S. *et al. Nat Methods* **9,** 1218–1225 (2012)

Optical schemes for wide-field color detection

Filter wheel

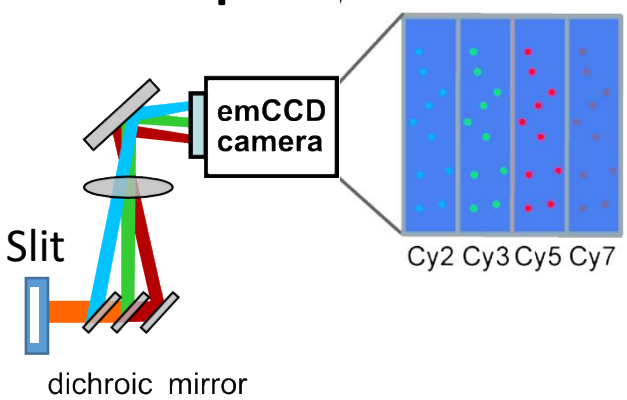




✓ Space

✗ Time

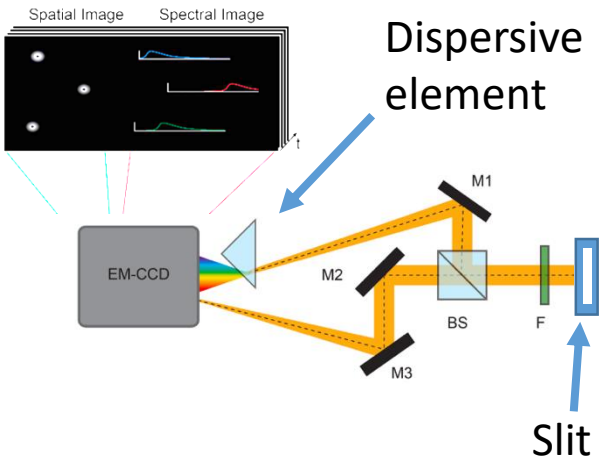
Color channel split-view



✓ Time

✗ Space

Spectral split-view



✓ Time

✓ More colors

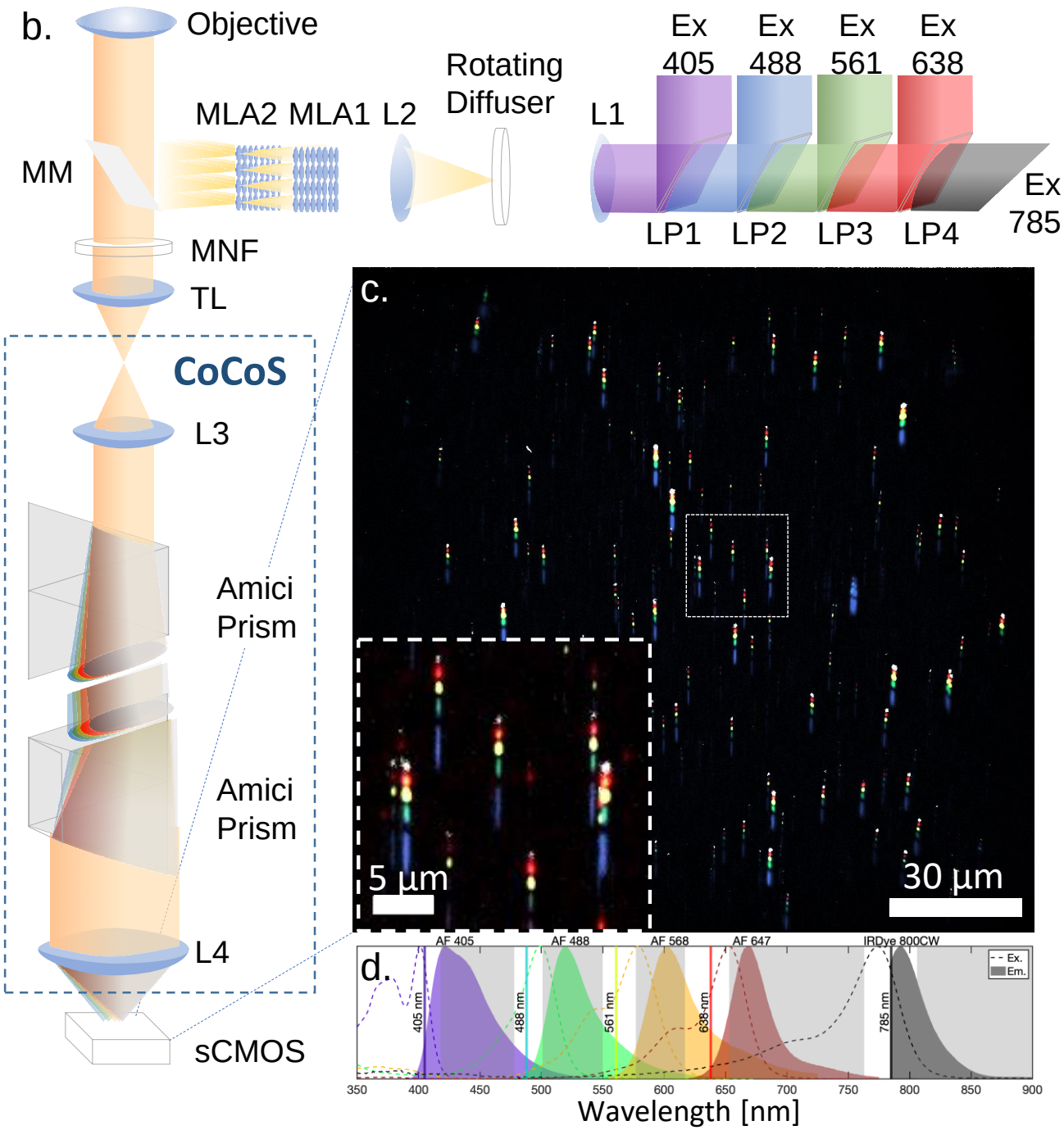
✗ Space ($<20\times20\mu m^2$)

✗ SNR

Zhang, Z. et al., *Nat. Methods* **12**, 935–938 (2015).
Dong, B. *et al., Nat. Commun.* **7**, 1–8 (2016).
Mlodzianoski, M. J. et al., *PLoS One* **11**, 1–12 (2016).

CoCoS Optical Setup

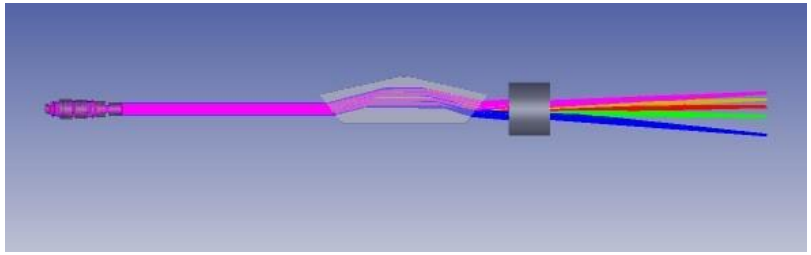
- ✓ **Large FOV** (130×130 μm²)
- ✓ Almost **no photon loss**
- ✓ **Simultaneous** multi-color acquisition
- ✓ Simple **add-on** (maintains optical axis)



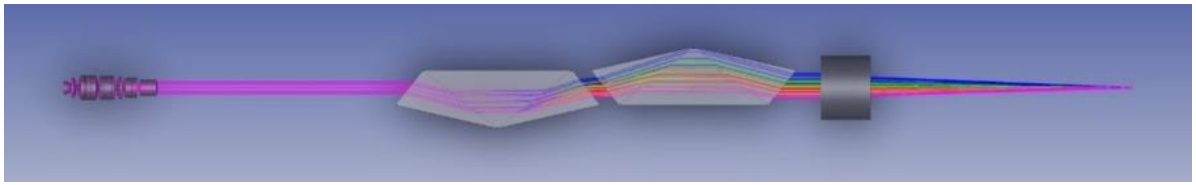
Operation principle

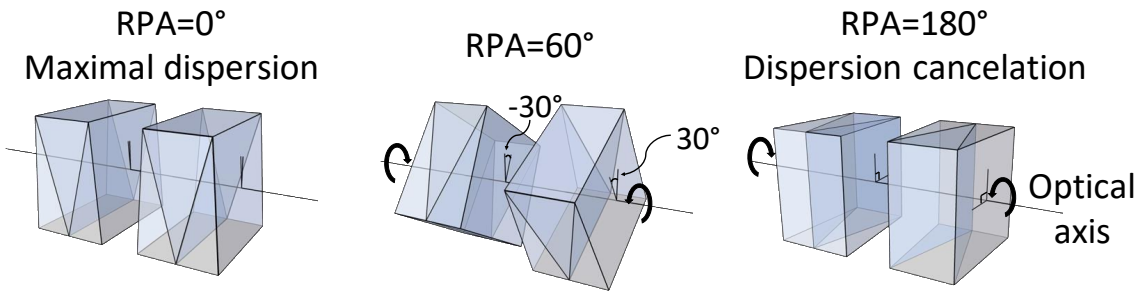
Double Amici prism

(direct vision prism)



Atmospheric chromatic aberration correction in astronomy



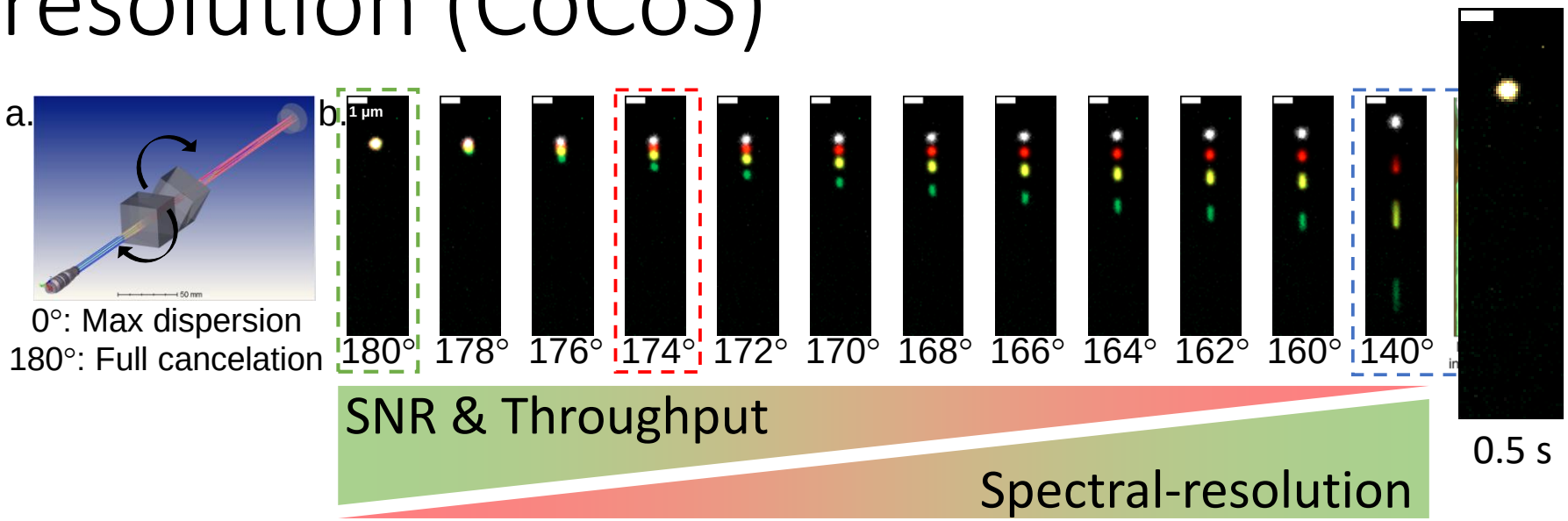


RPA = relative prism angle

$$D(\lambda) = 2\mathrm{D}_{\mathrm{SP}}(\lambda) \cdot \sin\left(\frac{180° - RPA}{2}\right)$$

Wynne, C. G. & Worswick, S. P., *Mon. Not. R. Astron. Soc.* **220**, 657–670 (1986).

Continuously Controlling Spectral-resolution (CoCoS)

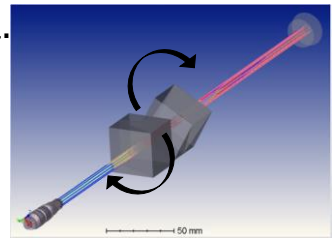


Three CoCoS modalities:

- Localization (no dispersion)
- Color detection (minimal dispersion)
- Spectroscopy (fully detailed spectra)

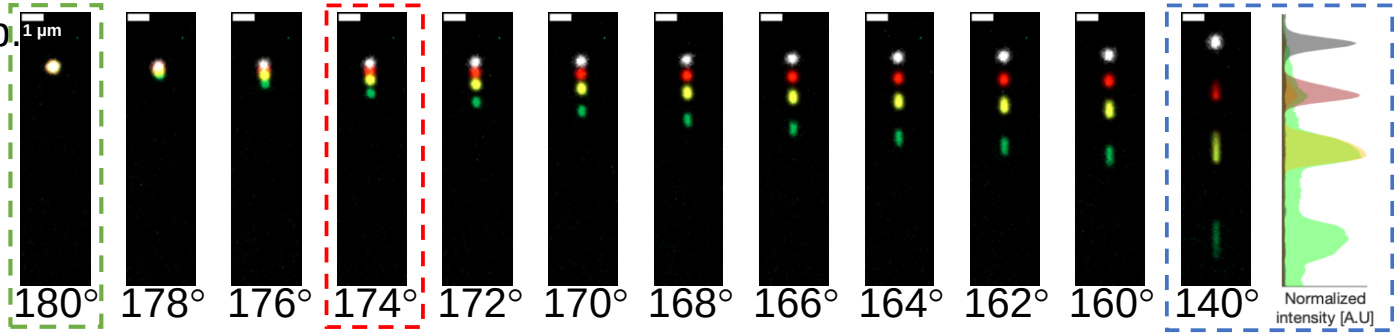
Continuously Controlling Spectral-resolution (CoCoS)

a.

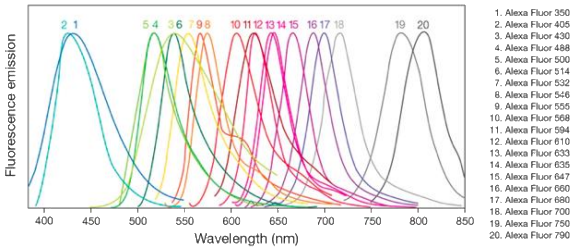


0°: Max dispersion
180°: Full cancelation

b.



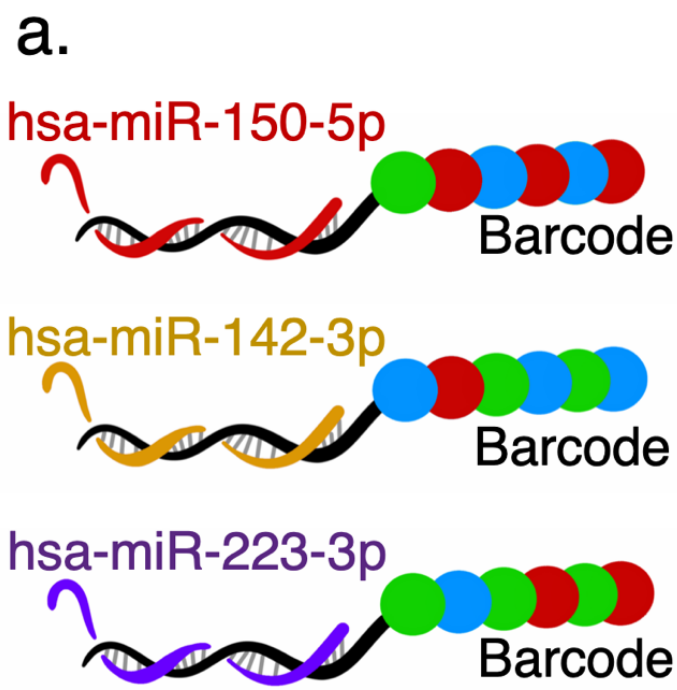
Choosing the ideal spectral-resolution for color detection:

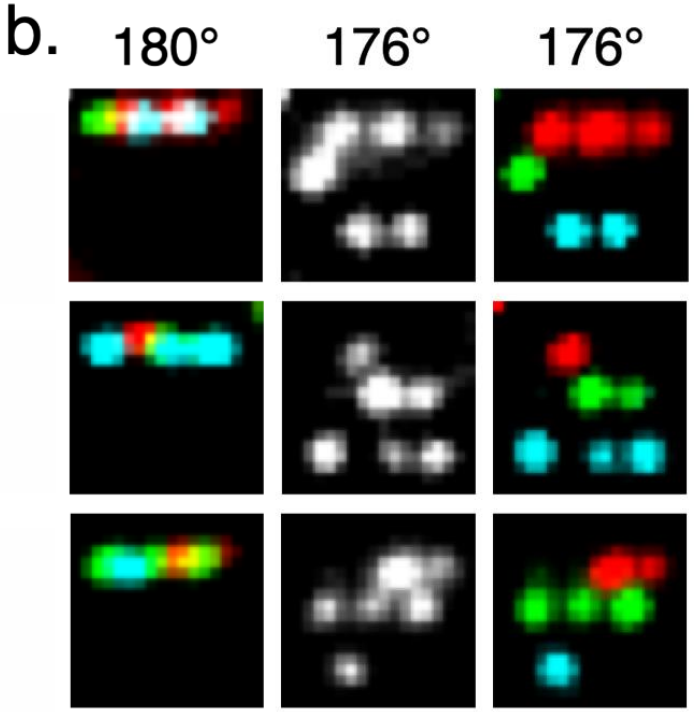


	AF 405	AF 488	CY 3	ATTO 550	AF 555	AF 568	AF 594	CF 640R	AF 647	CY 5.5	IRDYE 800CW
AF 405		178.3	178.7	178.7	178.7	178.8	178.9	179.0	179.0	179.1	179.2
AF 488	178.3		174.1	174.9	175.2	176.4	176.9	177.6	177.7	177.9	178.3
CY 3	178.7	174.1		152.2	161.4	173.2	175.0	176.0	176.2	176.8	177.7
ATTO 550	178.7	174.9	152.2		120.5	171.0	173.8	175.6	175.8	176.5	177.5
AF 555	178.7	175.2	161.4	120.5		169.3	173.1	175.3	175.5	176.3	177.4
AF 568	178.8	176.4	173.2	171.0	169.3		160.4	172.9	173.5	175.1	176.9
AF 594	178.9	176.9	175.0	173.8	173.1	160.4		170.3	171.3	173.9	176.5
CF 640	179.0	177.6	176.0	175.6	175.3	172.9	170.3		112.0	167.6	174.4
AF 647	179.0	177.7	176.2	175.8	175.5	173.5	171.3	112.0		164.6	174.0
CY 5.5	179.1	177.9	176.8	176.5	176.3	175.1	173.9	167.6	164.6		171.5
IRDYE 800CW	179.2	178.3	177.7	177.5	177.4	176.9	176.5	174.4	174.0	171.5	

Application 1: Color detection

Single microRNA identification using 4-color NanoString barcodes



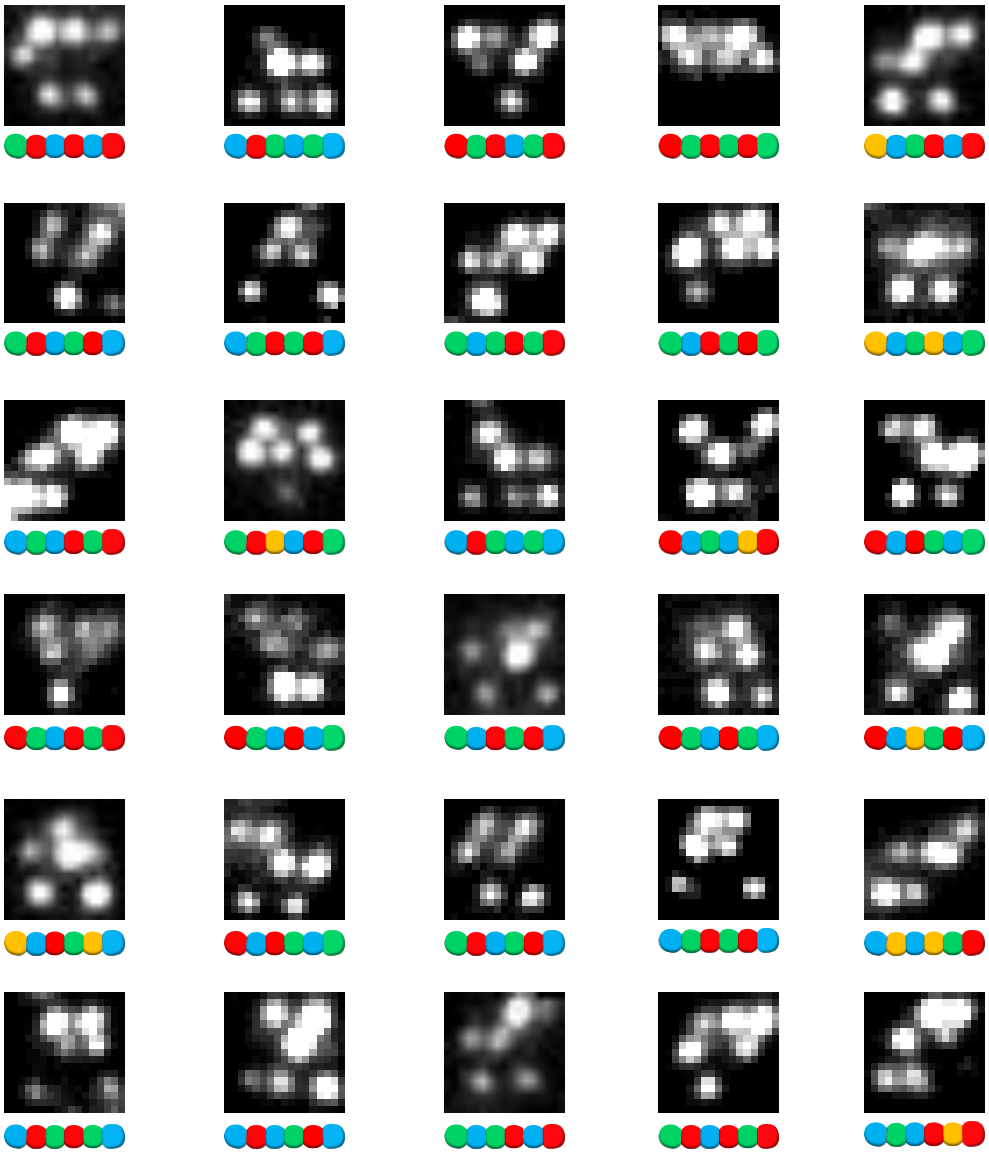


NanoString sequential acquisition

CoCoS single snapshot matrix decoding

In collaboration with the Shomron lab

Barcode decoding in a single snapshot



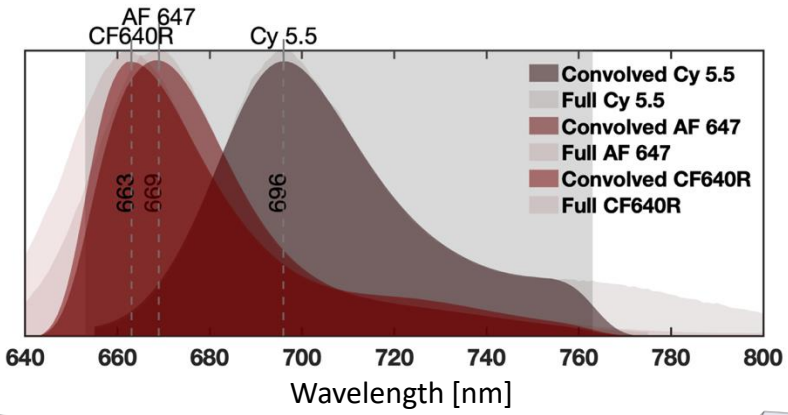
- ✓ Single-snapshot decoding: **4-fold** increased throughput
- ✓ More colors - No time cost: **>410-fold** increase in available barcode combinations

In collaboration with the Shomron lab

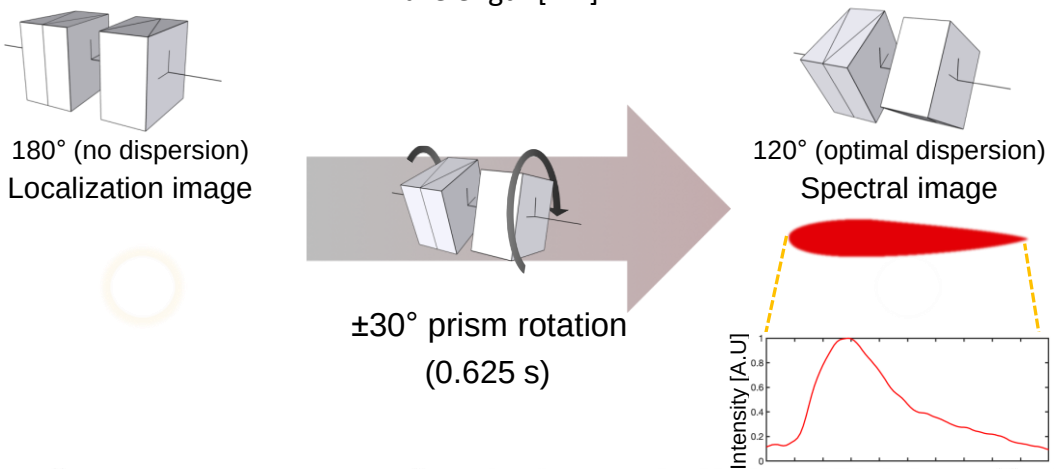
Application 2: Spectroscopy

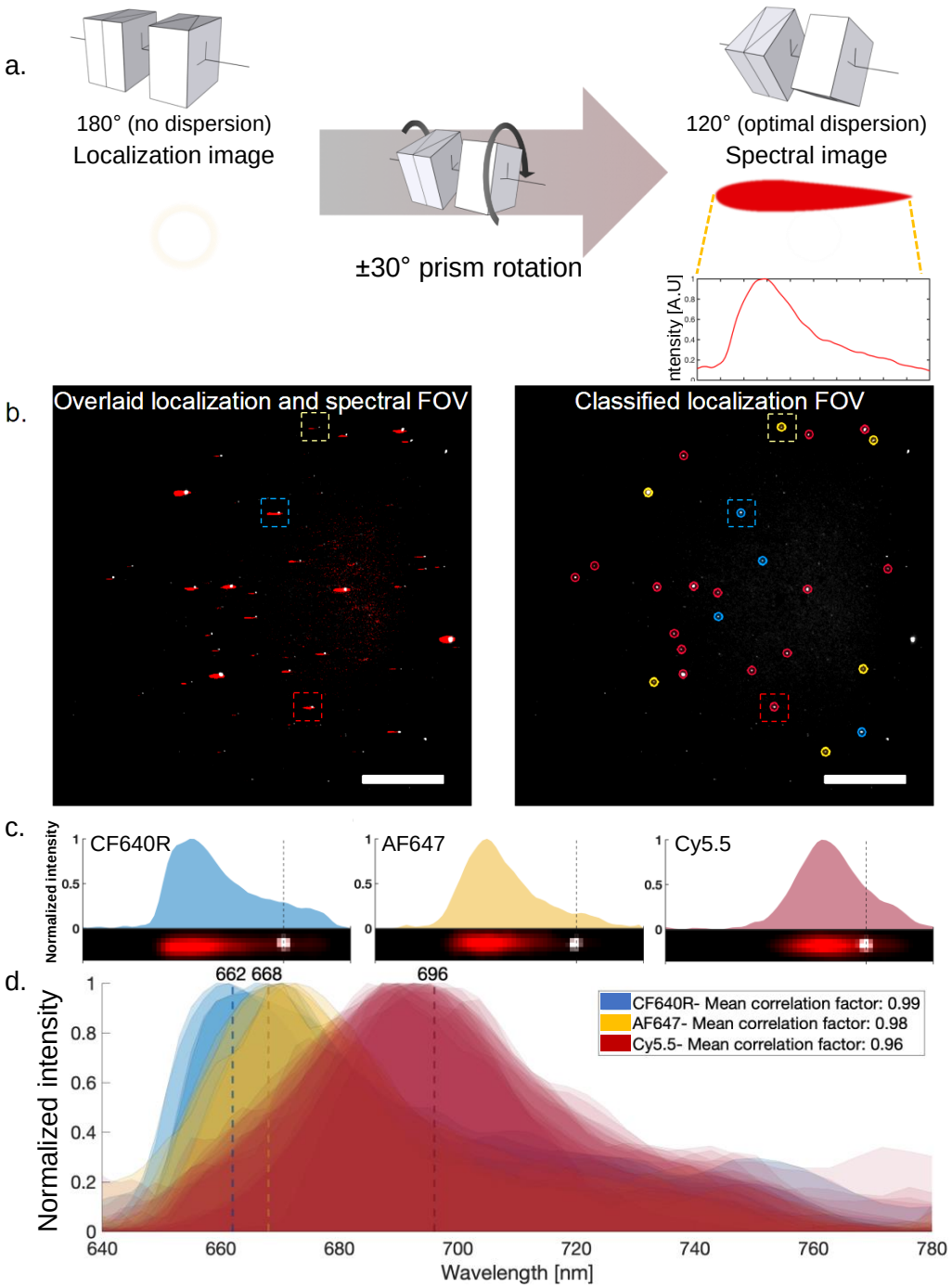
Resolving the shades of far-red dyes
(spectral detection with a single laser excitation)

Can we resolve multiple far-red dyes within the same color channel?

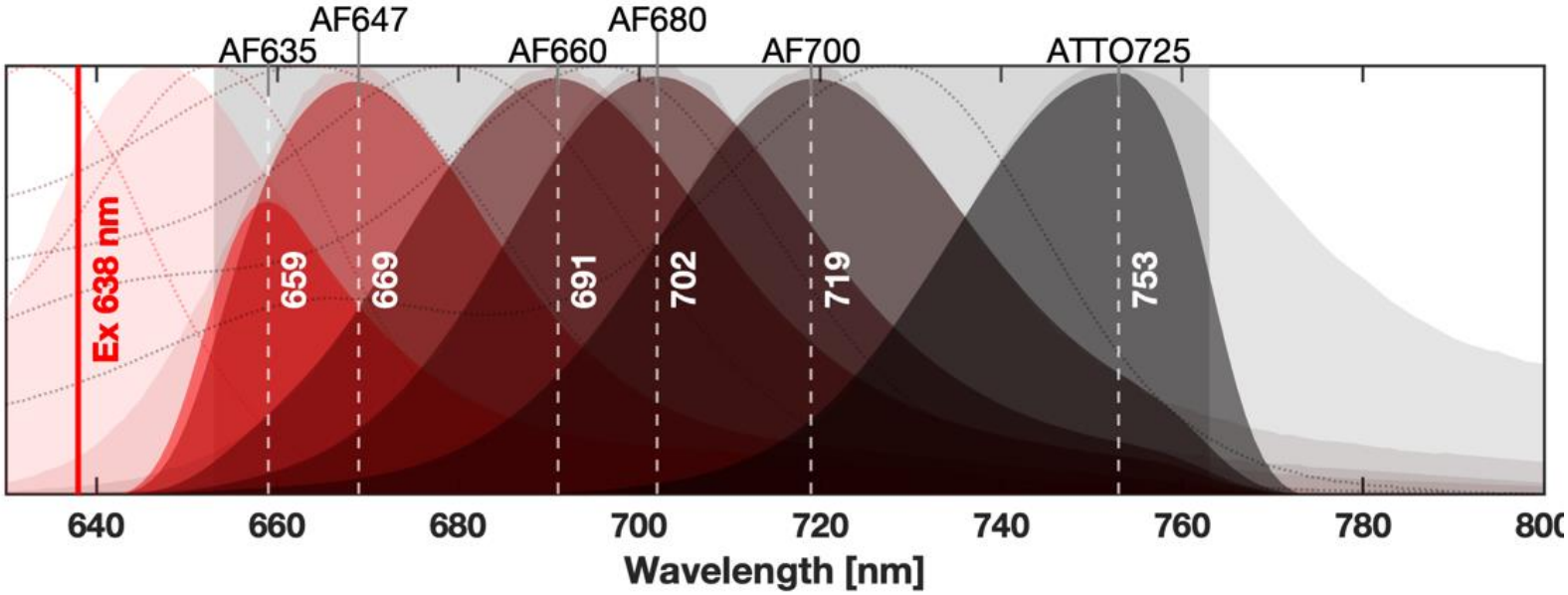


a.



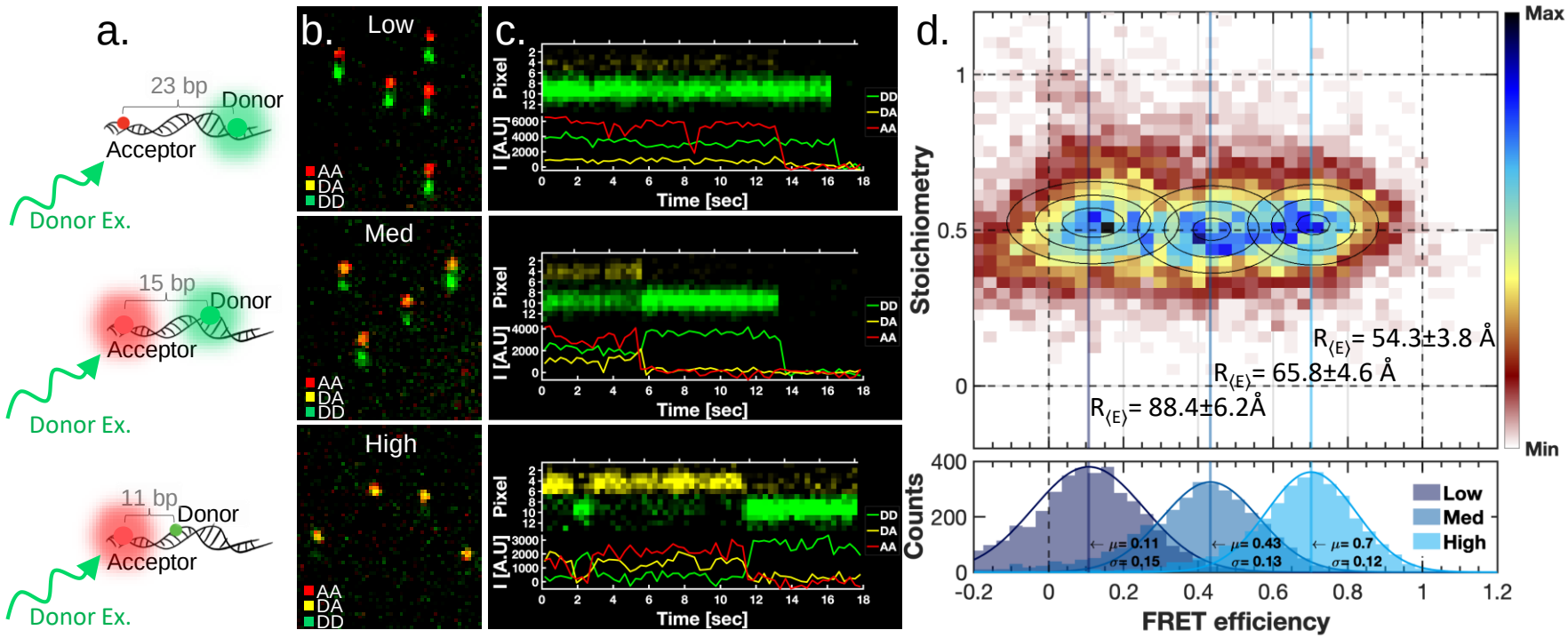

a.
180° (no dispersion)
Localization image
±30° prism rotation
120° (optimal dispersion)
Spectral image
ntensity [A.U]
b.
Overlaid localization and spectral FOV
Classified localization FOV
c.
Normalized intensity
CF640R
AF647
Cy5.5
d.
662 668
696
CF640R- Mean correlation factor: 0.99
AF647- Mean correlation factor: 0.98
Cy5.5- Mean correlation factor: 0.96
Normalized intensity
Wavelength [nm]

Future application:
6 targets with **a single excitation**



- ✓ <6nm spectral-resolution – full spectral characterization
- ✓ Reduced phototoxicity for live samples
- ✓ Reduced optical complexity and costs (single excitation, no chromatic aberrations)

Application 3:
Measuring nm distances with smFRET



- ✓ Single molecule sensitivity in Epi-illumination (no TIRF)
- ✓ >6-fold increase in throughput over standard dual-color smFRET
- ✓ >4-color smFRET with ultra-high throughput

In collaboration with the Craggs lab

Thanks!

Cold Spring Harbor Laboratory

bioRχiv

THE PREPRINT SERVER FOR BIOLOGY

New Results

Comment on this paper

Multi-modal Single-molecule Imaging with Continuously Controlled Spectral-resolution (CoCoS) Microscopy

Jonathan Jeffet, Yael Michaeli, Dmitry Torchinsky, Ifat Israel-Elgali, Noam Shomron, Timothy D. Craggs, Yuval Ebenstein

doi: https://doi.org/10.1101/2020.10.13.330910

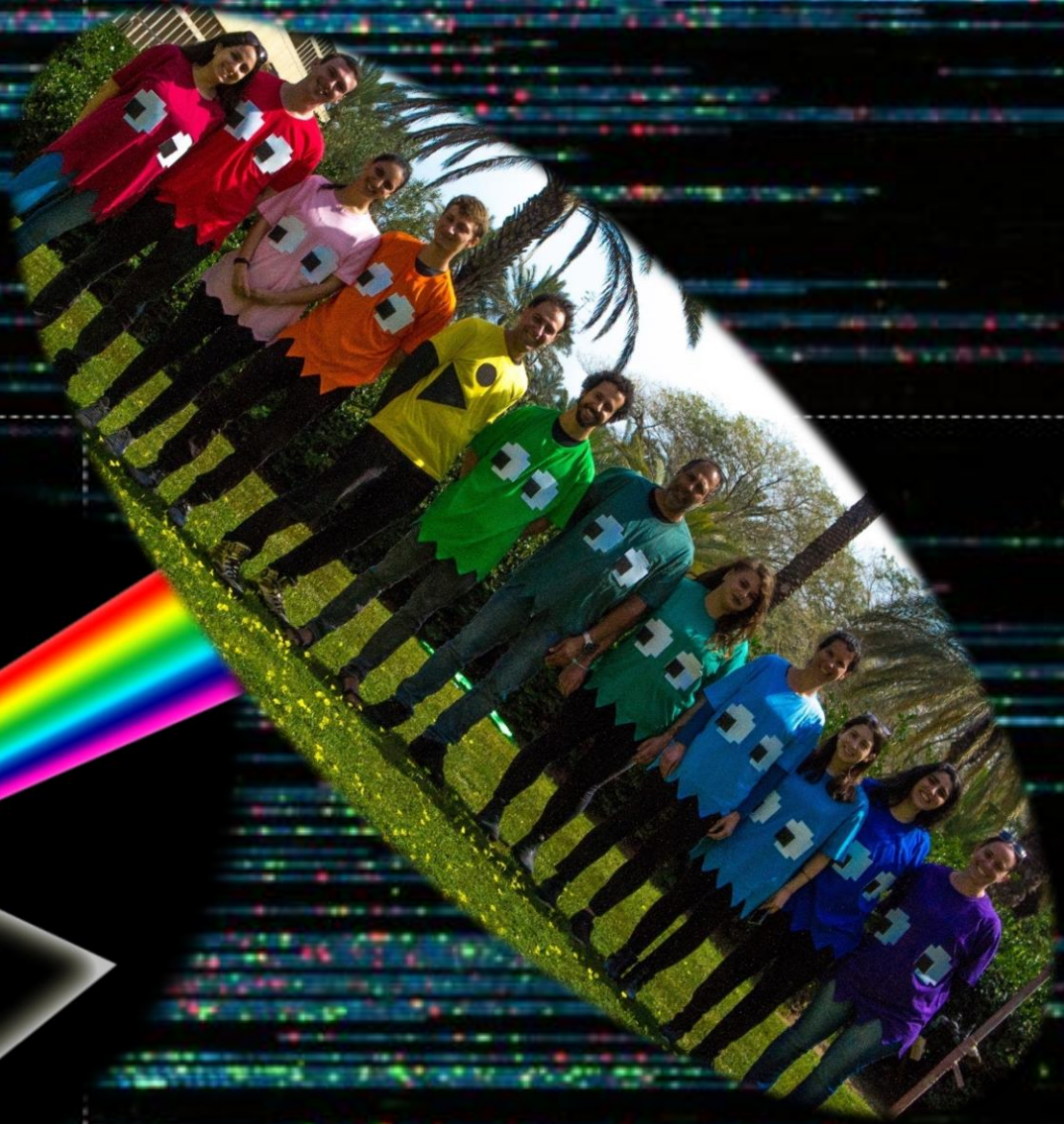
אוניברסיטת תל אביב

NanoBioPhotonixLab

קרן עזריאלי Azrieli Foundation

תכנית עמיתי עזריאלי Azrieli Fellows Program

Thanks!



אוניברסיטת תל אביב

NanoBioPhotonixLab

קרן עזריאלי
Azrieli Foundation
תכנית עמיתי עזריאלי
Azrieli Fellows Program

VL **Veronica Labrador** 5 minutes ago
How would this technique work in a high density fluorophore situation?

DD **Denitza Denkova** 5 minutes ago
Is there any limitation on how sparse/dense your sample labelling can be?

JC **Julien Colombelli** 5 minutes ago
In SMLM to Cocos combination, how is the maximum density of single molecules affected compared to a normal SMLM experiment: should it be n (?) times lower in order to not suffer overlap ?

ST **Sebastien Tosi** 4 minutes ago
Does the order of detection of the labels matters for barcode classfication? If so, how do you deal with barcode orientation?

JC **Julien Colombelli** 4 minutes ago
With aligned prisms for no dispersion, does the PSF suufer and downgrades the SMLM localization precision ?