Super-resolution microscopy using DNA-PAINT

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Introduction

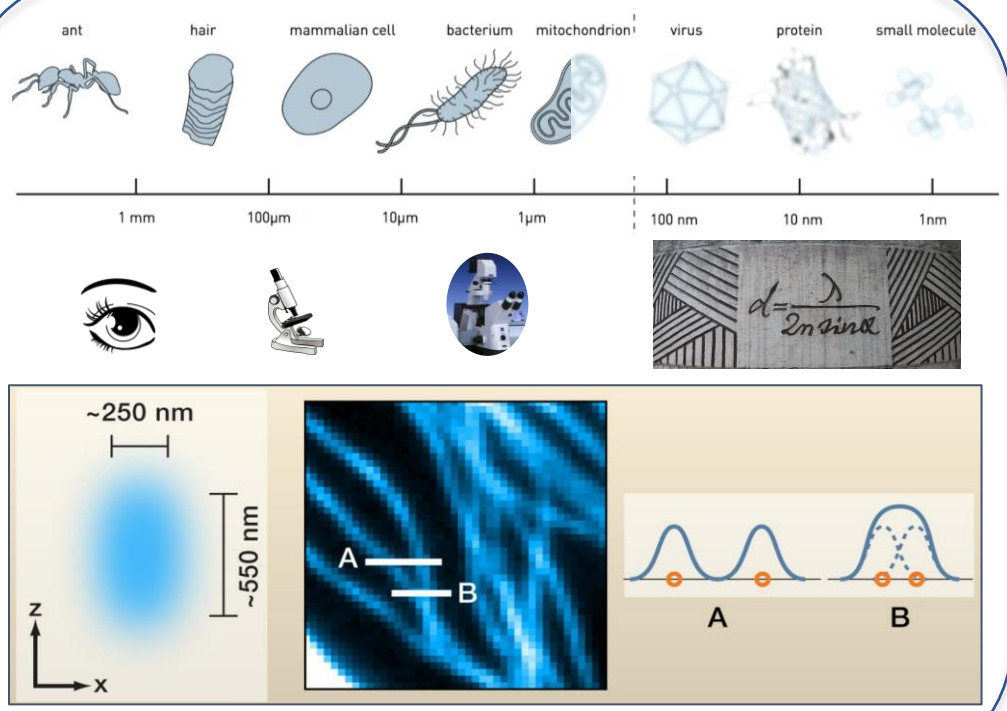


Fig: limitation offered by diffraction barrier

- Super-resolution imaging overcomes the diffraction barrier. They are mainly-

- Patterned Illumination based : STED, SSIM
- Single Molecule Localization based (Blinking based techniques) : PALM, STORM, PAINT

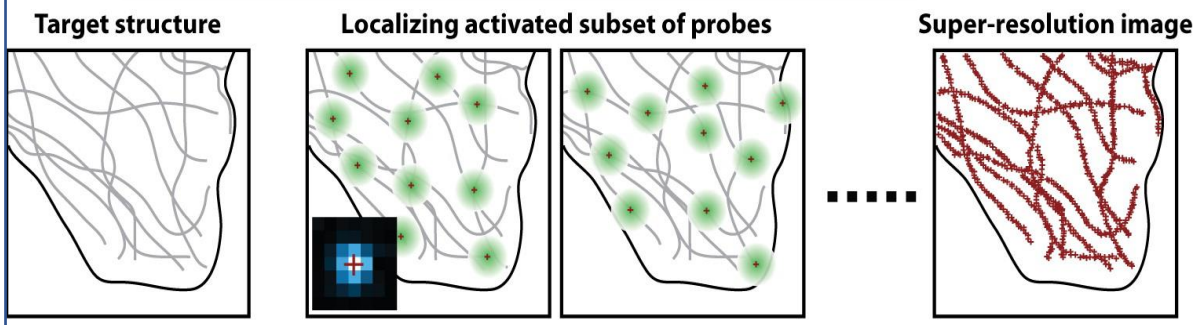


Fig: SMLM based super-resolution imaging of cellular targets

What is PAINT?

- PAINT stands for Point Accumulation in Nanoscale Topography
- Unlike the other SMLM method, PAINT exploits transient interactions between molecules for super-resolution

Why DNA-PAINT?

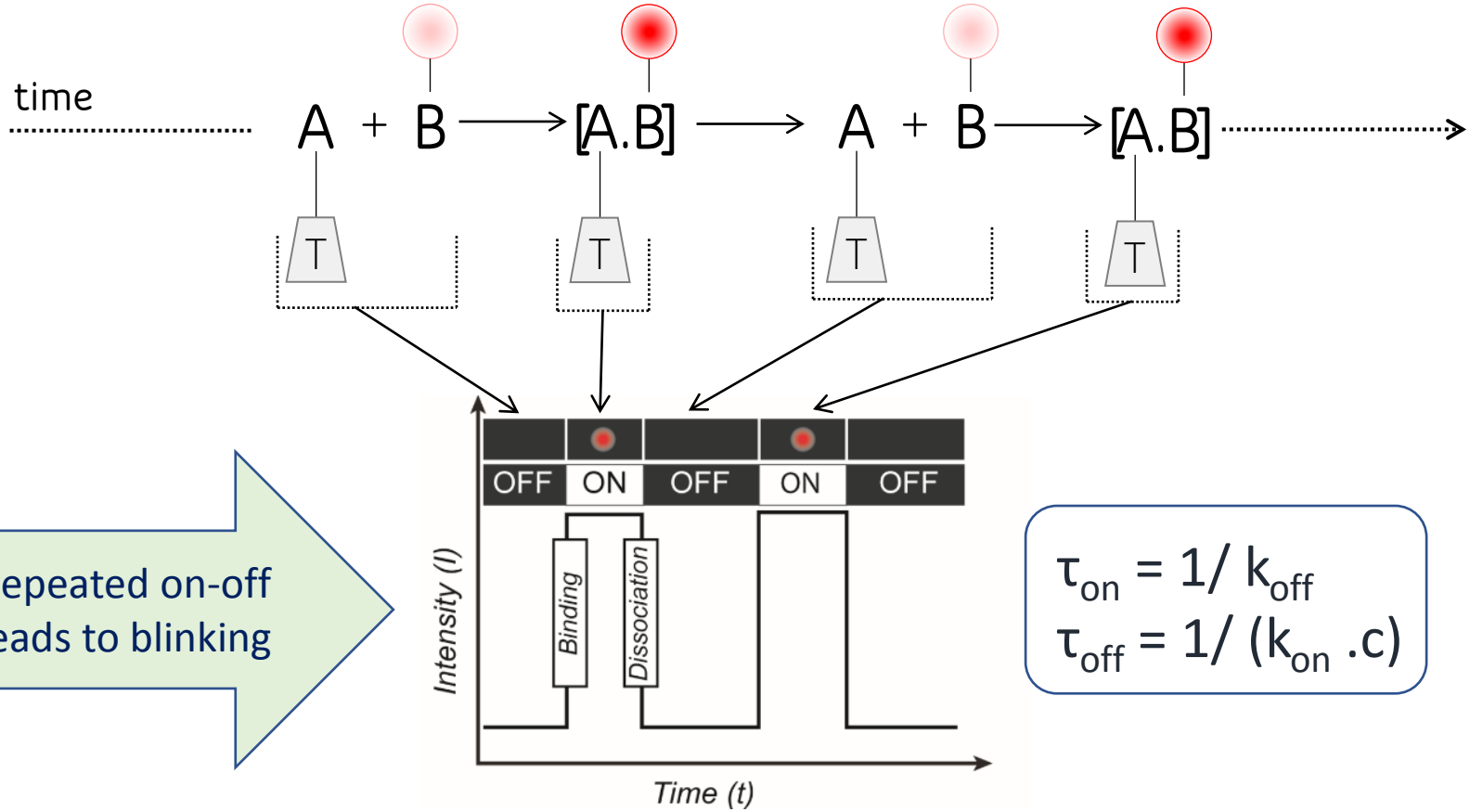
- Theoretically high number of multiplexing
- Immune to photobleaching
- Tuneable blinking kinetics

Huang B, Babcock H, Zhuang X. Cell. **(2010)** 143:1047–58
Hochstrasser et al, PNAS **2006,**103 (50), 18911-18916

Dynamic interaction of molecules for PAINT imaging

Increase in association constant, K_a

$A + B \rightleftharpoons [A.B]$ $\quad$ $A + B \rightleftharpoons [A.B]$ $\quad$ $A + B \rightleftharpoons [A.B]$



Repeated on-off leads to blinking

$\tau_{on} = 1/ k_{off}$

$\tau_{off} = 1/ (k_{on} .c)$

Agasti, S. et al, Chem.Comm., 2019, **55**, 14430-14433

Interaction between ssDNAs

For PAINT imaging-

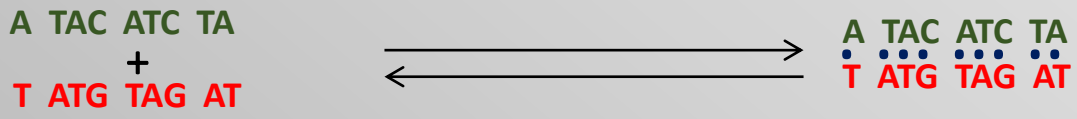
- Molecules should have tuneable association constant (K_a)
- Orthogonal interaction for multiplexing

DNA-based tags fulfil both these requirement-

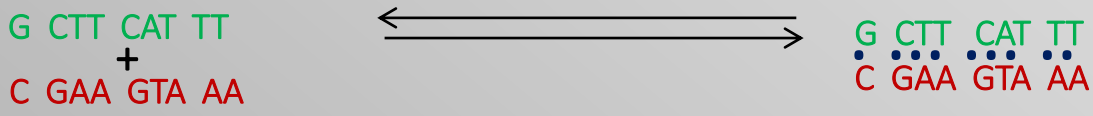
- Their interactions are programmable
- Non-Complementary strands provide orthogonal interaction

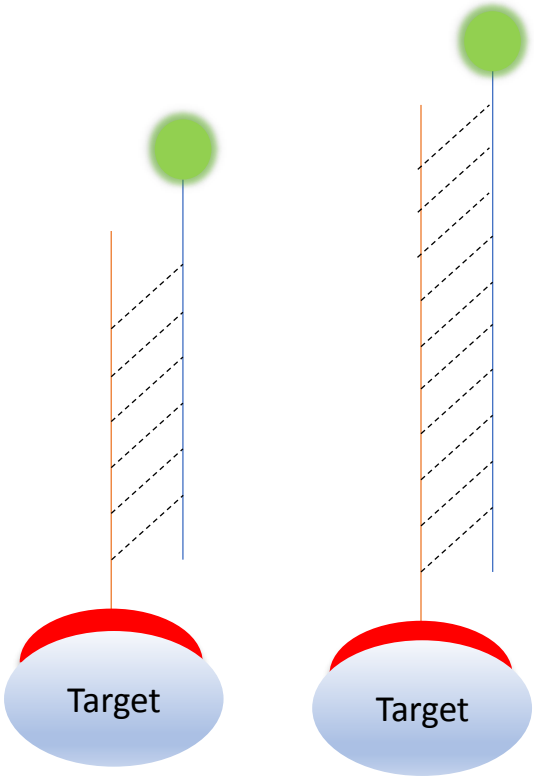
Easy to tune the binding interaction:

- ❑ By changing oligo length
- ❑ By programming the base sequence



Easy to design orthogonal sequence





Binding time increases as oligo length increases

Yin, P. et al., Nat Commun **8,** 2090 (2017)

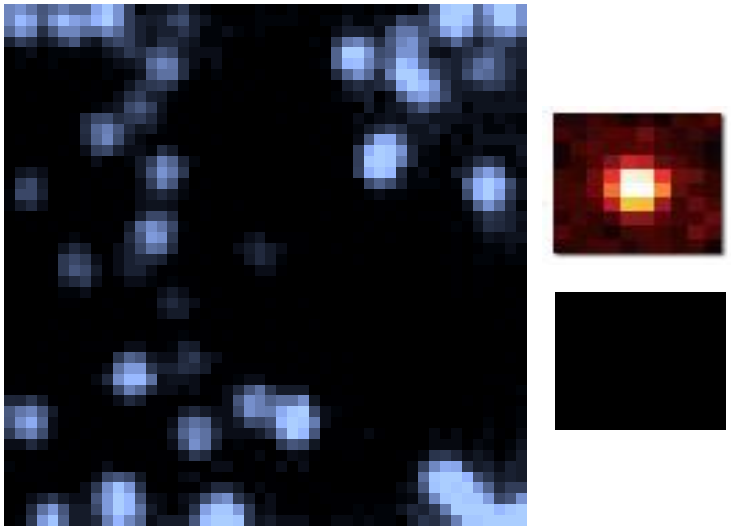
How does DNA-PAINT work?

DNA-PAINT depends on the transient hybridization and dissociation of two oligo strands.

A ssDNA strand is fixed on the cellular target and is called as docking strand

The other fluorophore labelled strand is called imager strand.

Transient binding event leads to fluorescence blinking, and it gives super-resolved images.



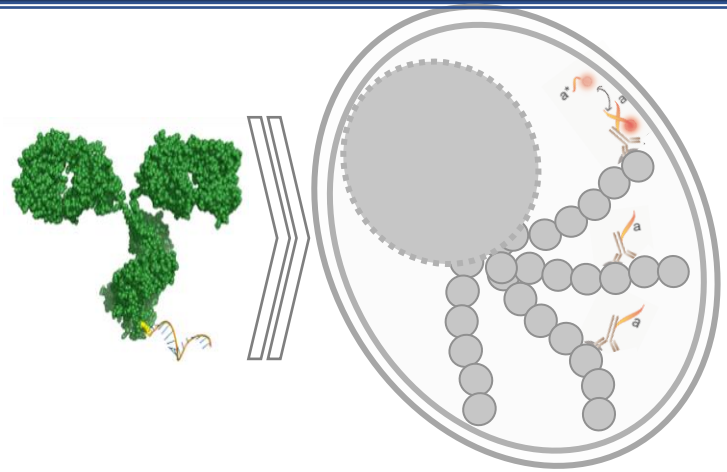


Fig: Immunostaining of cellular proteins

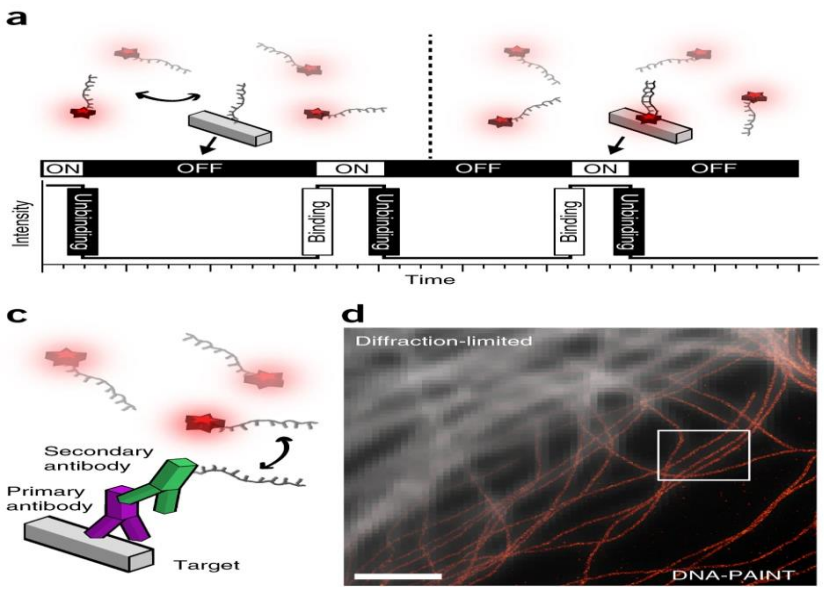


Fig: Pictorial depiction of DNA-PAINT working mechanism

Jungman, R. et al., Nano Lett. **10**, 4756–4761 **(2010) ,** *Nat Protoc,* **12**, 11982–128 **(2017)**

Image acquisition by DNA-PAINT

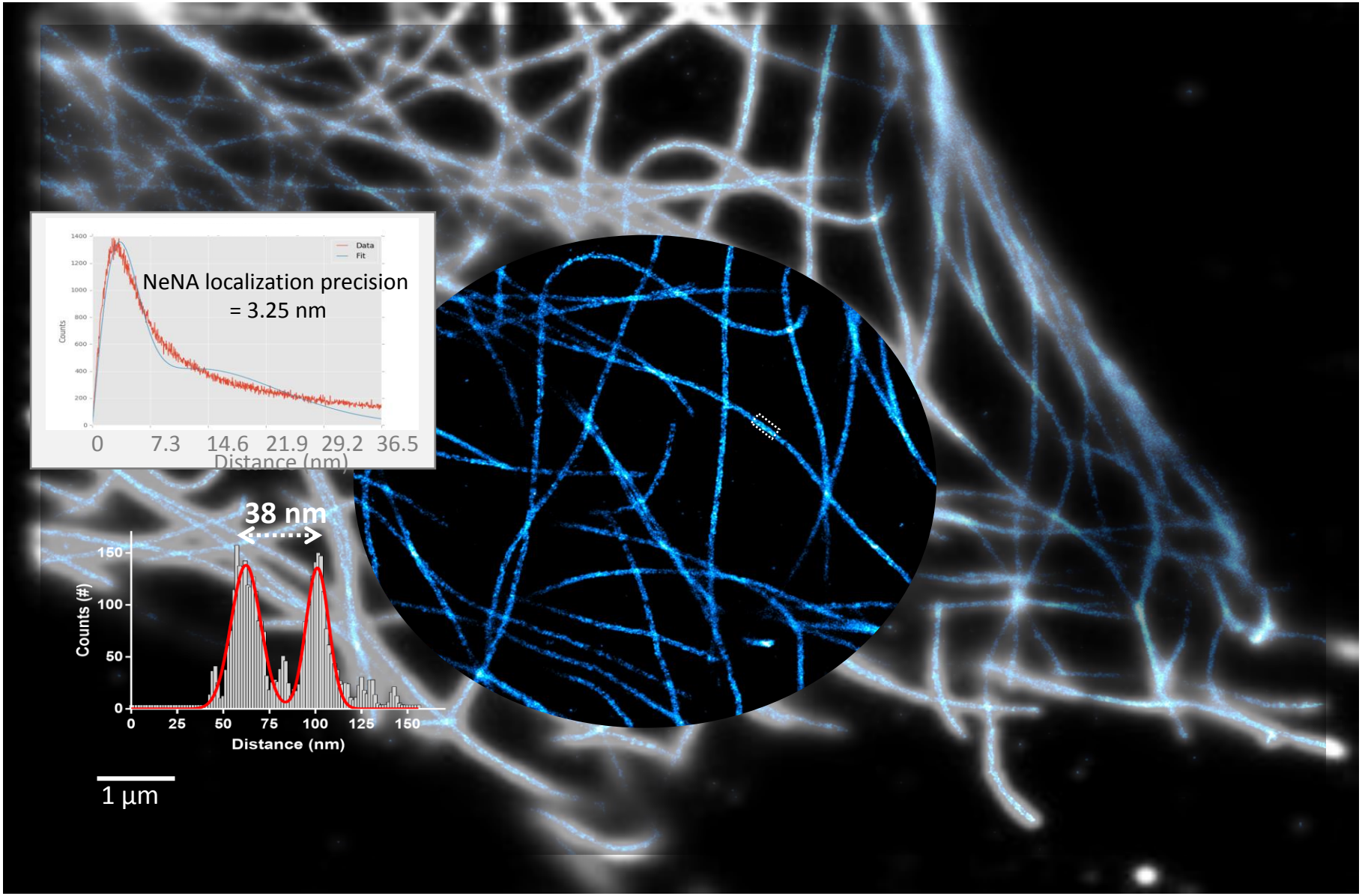


Fig: Super-resolution image of microtubule

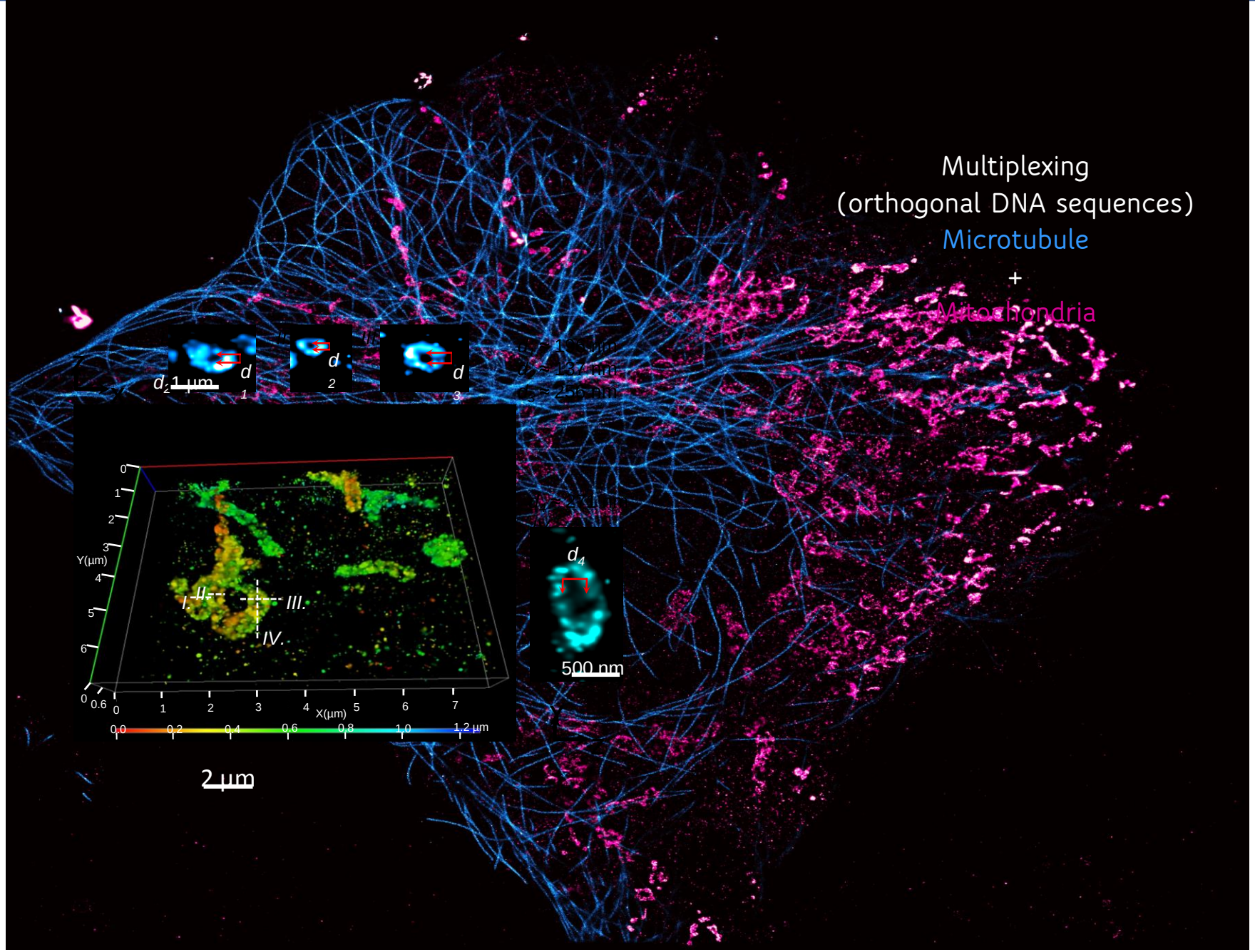


Fig: 3D multiplexed imaging of microtubule and mitochondria

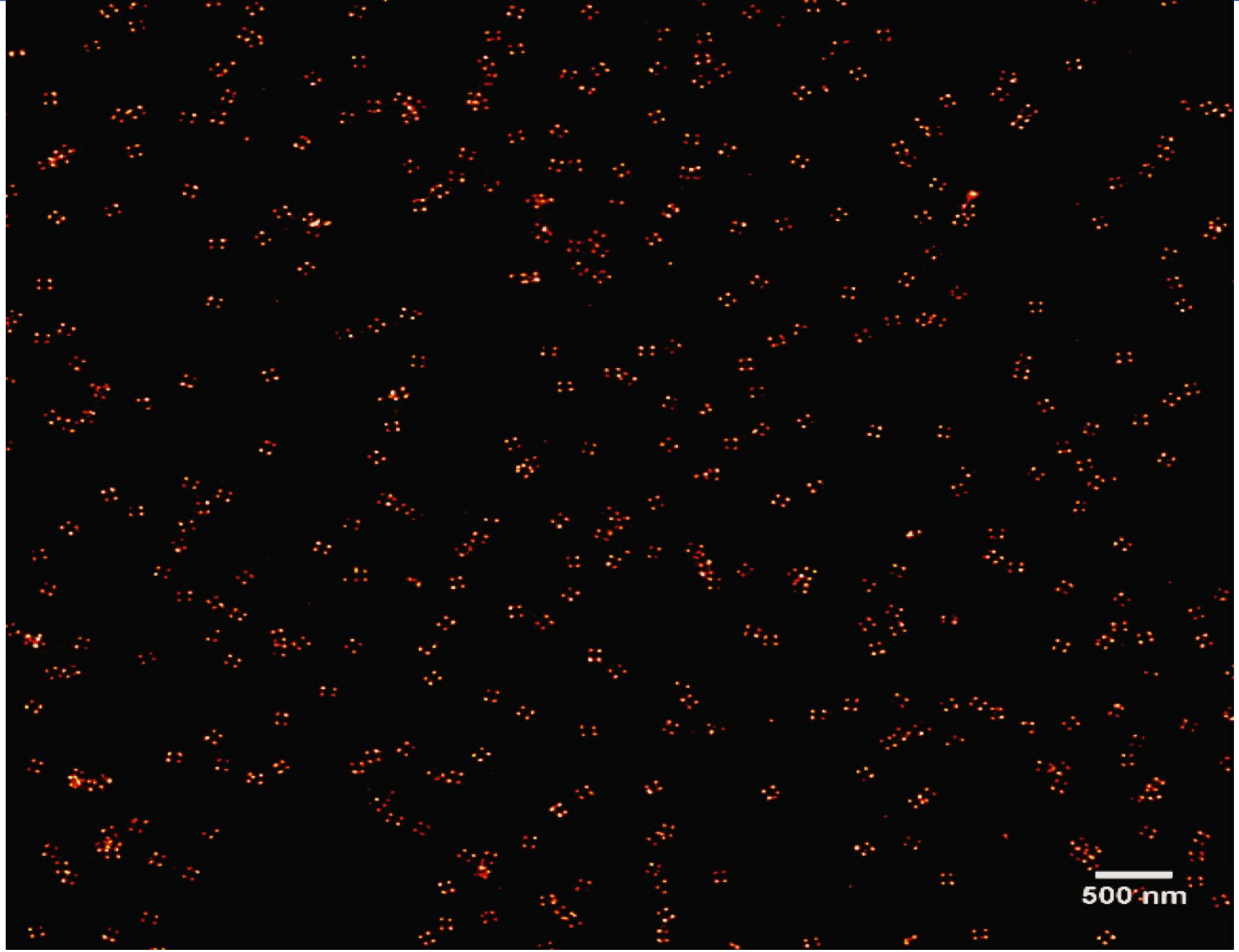


Fig: Super –resolution image of DNA-origami nanostructure

Conclusion

- DNA-PAINT has emerged as a powerful tool in recent years for biomedical research as it offers several benefits over other SMLM techniques
- DNA-PAINT have limitations also, such as live-cell incompatibility, high background at higher imager concentration and above all slower-image acquisition speed.
- This field still has a lot of space for improvement and has become a topic of research in various scientific community.

Acknowledgement

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- Agasti lab members