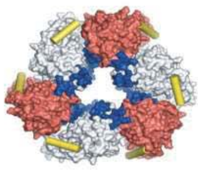
Nanomanipulator na osnovi TIRF in optične pincete

Ajasja Ljubetič

Research Assistant Prof., Department of Synthetic Biology and Immunology, National Institute of Chemistry, Slovenia

Dynamic single molecule method

Structure & composition

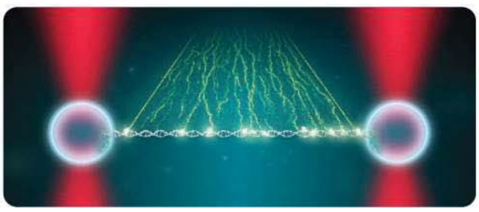


cryo-EM

Single-molecule resolution

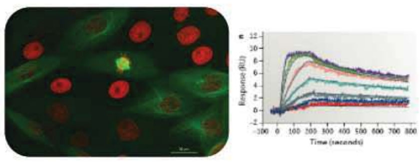
No dynamic information

Dynamic single-molecule



Dynamic single-molecule methods

Function



Live cell imaging

SPR

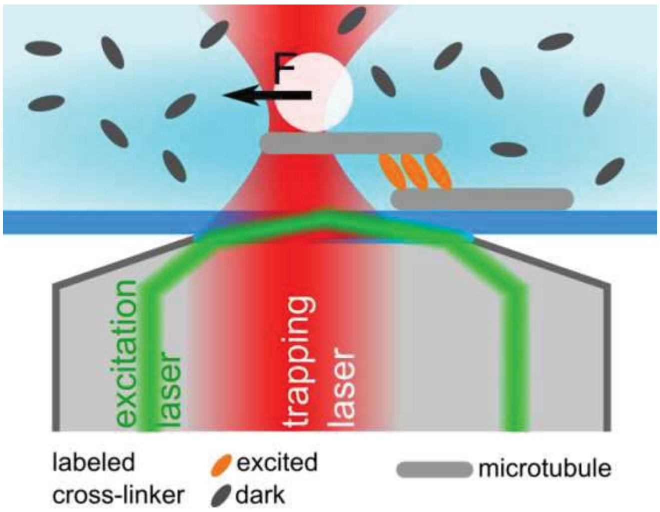
Dynamic information

Observation only

Averaged data

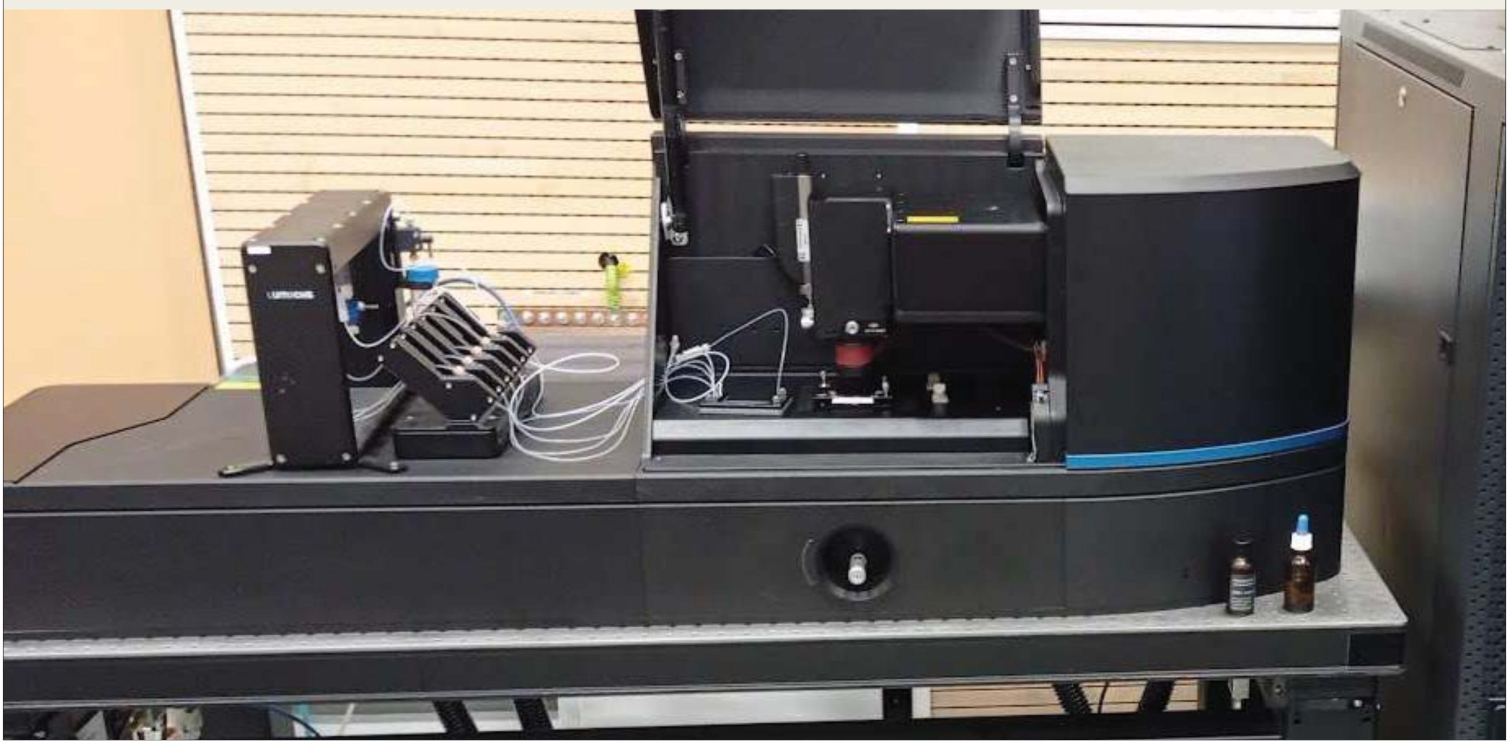
Nano-manipulator of single molecules based on TIRF microscopy and optical tweezers

- TIRF (Total internal reflection fluorescence)
    - Fluorescence method (488, 532, 638 nm)
    - Great signal to noise
    - Fast acquisition (20 fps)
    - Super resolution microscopy (<20nm)
- IRM (label free imaging)
- Brightfield
- Optical tweezer
    - Manipulation
    - Force measurement
    - Single molecule force measurement
- Microfluidics
- **Combination** opens new frontiers!



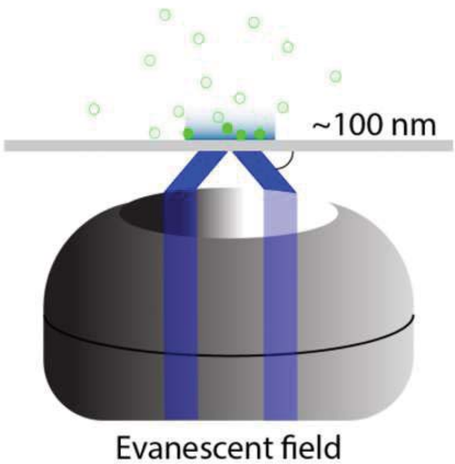
400k + two public calls needed ☺
User training in Nov 2023

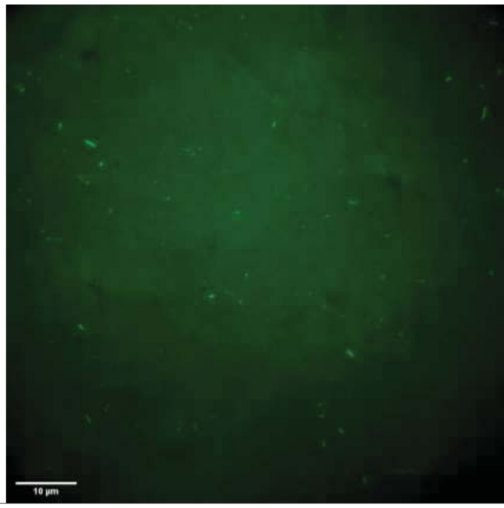
LUMICKS C-TRAP Edge 350



Total Internal Refelection Fluorescence

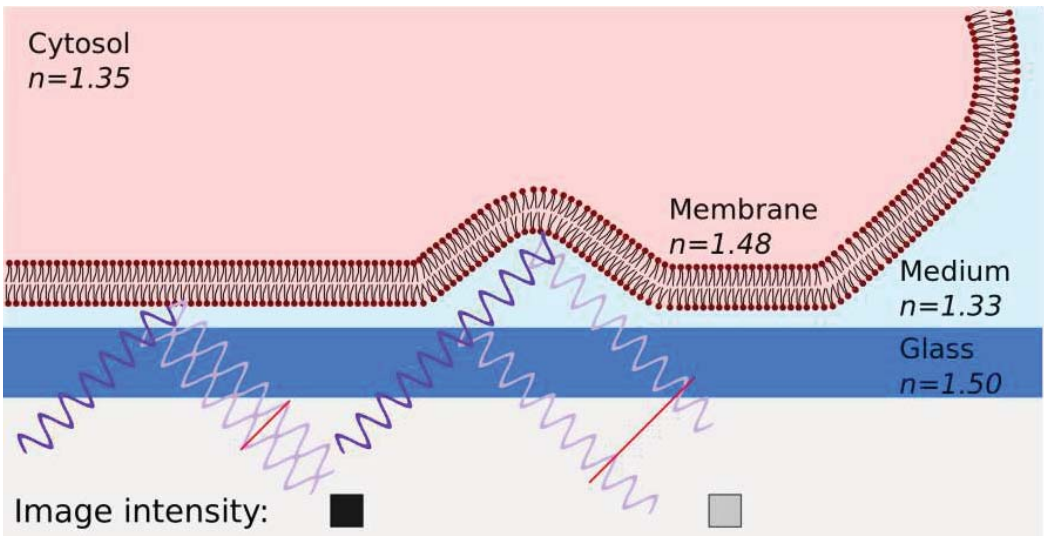
- Excites only 100-200 nm from the surface
- >10x better signal to noise
- Membrane proteins, attachment structures, cytoskeleton



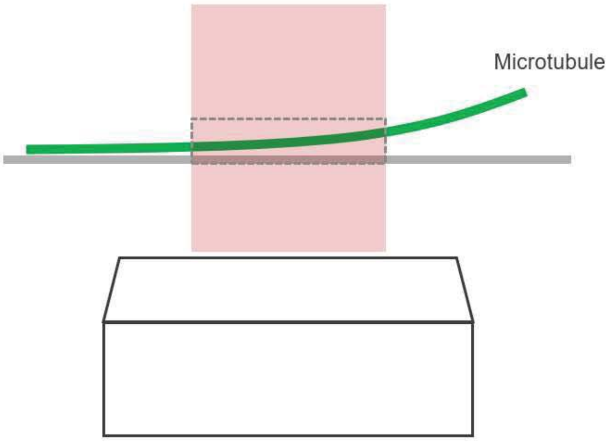


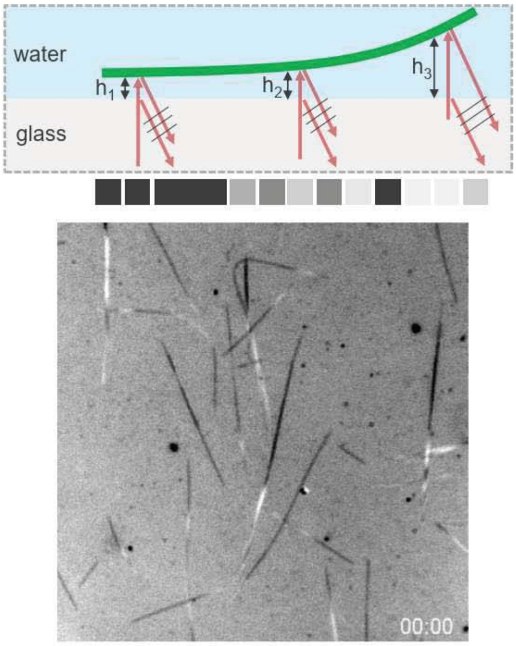
IRM: Interference reflection microscopy

- Works only near the surface
- No labels are needed
- Membranes and adhesion points
- Fibers
- Gas vesicles



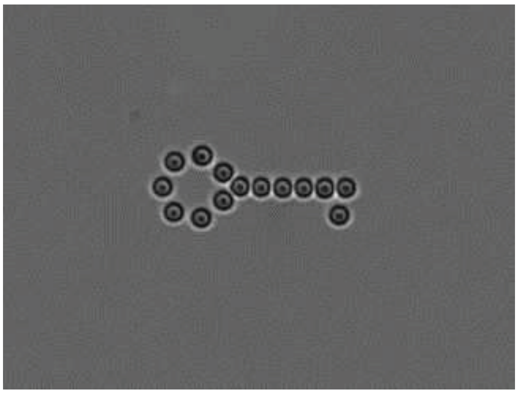
Microtubules

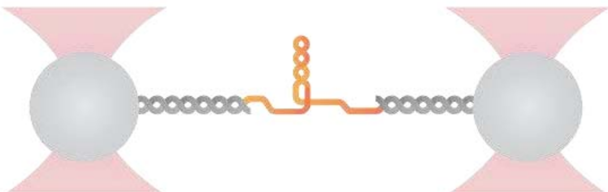




Optical tweezer

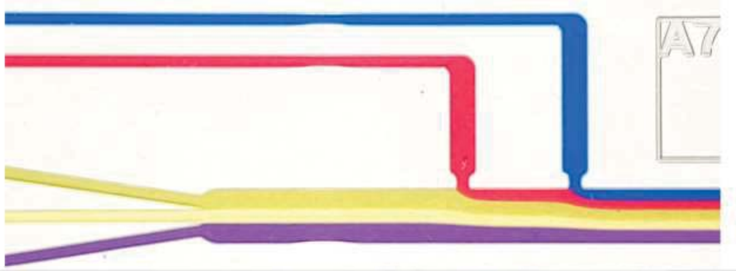
- Can grab small beads 0.8 - 3um and move them precisely.
- Uses 1065 nm laser
- Can have multiple traps at the same time (time sharing)
- We have polarization splitter (so "only" two traps)
- From displacement of where the trap is vs where it should be forces can be measured.

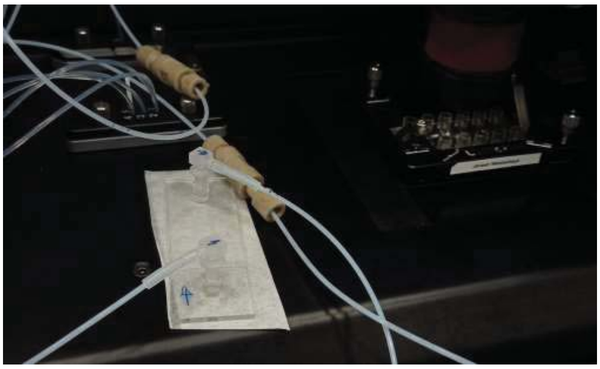


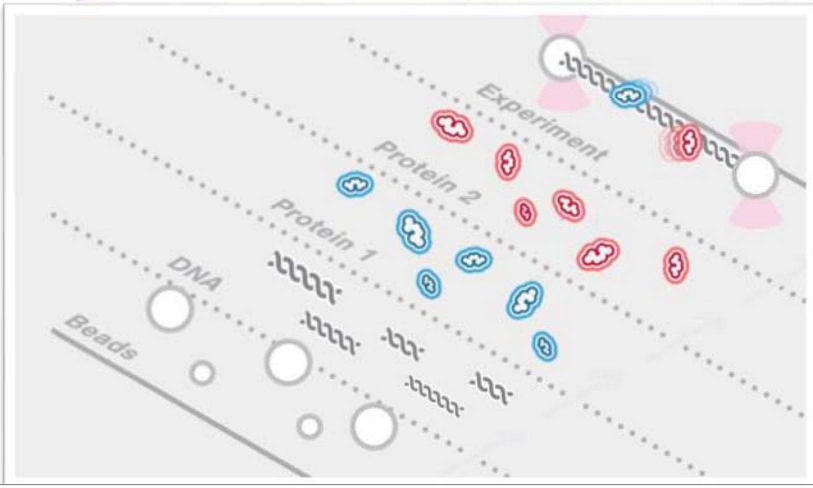


Microfluidics

- 6 channels (all at the same pressure)
- Automatic valves
- Microfluidics cell

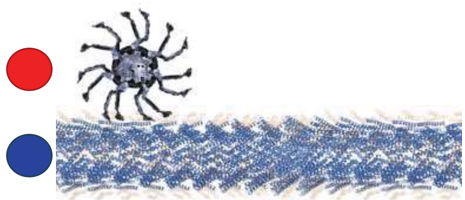


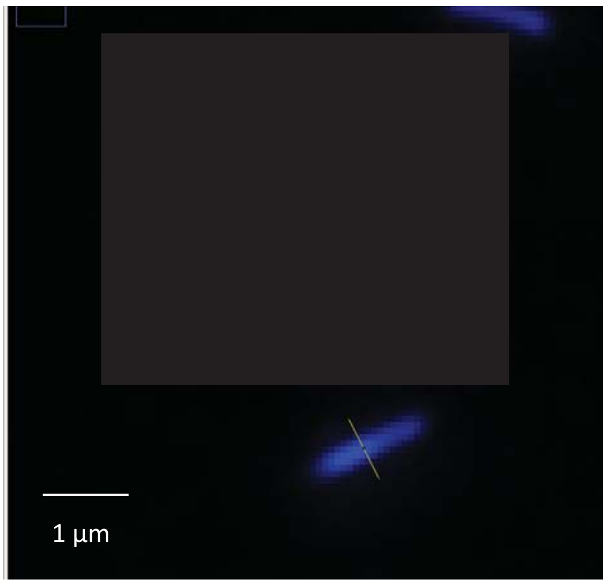


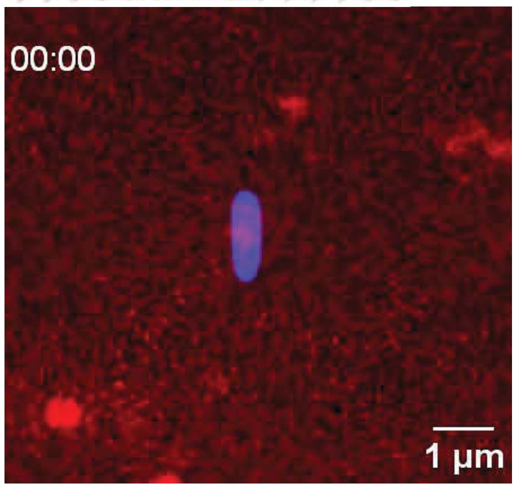


Single molecule tracking

Liza Ulčakar

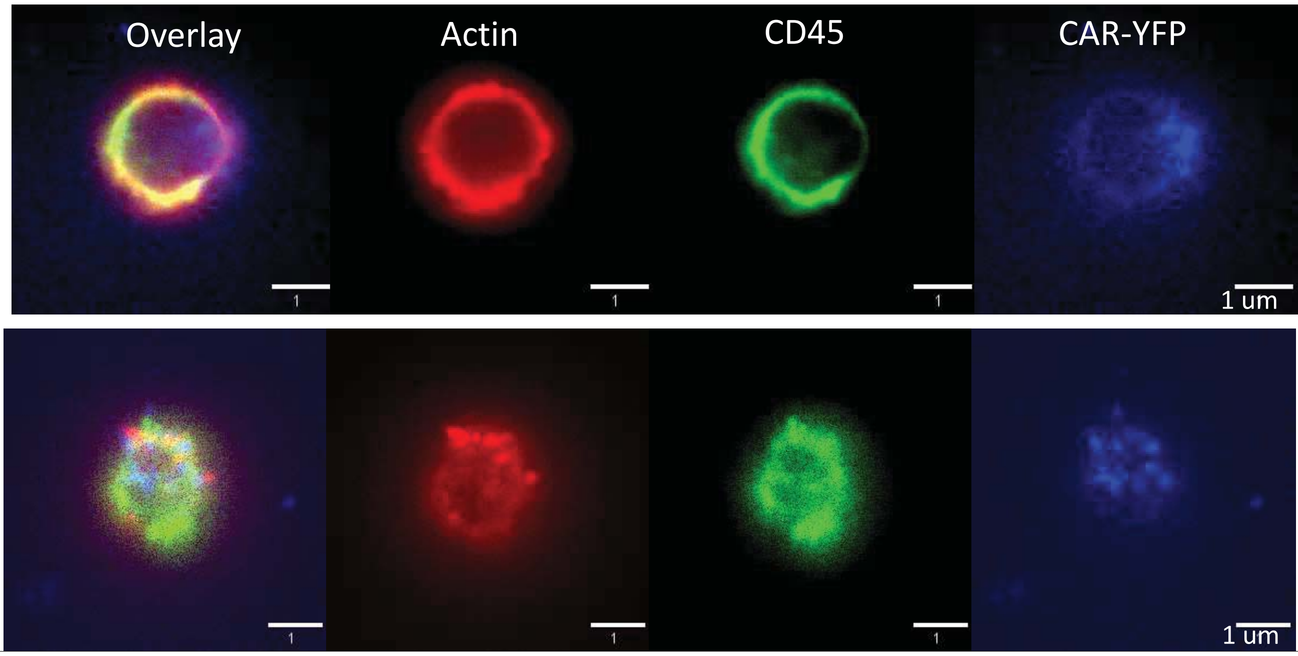




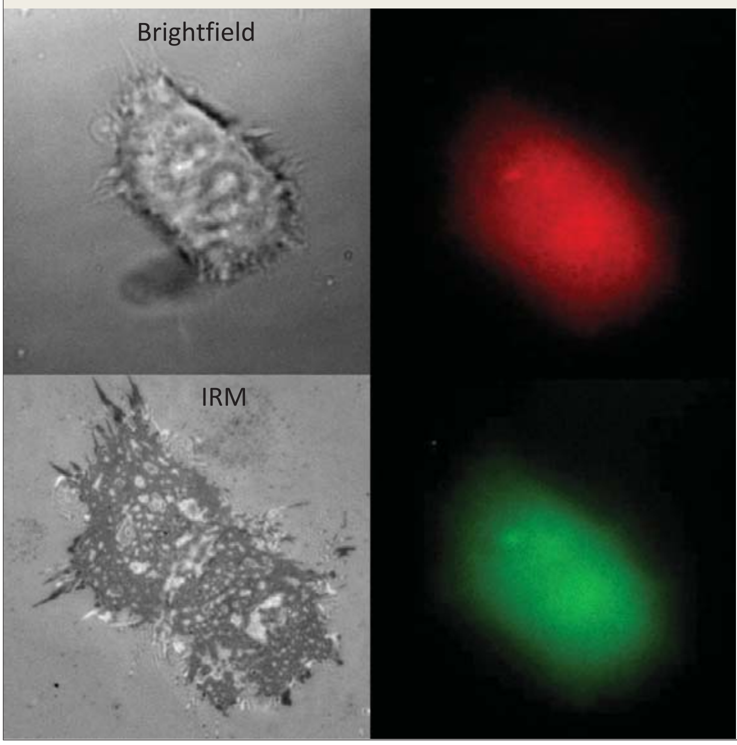


Immunological synapse

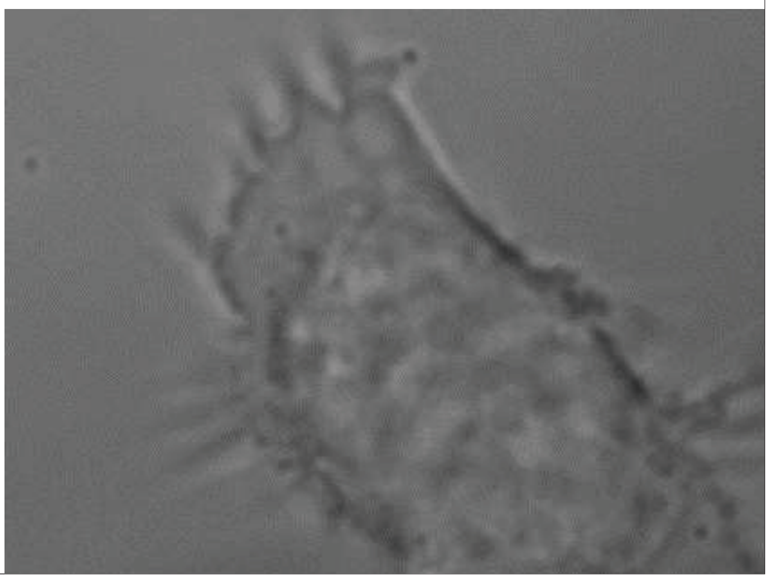
Wild type chimeric antigen receptors with YFP



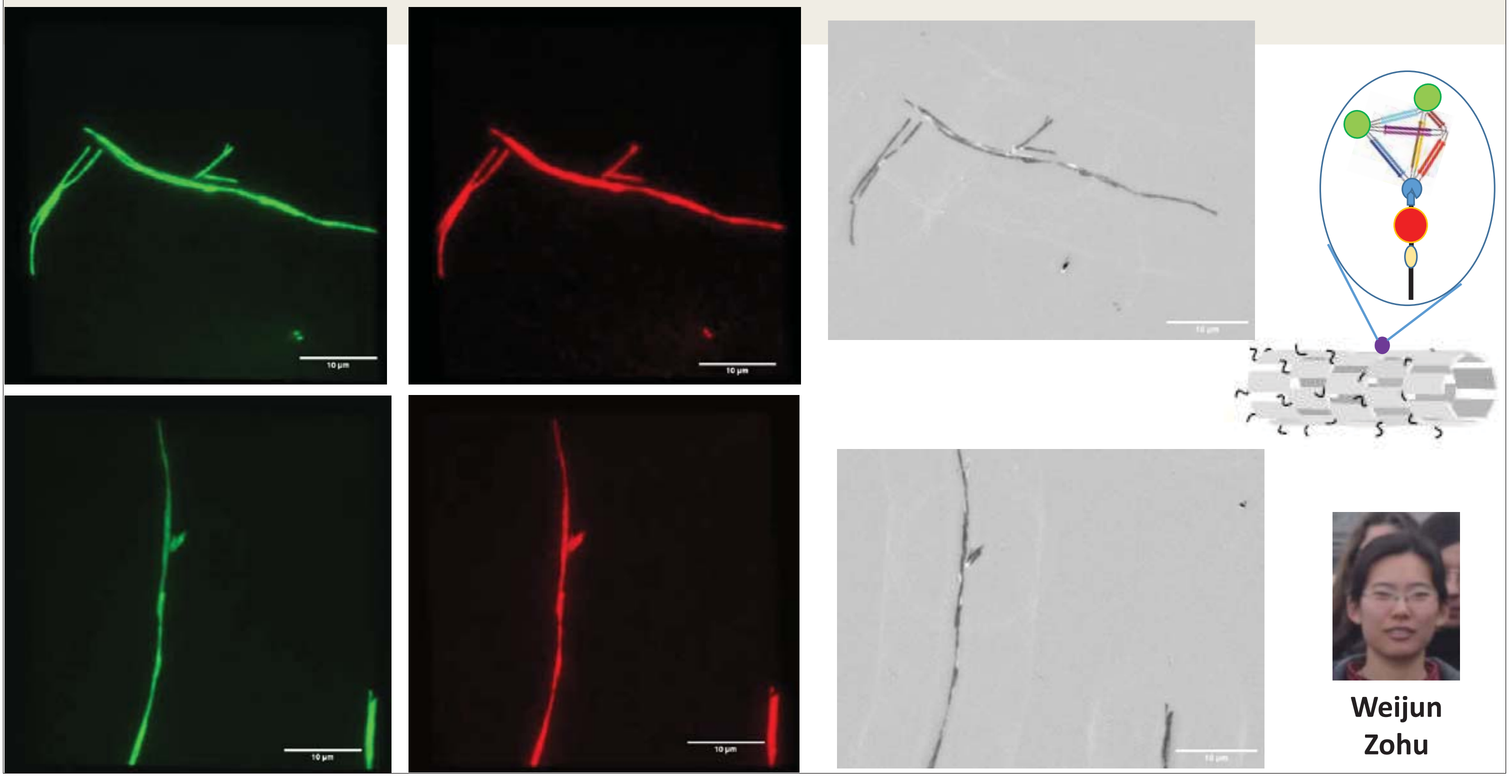
TIRF, IRM, brightfield , Tweezer



Brightfield + tweezer



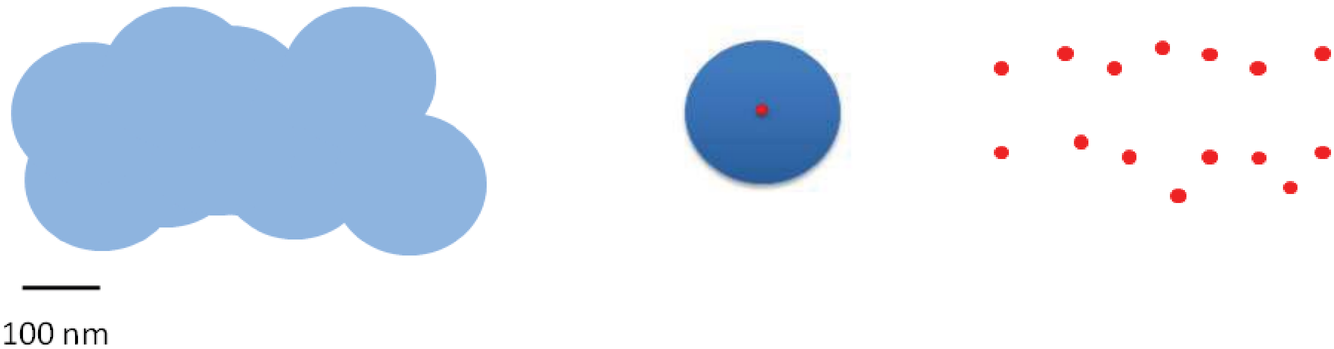
TIRF Ex: 488 nm | TIRF Ex: 532 nm | IRM



Weijun Zohu

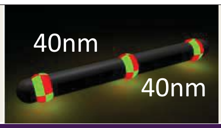
Super resolution methods!

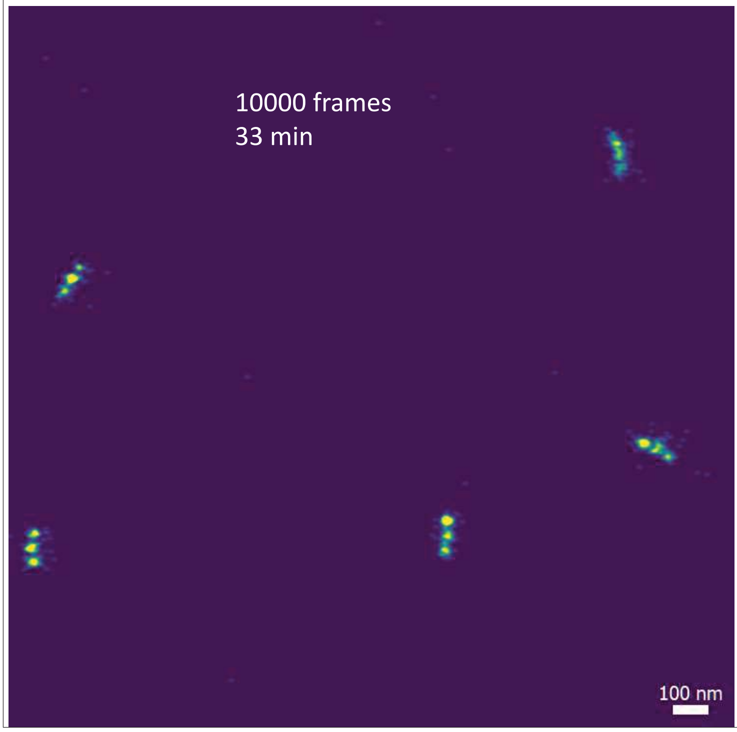
- Resolution of optical microscopy is ~300 nm
- But we can cheat!

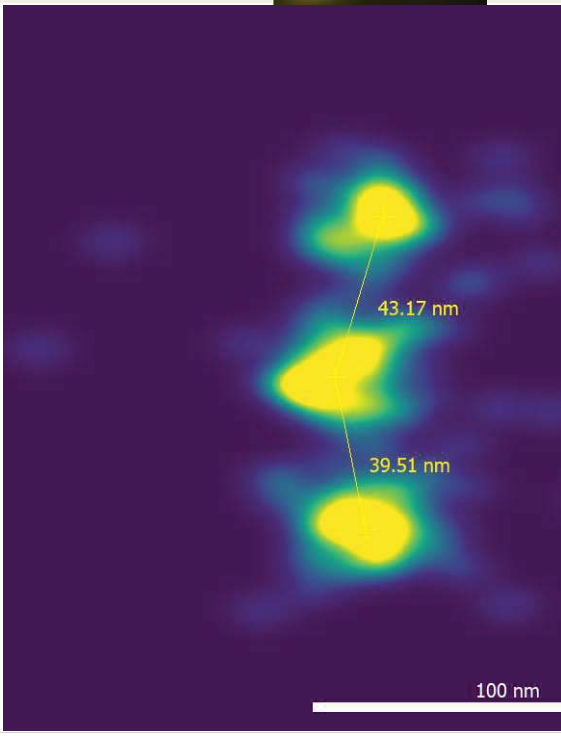


- **DNA/CC – PAINT**: Points accumulation for imaging in nanoscale topography
- 1 nm resolution is possible, **10 nm** is regularly achievable.

How close can we come to 1 nm? ☺



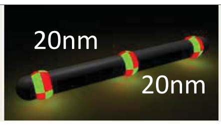


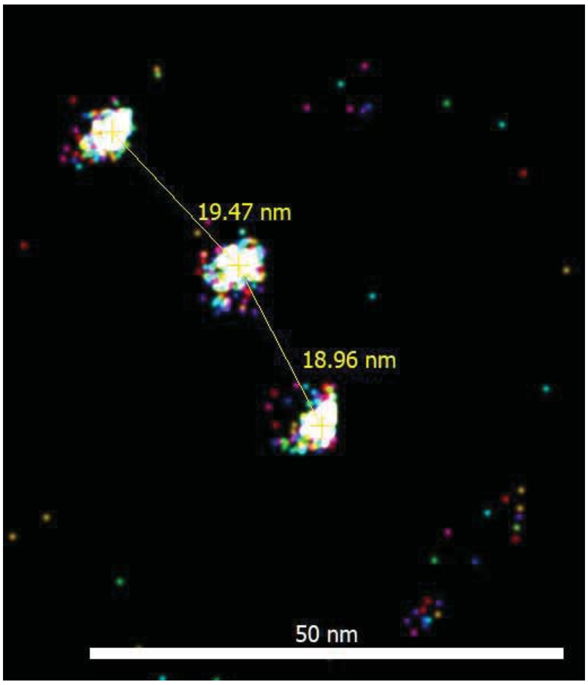


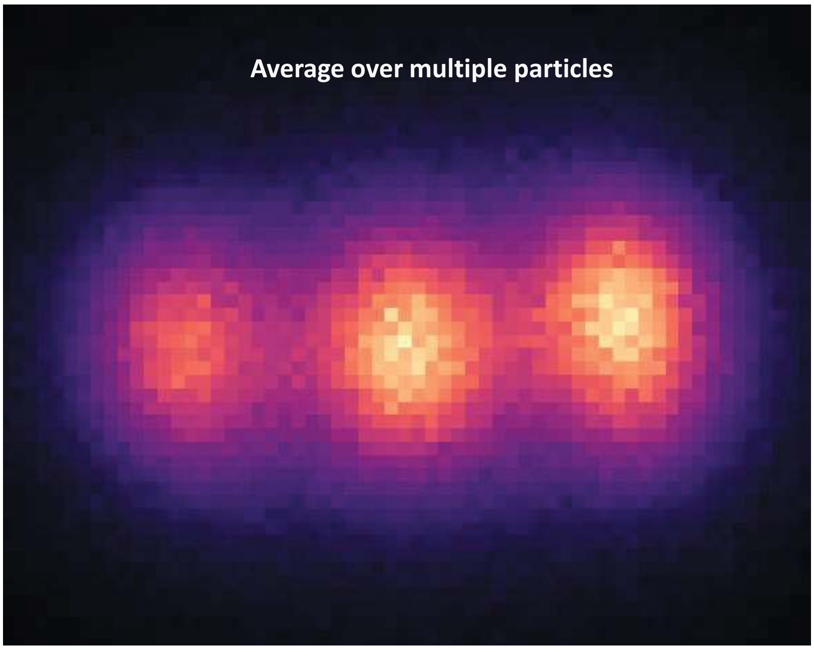

Federico Olivieri


Liza Ulčakar

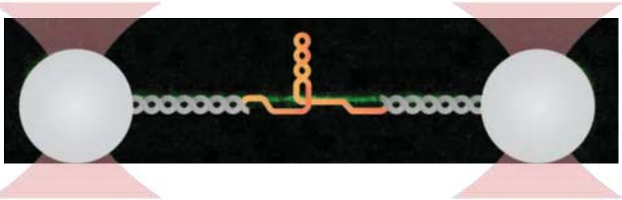
20 nm distance



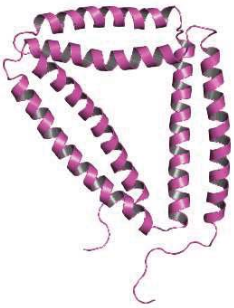


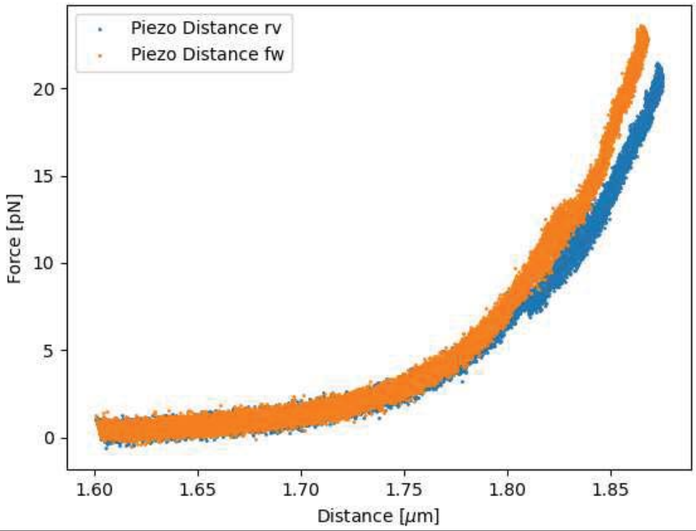


Pulling single molecules

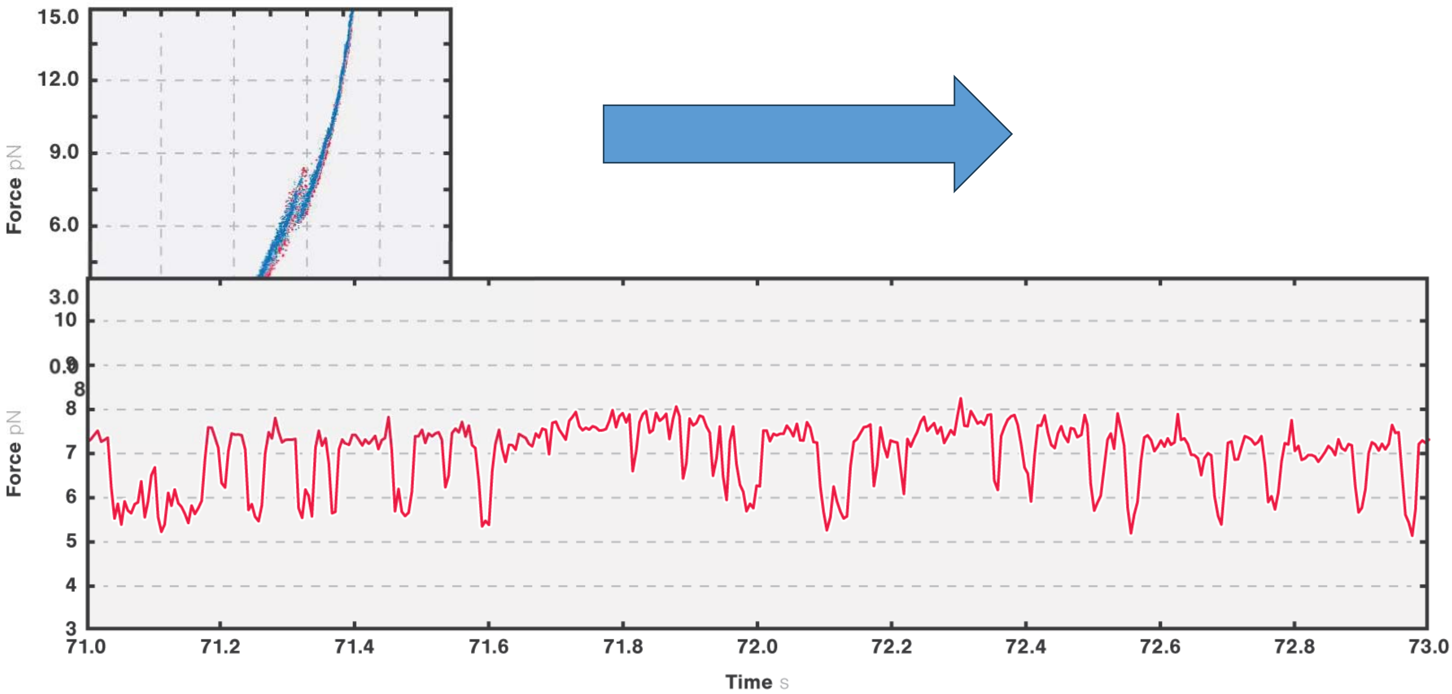


Federico Olivieri Weijun Zohu



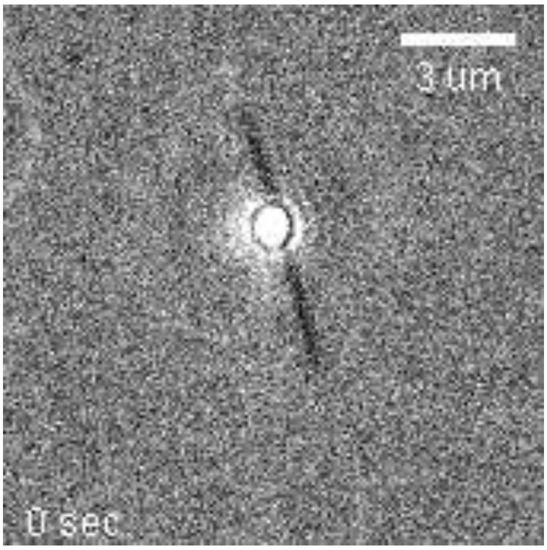


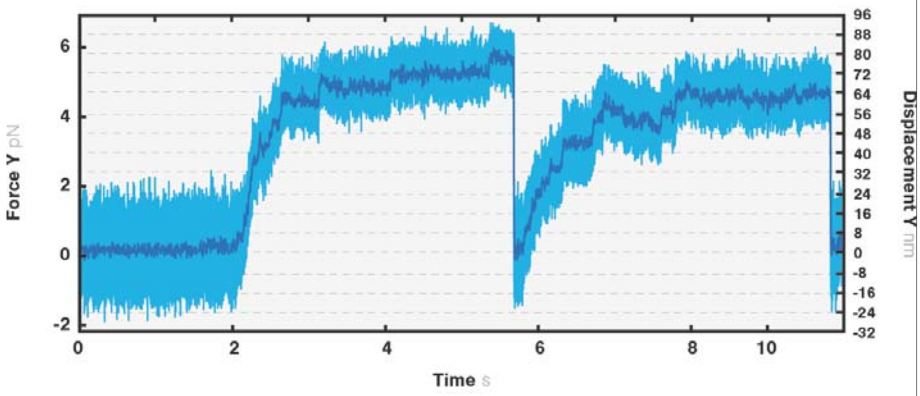
The future -> seeing descrete event

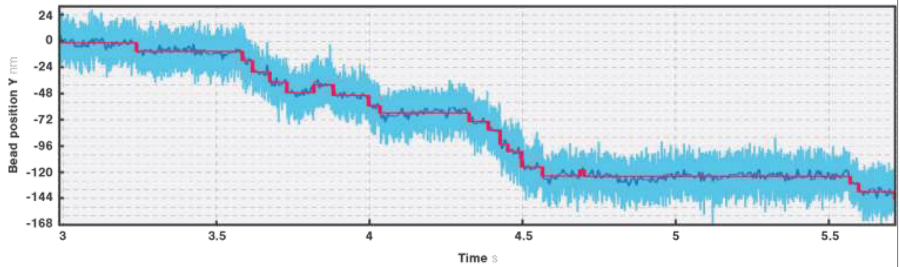


The future: forces that molecular robots make

IRM: Kinesin stepping







Conclusions

- Excellent machine that is super useful to our research!
- Only combination of TIRF+Tweezer in Slovenia!
  - Only multichannel TIRF that I know of
- Combination of different methods in the same microscope is the main advantage!