Journal Club – Optics

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SCIENCE ADVANCES | RESEARCH ARTICLE

BIOPHYSICS

Ultraprecise single-molecule localization microscopy enables in situ distance measurements in intact cells

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https://advances.sciencemag.org/content/6/16/eaay8271

Scope/Claim of the paper

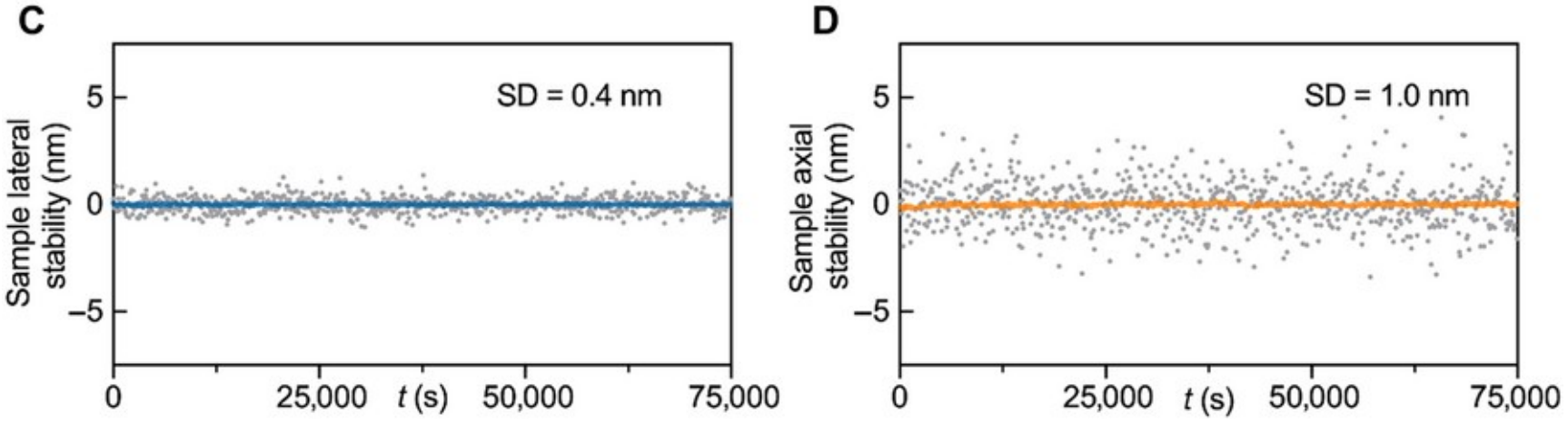
- direct distance measurements between molecules in intact cells on the scale between 1 and 20 nm
  - biological relevant length scales
- stabilization of <1 nm and localization precision of ~1 nm
  - “drift is reduced below the photon-limited localization precision and causes the fluorescent positions to be prealigned”

Intro

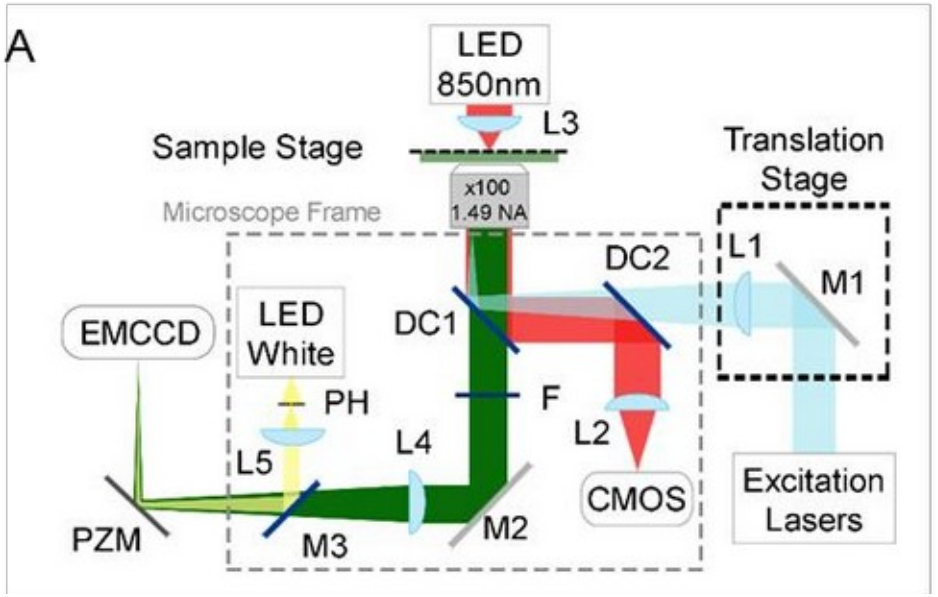
- engineering solution for SMLM, termed Feedback SMLM
  - SMLM: long duration of experiment (6h), but also short binding events (200ms)
- No post-acquisition processing including drift correction, grouping, filtering, averaging, or summation
  - Post-acquisition processing has problems with high densities of detections such as proteins in clusters with and with rare events

Method

- real-time drift correction in 3D, using three types of corrections
  - nonfluorescent fiducials are used outside the field of view of the camera for SMLM
  - autonomous optical feedback loop in the emission path
  - Reduction of the variation registered across the EMCCD

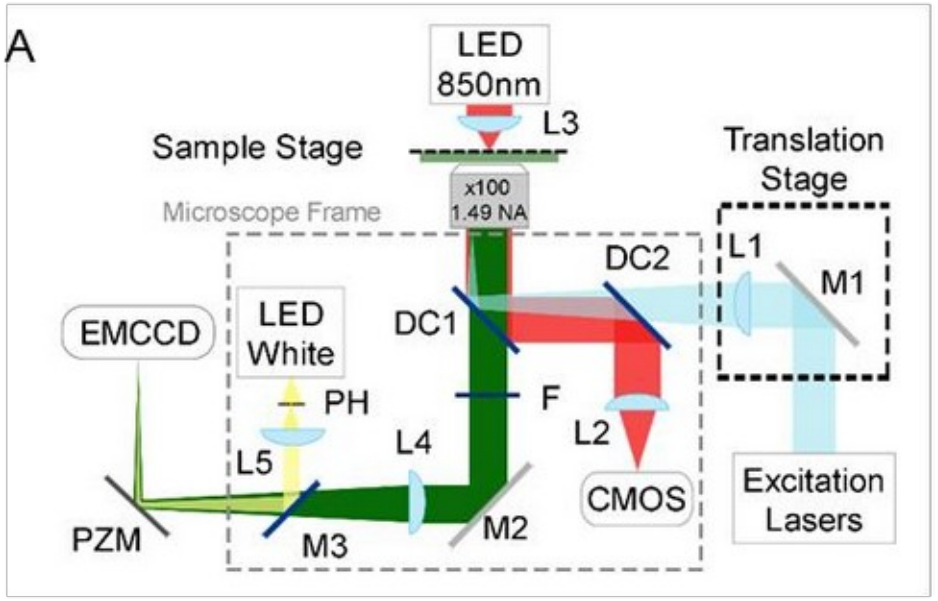


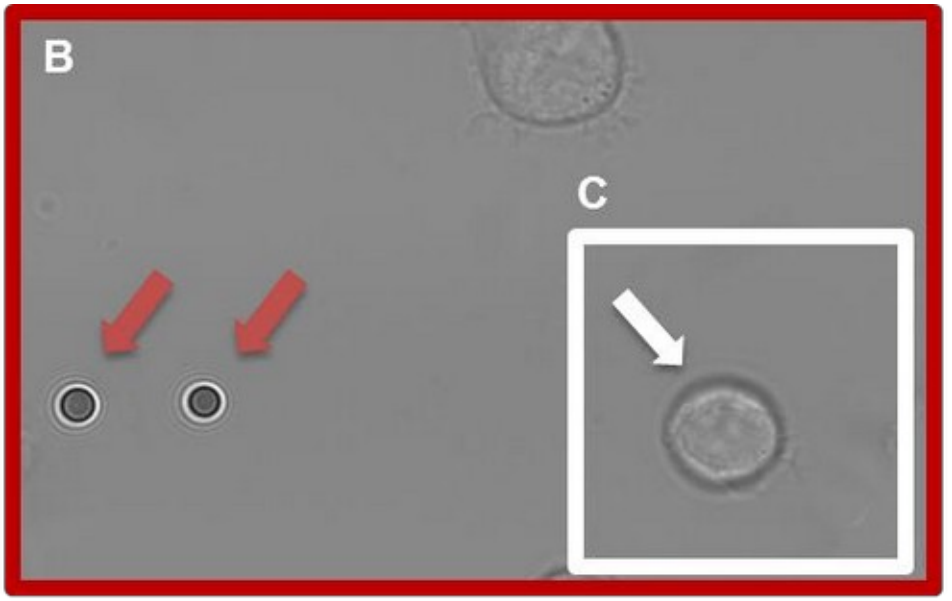
Setup

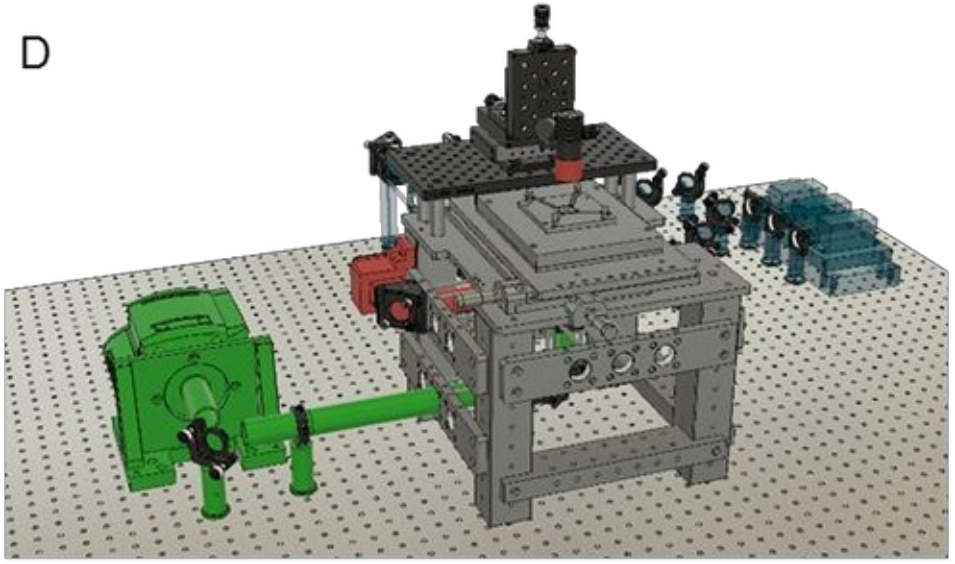

A
LED
850nm
L3
Sample Stage
Translation
Stage
Microscope Frame
x100
1.49 NA
L1
M1
DC2
EMCCD
LED
White
DC1
PH
F
L2
L4
L5
CMOS
Excitation
Lasers
PZM
M3
M2

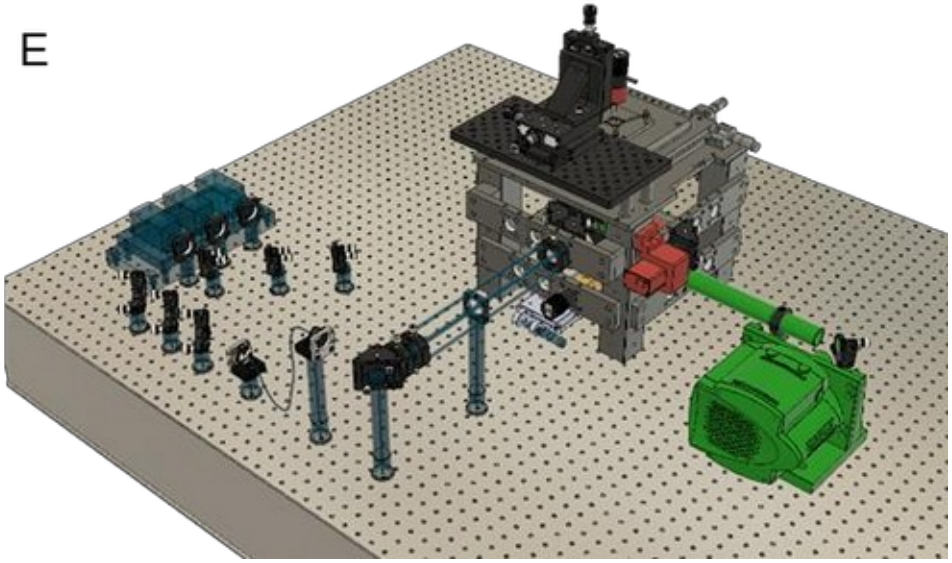
Setup

EMCCD with 40µm × 40µm FoV (pixel size, 80 × 80 nm)
100× 1.49 NA TIRF objective (Nikon, CFI Apochromat)









Fiducials

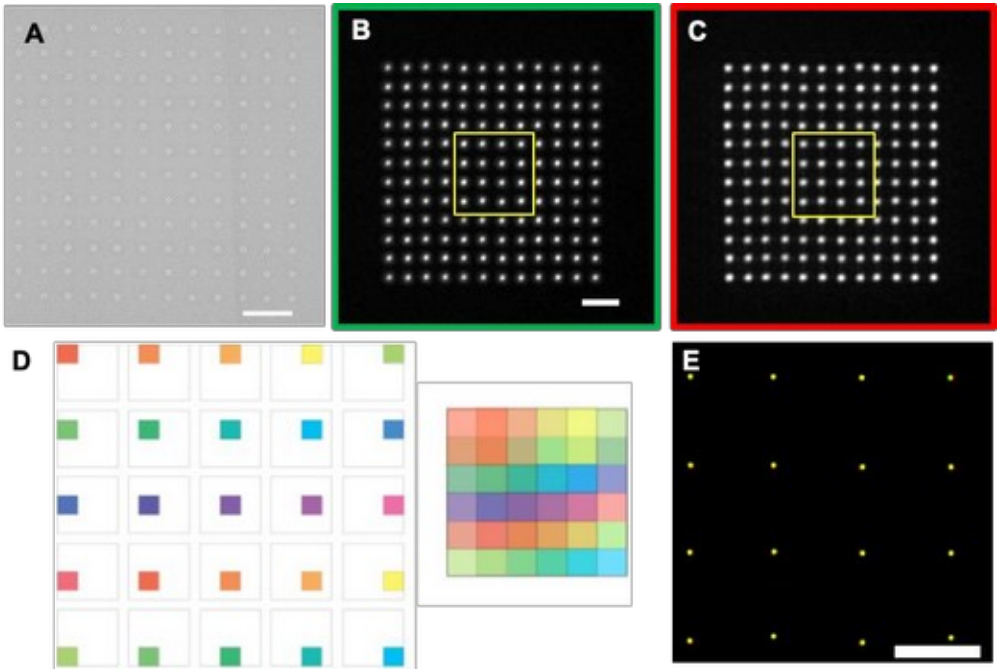
- 3µm polystyrene beads that are illuminated with an infrared LED to create diffraction rings
  - Provide information of both the x-y and z position
  - Look-up-table for the z direction
- CMOS camera (112µm × 70 µm FoV, about fivefold larger than the fluorescence camera)
- stage corrections at 15 Hz, independent of imaging
- stabilization of 0.4 and 1 nm (SD) in the lateral and axial directions

Feedback loop in the emission

- White LED, integrated in the microscope body, creates an optical fiducial on the EMCCD
  - position of LED is localized with a precision of 0.05 nm
  - piezoelectric mirror is used to account for long-term drift of the microscope (i.e., mechanical instabilities)
  - reduces the image drift to 0.22 nm
  - LED spot was positioned on the periphery of the EMCCD

EMCCD characterization and chromatic correction

- “nanohole array”
  - holes with a diameter of 100 nm nanofabricated on an aluminum-coated coverslip
  - filled with red (Alexa Fluor 647) and green (Alexa Fluor 488) dyes at ~100 µM concentration



Only chromatic aberration or also other aberrations corrected?

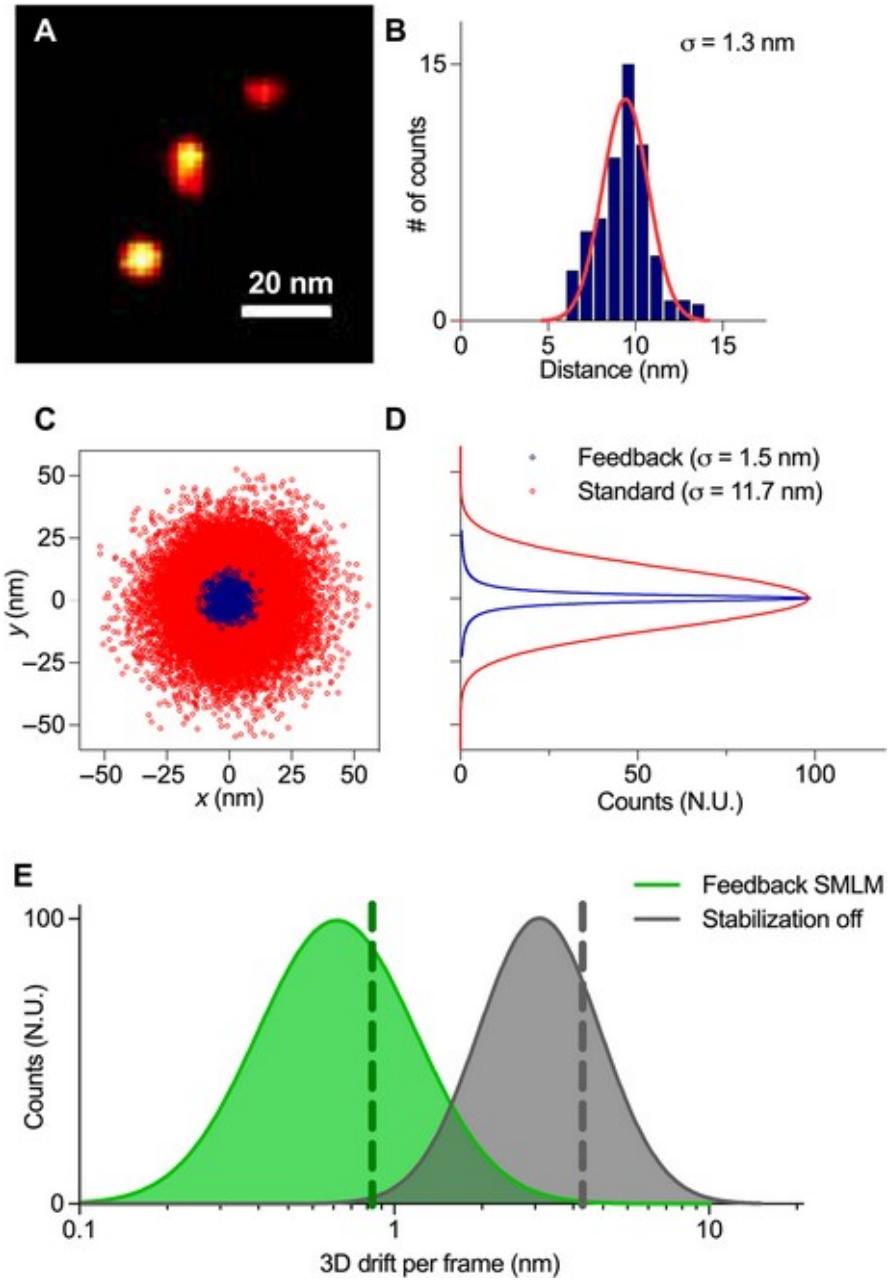
Additional info

- environmental control box was installed around the optical setup to control temperature fluctuations (peltier elements)
  - custom-built
  - temperature and humidity have a standard deviation of 0.02°C and 0.88%, respectively
- data acquisition and instrument control were performed using an Xbox One controller

DNA-PAINT

DNA origami w/ 3 docking sites
“Standard” is actually a commercial Zeiss microscope

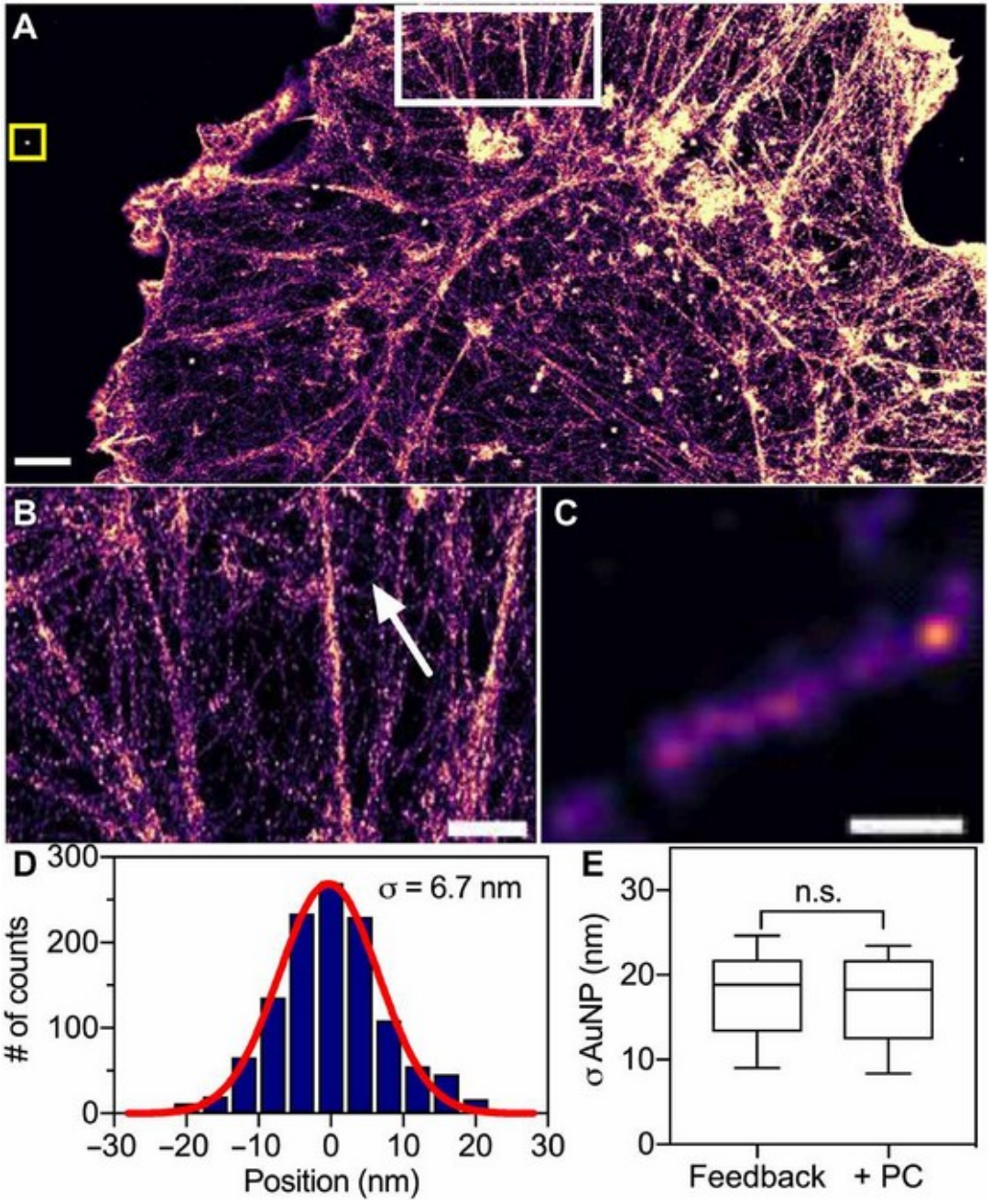
- No post-acquisition processing
- ~1 nm localization precision
- No 3D localization, statistics?



actin filaments

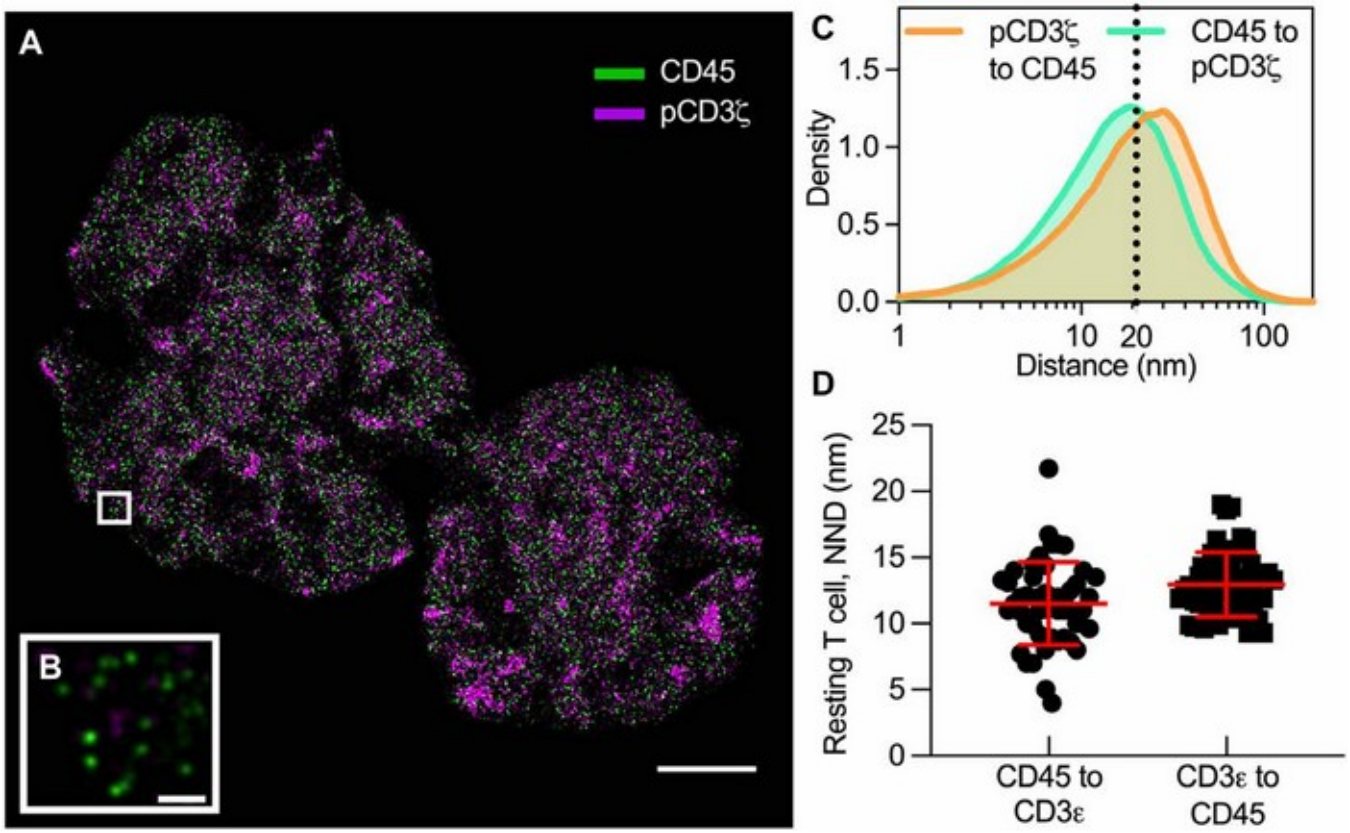
- identify single filaments and determine their widths as 5 to 9nm (again no post-acquisition processing)
- No residual drift when using gold nanoparticles

phalloidin in a COS-7 cell



active and resting T cells in situ

- DNA-PAINT acquisitions of CD45 and phosphorylated CD3ζ
- Difference between dist in C and D



Conclusion

- Claims could be better backed by data
  - Only 1 image of the DNA origami. A bit the same for all other analysis/samples.
  - Distance measurements are confuse. The used system is really not ideal.
- How does the LED spot on the EMCCD look like?
- Not easy to mount an LED in a normal microscope body