Improving resolution of fibrillar biomolecules from AFM image

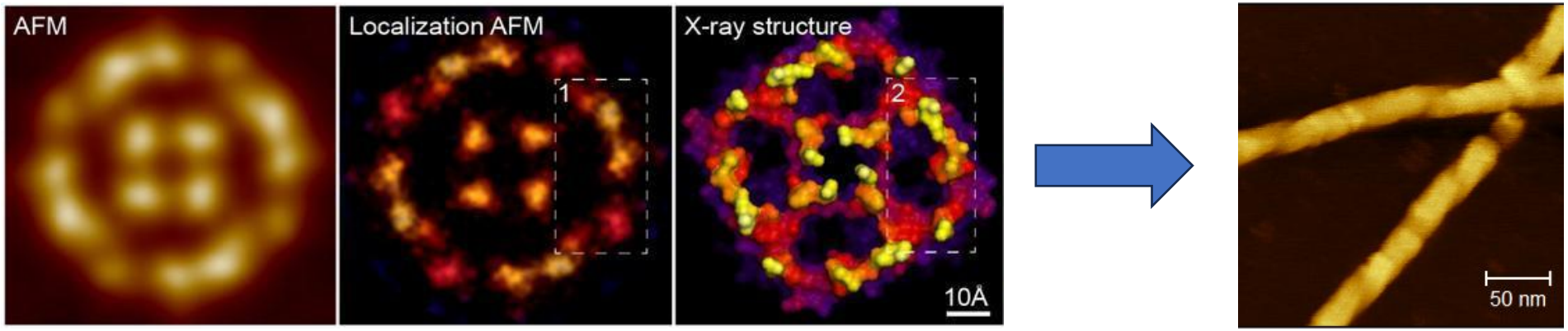
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Masaryk university, Brno, Czech Republic
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The idea

- **Tau protein** – AFM imaging – resolution limit enhancement
- Application of **LAFM** (Localization atomic force microscopy):

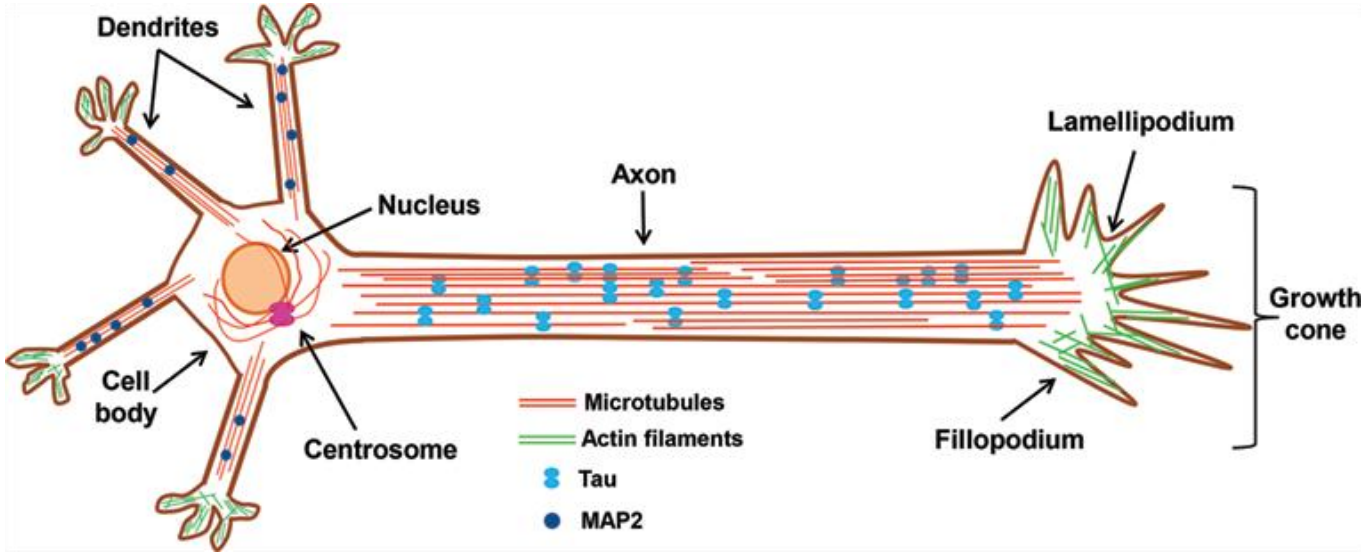


- From blurred image to detailed structure
  - comparable to X-Ray structure or PDB model

Heath, G.R., Kots, E., Robertson, J.L., Lansky, S., Khelashvili, G., Weinstein, H., Scheuring, S., 2021. Localization atomic force microscopy. Nature 594, 385–390. https://doi.org/10.1038/s41586-021-03551-x

Tau protein biology

- Tubulin associated unit
- Stabilizes microtubules in axons of neurons

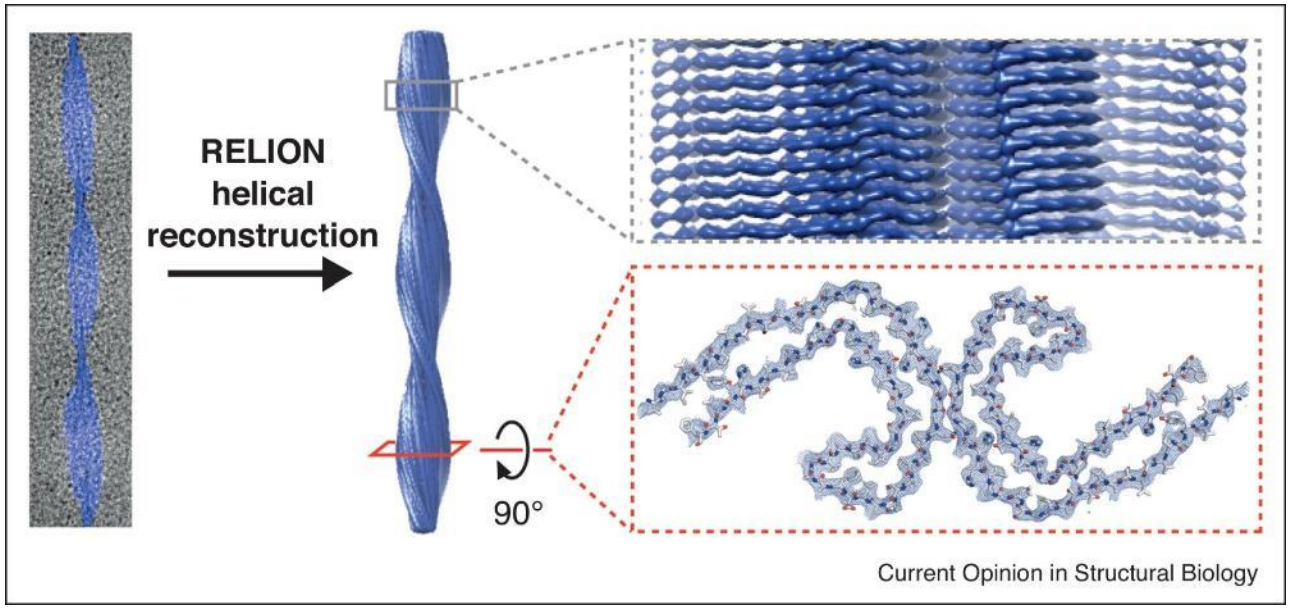


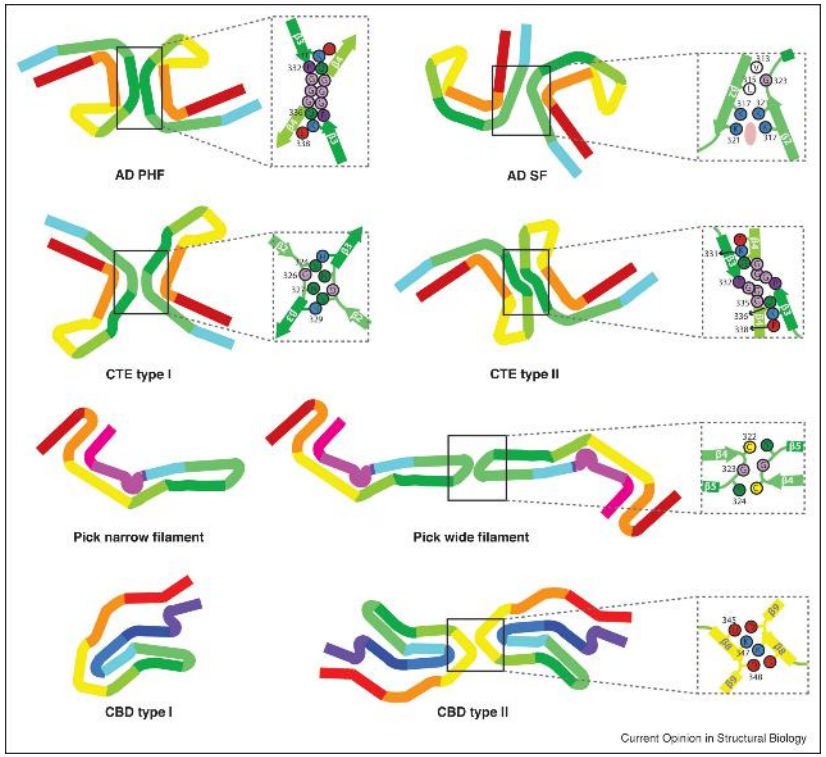
- By phosphorylation: From disordered to specific secondary structure
- Hyperphosphorylated: Insoluble neurotoxic aggregates

Roychowdhury, S., Sierra-Fonseca, J.A., Roychowdhury, S., Sierra-Fonseca, J.A., 2017. Heterotrimeric G Proteins and the Regulation of Microtubule Assembly, in: Cytoskeleton - Structure, Dynamics, Function and Disease. IntechOpen. https://doi.org/10.5772/66929

Tau protein structure

- Monomers → Protofilaments → Filaments



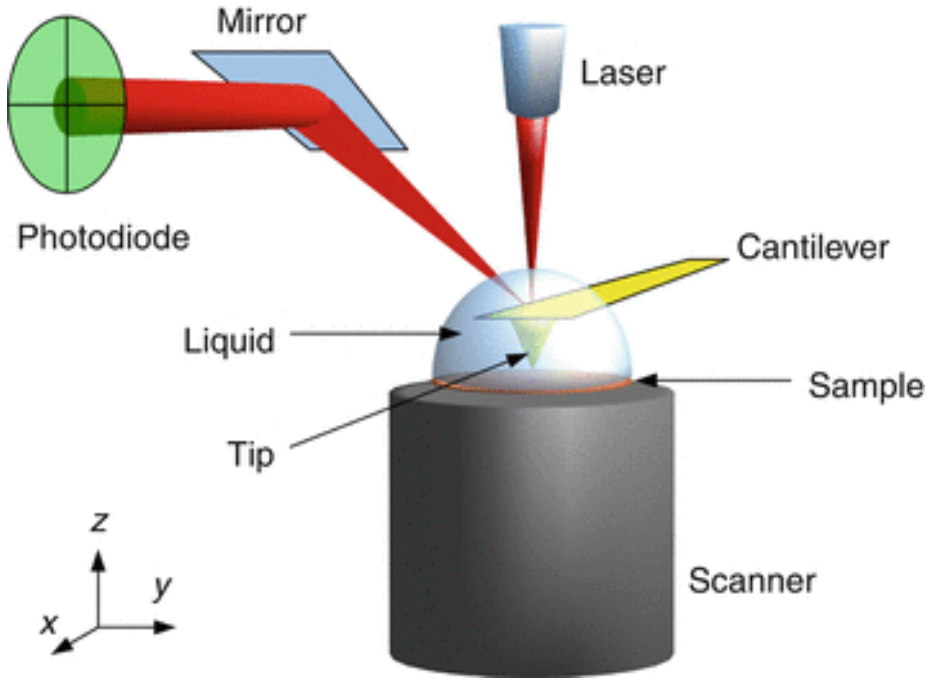


- Unique structure of filaments in each tauopathy

Sjors HW Scheres, Wenjuan Zhang, Benjamin Falcon, Michel Goedert, Cryo-EM structures of tau filaments, Current Opinion in Structural Biology, Volume 64, 2020, Pages 17-25, ISSN 0959-440X, https://doi.org/10.1016/j.sbi.2020.05.011

Sample of Tau for AFM and measuring

- Tau filaments made from monomers by phosphorylation by GSK3β
- Filament parameters:
  - Negative charge from phosphorylation
  - Helical rise of amyloid filament: 0,47 nm
  - Diameter: 7 – 20 nm
- Measured in MQ-Water
  - Applied to silane treated mica
- AFM: Bruker's Dimension FastScan
- Mode: PeakForce QNM
- Cantilever: ScanAsyst-Fluid+ (Tip radius: 2 nm)

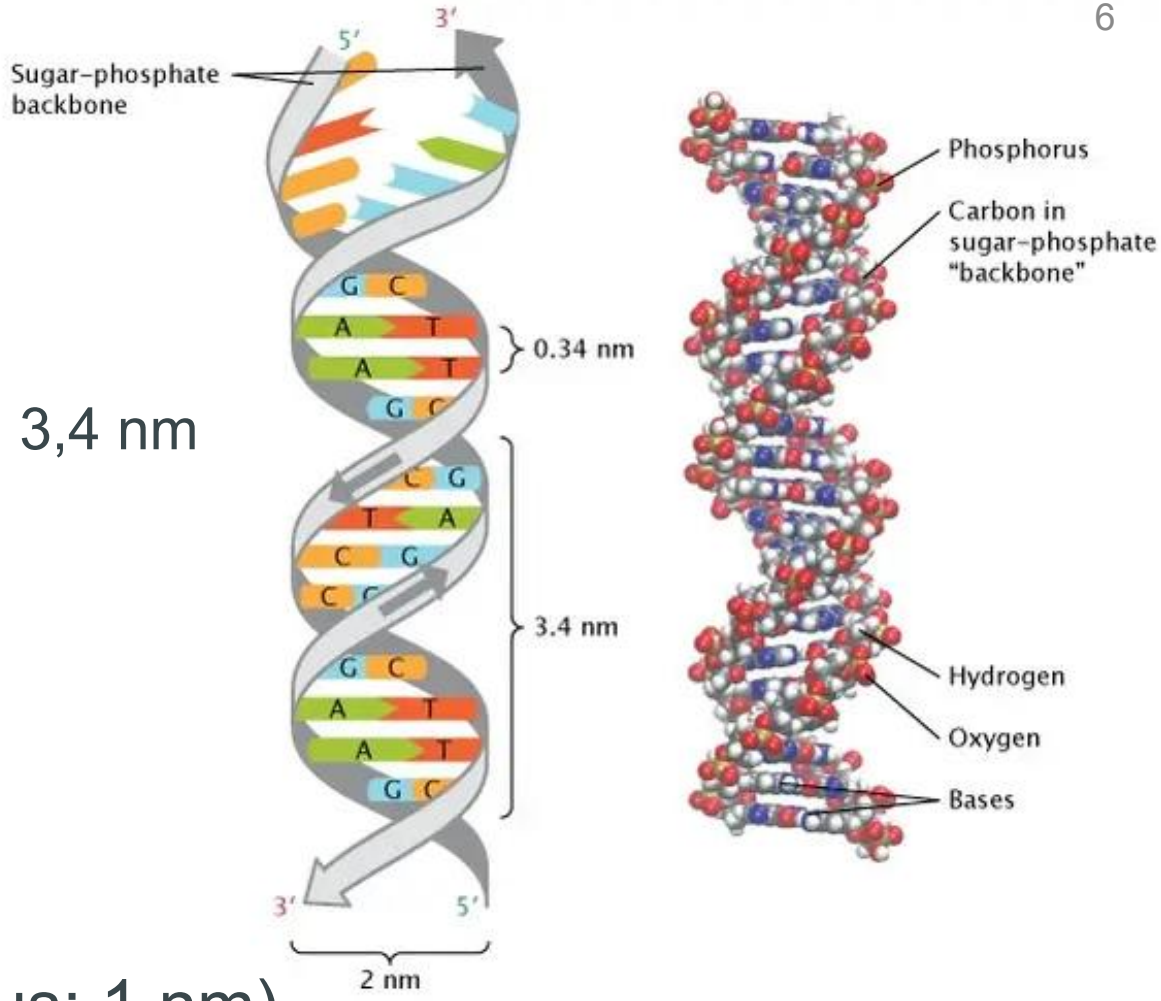


Pyne, A.L.B., Hoogenboom, B.W. (2016). Imaging DNA Structure by Atomic Force Microscopy. In: Leake, M. (eds) Chromosome Architecture. Methods in Molecular Biology, vol 1431. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-3631-1_5

DNA for comparison

- DNA filaments of 200 base pairs
- Filament parameters:
  - Negative charge from phosphate
  - Turn of B-DNA double helix: 2,2 + 1,2 = 3,4 nm
  - Diameter: 2 nm

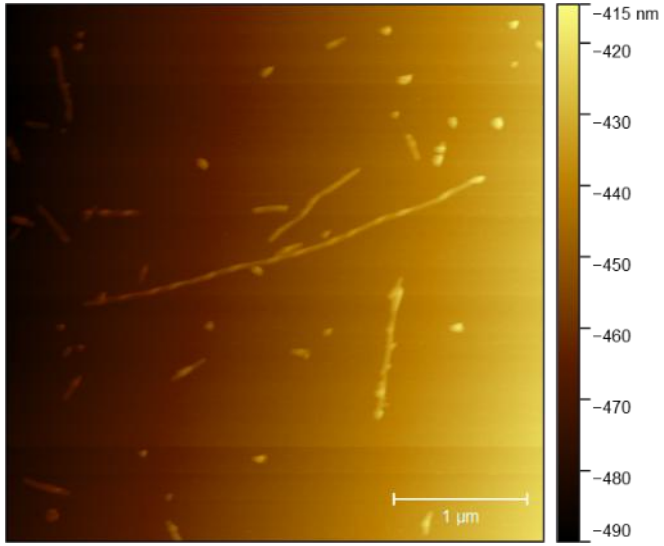
- Measured in HEPES
  - Mica with copolymer PLL-PEG
- AFM: Bruker's Dimension FastScan
- Mode: PeakForce QNM
- Cantilever: FastScan-D-SS (Tip radius: 1 nm)



Alketbi, Salem K. (2023). The role of DNA in forensic science: A comprehensive review. International Journal of Science and Research Archive. 9. 814-829. 10.30574/ijsra.2023.9.2.0624

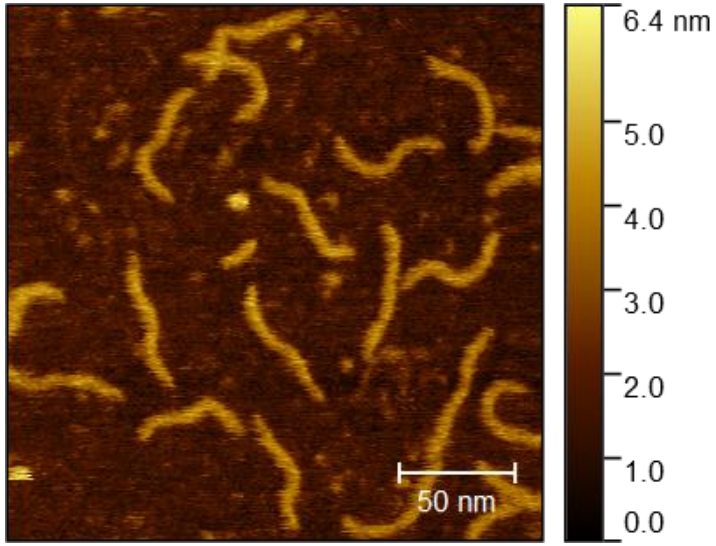
Captured images

- 35 images of Tau filament



Scan size: 4,0 µm; Lines: 512
Scan rate: 2 Hz
Peak Force Setpoint: 1,000 nN
Peak Force Amplitude: 10,0 nm

- 40 images of DNA

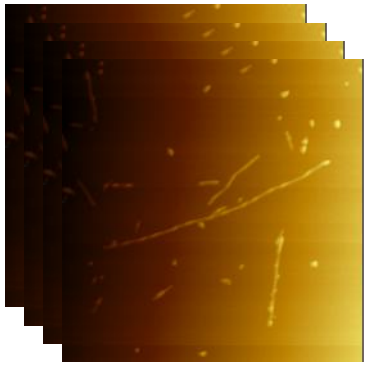


Scan size: 230 nm; Lines: 304
Scan rate: 10,5 Hz
Peak Force Setpoint: 0,066 nN
Peak Force Amplitude: 5,0 nm

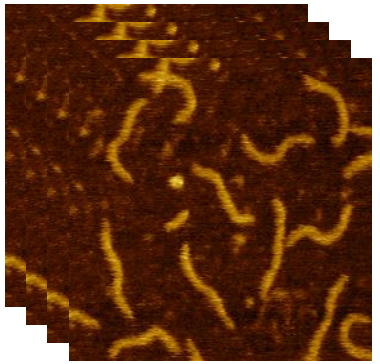
NanoLocz

- Free interactive AFM image viewer and analysis platform
- Workflow to get single molecule LAFM image
  - Level → Detect → Fine Align → Localize

- From many images of single molecule:

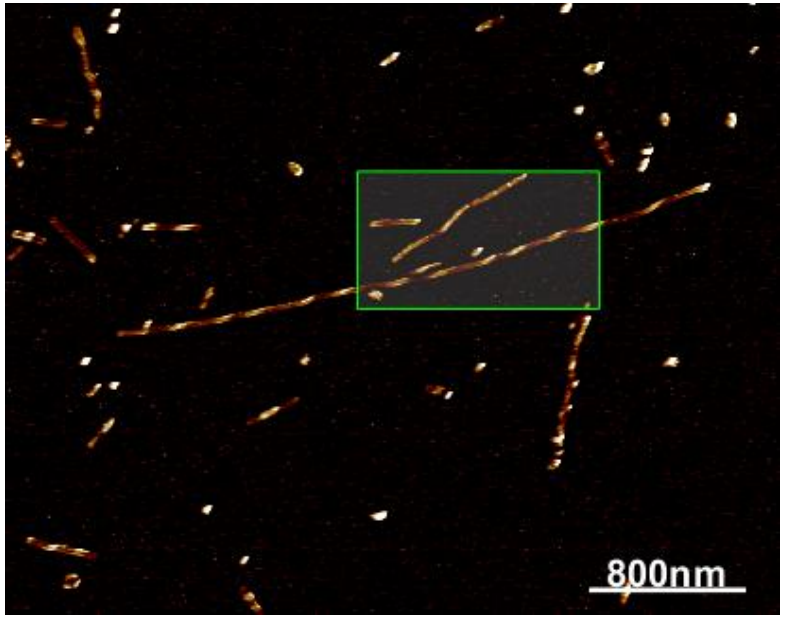


Tau: 0,128 px/nm



DNA: 1,32 px/nm

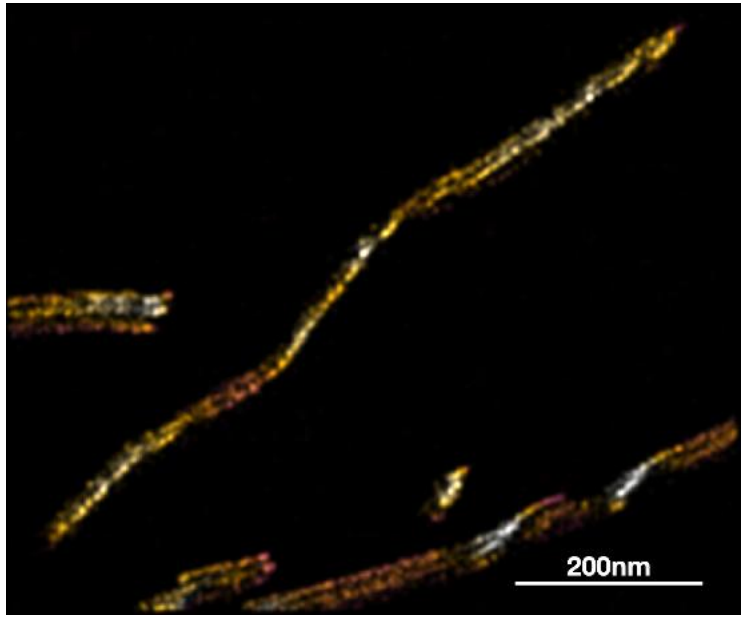
NanoLocz Tau LAFM



a) One of 35 images, leveled, with selected ROI

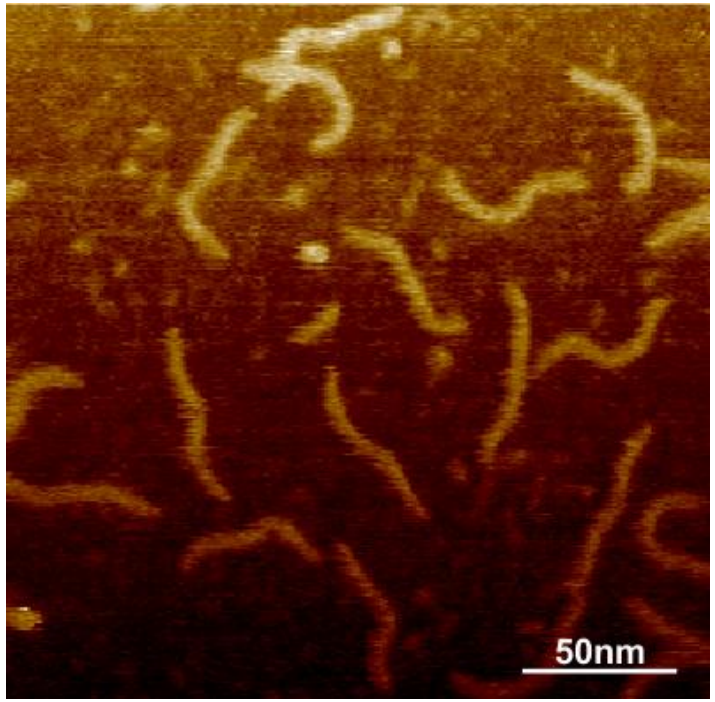


b) LAFM map from original image sequence

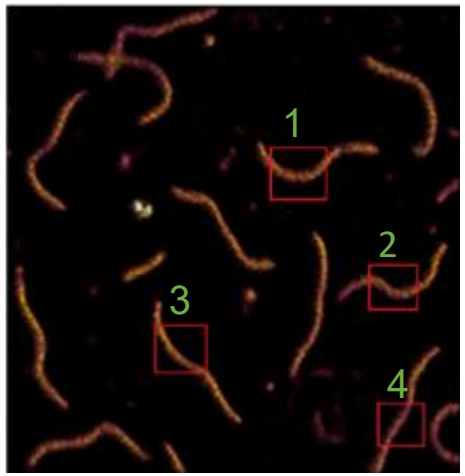


c) „Secondary“ LAFM map by repeating alignment based on first LAFM map

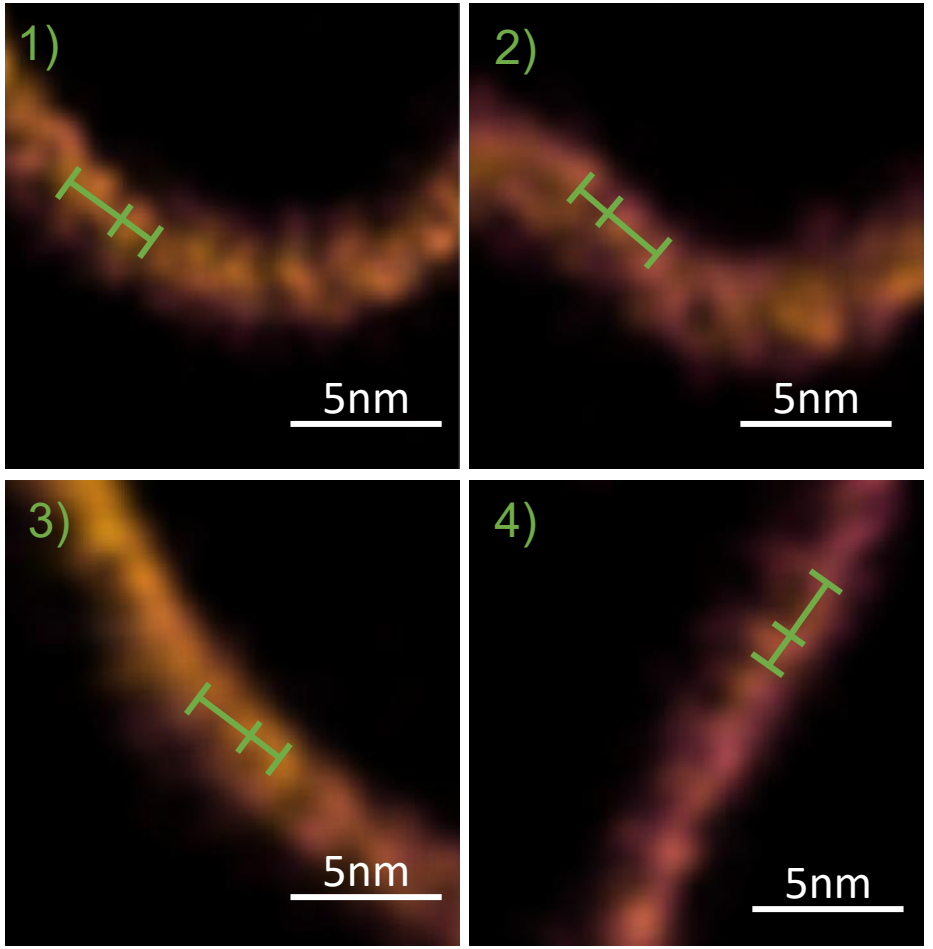
NanoLocz DNA LAFM



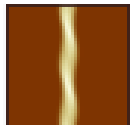
a) One of 40 original images



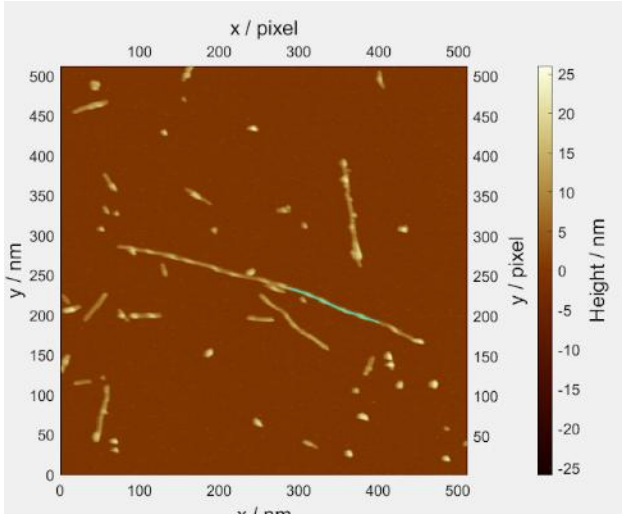
b) LAFM map with 4 selected areas



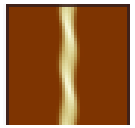
c) Zoomed in at each of 4 areas with ilustrative size of major and minor groove.

Trace_y 

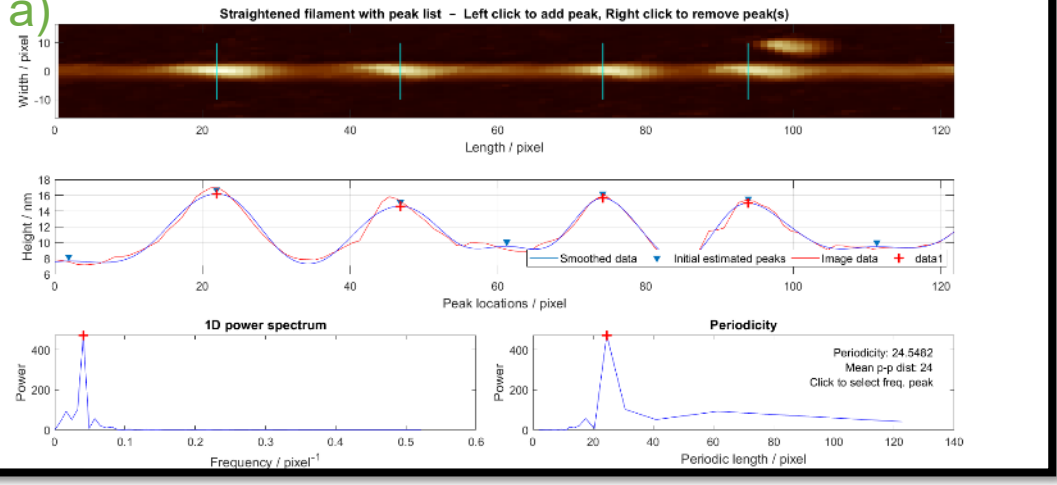
- Software algorithms for structural analysis of individual helical filaments by 3D contact point reconstruction AFM
- Workflow to get 3D model of filament
  - Import image → trace filament → reconstrucion of 3D helical model
- From a single image:



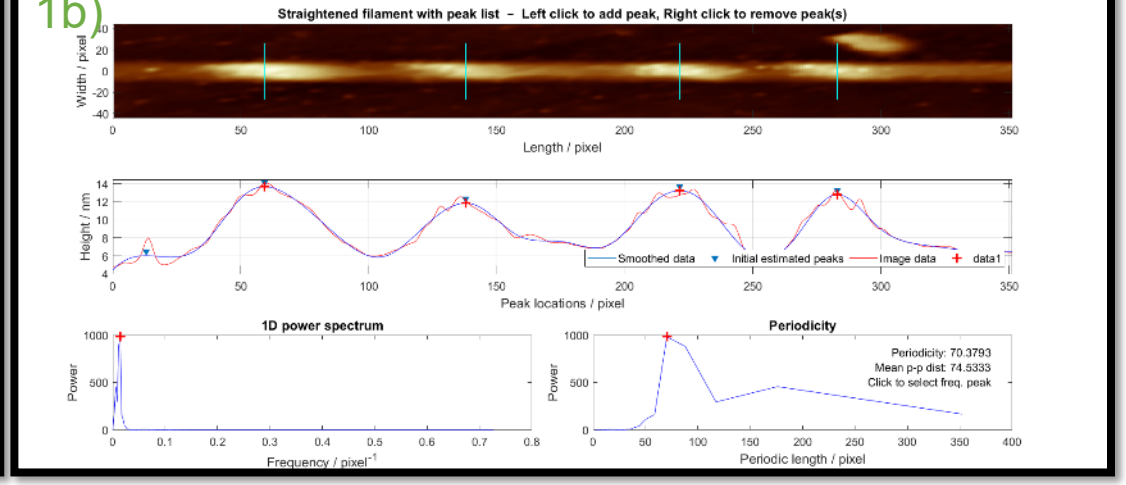
- 1st: 0,128 px/nm, 2nd (after 3x bicubic interpolation) 0,384 px/nm

Trace_y  original vs. 3x interpolated image

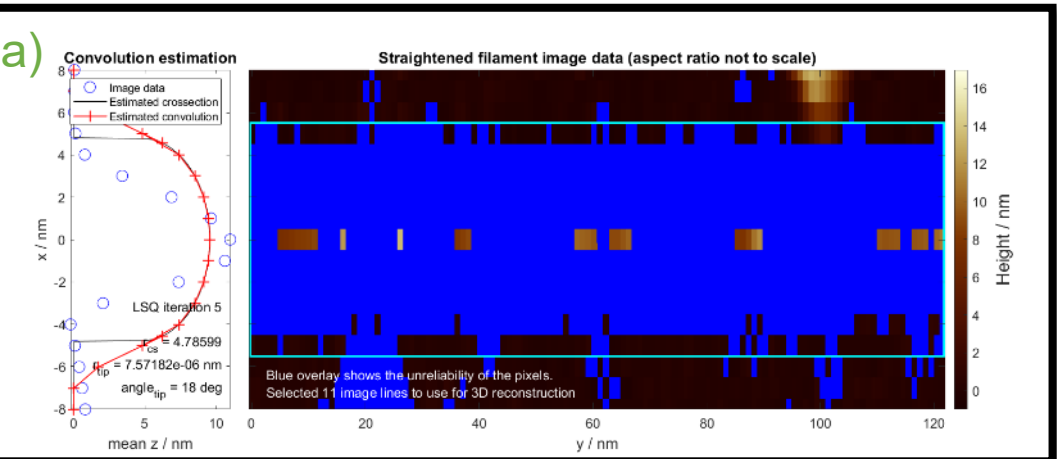
1a)



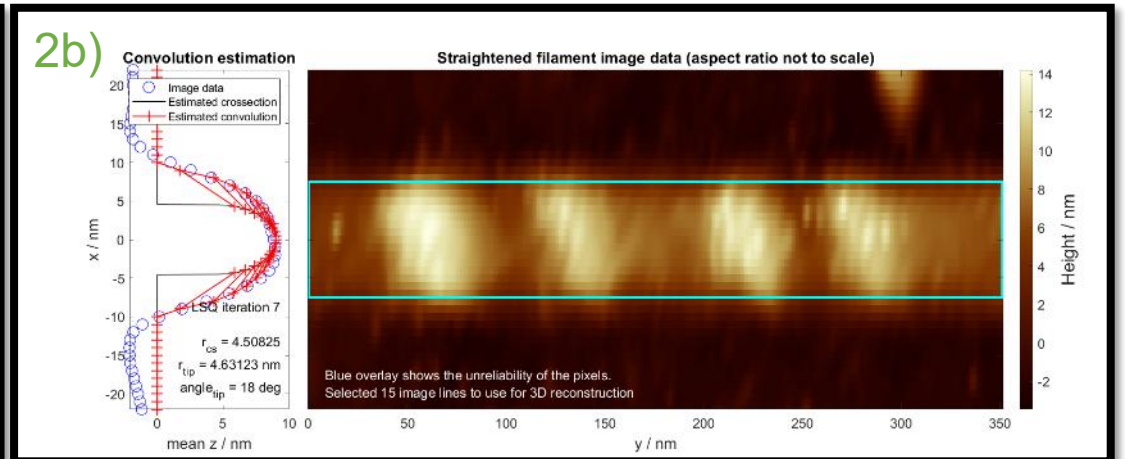
1b)



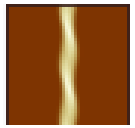
2a)



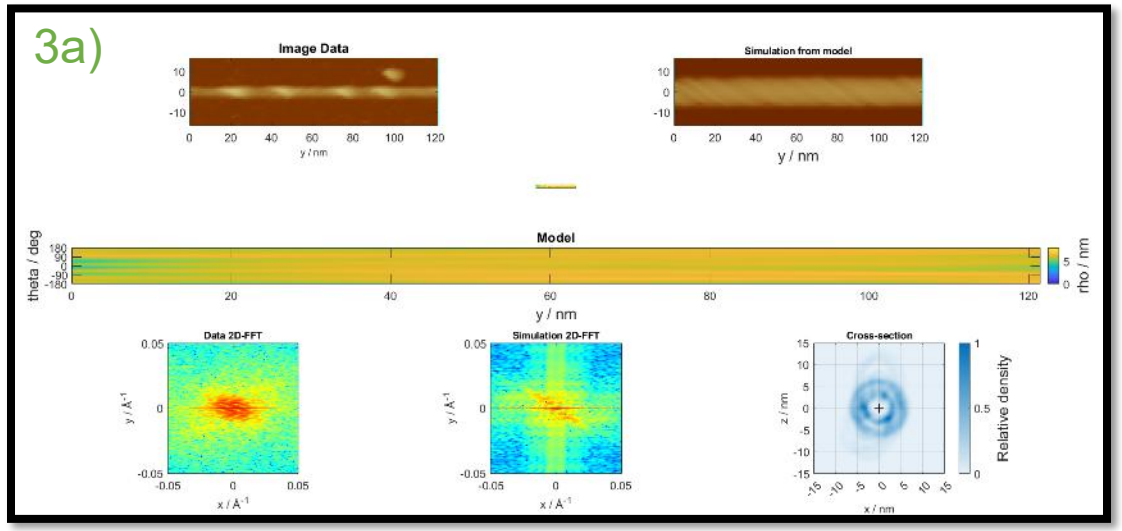
2b)



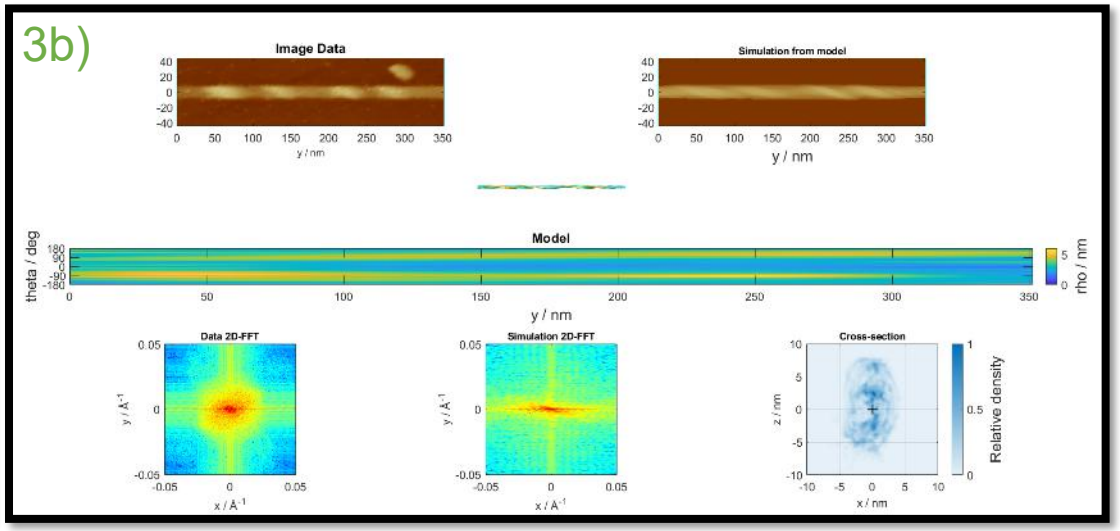
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Trace_y  original vs. 3x interpolated image

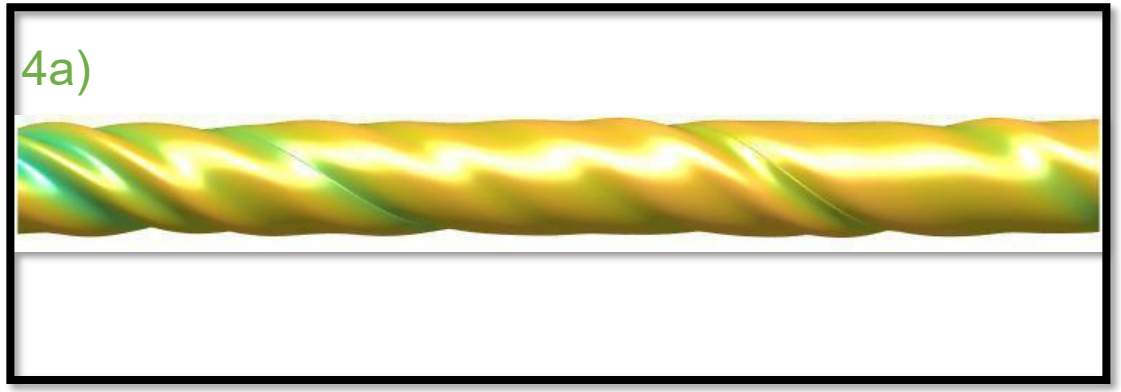
3a)



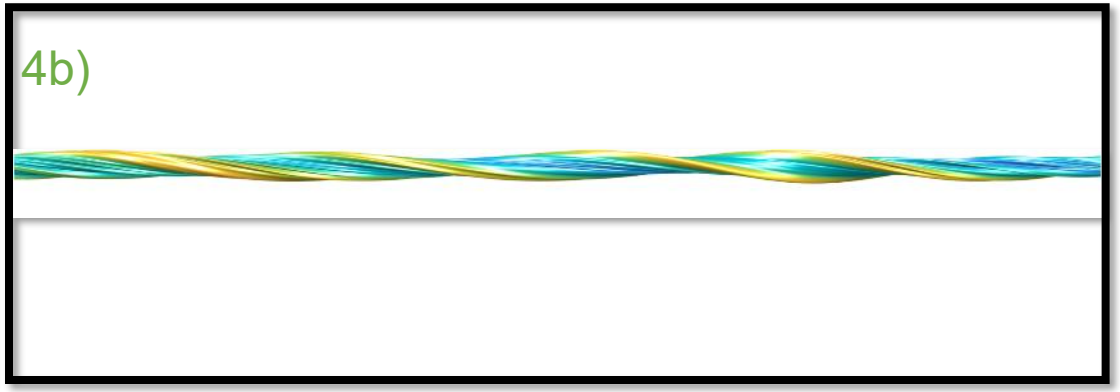
3b)



4a)



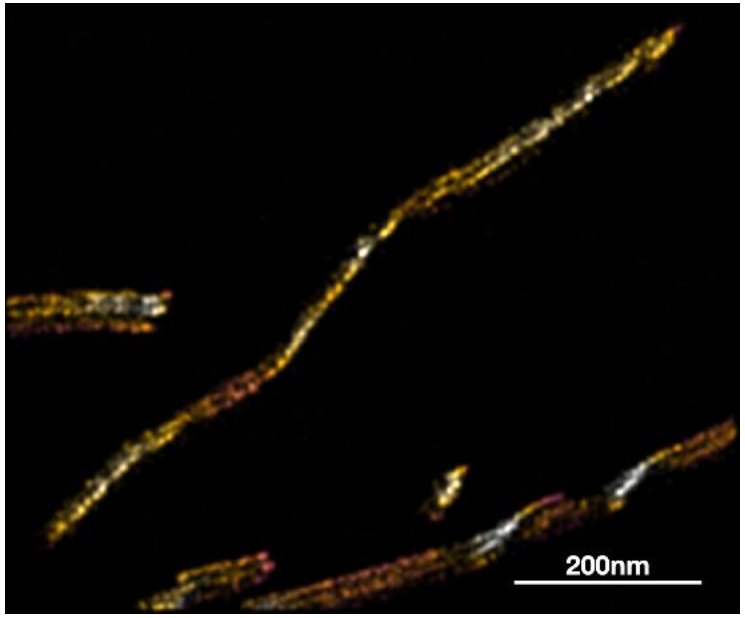
4b)



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The results

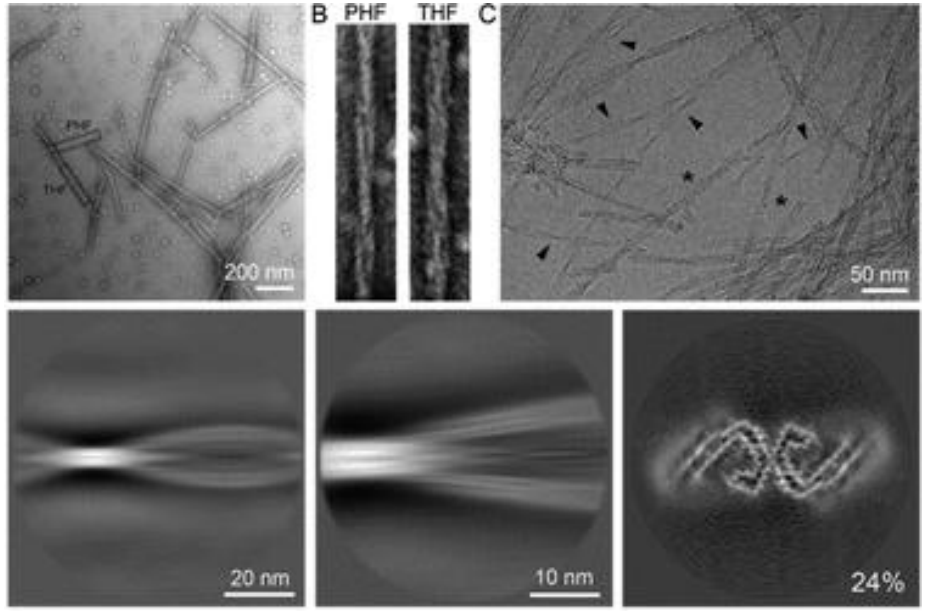
- NanoLocz



- Trace_y



- Electron microscopy
  (Harvard Medical School)



Glynn, Calina & Chun, Joshua & Donahue, Cameron & Nadler, Monica & Fan, Zhanyun & Hyman, Bradley. (2023). Reconstitution of the Alzheimer's Disease Tau Core Structure from Recombinant Tau 297–391 Yields Variable Quaternary Structures as Seen by Negative Stain and Cryo-EM. Biochemistry. 63. 10.1021/acs.biochem.3c00425


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Thank you for your attention!
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