HIGH LET RADIATION EFFECTS ON DNA IN WATER

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MOTIVATION, GOAL, APPROACH & 1ST RESULTS

- Clustered DNA damage is a cell marker of cancer risk
- Quantitative experimental characterization of heavy ions induced clustered damage of DNA in water as a basis for computational models.
- Using agarose gel electrophoresis and Atomic Force Microscopy (AFM) in order to extract a detailed analysis of the DNA damage.
- A size distribution of DNA fragments is extracted.

METHOD 1

- The measurement is difficult – the most of the methods are not able to detect short DNA fragments

  → underestimation of the actual state

- One of the few possibilities is atomic force microscopy (AFM)

  → so far only *in vitro* (extraction from the cells is challenging)

METHOD 2

- Plasmid DNA in scavenged solution = simplified *in vitro* model of cell nucleus
  - no repair processes
  - for study of primary DNA damage
  - control of indirect effects of radiation with the scavengers

- Radiation-induced DNA plasmid damage measured with
  - agarose gel electrophoresis – reveals strand breaks
  - agarose gel electrophoresis after treatment with base excision repair enzymes – reveals damaged base lesions
  - atomic force microscope – reveals short DNA fragments

METHOD 3

pBR322: 4361 base pairs

- Plasmid DNA in scavenged solution = simplified model of cell nucleus

  no reparation processes

  for study of primary DNA damage

  control of indirect effects of radiation with the scavengers

TRIS, C3CA

Scavenging capacity in $M^{-1}s^{-1}$	Scavenger and concentration in mM	
	C3CA	Tris
10^5		0.14*
10^6	0.15	0.67
10^7	1.47	6.67
10^8	14.71	66.67

- Radiation-induced DNA plasmid damage measured with

  agarose gel electrophoresis – reveals strand breaks

  agarose gel electrophoresis after treatment with base excision repair enzymes – reveals damaged base lesions

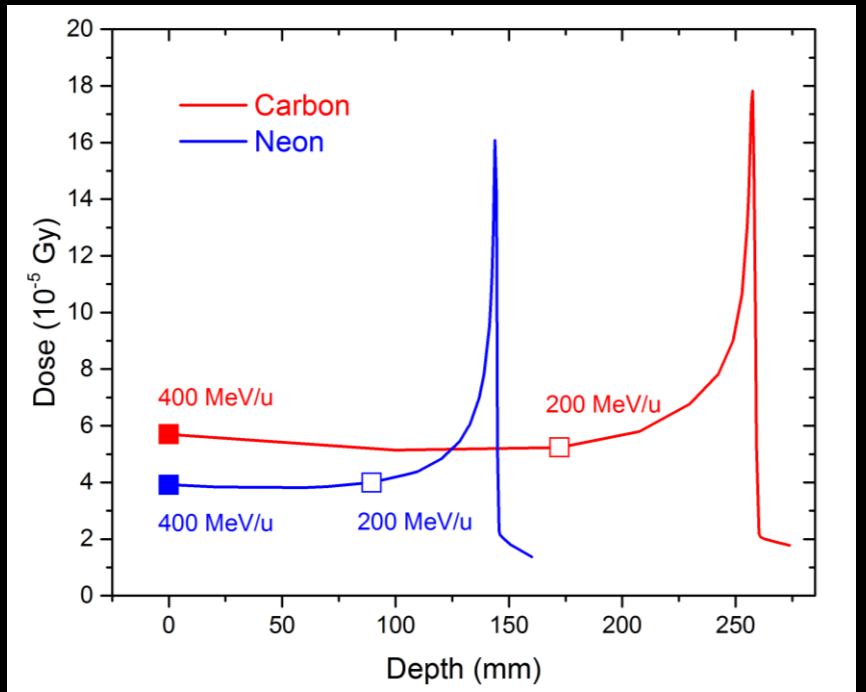
Nth, Fpg

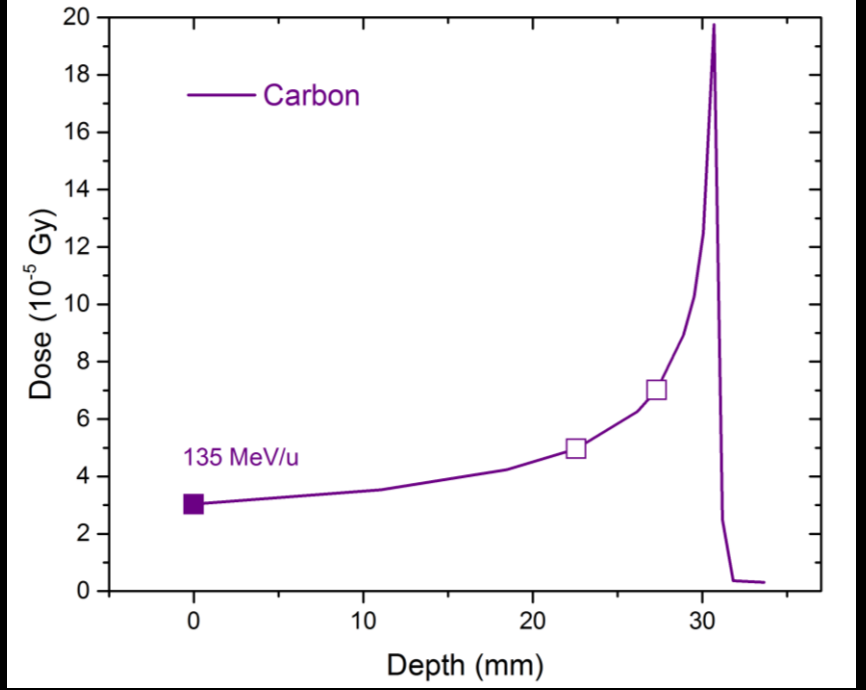
  atomic force microscope – reveals short DNA fragments

RADIATION SOURCES

- HIMAC: Heavy Ion Medical Accelerator in Chiba (Japan)
- 2015: 400 MeV/u carbon and 400 MeV/u neon beam
- 2016: 135 MeV/u carbon beam
- Entrance of Bragg curve (full marks)
- Plateau of Bragg curve (empty marks) – energy lowered with PMMA shielding

→ LET in range 11-45 keV/µm

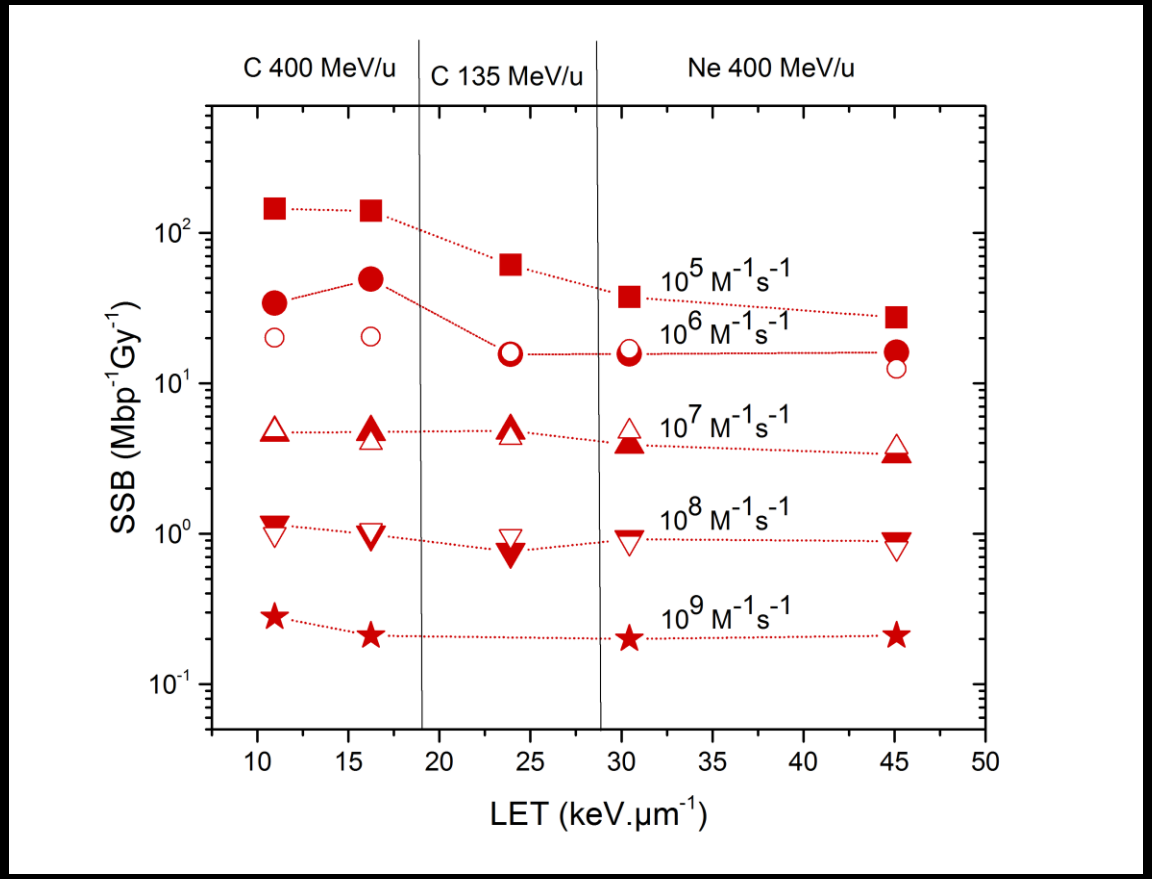


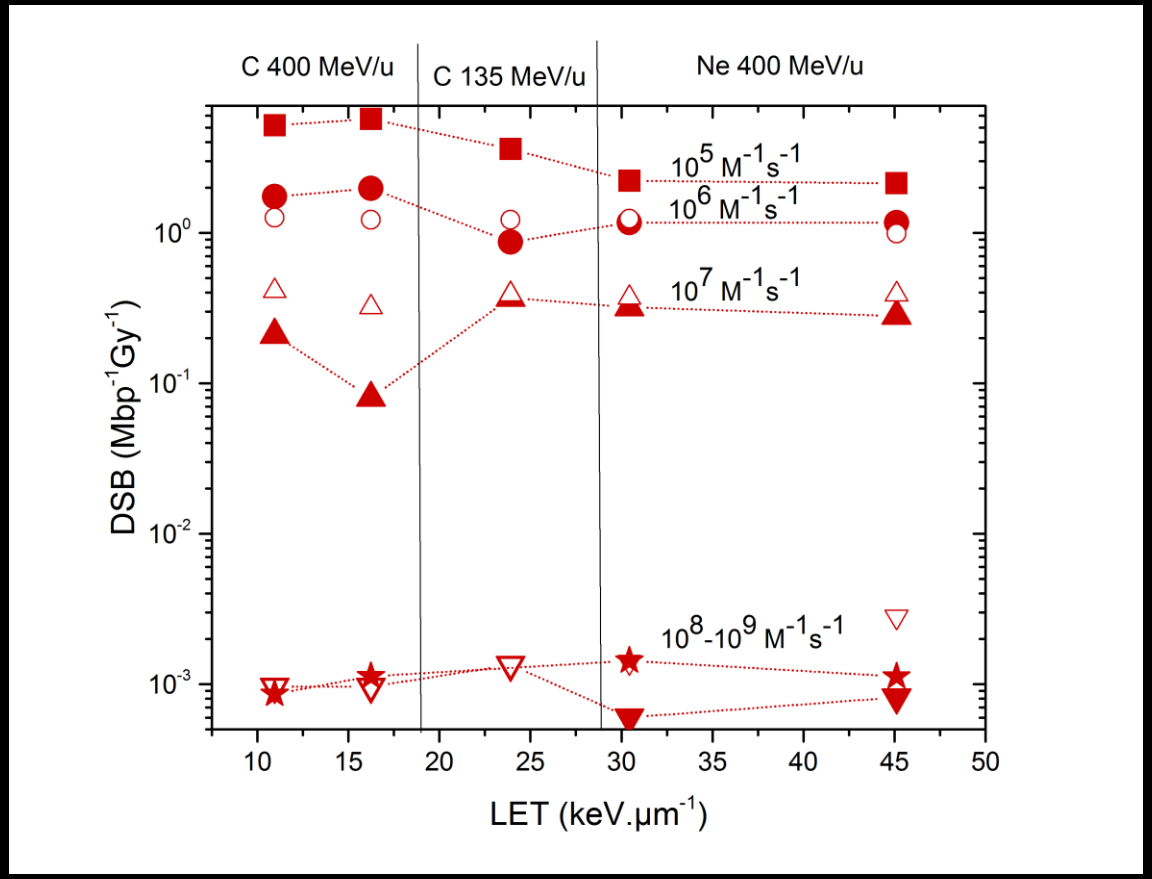


REVEALED STRAND BREAKS

TRIS: full symbols

C3CA: open symbols

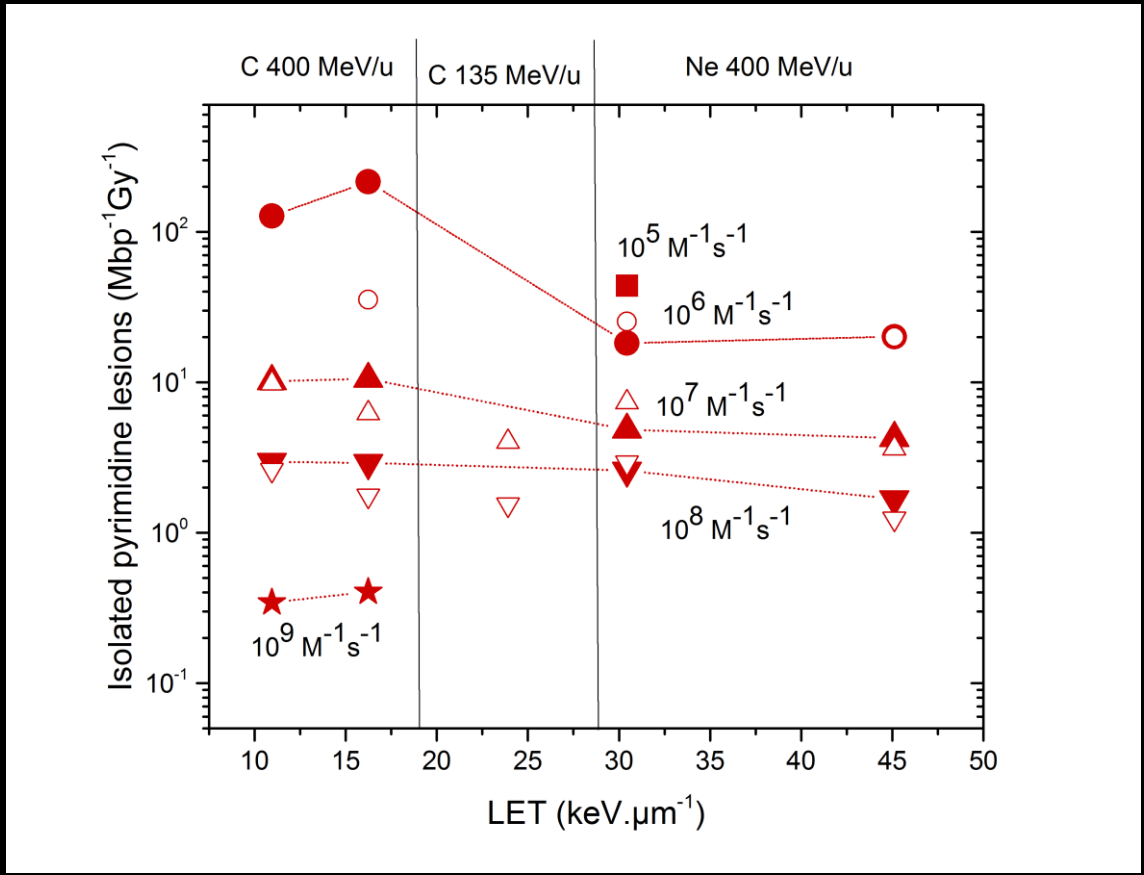


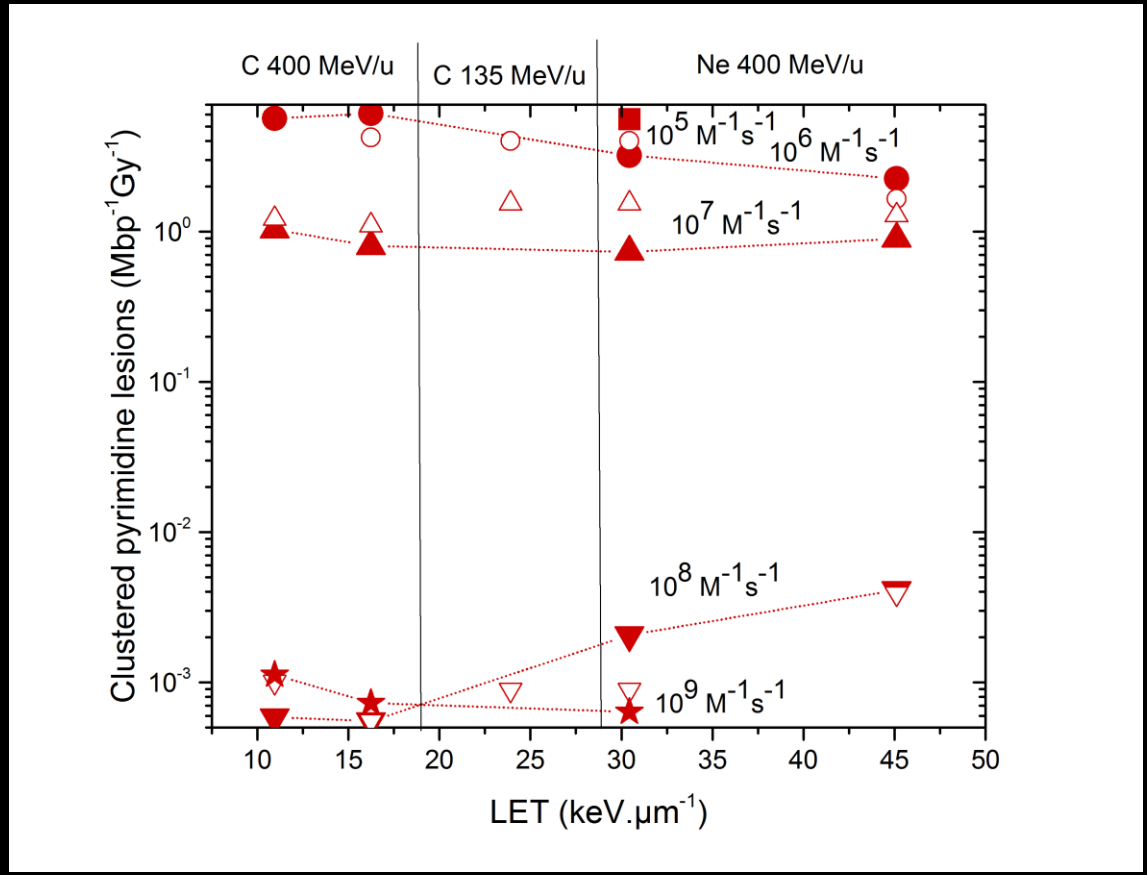


REVEALED FPG-LESIONS

TRIS: full symbols

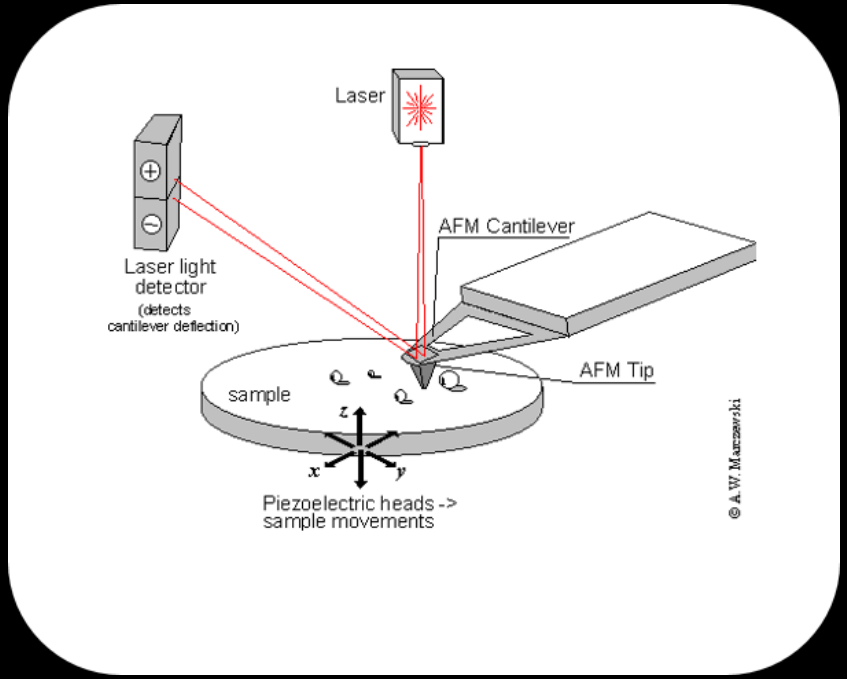
C3CA: open symbols





ATOMIC FORCE MICROSCOPY (AFM)

- Binnig G., Quate C.F. and Gerber Ch., Physical Review Letters 56, 1986
- Sample position controlled by piezoelectric table
- Cantilever position controlled by laser light
- Measuring of deflections of a cantilever due to attractive and repulsive forces between atoms on the sample surface and atoms at the cantilever tip

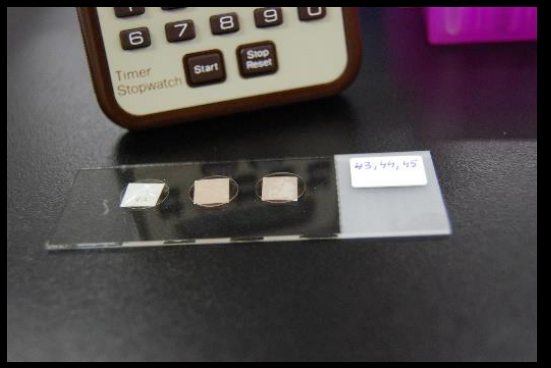


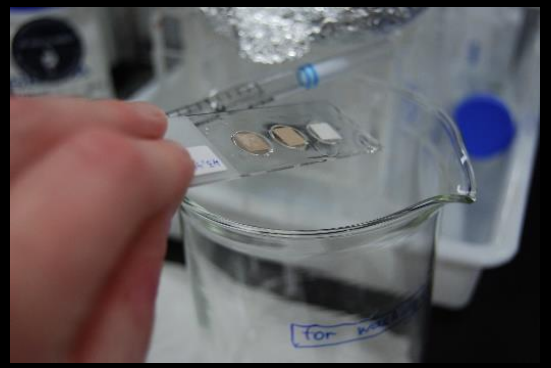
DNA SAMPLES FOR AFM

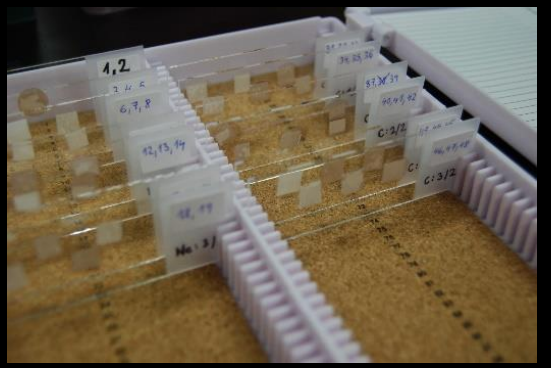
- Require extremely flat substrate for adsorption – mica (silicate mineral)
- Mica cannot bound DNA directly – is negatively charged, as well as DNA

  → DNA is adsorbed with Mg^{2+} buffer
- Buffer washed away and dried

  → imaging in the air

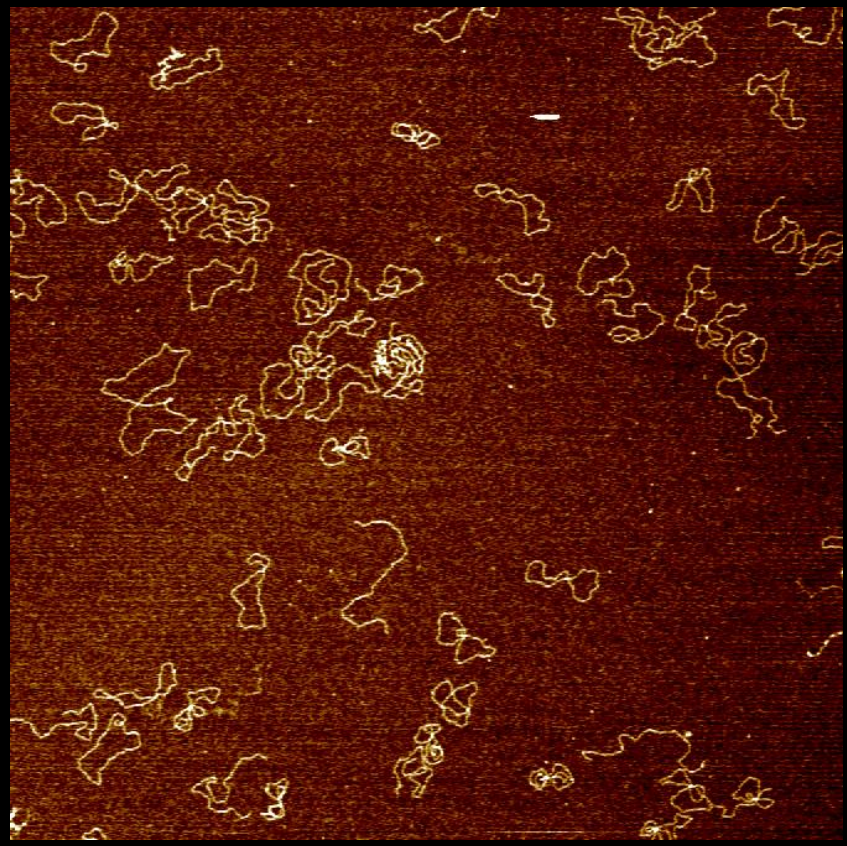






IMAGING WITH AFM

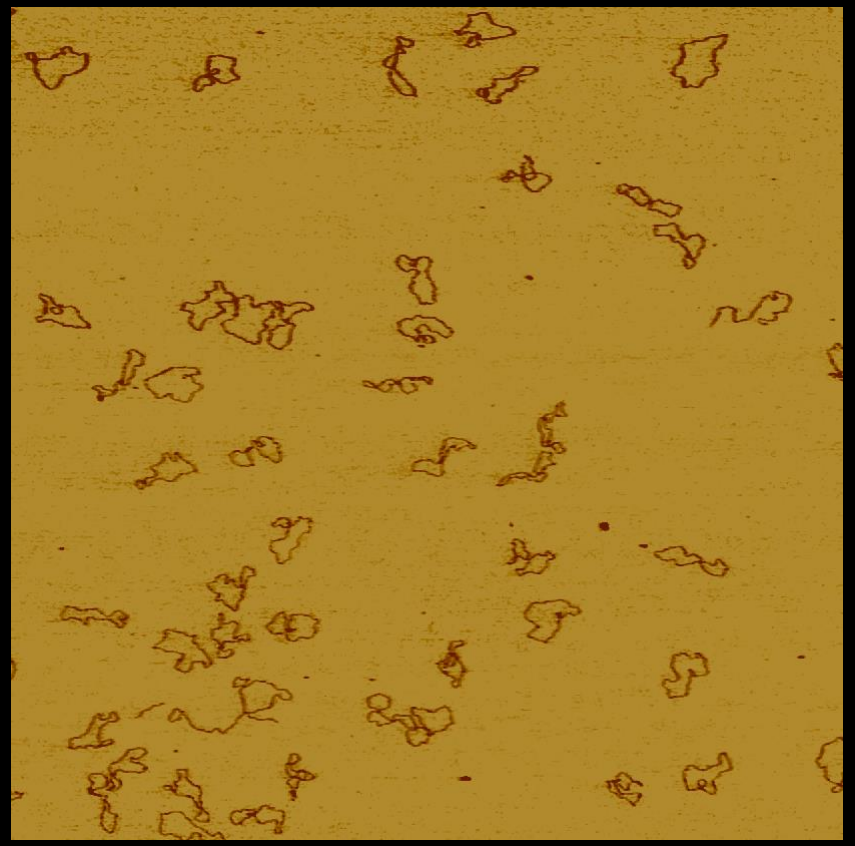
- AFM from Bruker, in the air
- Tapping mode (height profile)



5x5 µm

IMAGING WITH AFM

- AFM from Bruker, in the air
- Tapping mode (height profile)
- Peak force mode (adsorption profile)



5x5 µm

PROCESSING OF AFM IMAGES

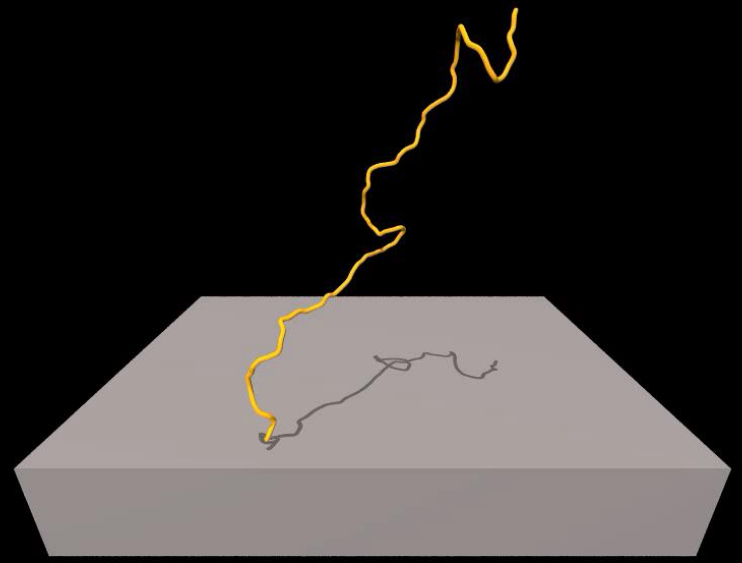
- We want measure lengths of molecules
- DNA molecule: 3D conformation in solution, 2D on the surface

- In house algorithm implemented in Matlab:

Image segmentation - isolation of pixels which describe DNA molecule

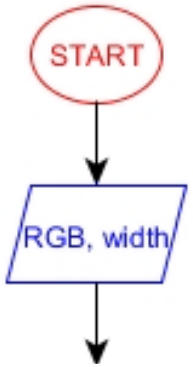
Pixel length - calculation of the length of isolated pixels

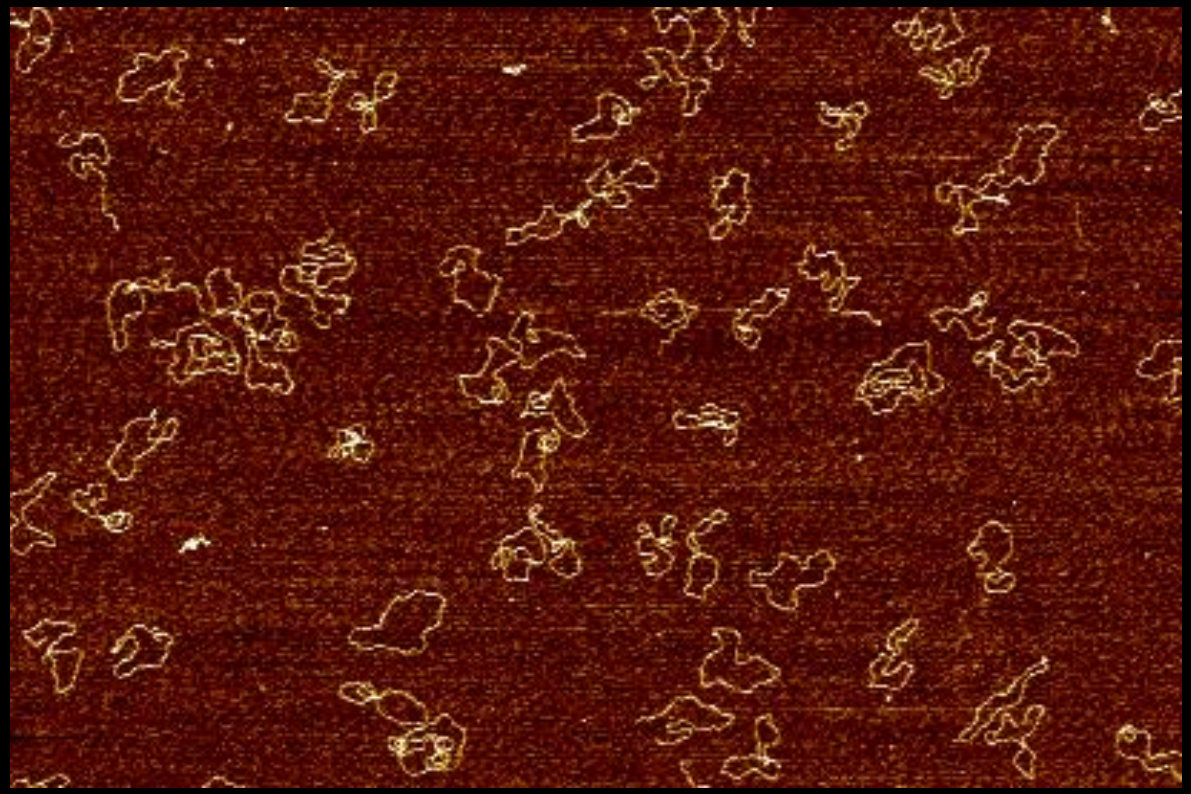
Calibration - transformation of the pixel length to the real units



Mücke N et al. (2009) PLoS ONE 4(11): e7756. doi:10.1371/journal.pone.0007756

ALGORITHM: IMAGE SEGMENTATION





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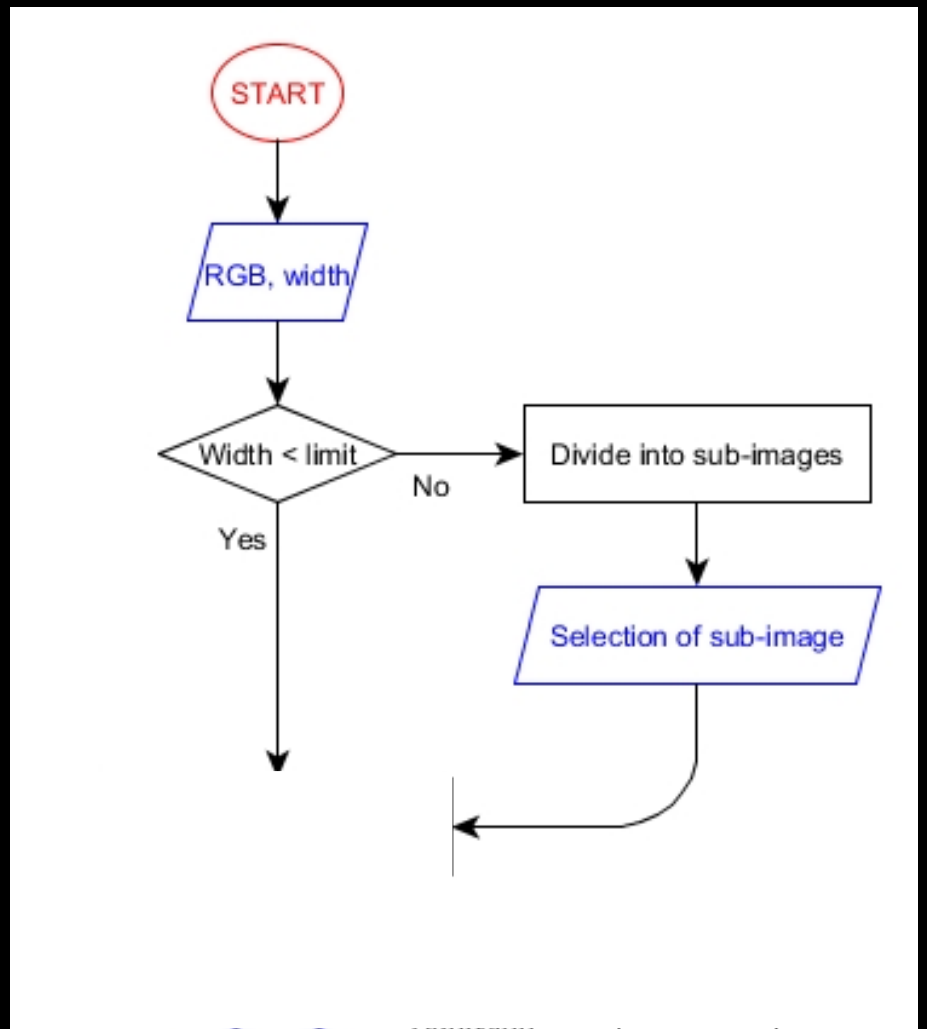

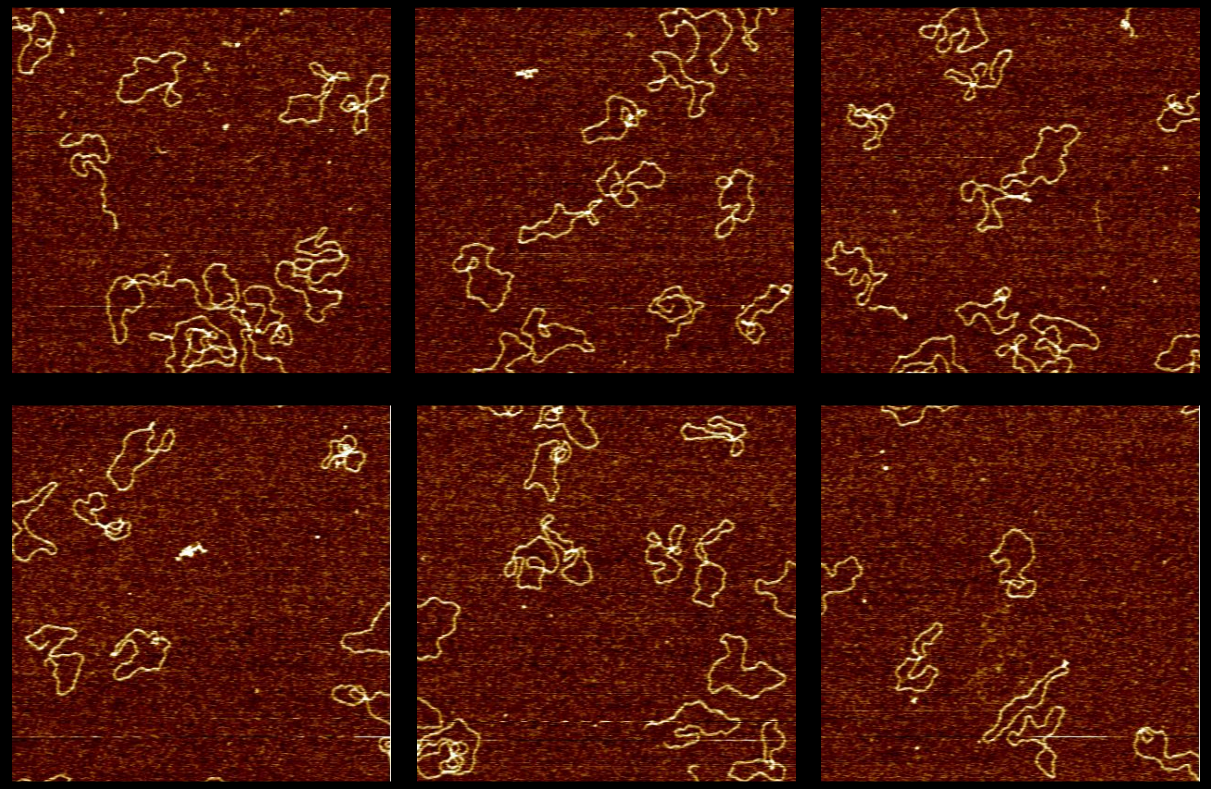

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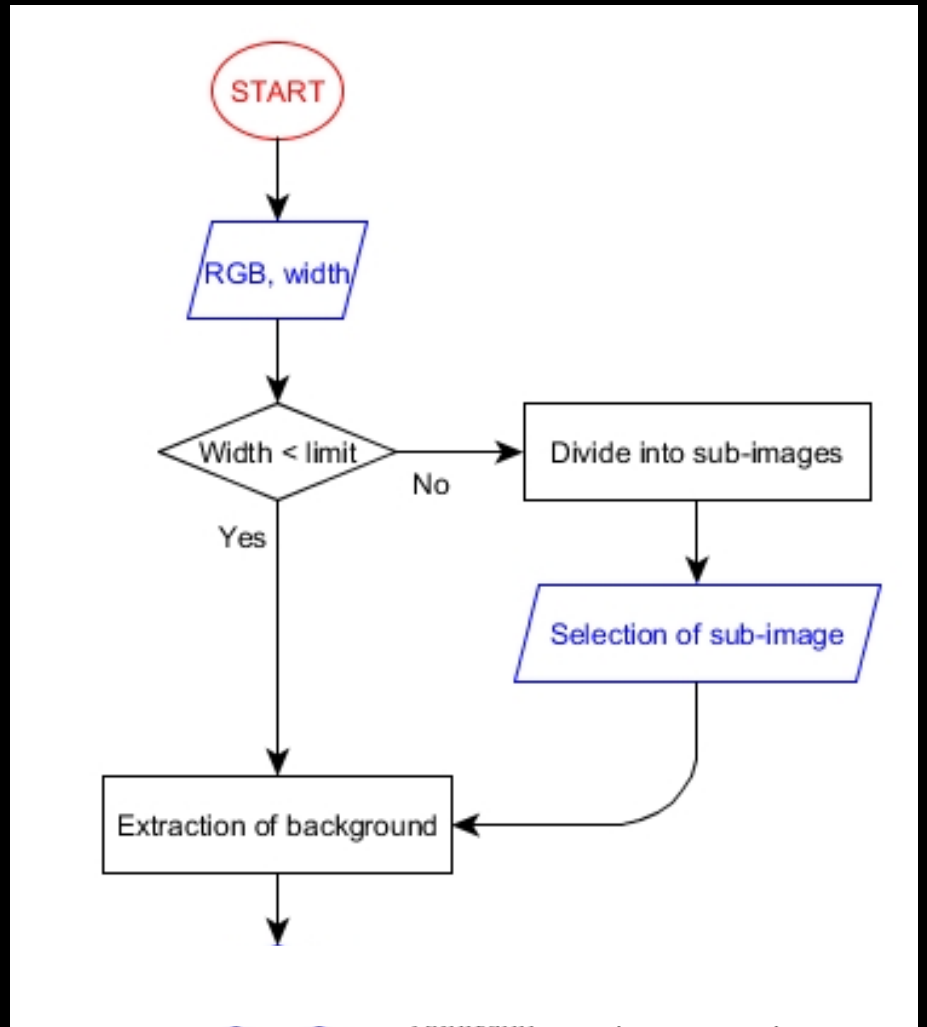

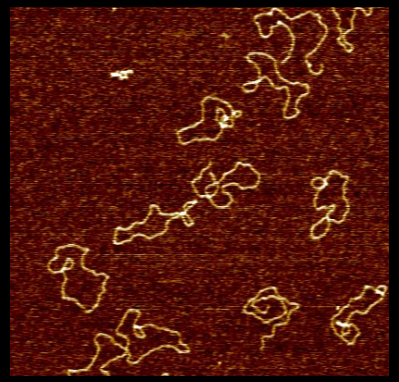

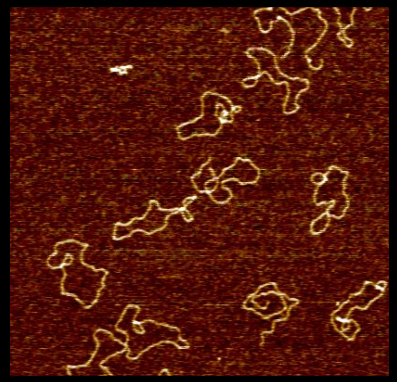

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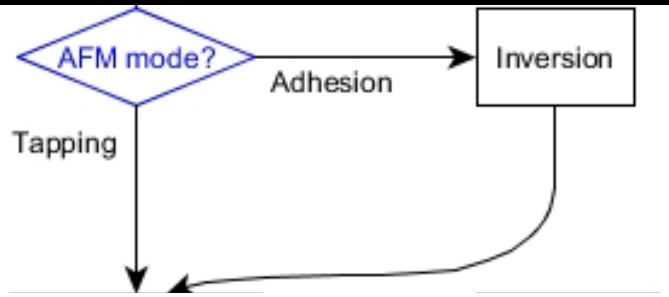

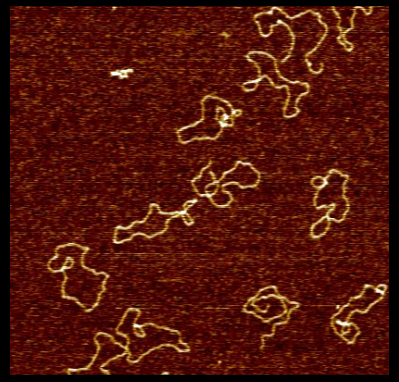

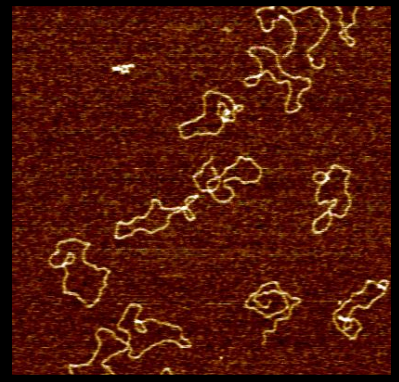

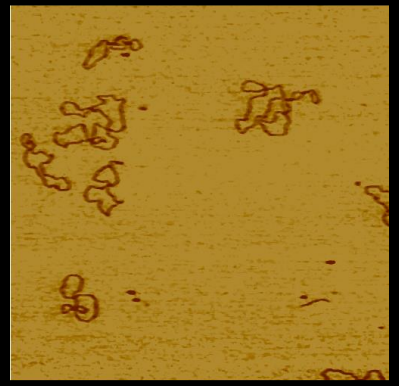

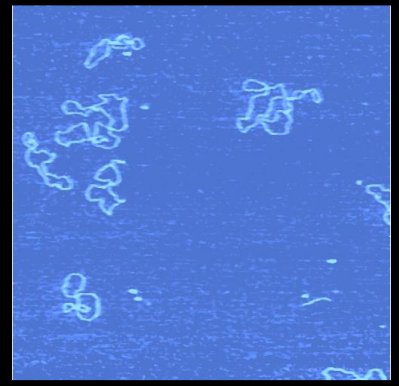

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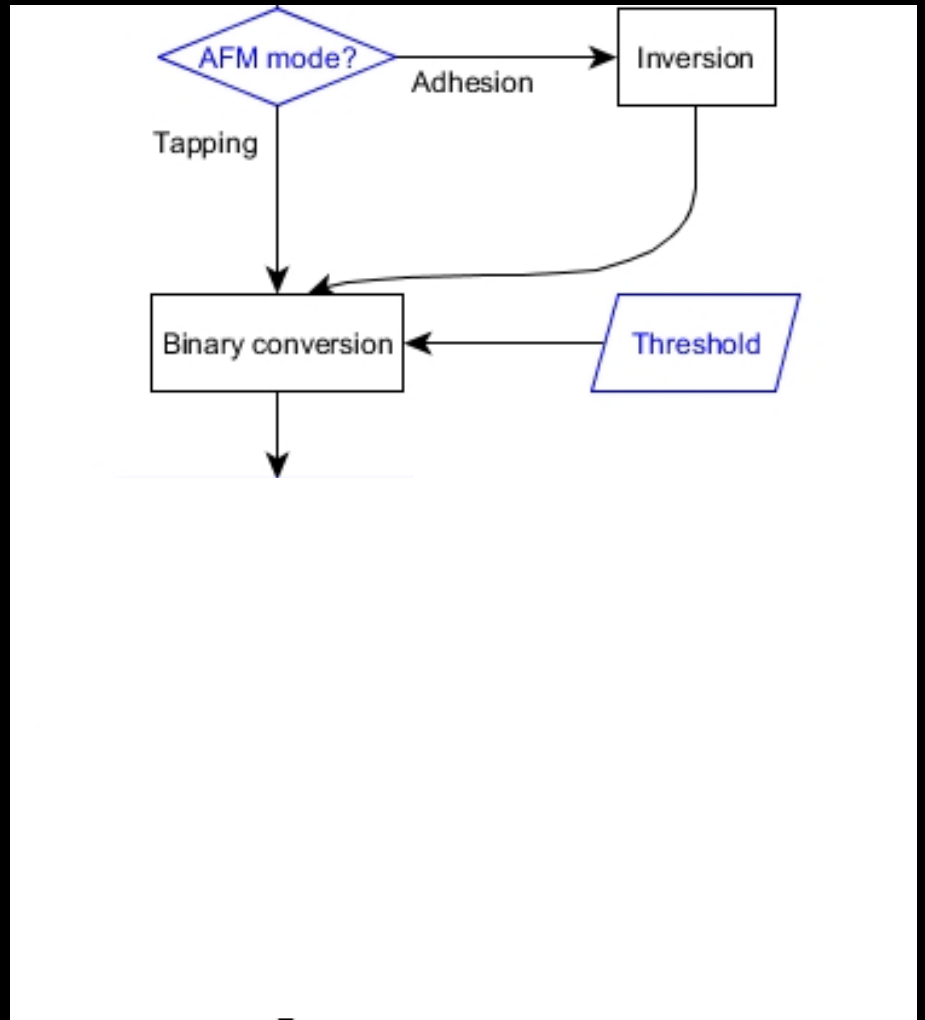

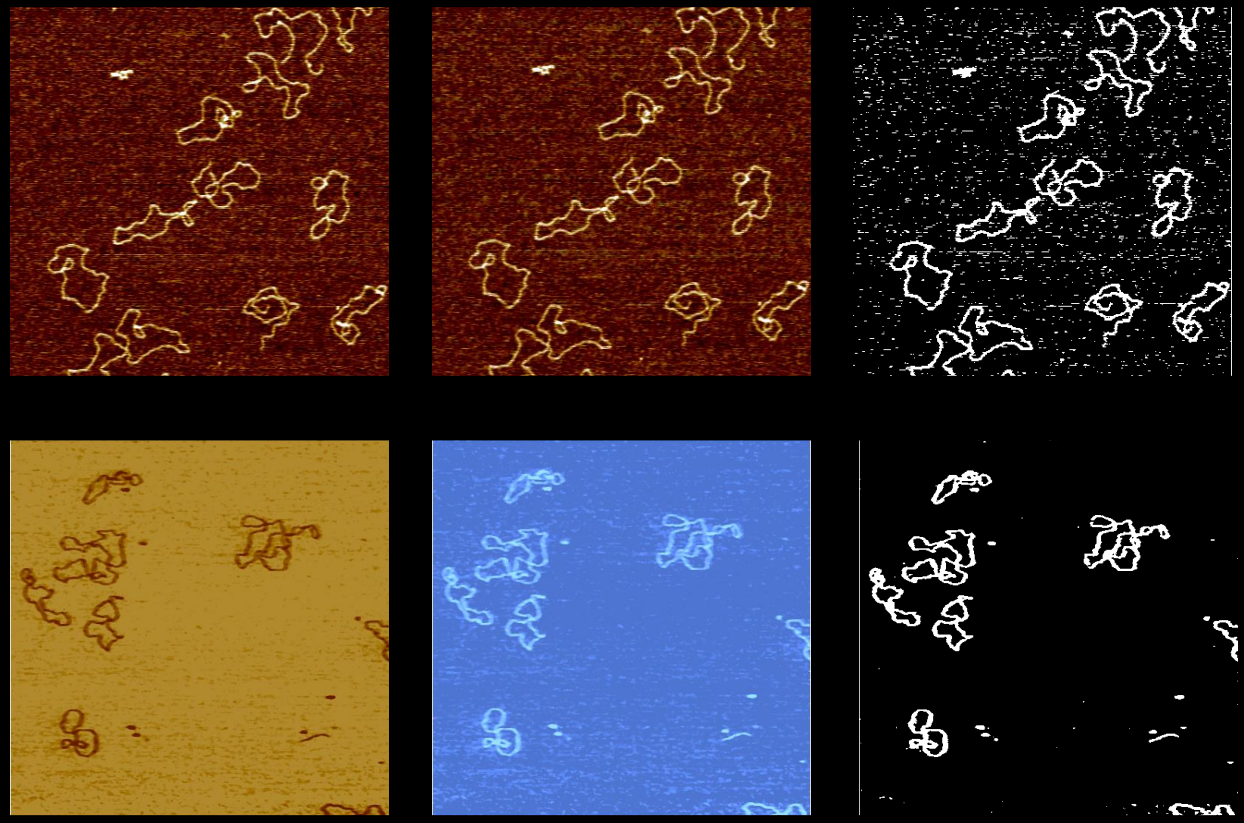

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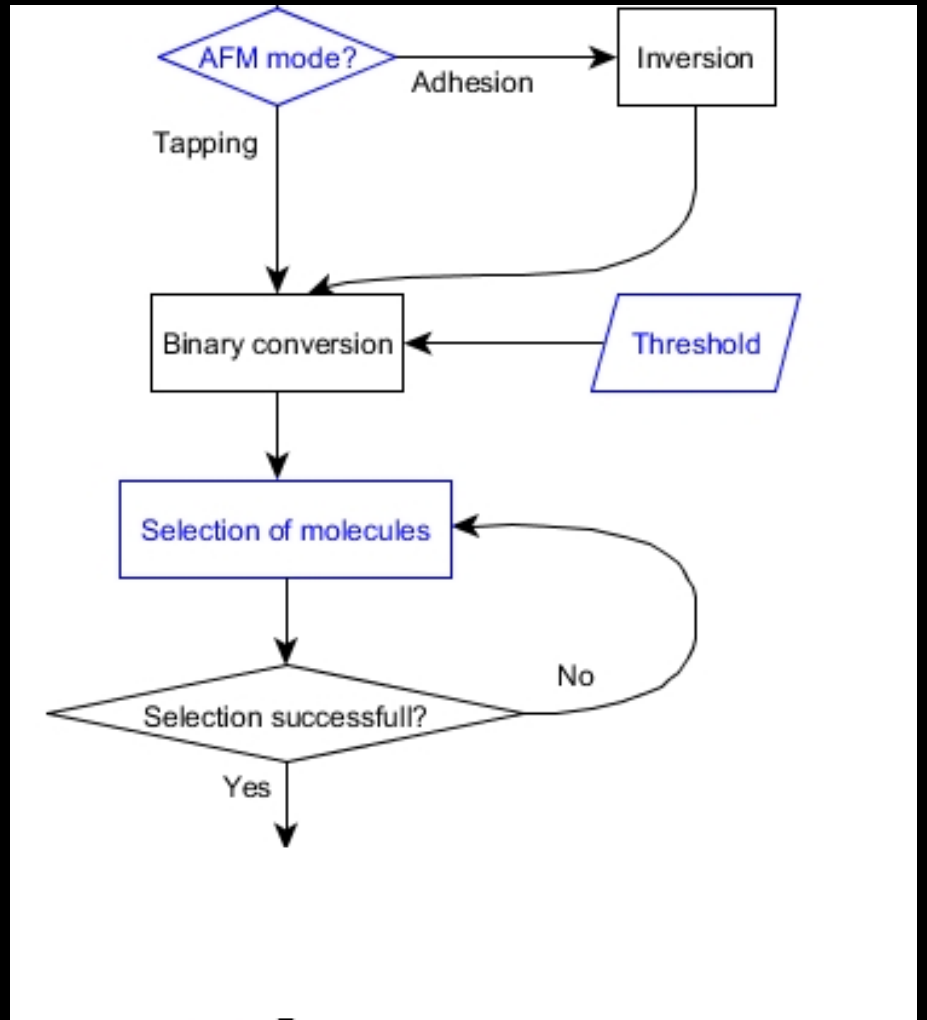

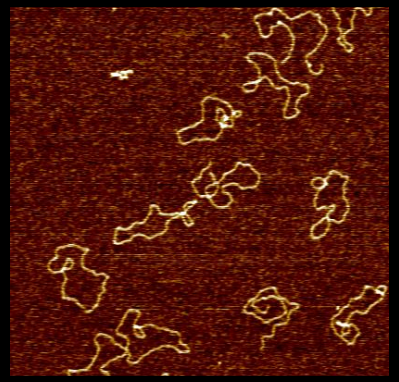

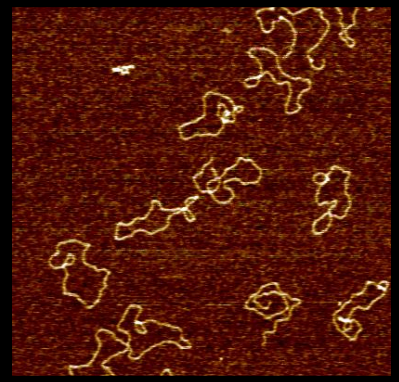

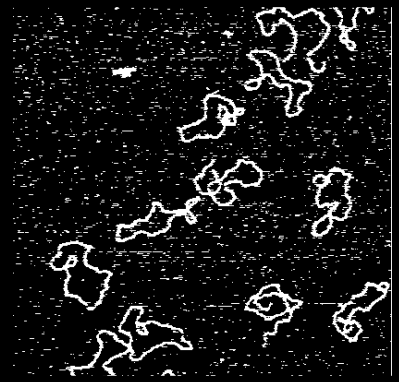

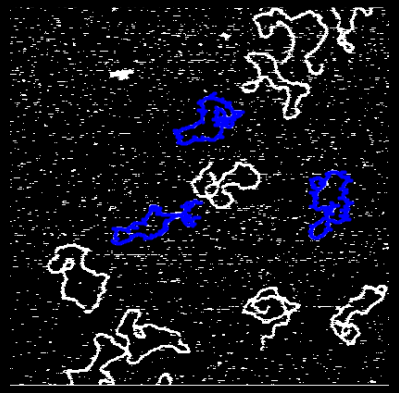

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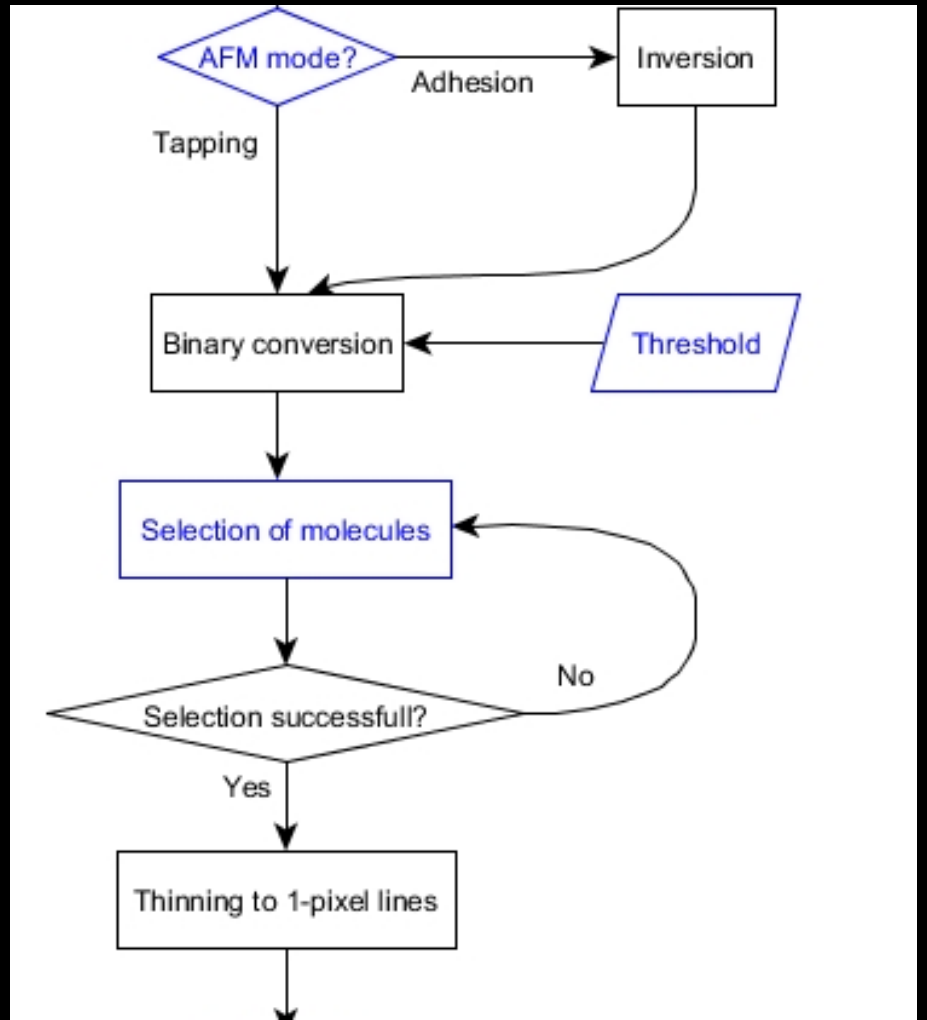
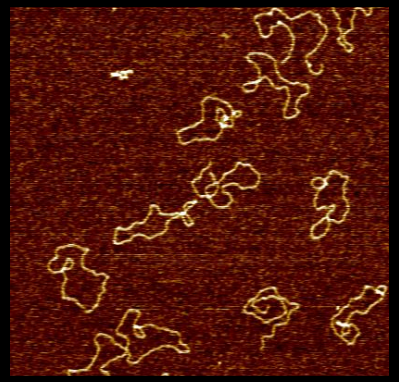
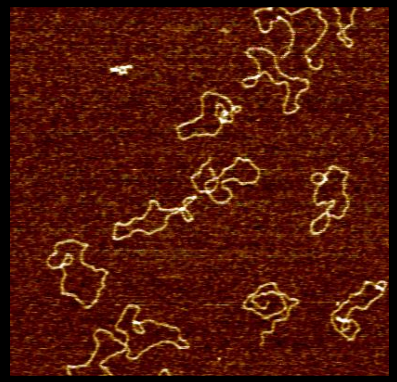
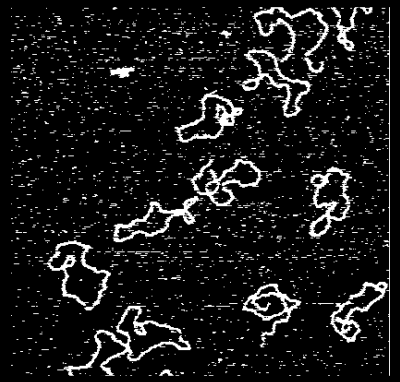
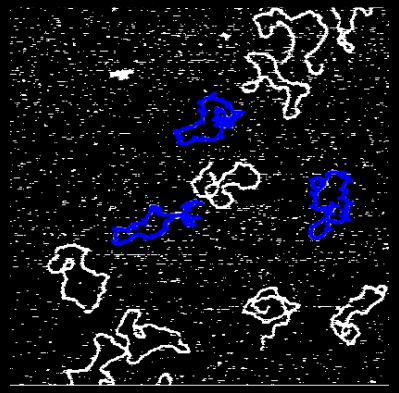








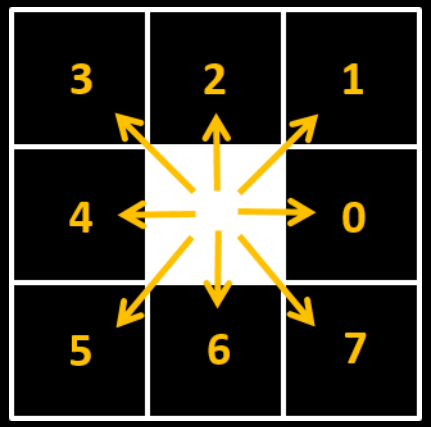


PROCESSING OF AFM IMAGES

Pixel length - calculation of the length of isolated pixels

- one-pixel line
- 8-connected Freeman chain code: *54555554554556677001O1*
- $n_{even} = 8$, $n_{odd} = 13$
- Freeman estimator:

$$L_{pixel} = n_{even} + \sqrt{2}\, n_{odd}$$

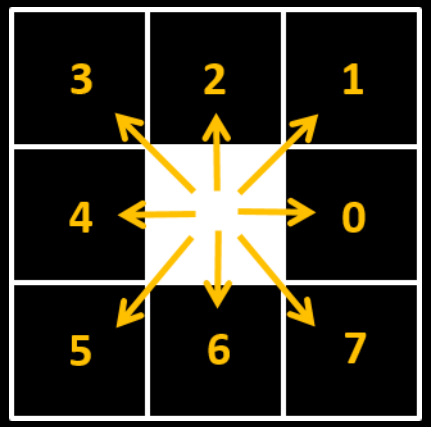


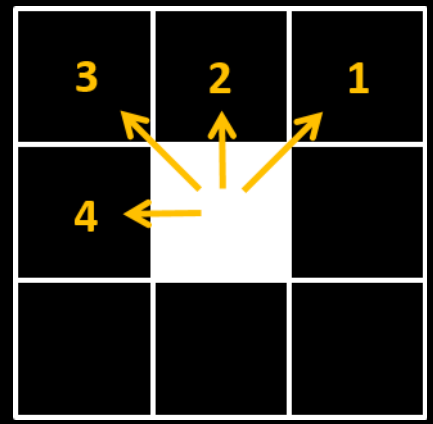
PROCESSING OF AFM IMAGES

Pixel length - calculation of the length of isolated pixels

- one-pixel line with crossings
- 4-connected Freeman chain code: *5455555454556677001 01*
- $n_{even} = 110$, $n_{odd} = 113$
- Freeman estimator:

$$L_{pixel} = n_{even} + \sqrt{2}\, n_{odd}$$





PROCESSING OF AFM IMAGES

Calibration - transformation of the pixel length to the real units

- crystallography studies: 1 bp (base pair) ~ 0,34 nm
- plasmid pBR322 has 4361 bp ~ 1482.74 nm

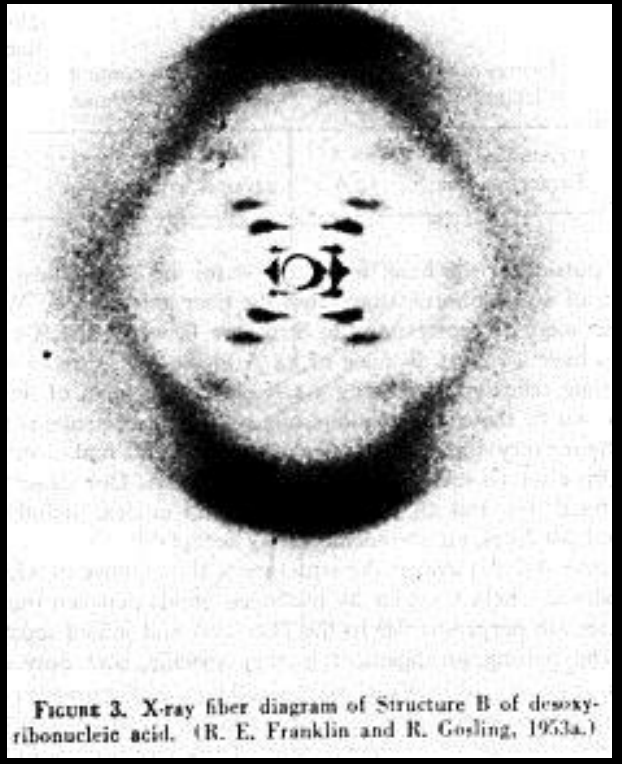
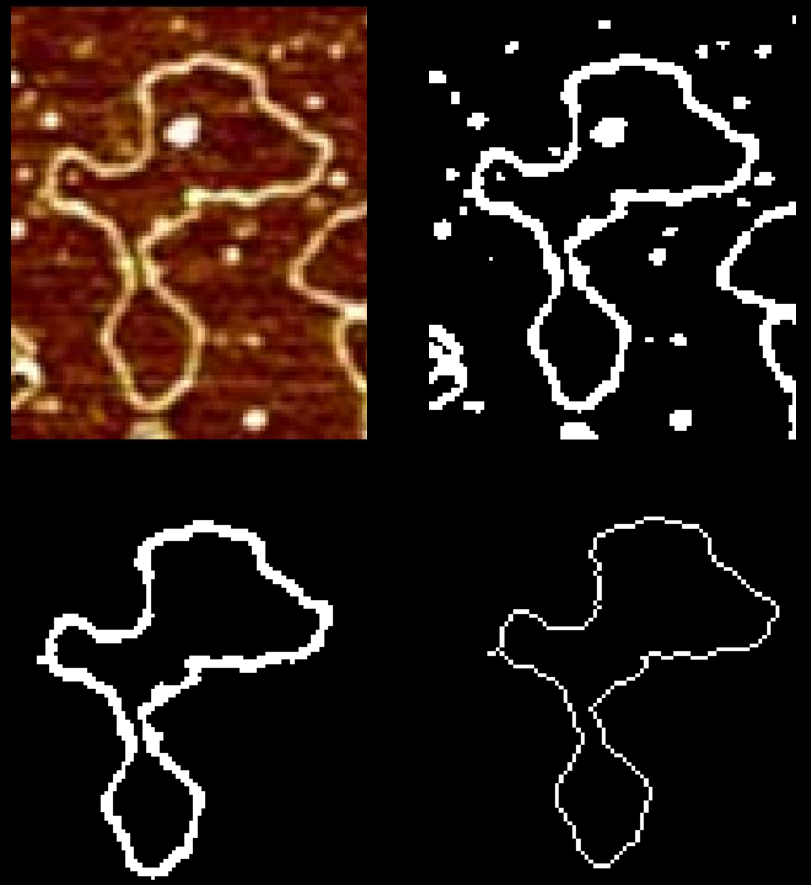


FIGURE 3. X-ray fiber diagram of Structure B of deoxyribonucleic acid. (R. E. Franklin and R. Gosling, 1953a.)

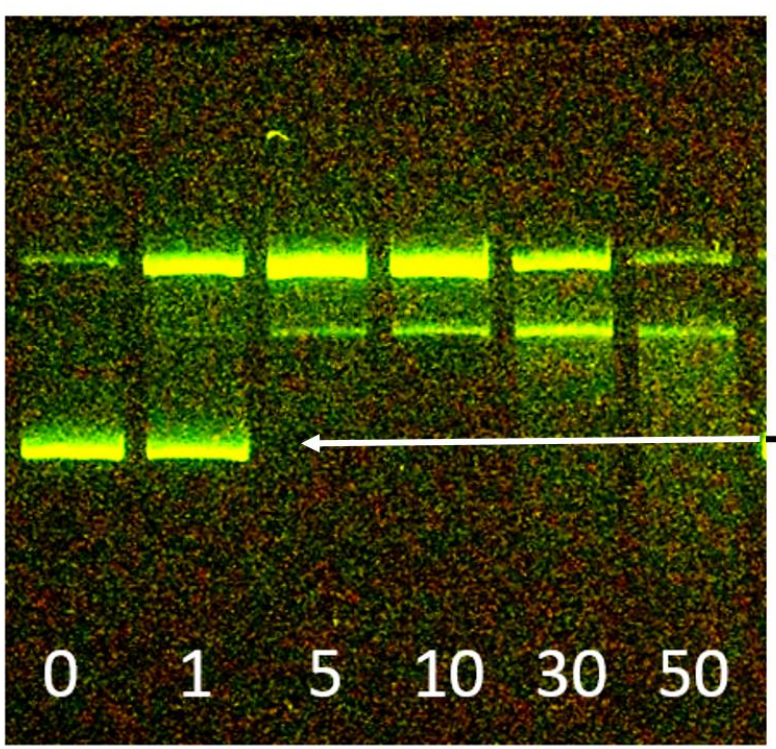
PROCESSING OF AFM IMAGES

Calibration - transformation of the pixel length to the real units

- crystallography studies: 1 bp (base pair) ~ 0,34 nm
- plasmid pBR322 has 4361 bp ~ 1482.74 nm
- estimation of pixel length for the molecule of full length (only circular, not linear – can be fragmented)



COMPARISON AGE AND AFM

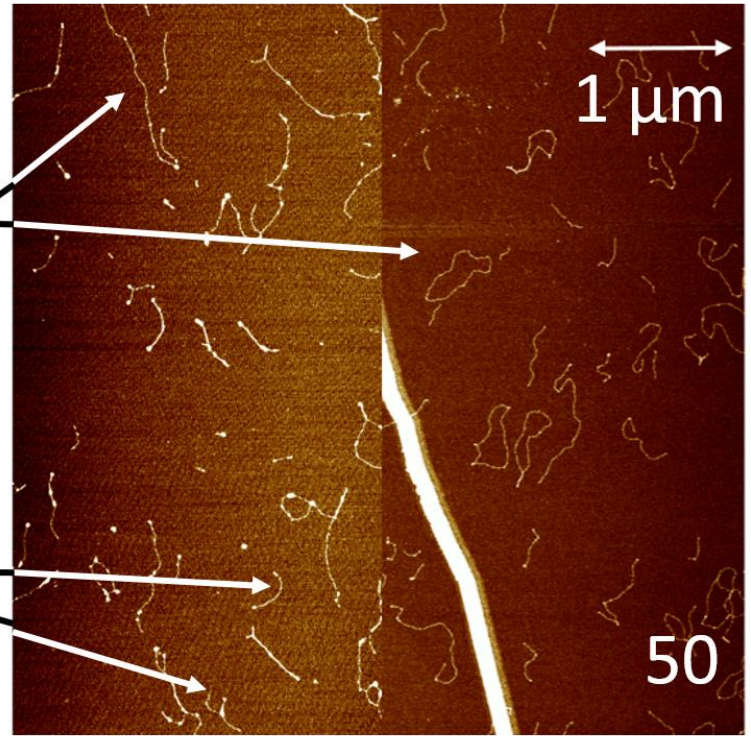


circular

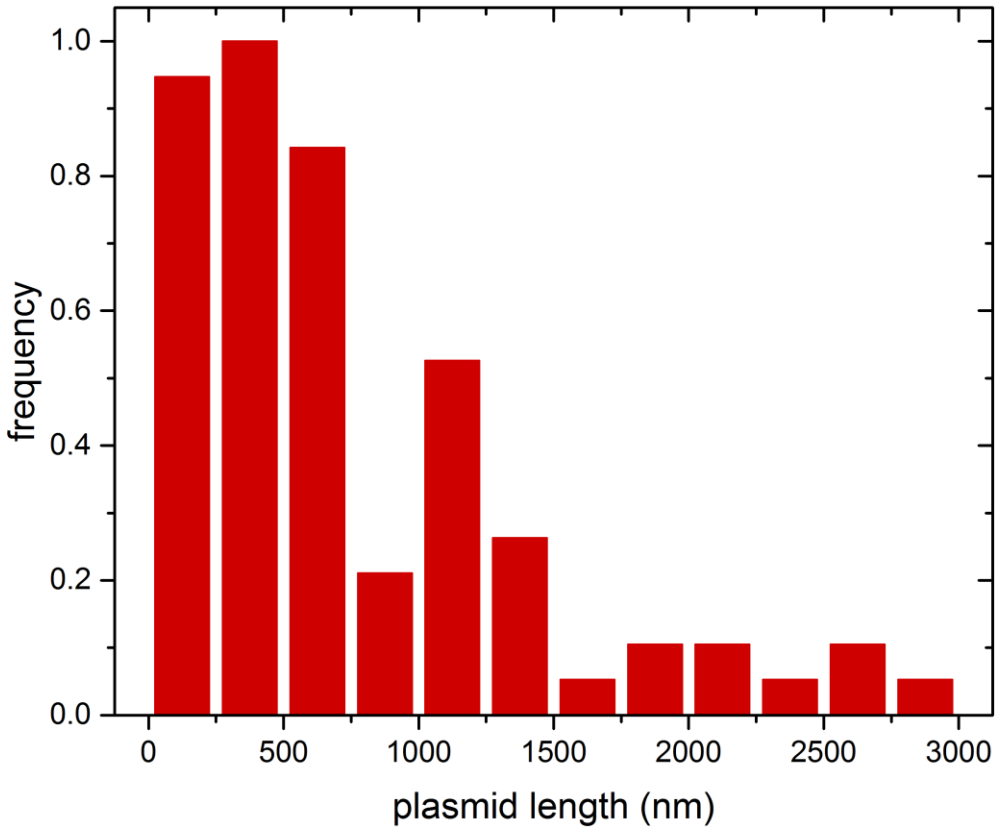
linear

supercoiled

fragments



REVEALED FRAGMENTATION



CONCLUSIONS

- Neglecting DNA fragments lead to probably large underestimation of clustered DNA damage
- Method is time consuming – analysis continues

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