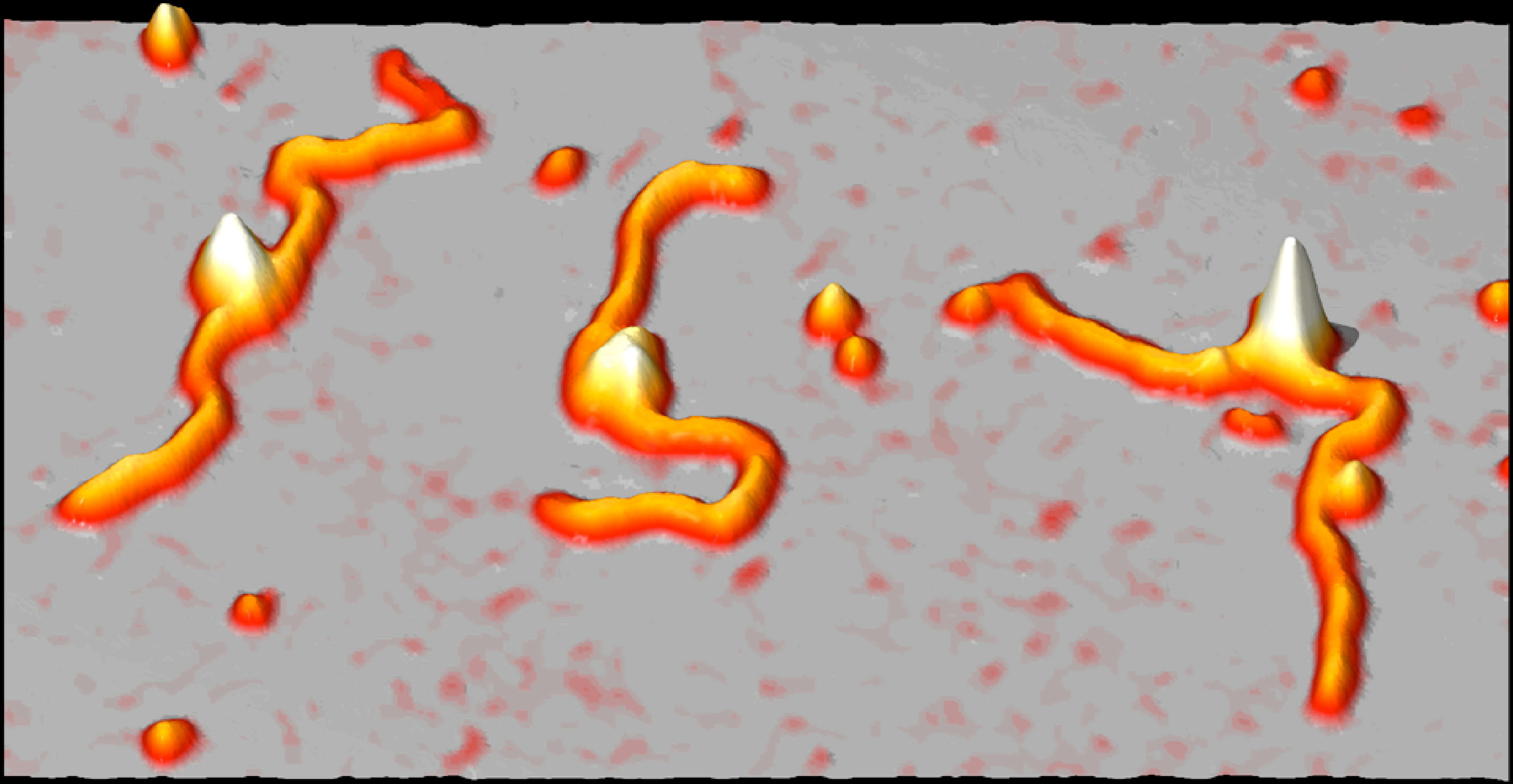
Scanning probe microscopy of protein-DNA complexes



Karsten Rippe, Kirchhoff-Institut für Physik, Molecular Biophysics Group
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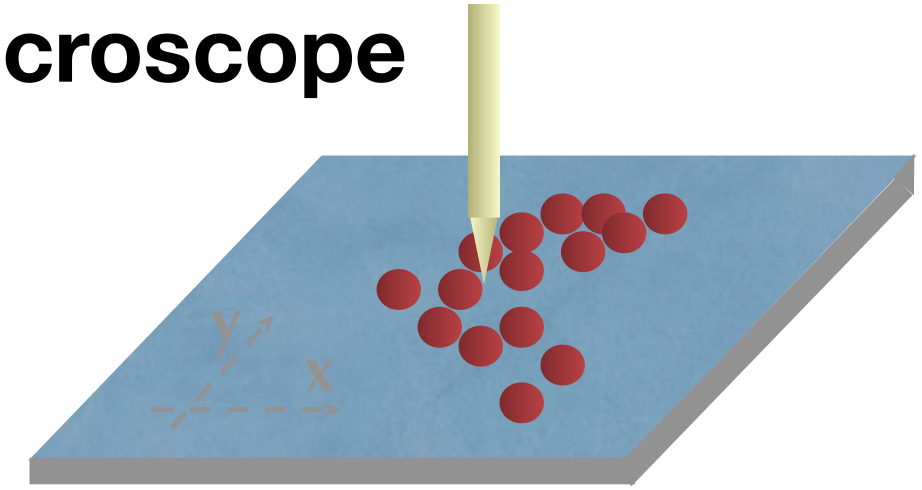
Scanning Probe Microscopy

STM: scanning tunneling microscope

tunneling of electrons between probe and surface

SFM/AFM: scanning/atomic force microscope

measuring of the force on the probe



MFM: magnetic force microscope

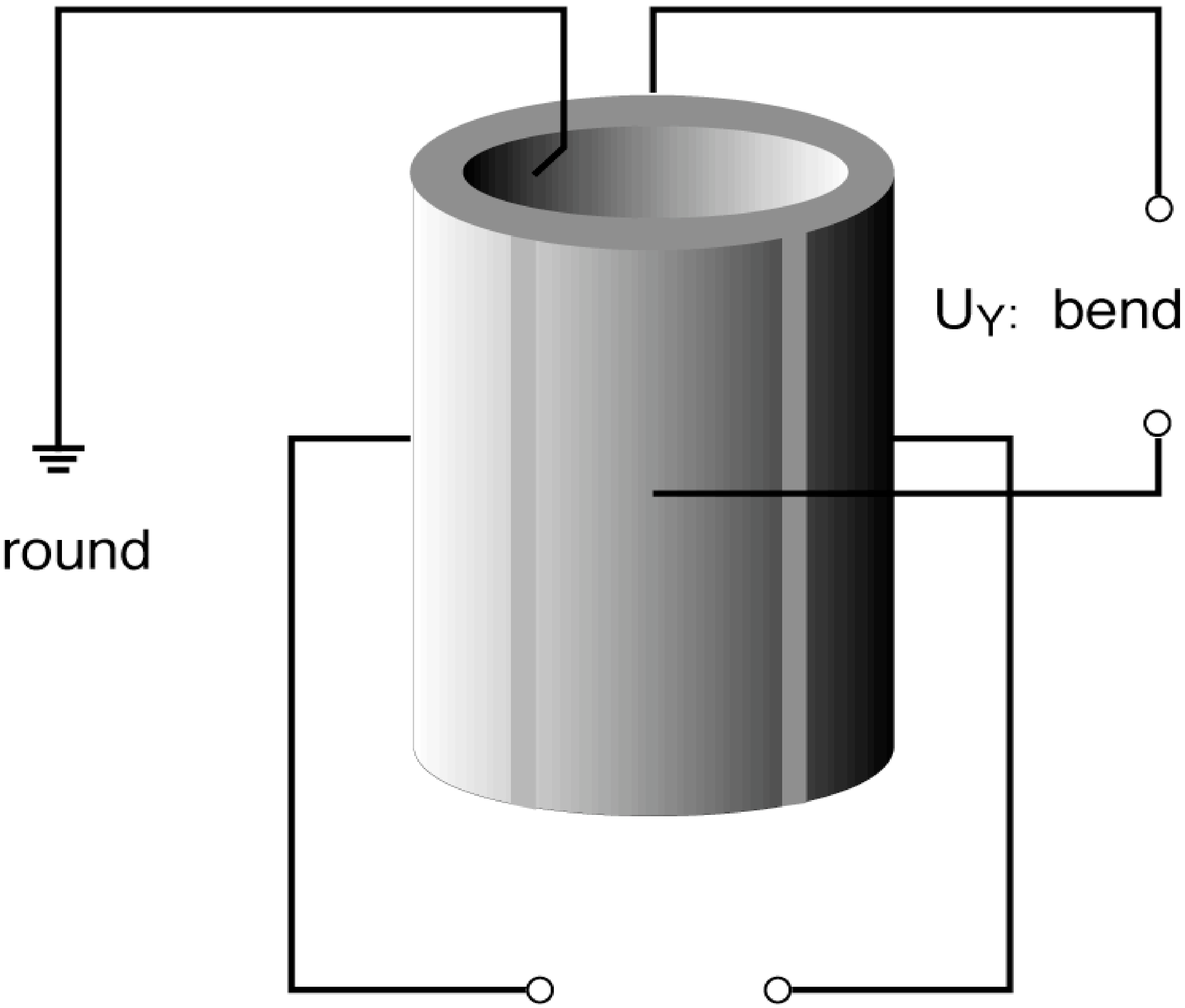
SFM with magnetical probe

SNOM: scanning near-field optical microscope

probe is a fiber; tunneling of photons

Piezo electric tube as used in SPM
1 mV -> 0.03 Å displacement!

U_Z: stretching in Z direction



U_Z-U_Y

U_Y: bending in Y direction

U_Z+U_Y

Ground

U_Z+U_x

U_Z-U_x

U_x: bending in X direction

Different Types of **S**canning **P**robe **M**icroscopes (**SPM**)

type	**probe**	**signal**	**resolution**	**samples**	**environment**	**application**
STM	conductive tip	tunneling current	1-2 Å	solid conductor	air, fluid	semi conductor metals, DNA (?)
SFM / AFM	flexible	tip-sample force	2-4 Å height 50-80 Å lateral	solid surfaces	air, fluid	multiple
SNOM	sharp wave guide	photon flux	200-400 Å	multiple	air, fluid	multiple
SICM	micro-pipette	ion flux	300 Å	surface	ionic fluid	porous membranes biomembranes
SECM	micro-electrode	faradaic current	~1000 Å	surface	fluid	electro-chemistry
STPM	micro-thermo couple	heat flux	~1000 Å	survace	air, fluid	multiple

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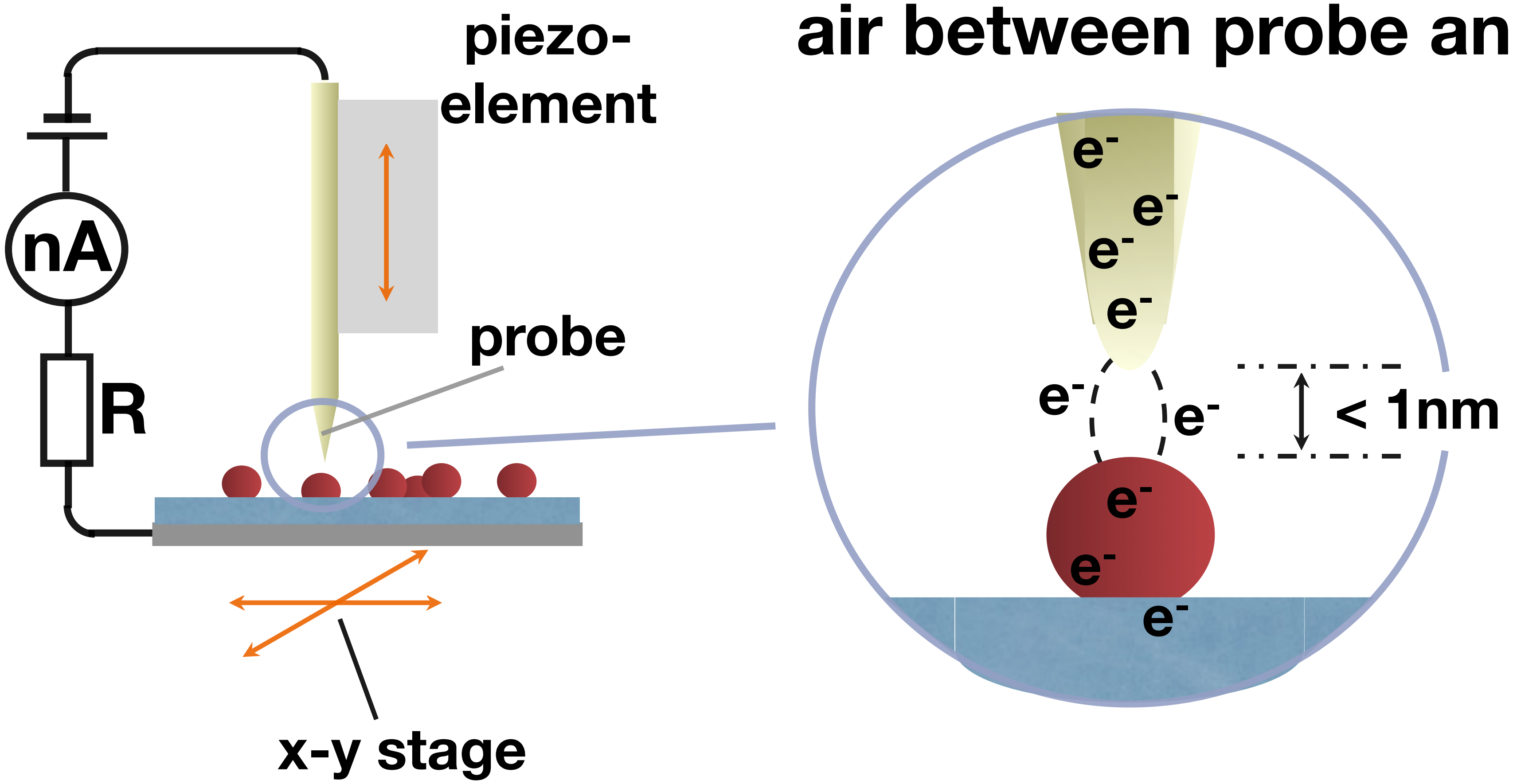
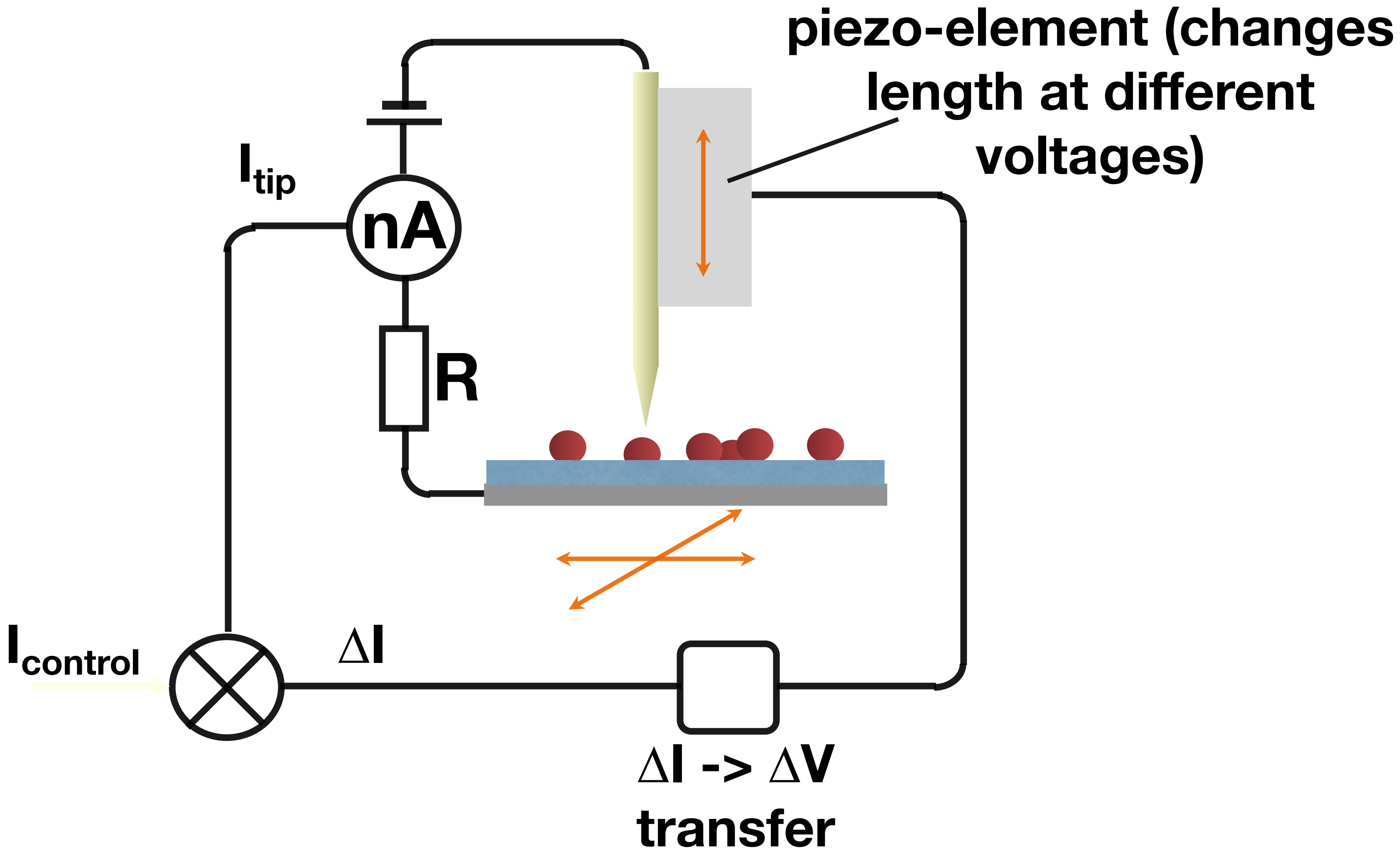
STM: scanning tunneling microscope

tunneling of electrons through air between probe and surface

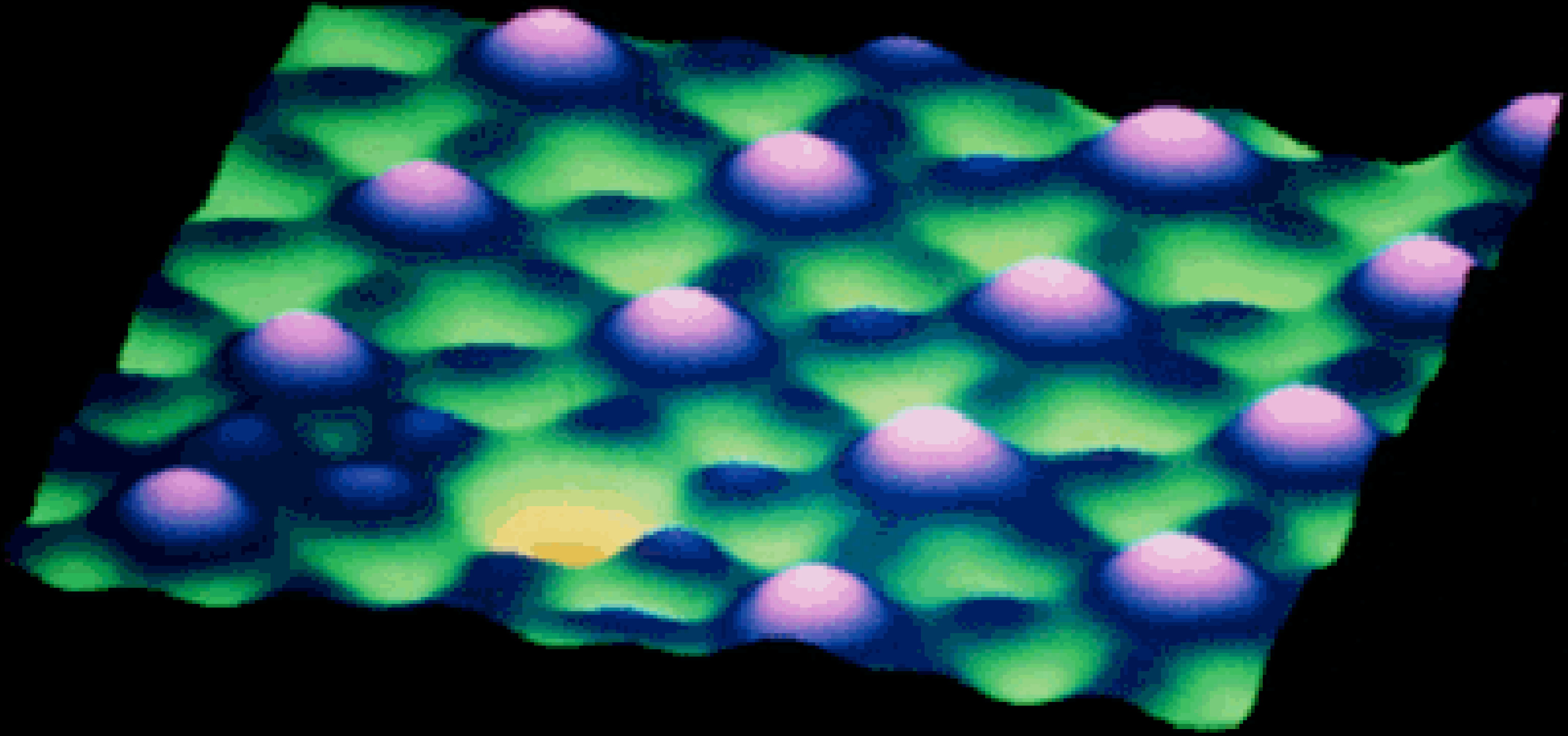


only conducting material

STM: scanning tunneling microscope



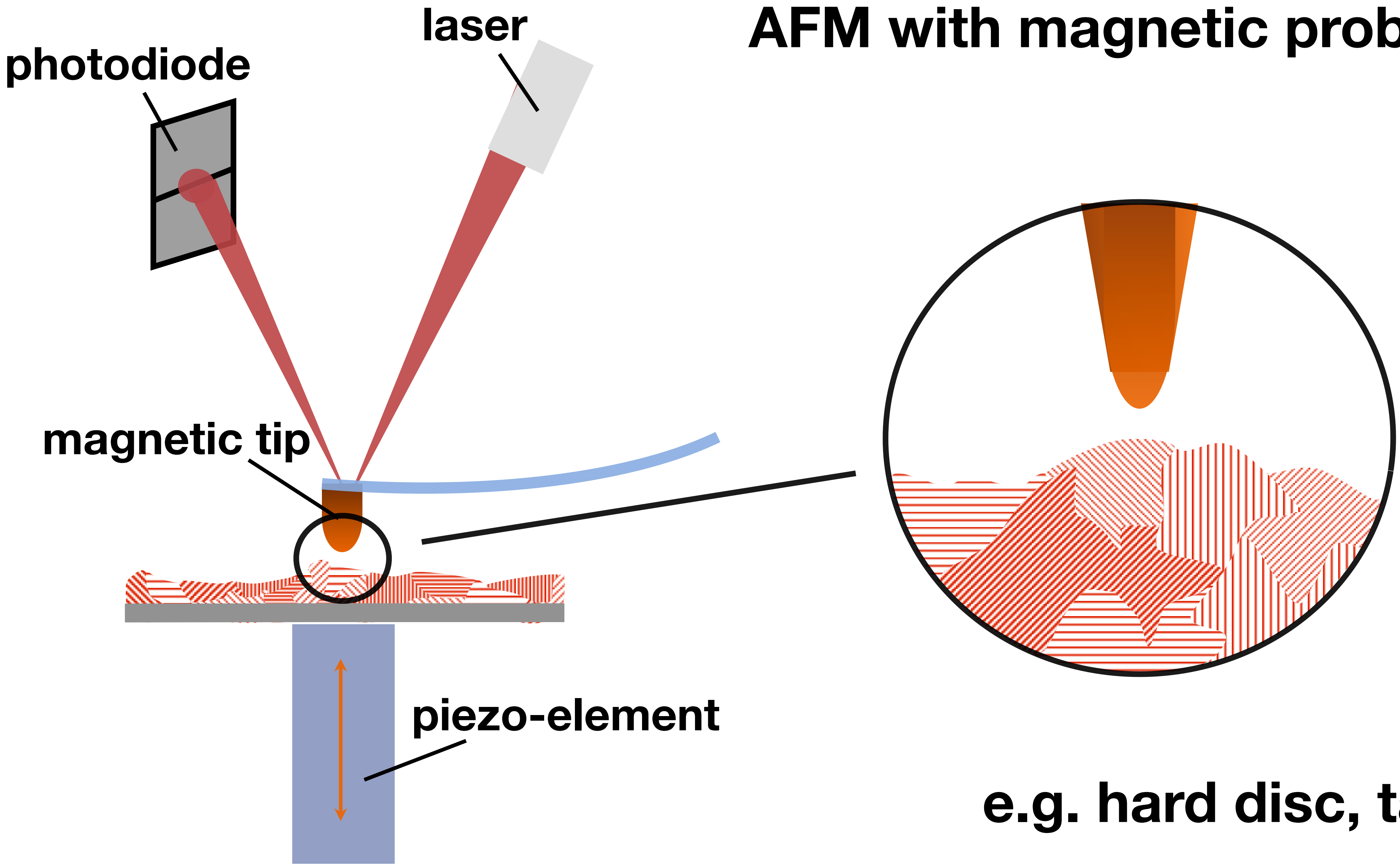
STM image showing single-atom defect in iodine adsorbate lattice on platinum



2.5 x 2.5 nm scan

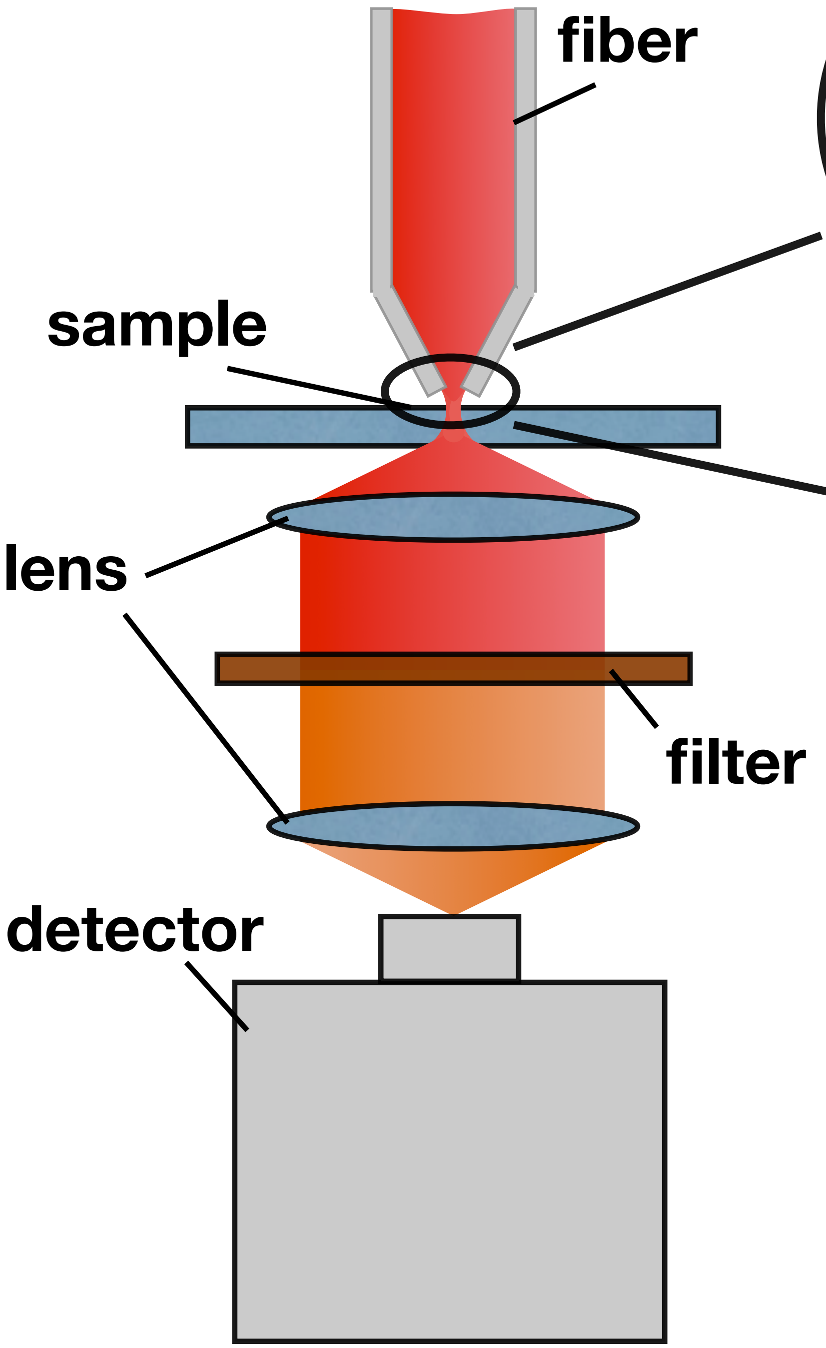
MFM: Magnetic Force Microscope

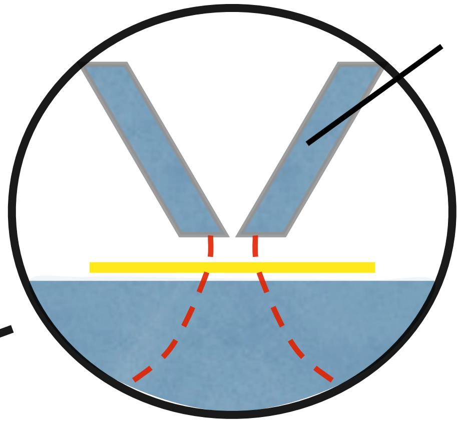
AFM with magnetic probe



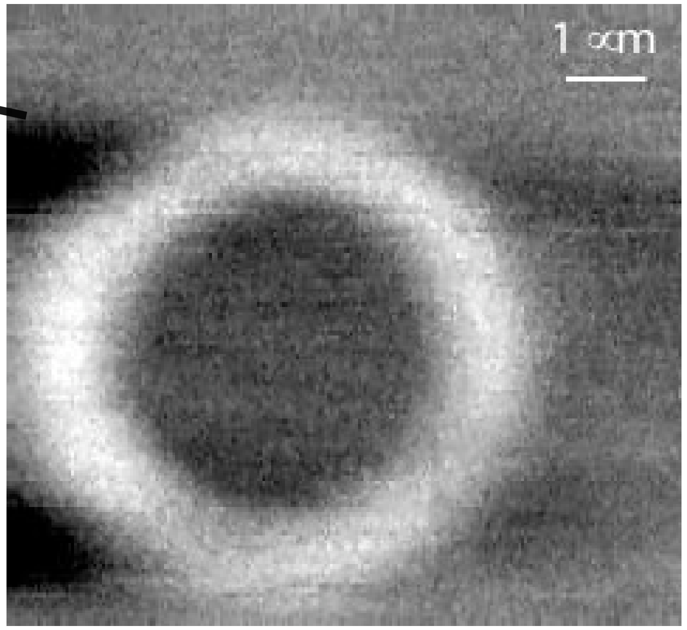
e.g. hard disc, tape

SNOM: Scanning Near-field Optical Microscope





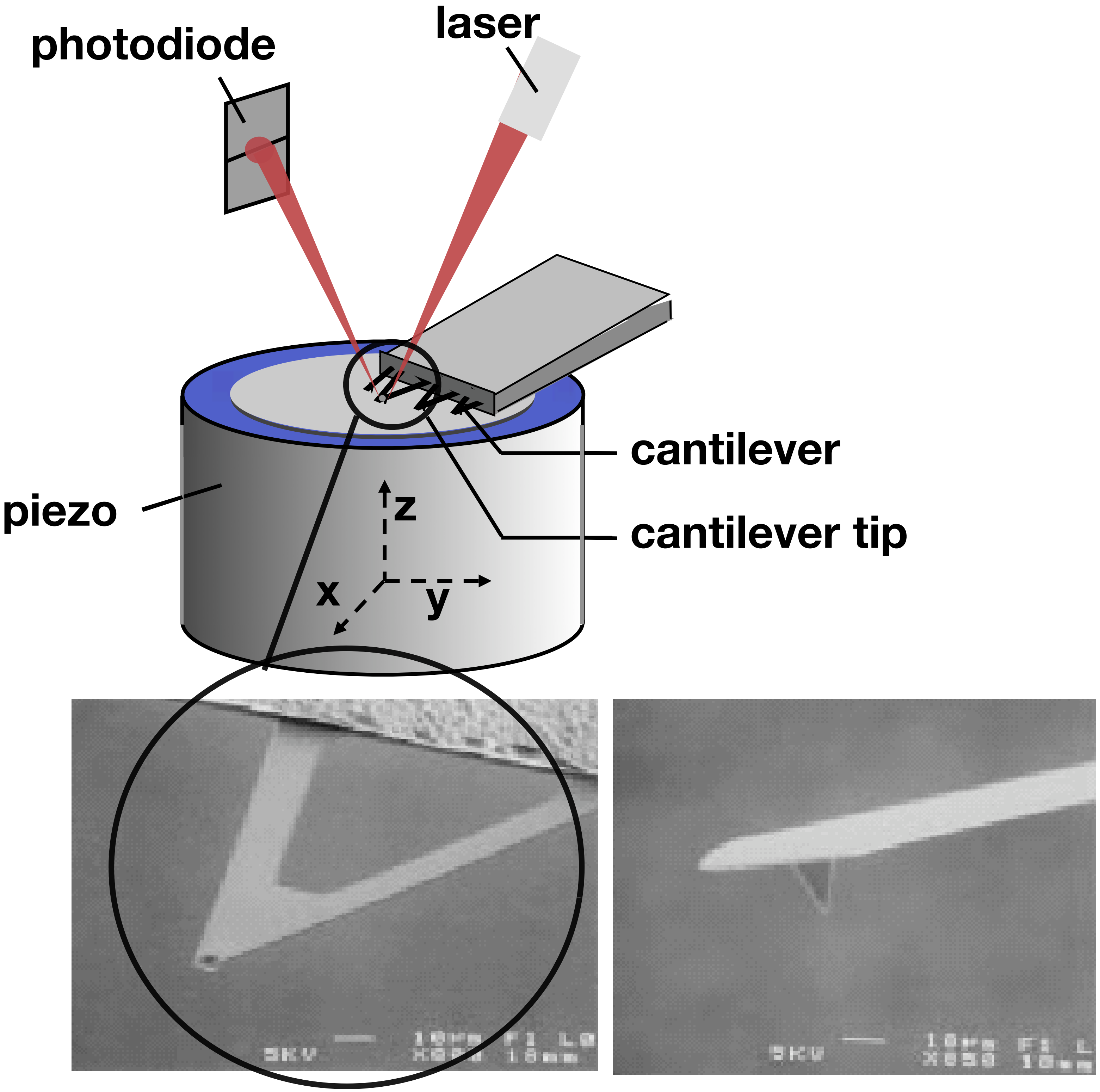
tunneling of photons between probe and surface

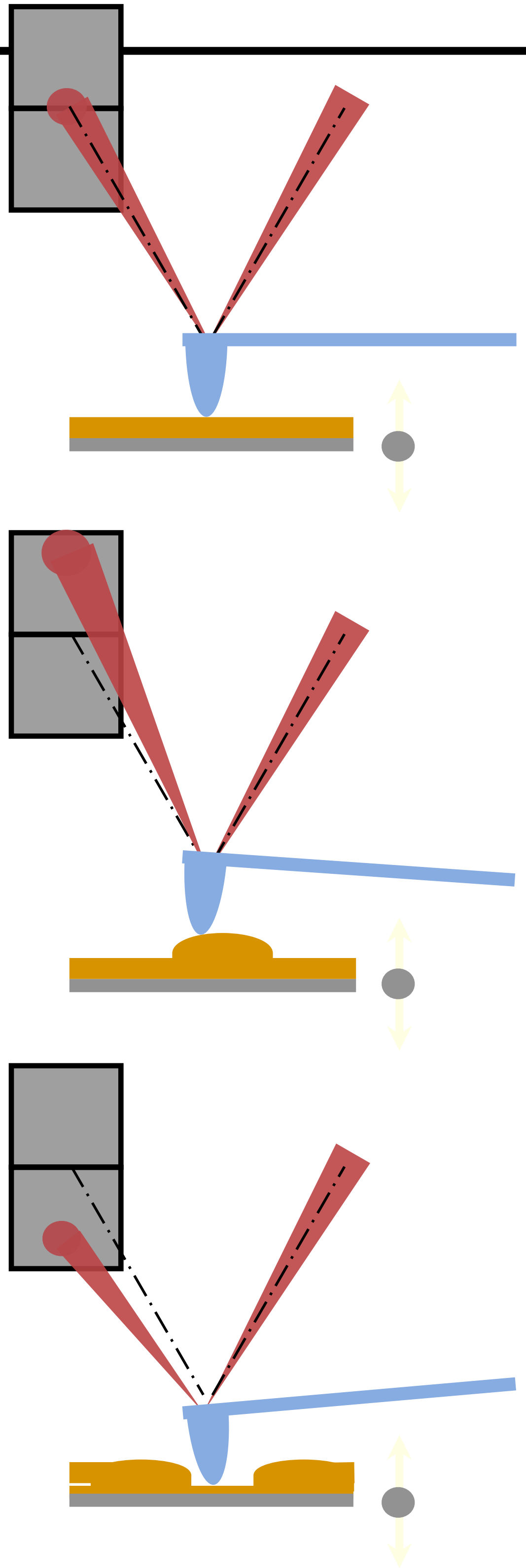


shows the amount of light that is absorbed/ transmitted for different colors

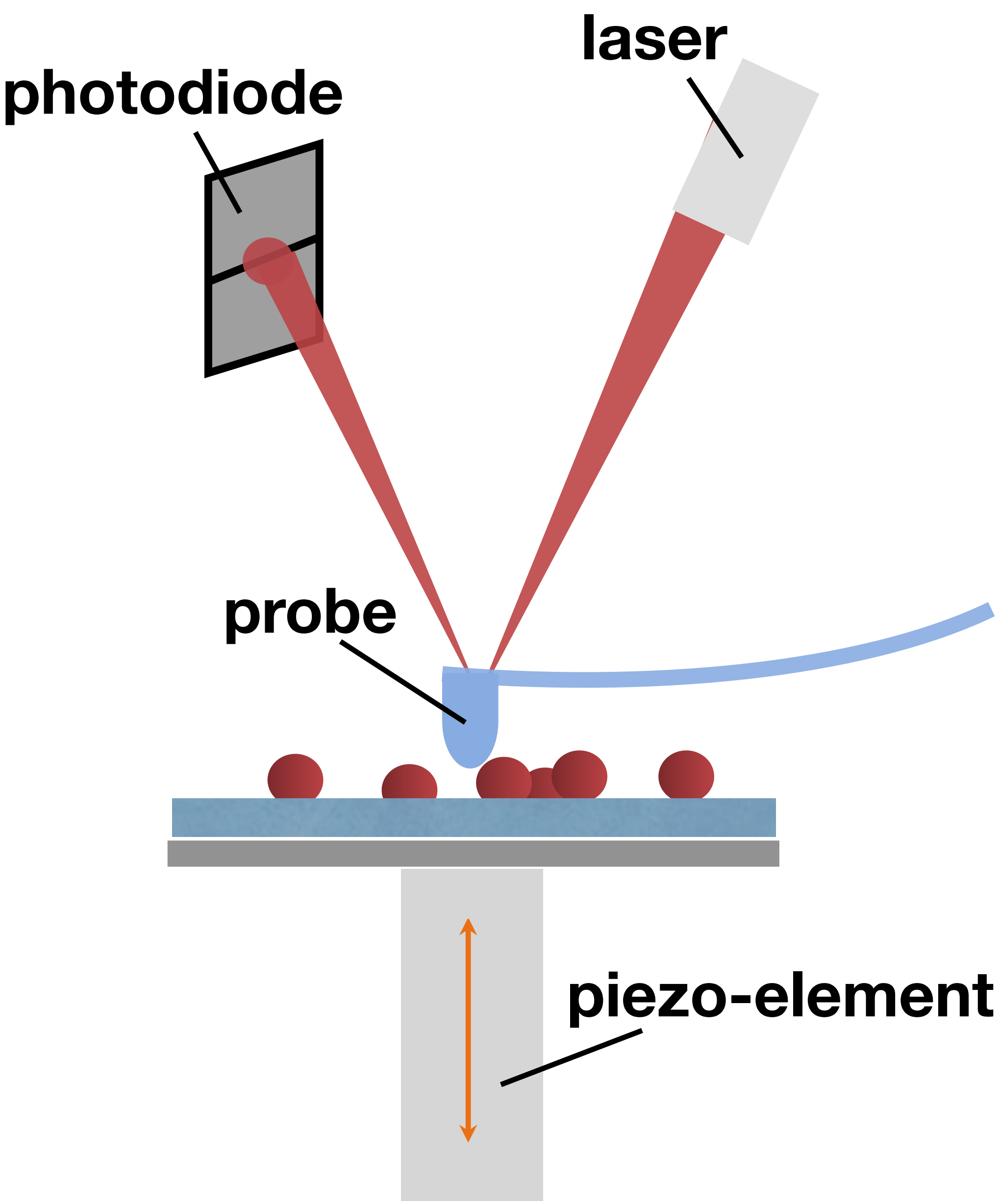
e.g. fluorescent molecules

Scanning/Atomic Force Microscope (SFM/AFM)

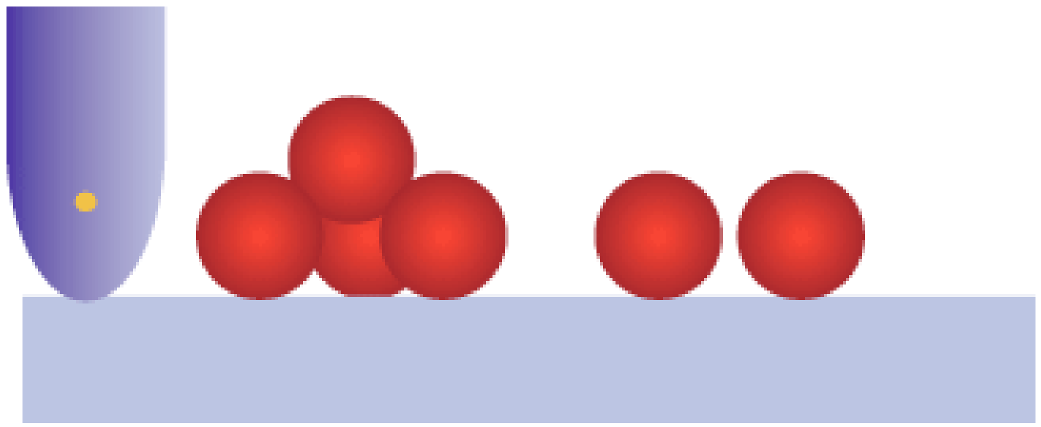




SFM/AFM: Scanning/Atomic Force Microscope

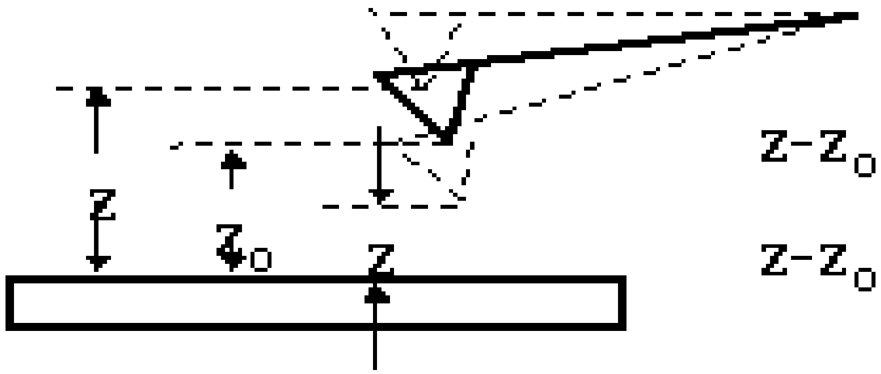


SFM probe scans over the surface (in contact)



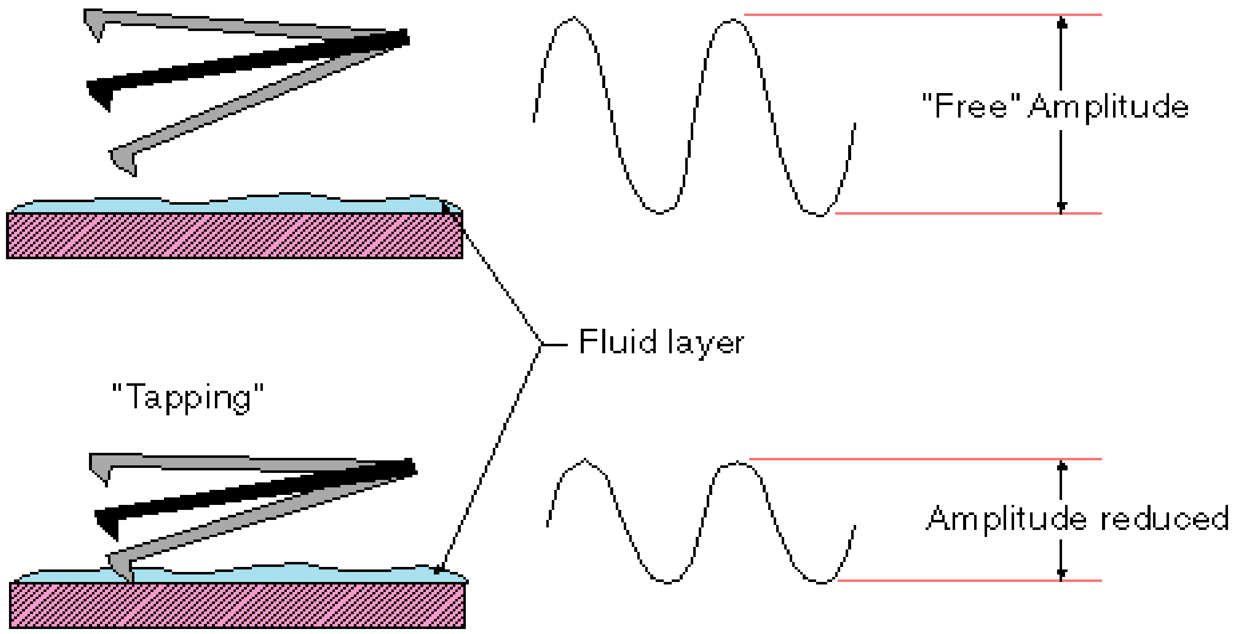
e.g. DNA, proteins, chromatin fibers, membranes, living cells, etc.

SFM imaging in “tapping mode“

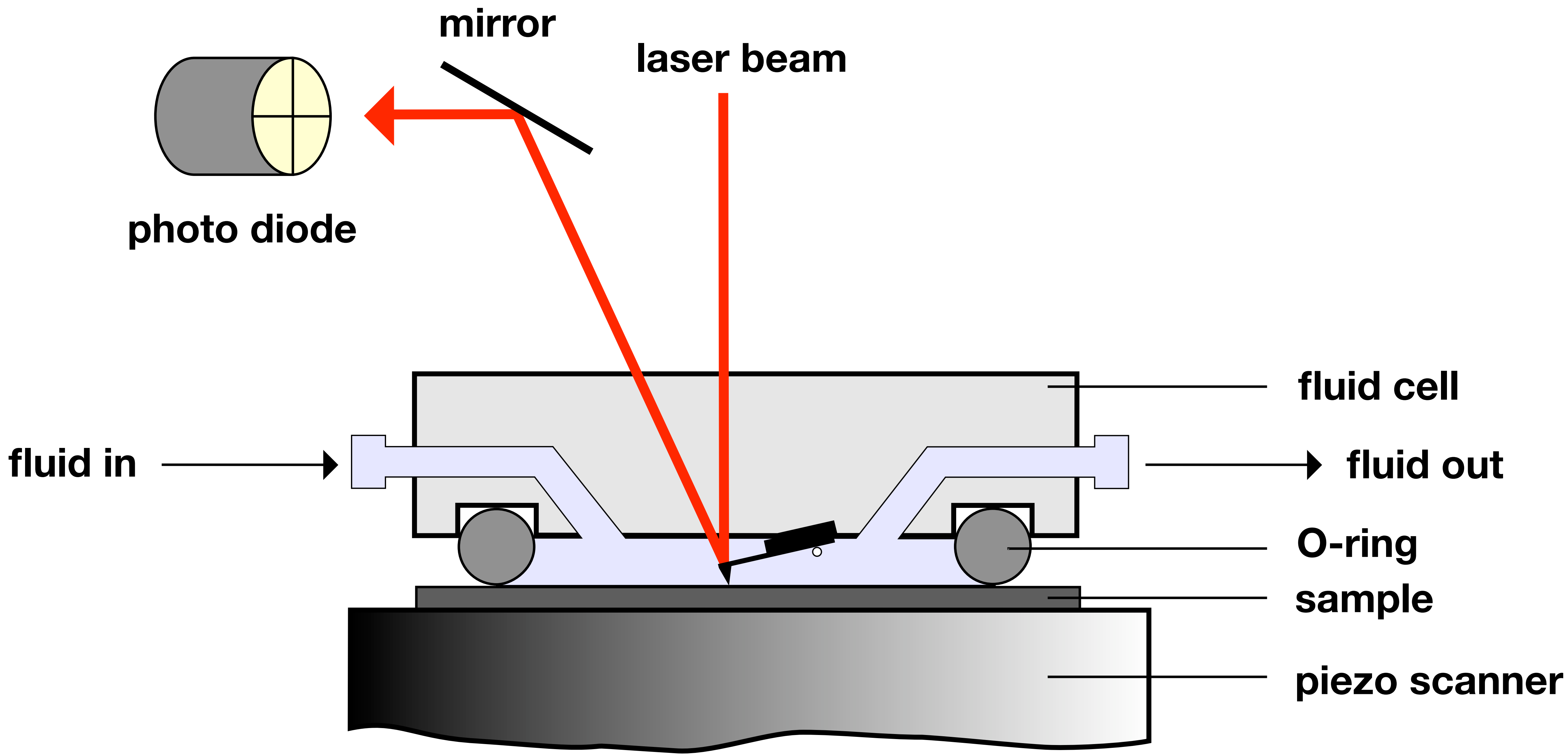


$z-z_0 > 0,\ F<0$

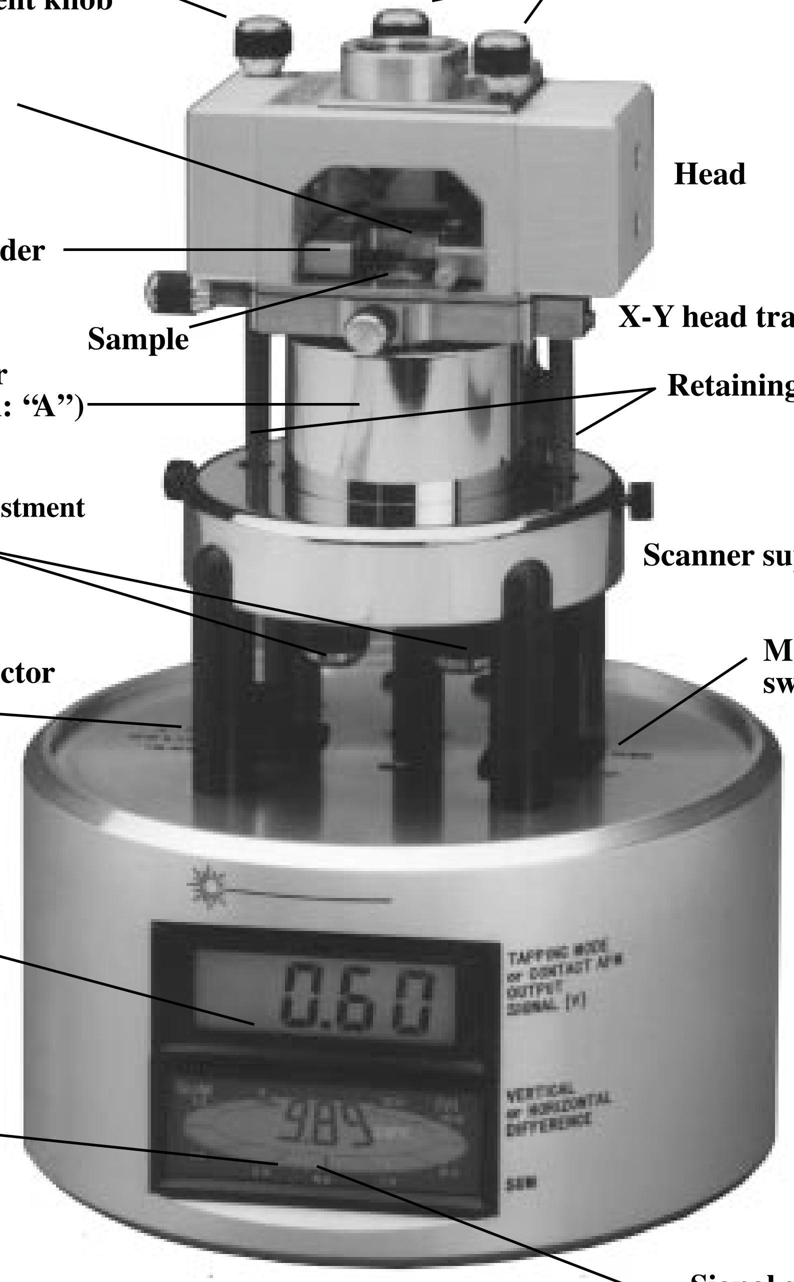
$z-z_0 < 0,\ F>0$



Scanning/atomic force microscope (SFM/AFM)



MultiMode SPM (Digital Instruments)



Photodiode adjustment knob

Laser adjustment knobs

SPM tip

Head

Tipholder

X-Y head translator

Sample

Scanner (Shown: "A")

Retaining springs

Coarse adjustment screws

Scanner support ring

Mode selector switch

Motor control switch

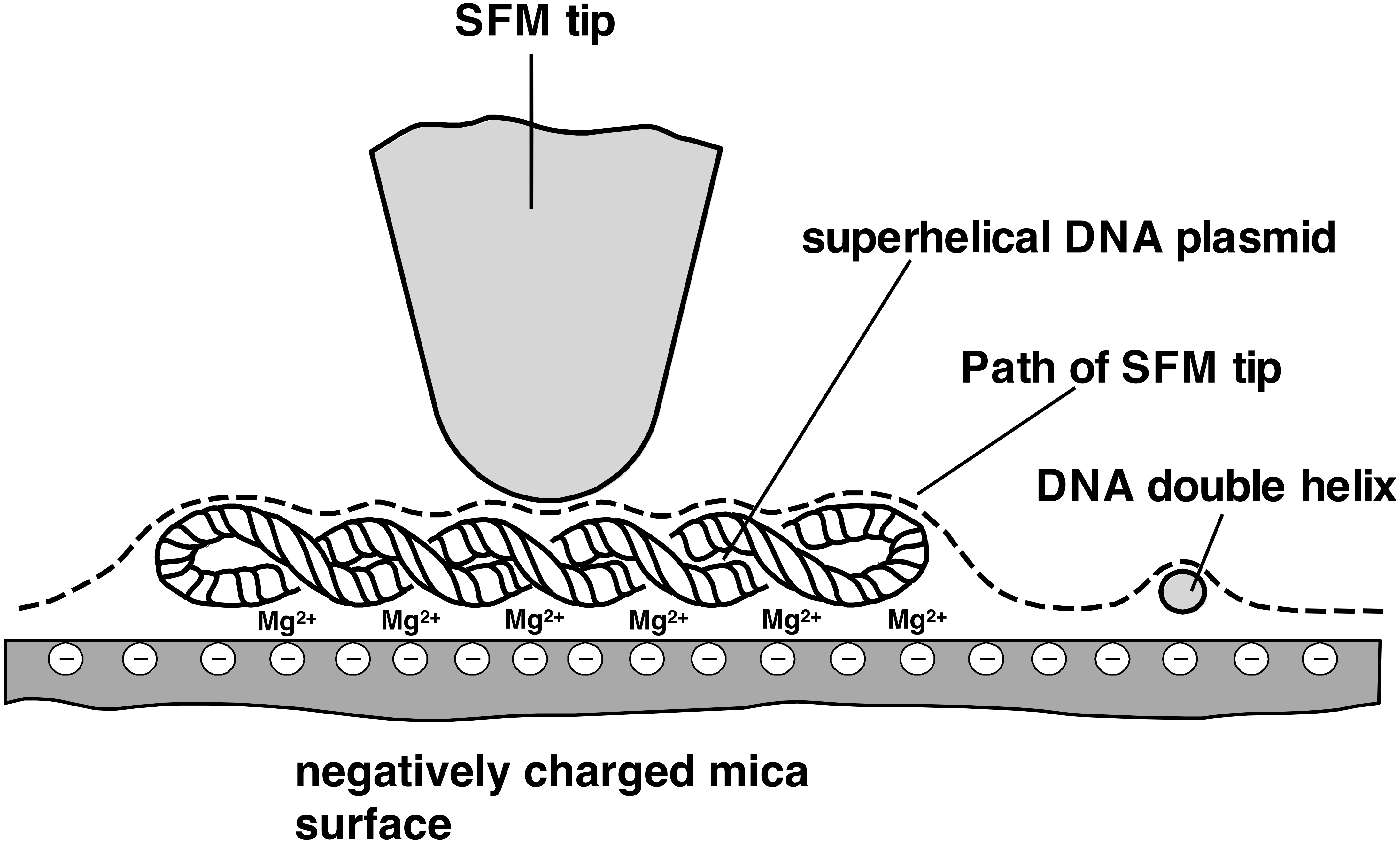
$\frac{A-B}{A+B}$ display

Base

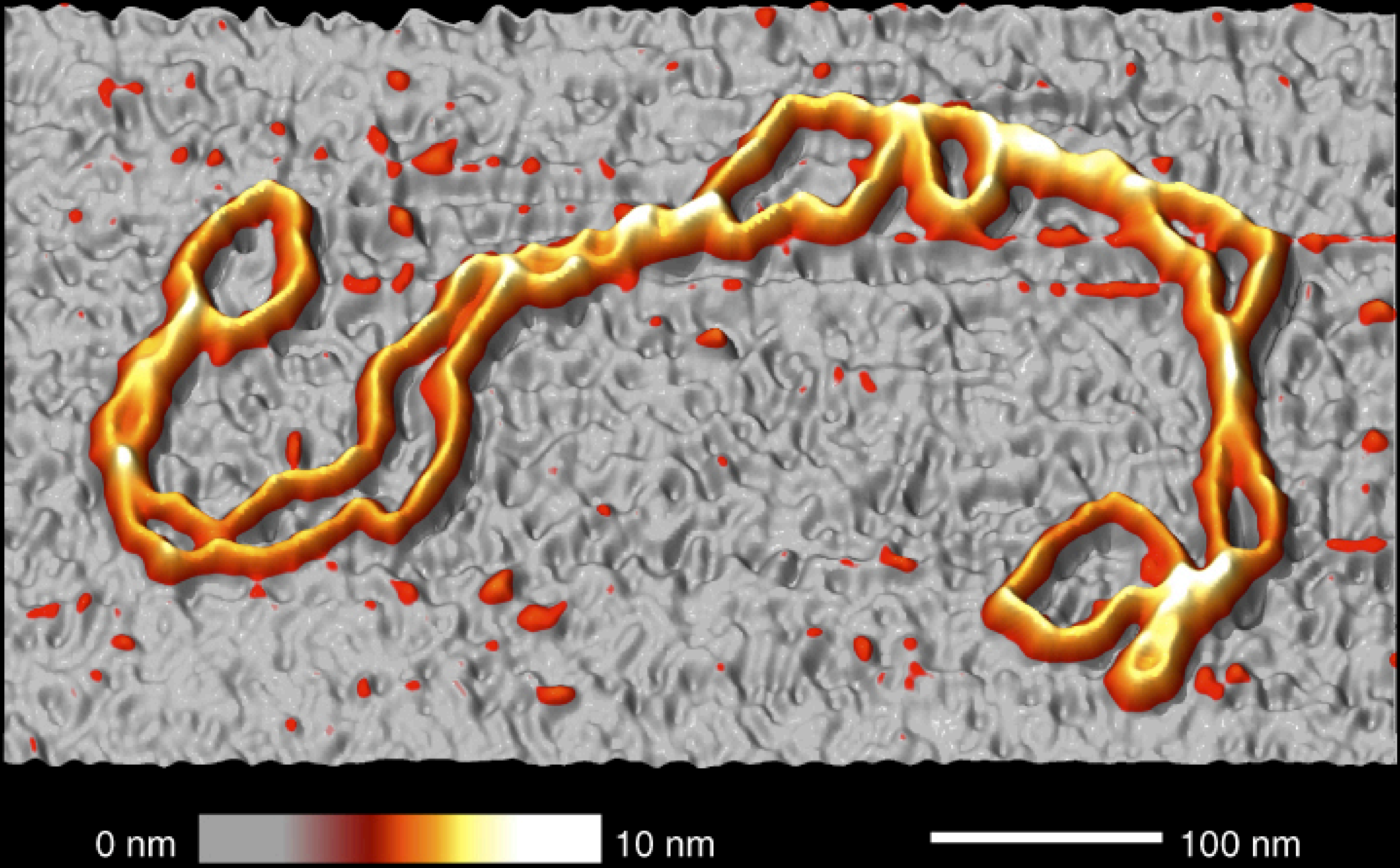
$\frac{(A+C)-(B+D)}{A+B+C+D}$ display

Signal sum display (elliptical)

Movement of the SFM tip along the sample



SFM image of a 6.8 kb superhelical plasmid

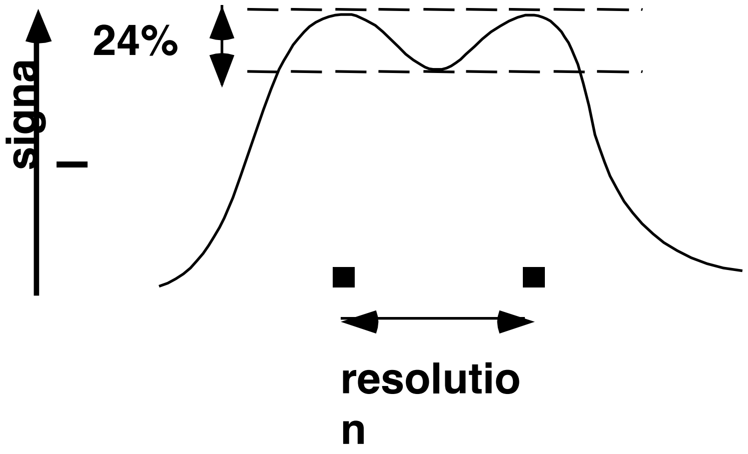


Performance of a microscope

magnification

$$M = \frac{\text{image size}}{\text{object size}}$$

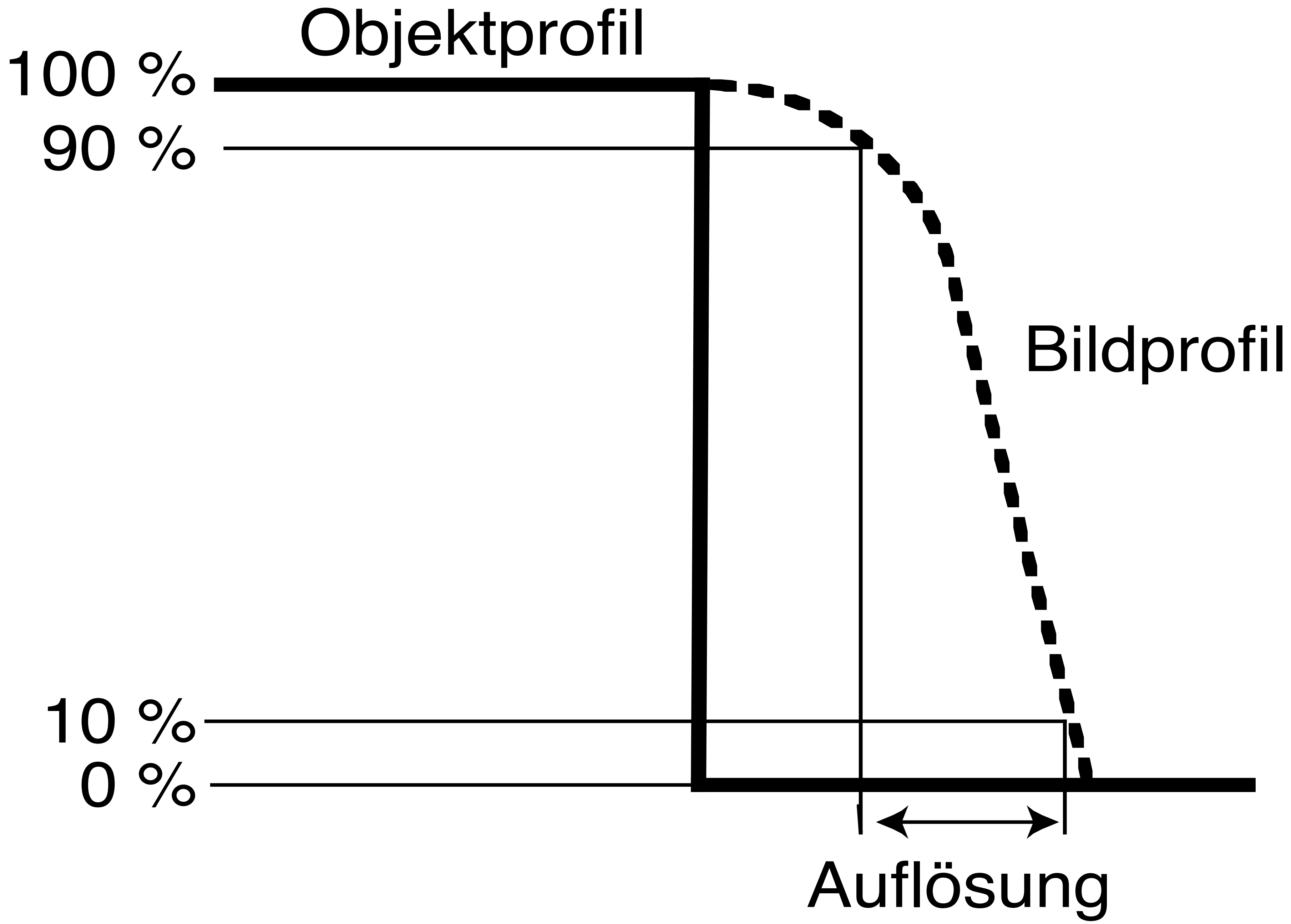
resolution



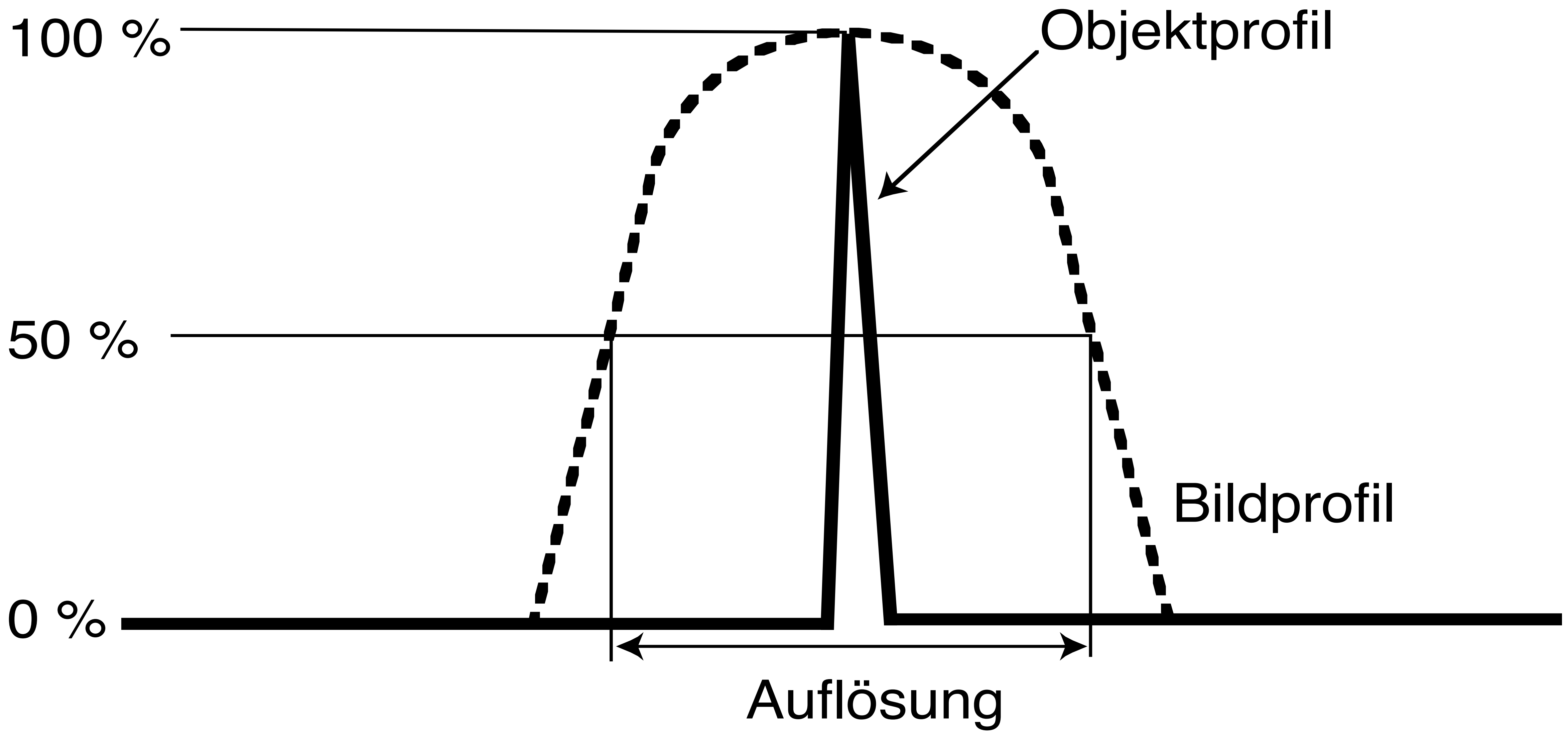
contrast

$$K = \frac{\text{object signal} - \text{background signal}}{\text{background signal}}$$

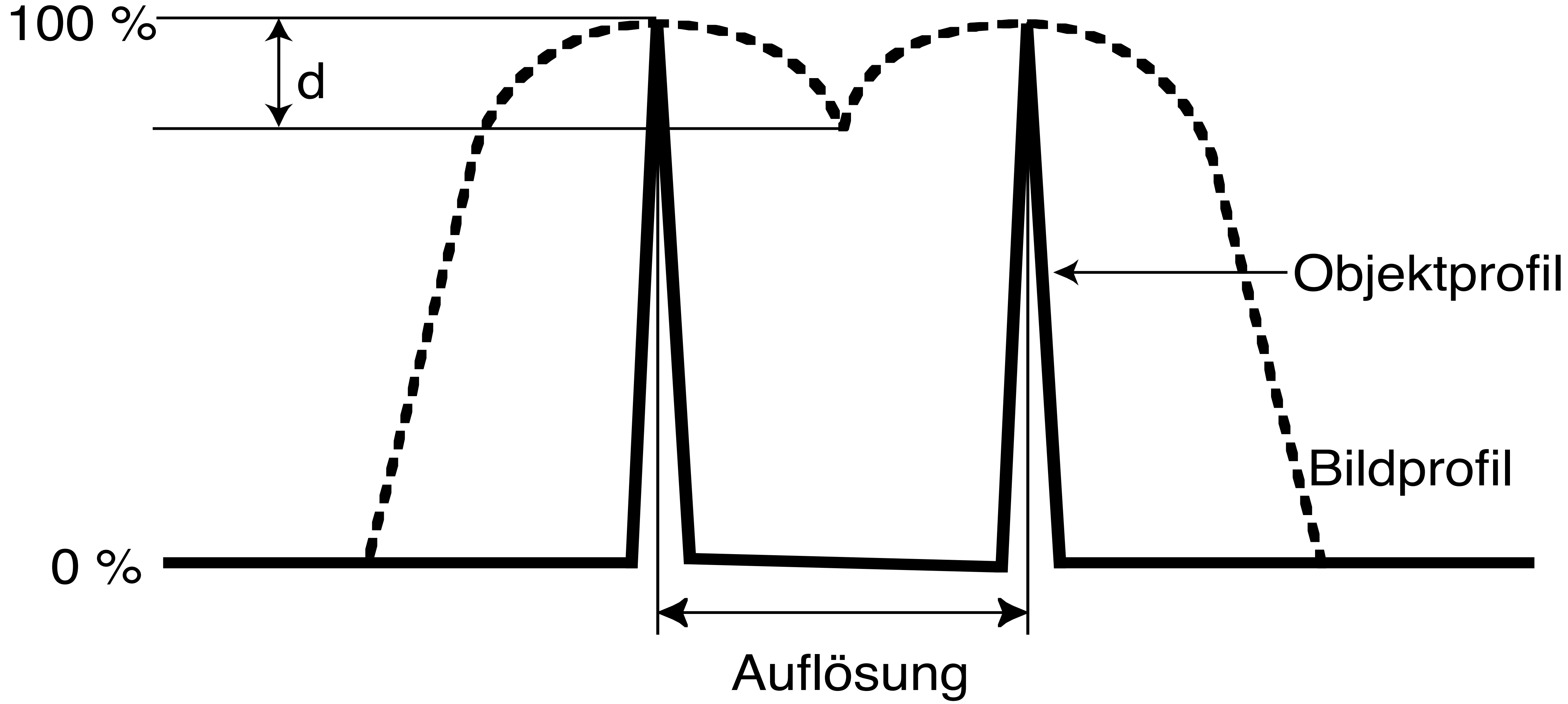
SFM resolution criteria - part 1



SFM resolution criteria - part 2



SFM resolution criteria - part 3



What limits the resolution in SFM - Vibrational Noise

- mechanical
- acoustical
- thermal

mechanical and acoustic noise:
=> building the SFM stiff with high frequency resonances
=> avoid resonance frquencies below ~10 kHz
=> put SFM on an isolation system that is soft with resonance frequencies below 1-2 Hz
=> e.g. bungee-cord/concrete block

What limits the resolution in SFM, cont.

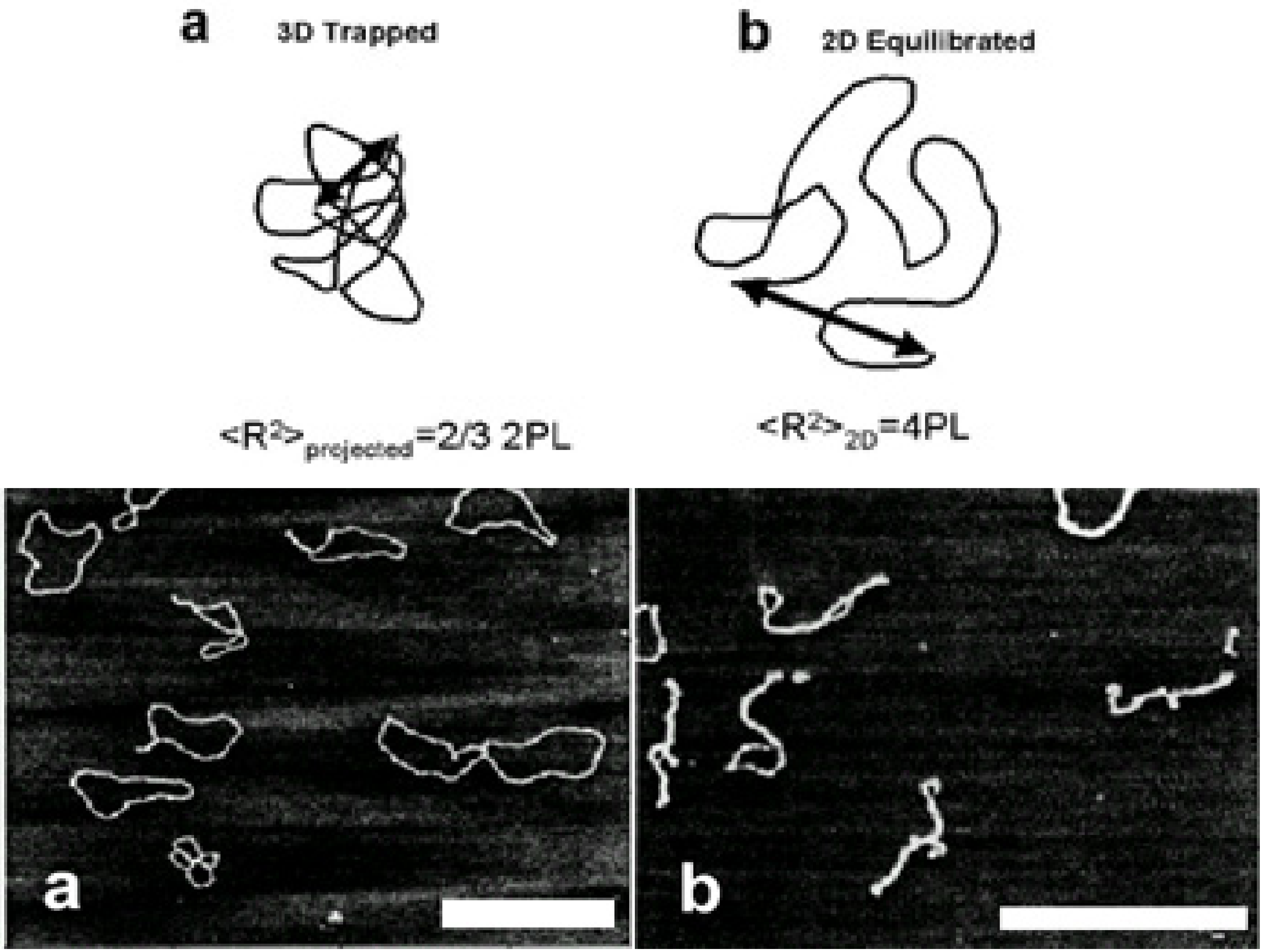
Piezo scanner nonlinearities and hysterisis
better than 10% accuracy is obtained after calibration with the new instruments

Image distortions
- compression of soft samples
- movement of samples by the scan process
- interactions between sample and tip: Adhesion will reduce the apparent size of the image. Repulsion will enlarge the apparent image size

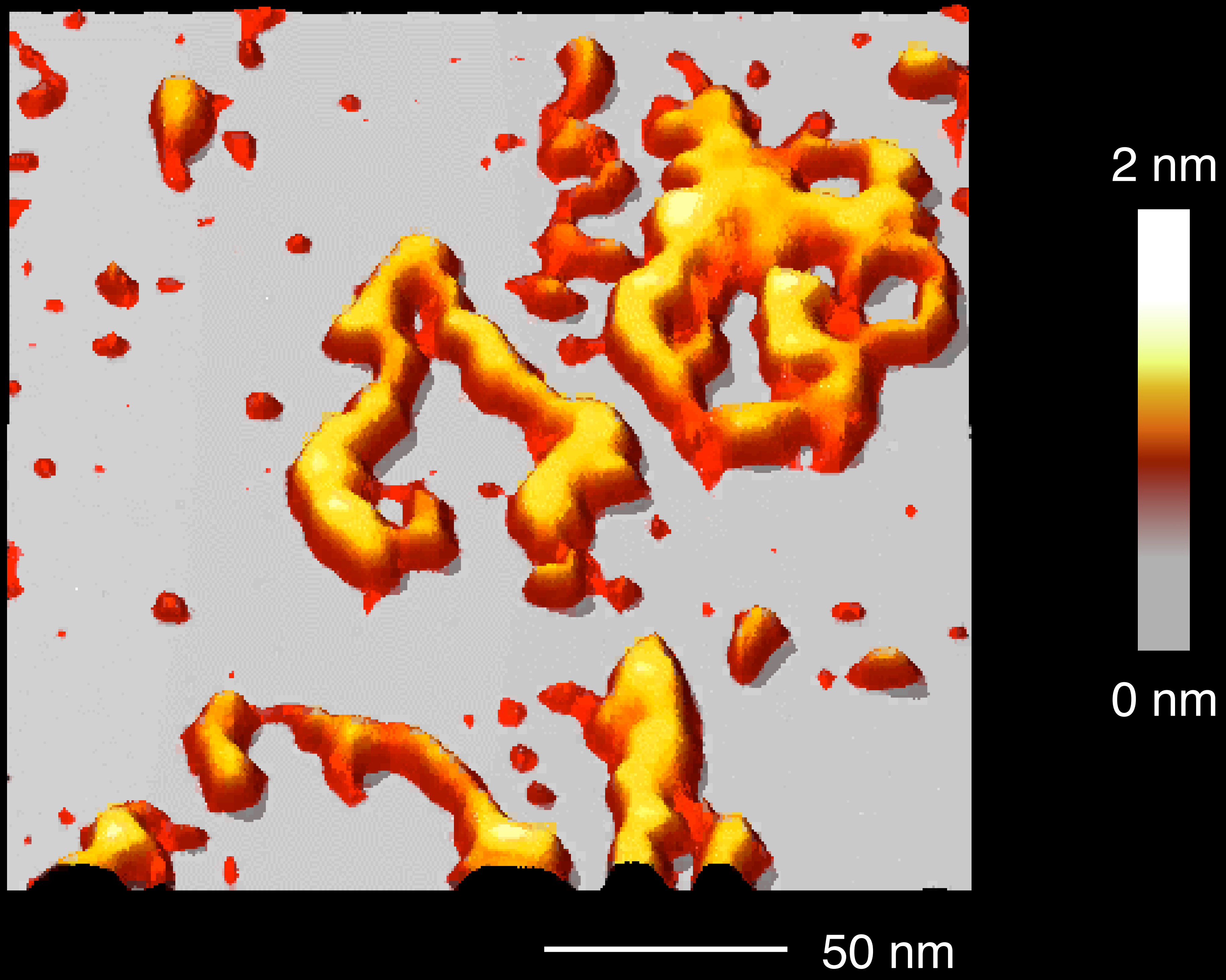
=> minimized by using the tapping mode

Geometric effects of the tip size

The DNA - surface interaction strength changes the apparent plasmid conformation (a) Ni^{2+} (b) Mg^{2+}

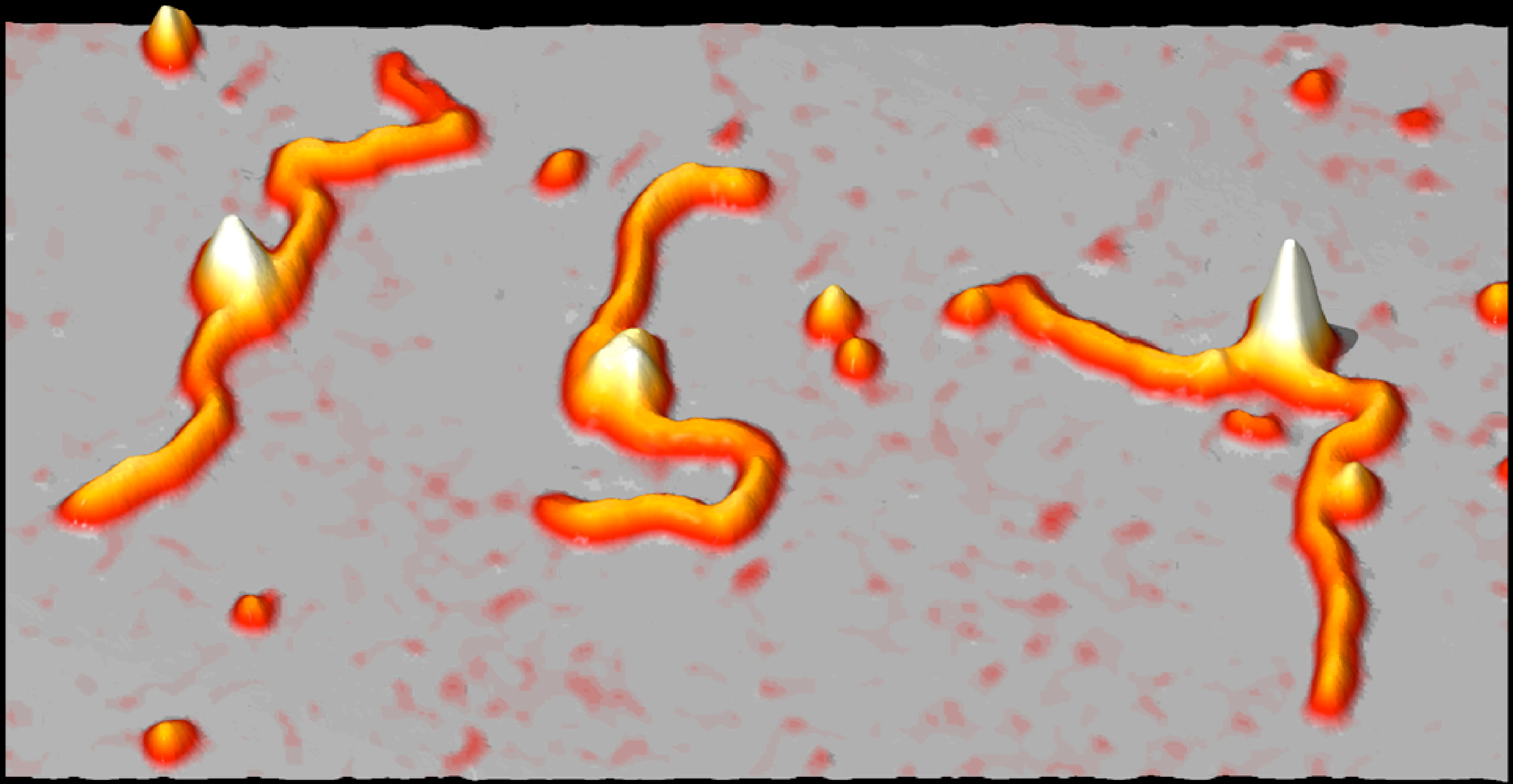


Movement of a 600 bp DNA fragment on a mica surface visualized by scanning force microscopy (SFM/AFM)

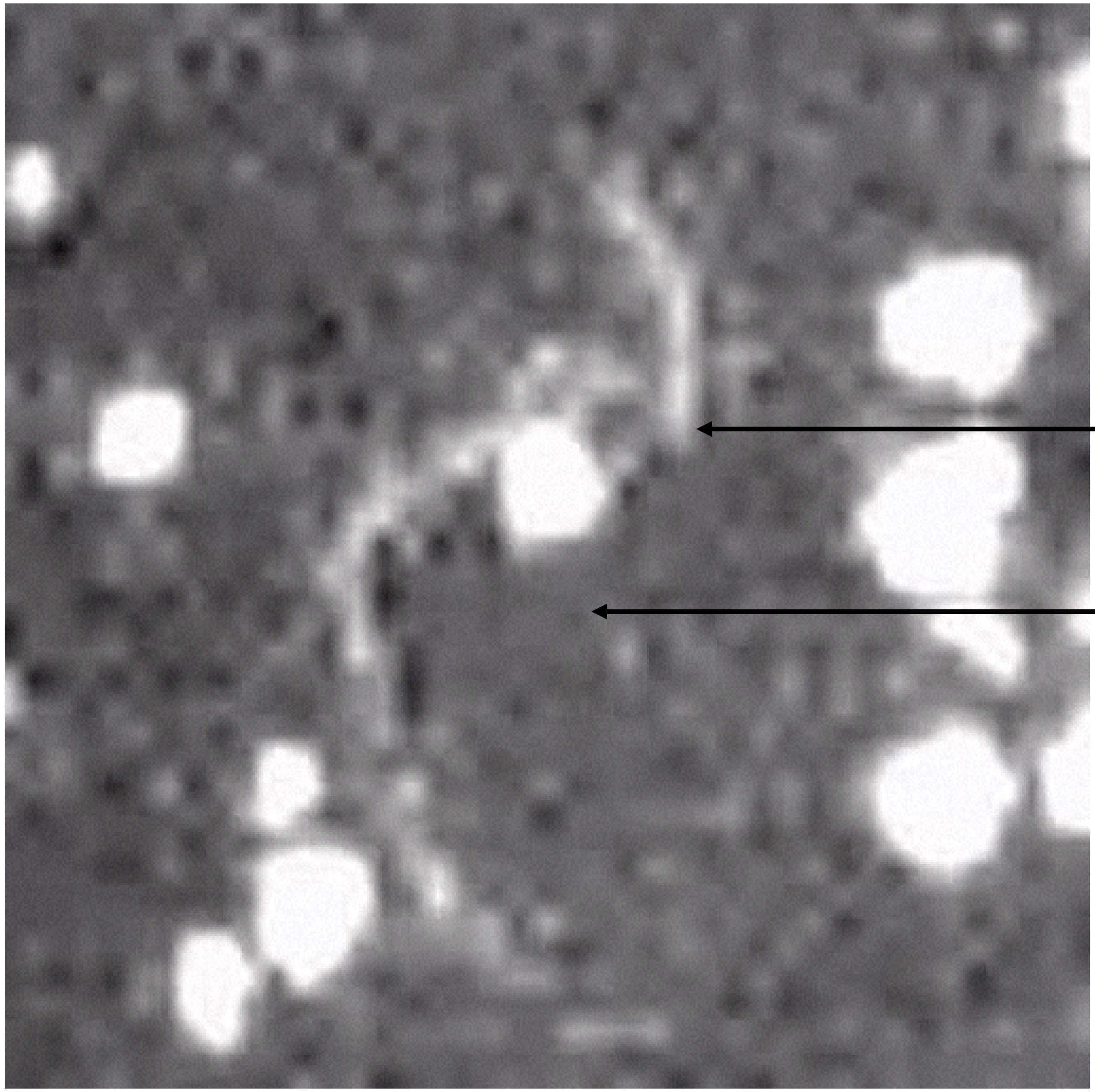


Movements observed by SFM occur in a random fashion as demonstrated by center of mass displacement of DNA

E. coli RNA polymerase at the promoter of a 1036 bp DNA



RNA polymerase finds its promoter by “sliding” along the DNA as visualized by SFM

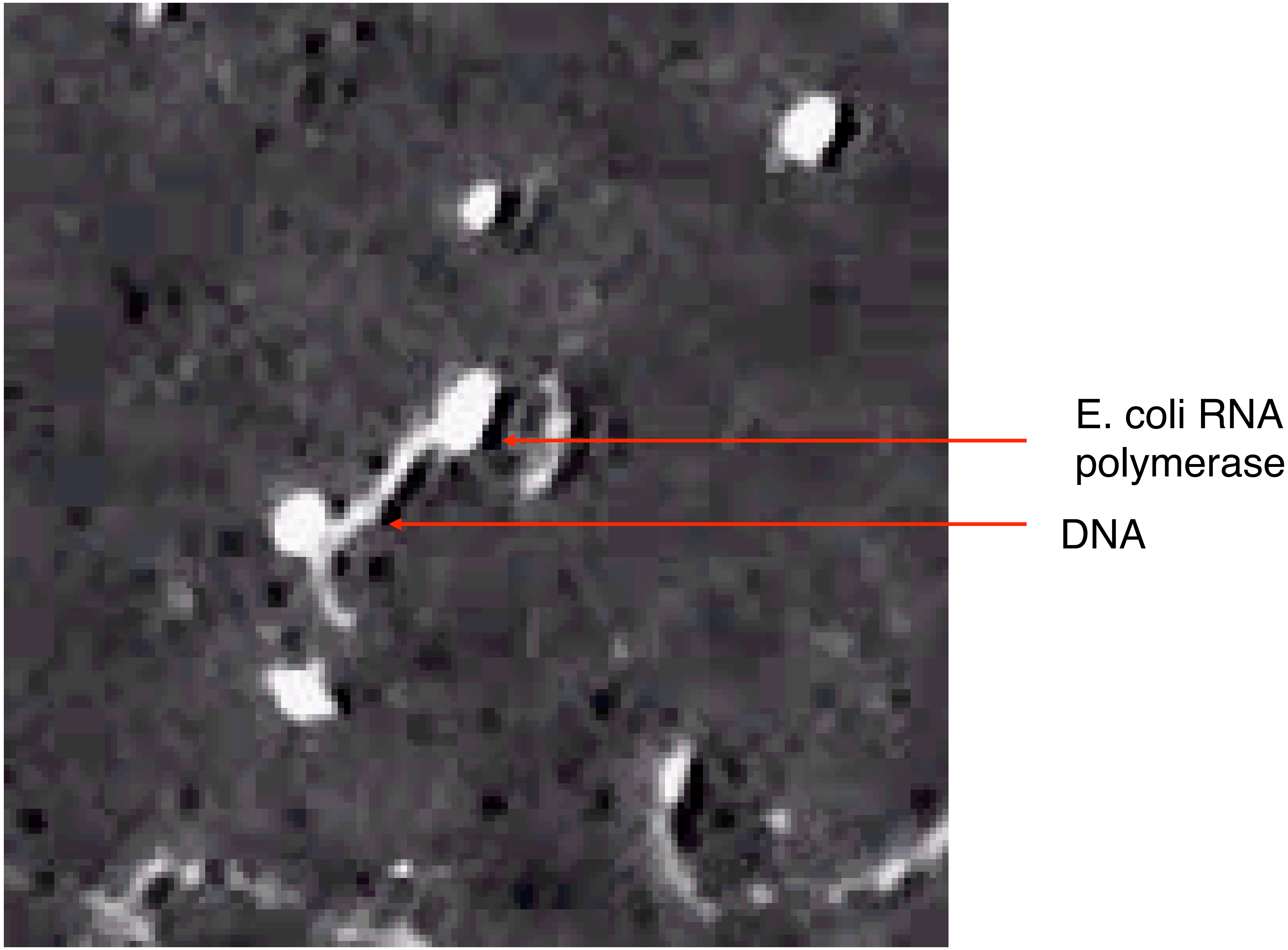


DNA

E. coli RNA polymerase

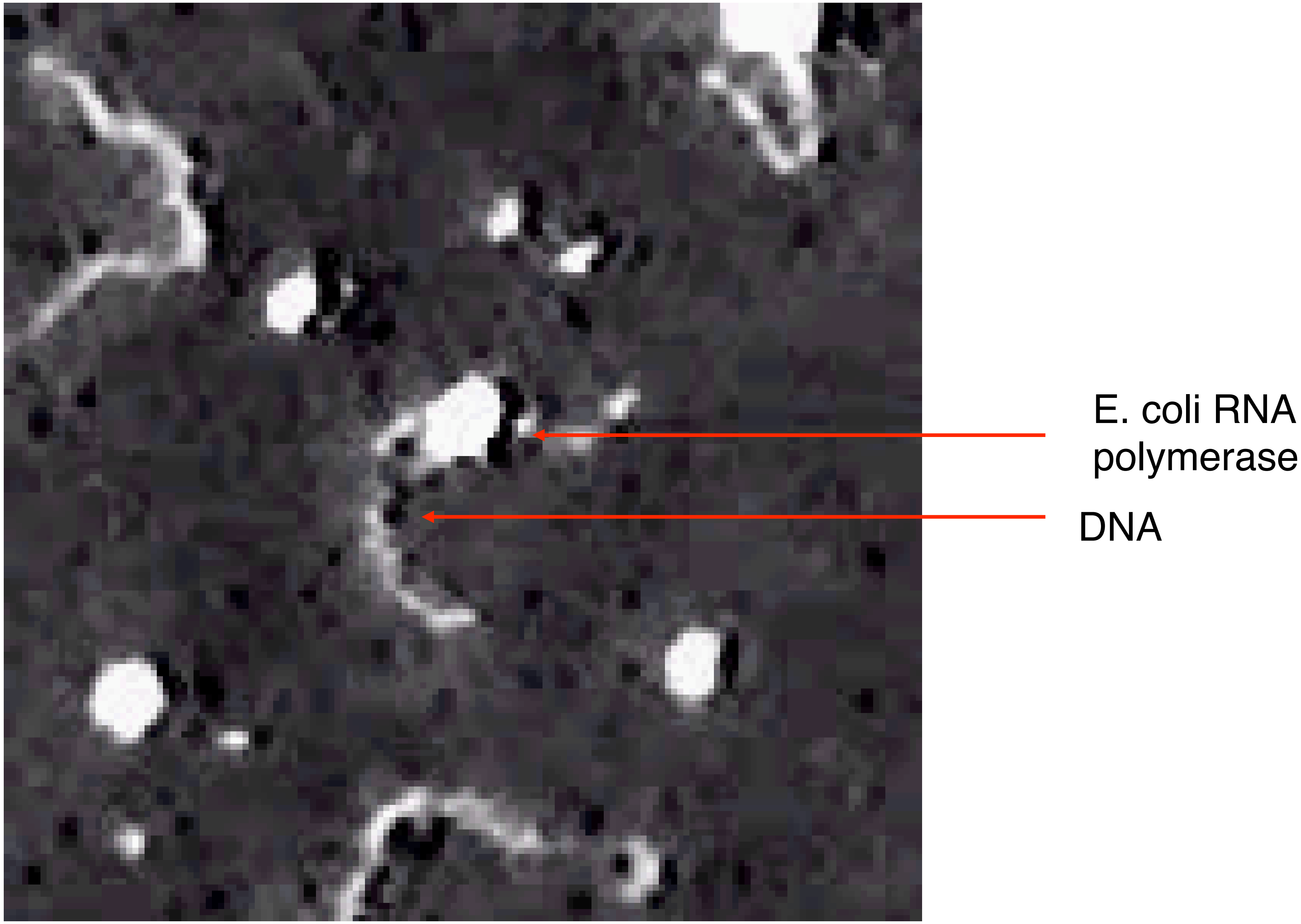
Guthold, M. et al. (1999). Direct observation of one-dimensional diffusion and transcription by escherichia coli RNA polymerase. Biophys J 77, 2284-2294.

Synthesis of RNA by E.coli RNA polymerase immobilized on the surface, Example 1



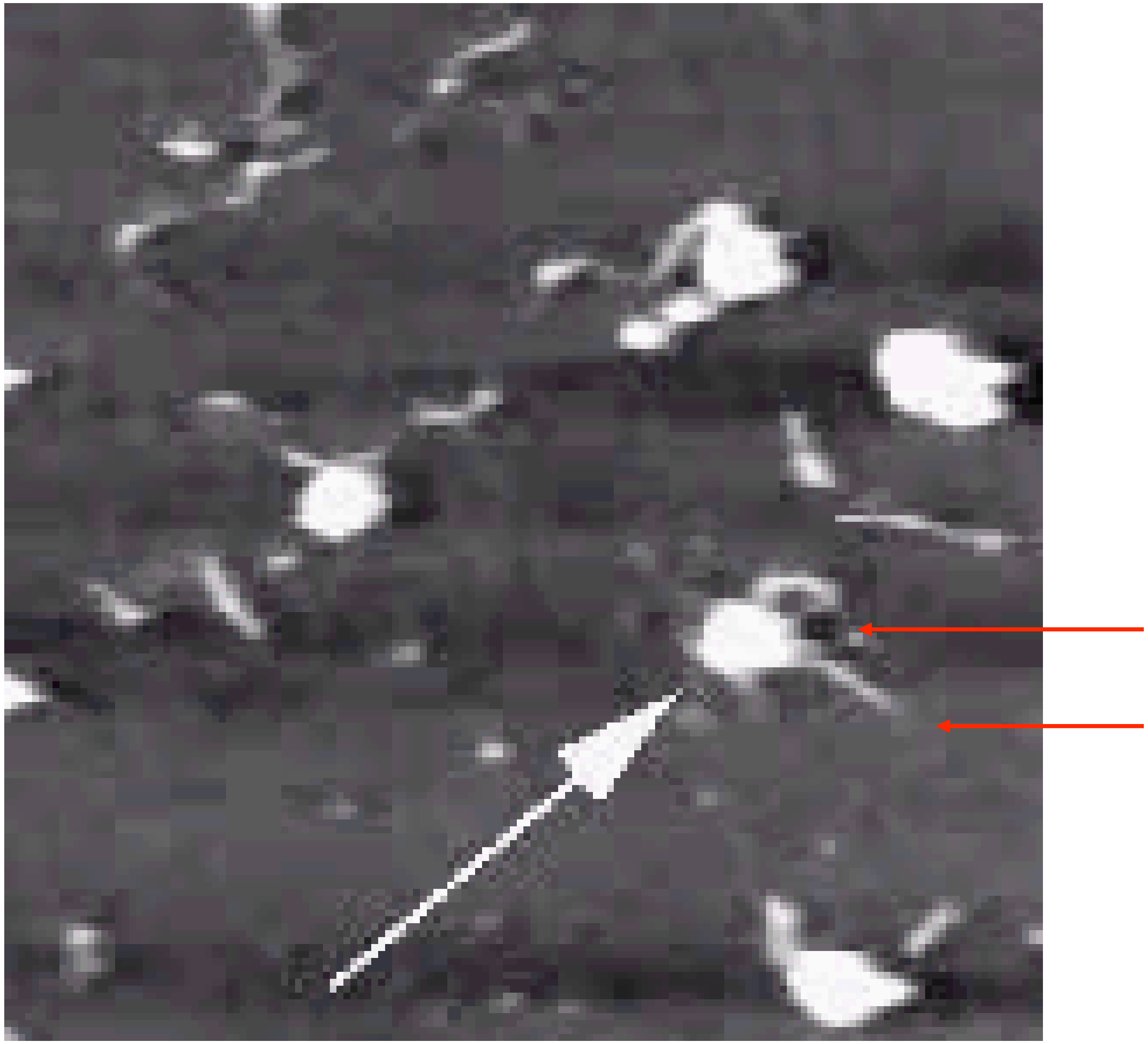
Kasas, Guthold, Bustamante, C.

Synthesis of RNA by E.coli RNA polymerase immobilized on the surface, Example 2



Kasas, Guthold, Bustamante, C.

Synthesis of RNA by E.coli RNA polymerase immobilized on the surface, Example 3

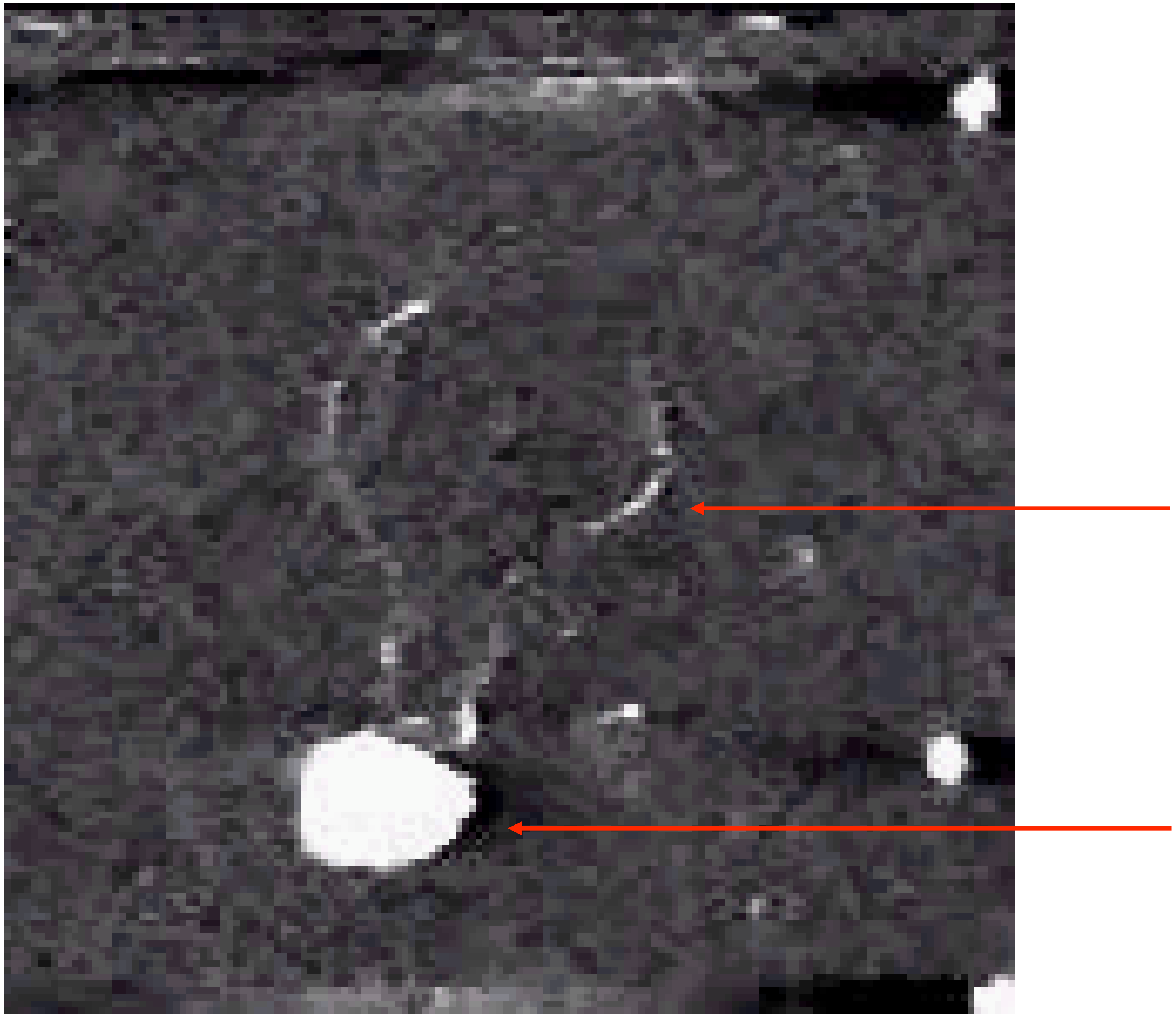


E. coli RNA polymerase

DNA

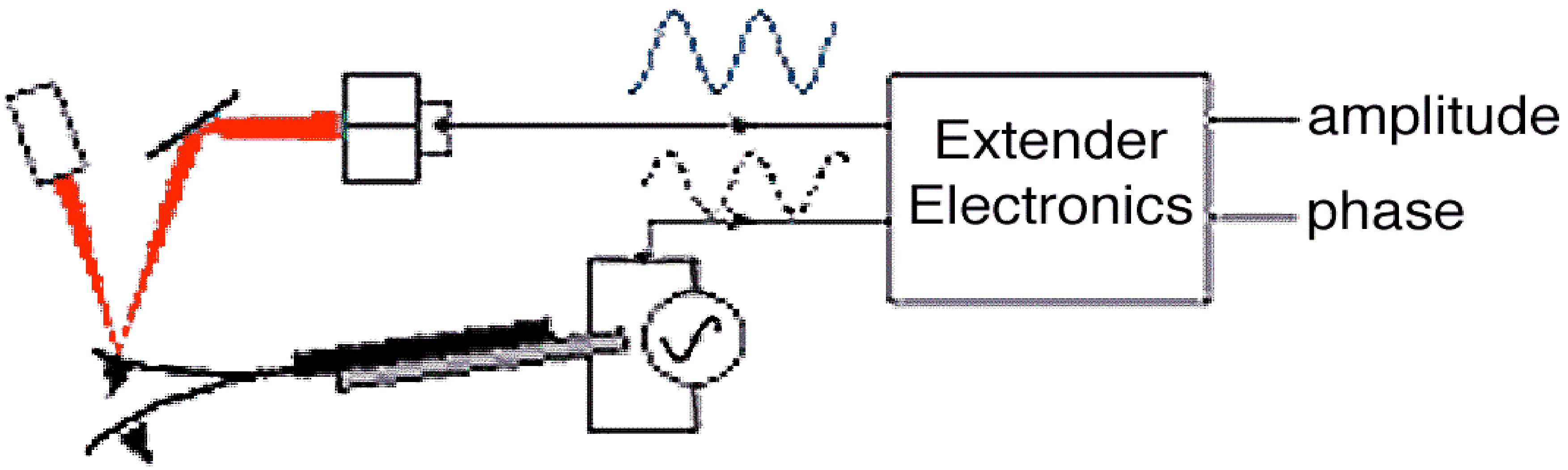
Kasas, Guthold, Bustamante, C.

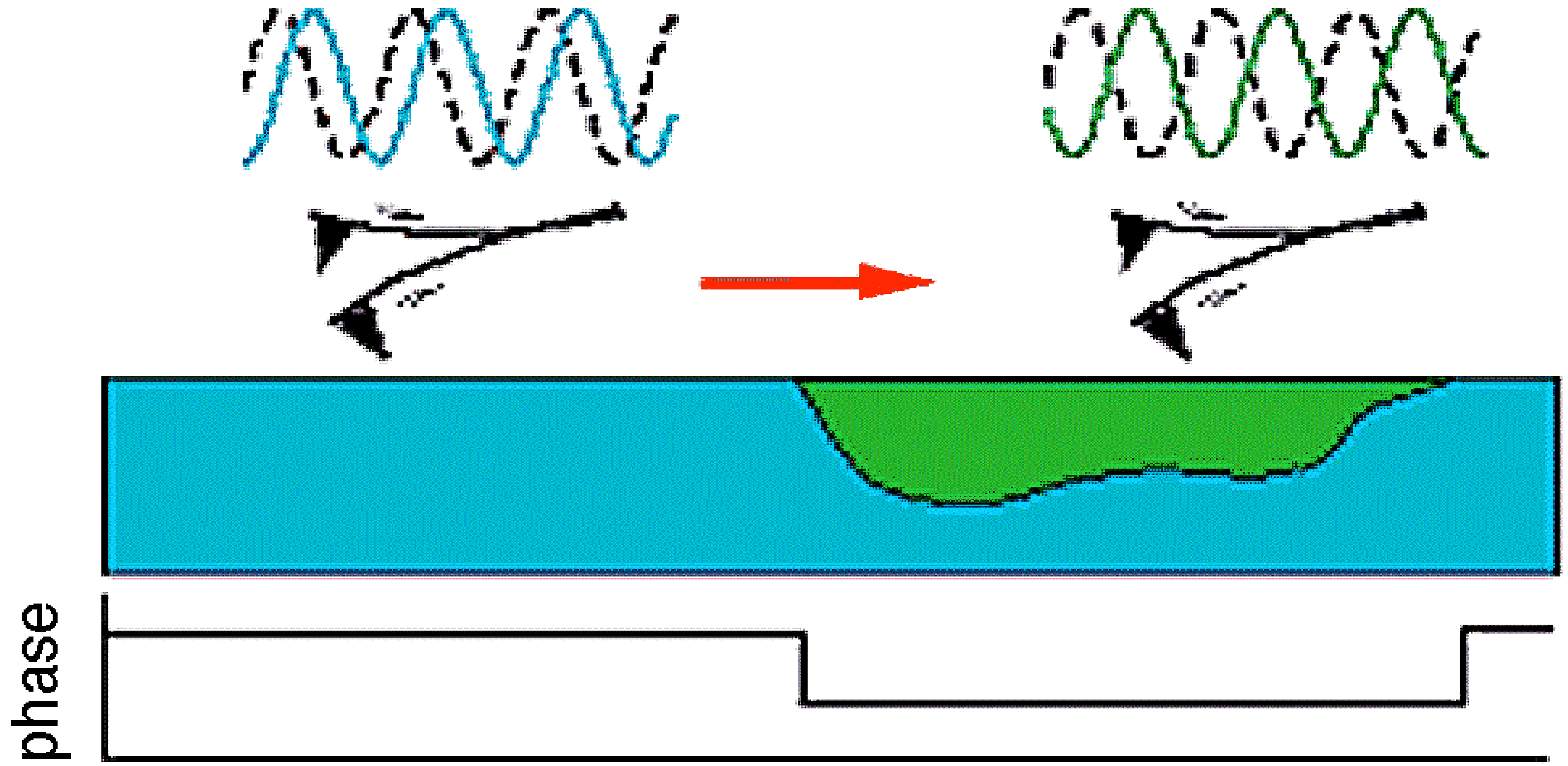
Synthesis of RNA by E.coli RNA polymerase immobilized on the surface, Example 4



Kasas, Guthold, Bustamante, C.

Phase imaging force microscopy





Phase imaging of DNA

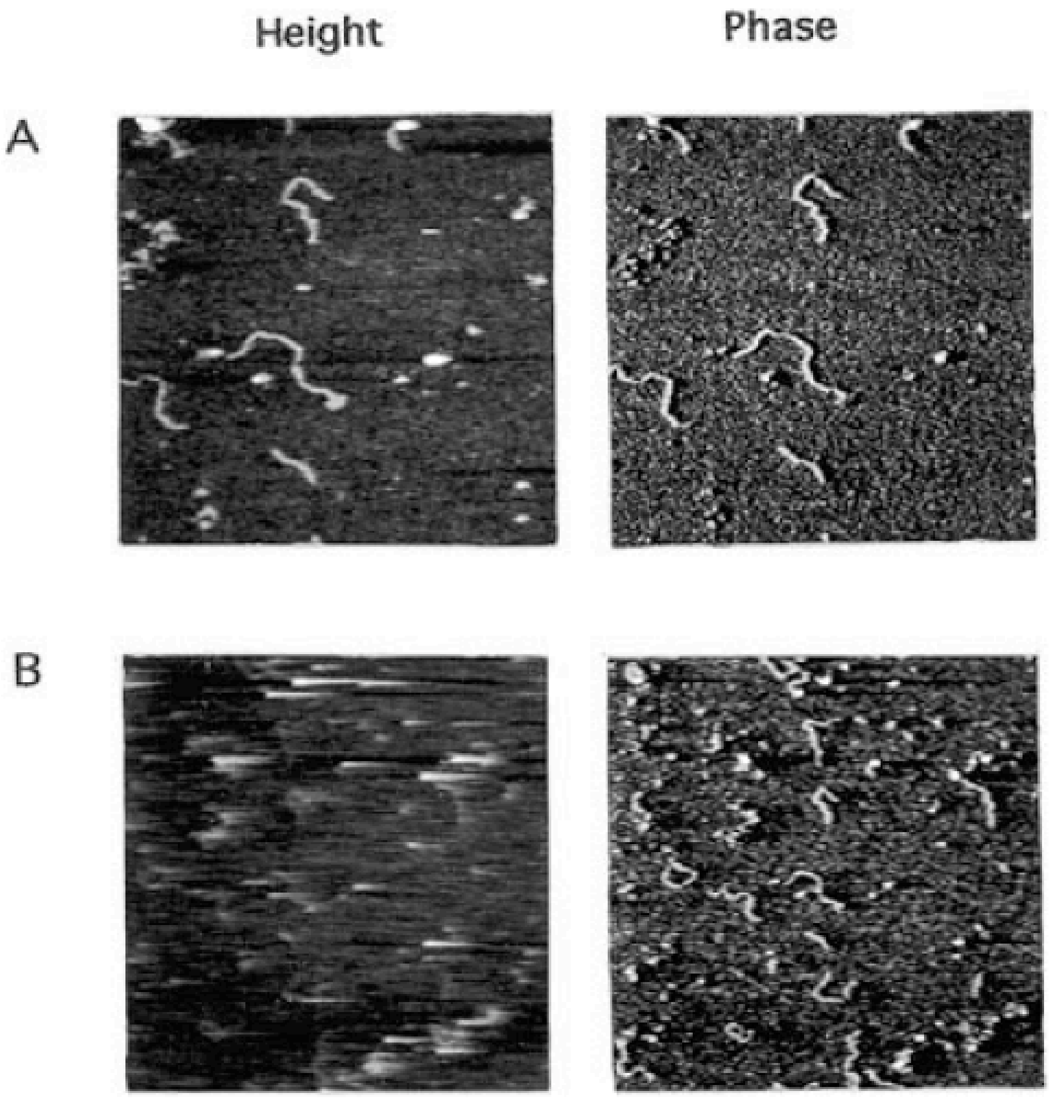


Figure 2. Effect of scan rate on height and phase images with tapping mode AFM. Simultaneously acquired height and phase images are of DNA deposited onto freshly split mica in a complex with RNAP and imaged in Zn buffer (20 mM Tris, 5 mM $MgCl_2$, 50 mM KCl, 1 mM β-mercaptoethanol, 2 mM $ZnCl_2$, pH 7.9). An EBD tip was used. (**A**) Scan rate 9.2 Hz (tip speed 18.4 Hz), image size 1 × 1 µm. (**B**) Scan rate 27.5 Hz (tip speed 110 Hz), image size 2 × 2 µm.

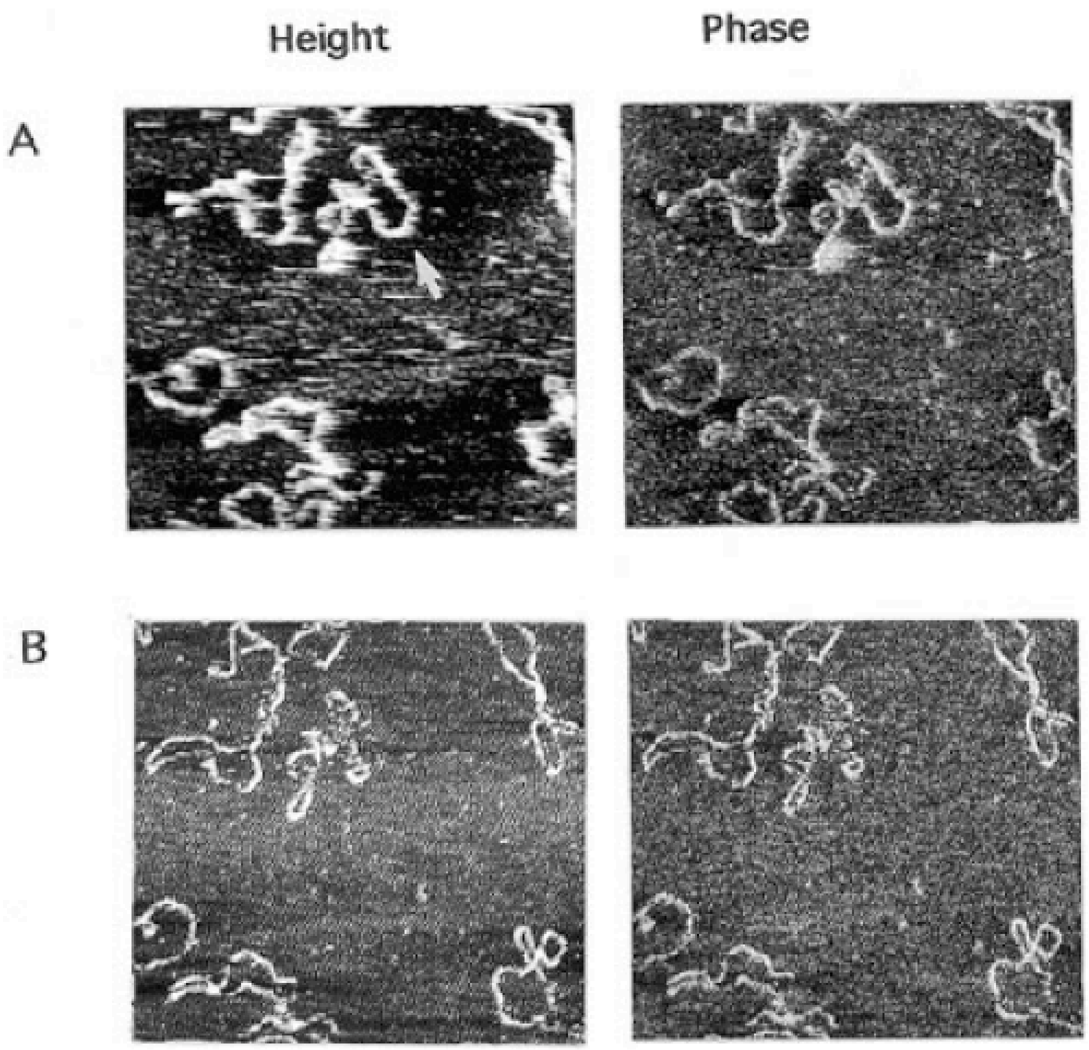
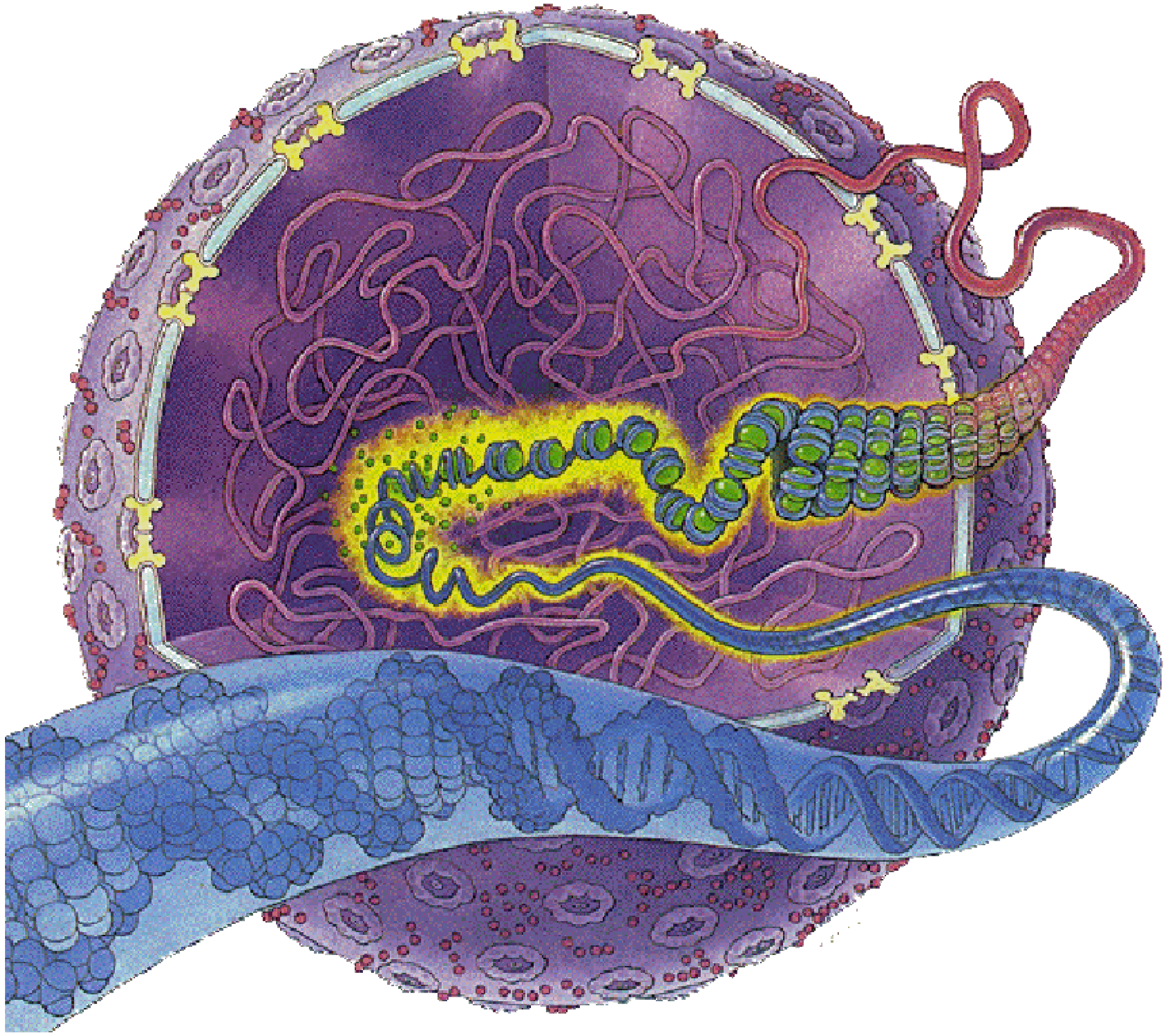


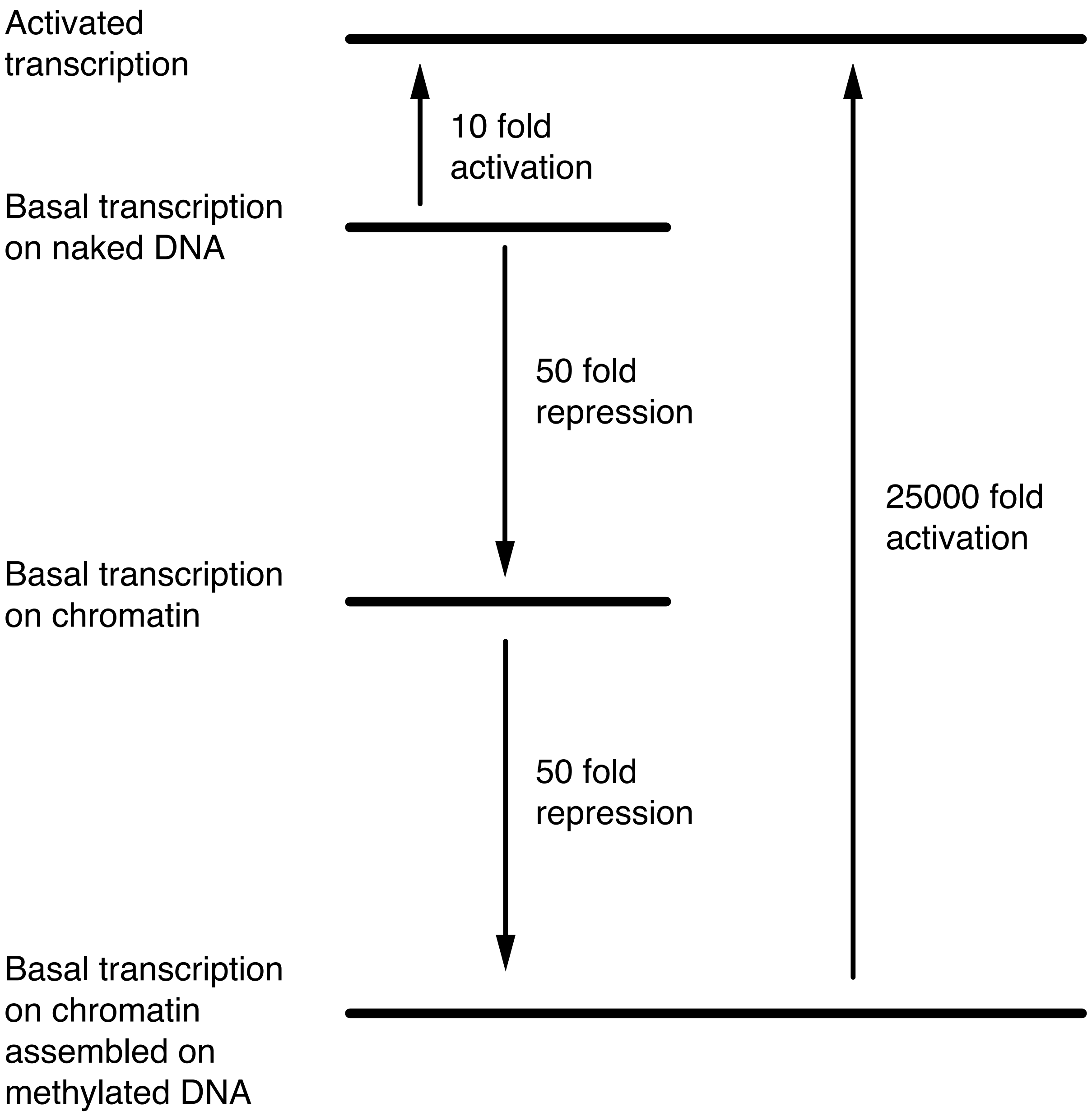
Figure 3. Effect of imaging force on height and phase images with tapping mode AFM. Simultaneously acquired phase and height images are of plasmid Bluescript on Ni^{2+}-coated mica in a 5 mM HEPES, 5 mM KCl and 2 mM $MgCl_2$ buffer. Images in (**A**) were acquired at a lower force than images in (**B**). The set point was lowered 6% between (A) and (B). The arrow in (A) shows a loop of DNA that has moved in (B). The sample was scanned with an EBD tip. Scan rate 6.1 Hz, scan size 1 × 1 µm.

Nucleic Acids Research, 1997, Vol. 25, No. 21 ***4381***

Chromatin conformation and gene expression are tightly connected



- 2 m DNA
- nucleus ≈ 10 μm diameter
- at least 30 000 genes
-10 000 different nuclear proteins



Activated transcription

10 fold activation

Basal transcription on naked DNA

50 fold repression

25000 fold activation

Basal transcription on chromatin

50 fold repression

Basal transcription on chromatin assembled on methylated DNA

chromatin conformation can change gene expression by a factor of ~25000

Factors that change the chromatin compaction state and affect gene expression

active, open conformation

Chromatin assembly factors (ACF)

Histone chaperone NAP1

inactive, compact conformation

<u>Histone variants:</u>

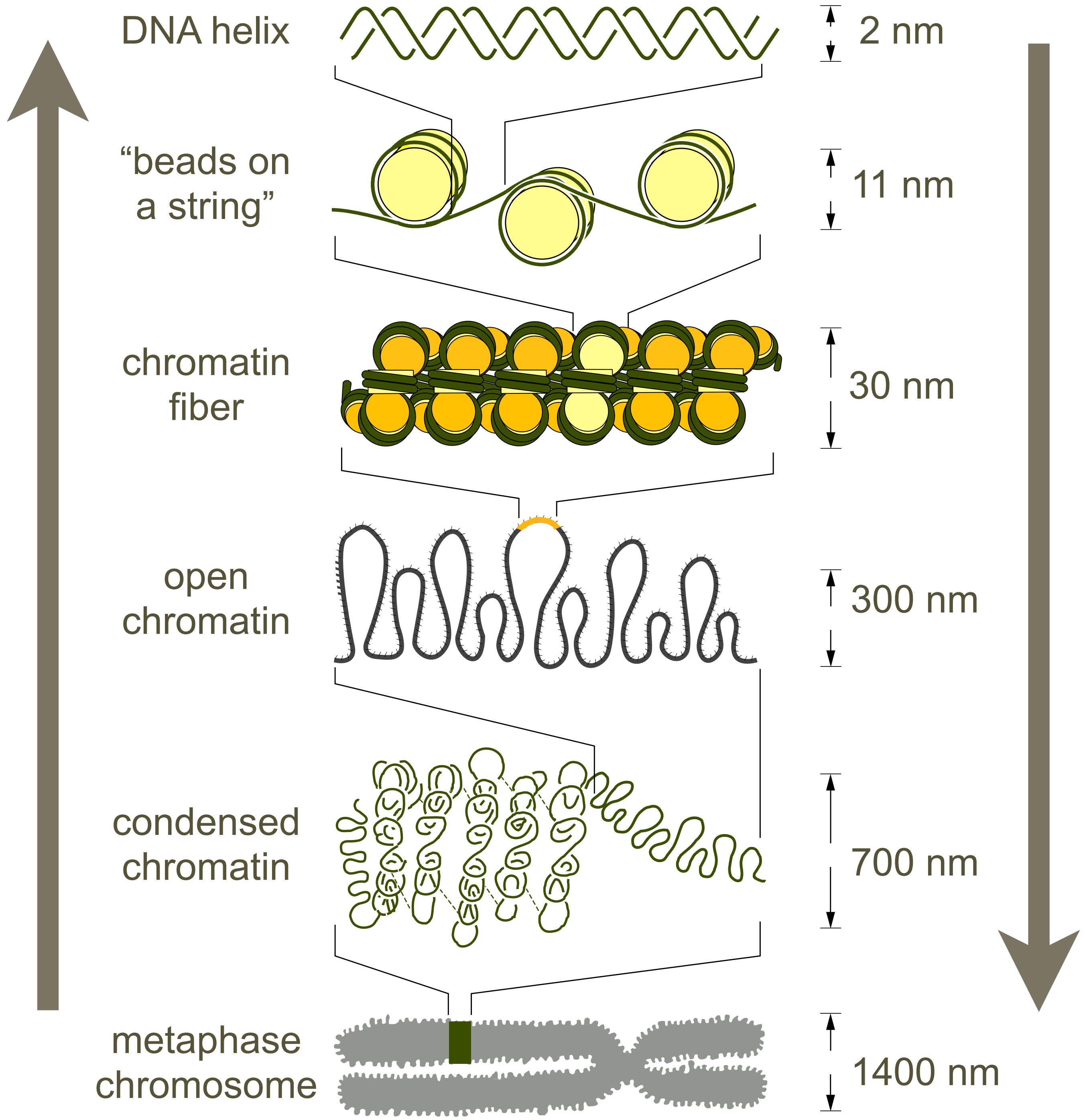
- H3.3
- H2A.Z

<u>Histone modifications:</u>

- acetylation
- methylation
- phosphorylation

<u>other factors:</u>

- HMG proteins
- chromatin remodellers



<u>Histone variants/H1:</u>

- CENP-A
- macroH2A
- H1

<u>Histone modifications:</u>

- deacetyation
- methylation
- phosphorylation

<u>DNA methylation</u>

<u>other factors:</u>

- HP1
- Sir proteins
- MeCP2
- non-coding RNA

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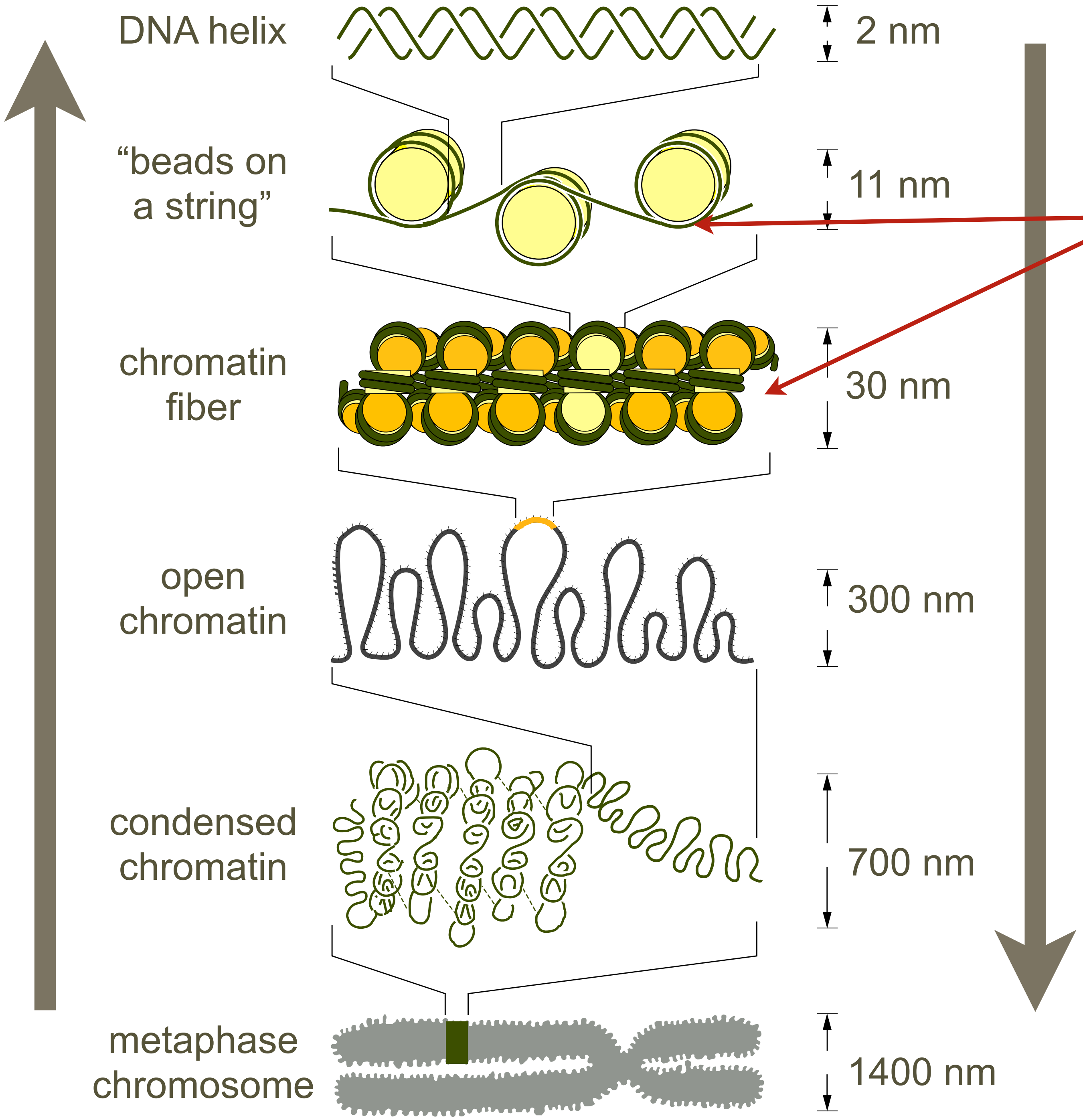

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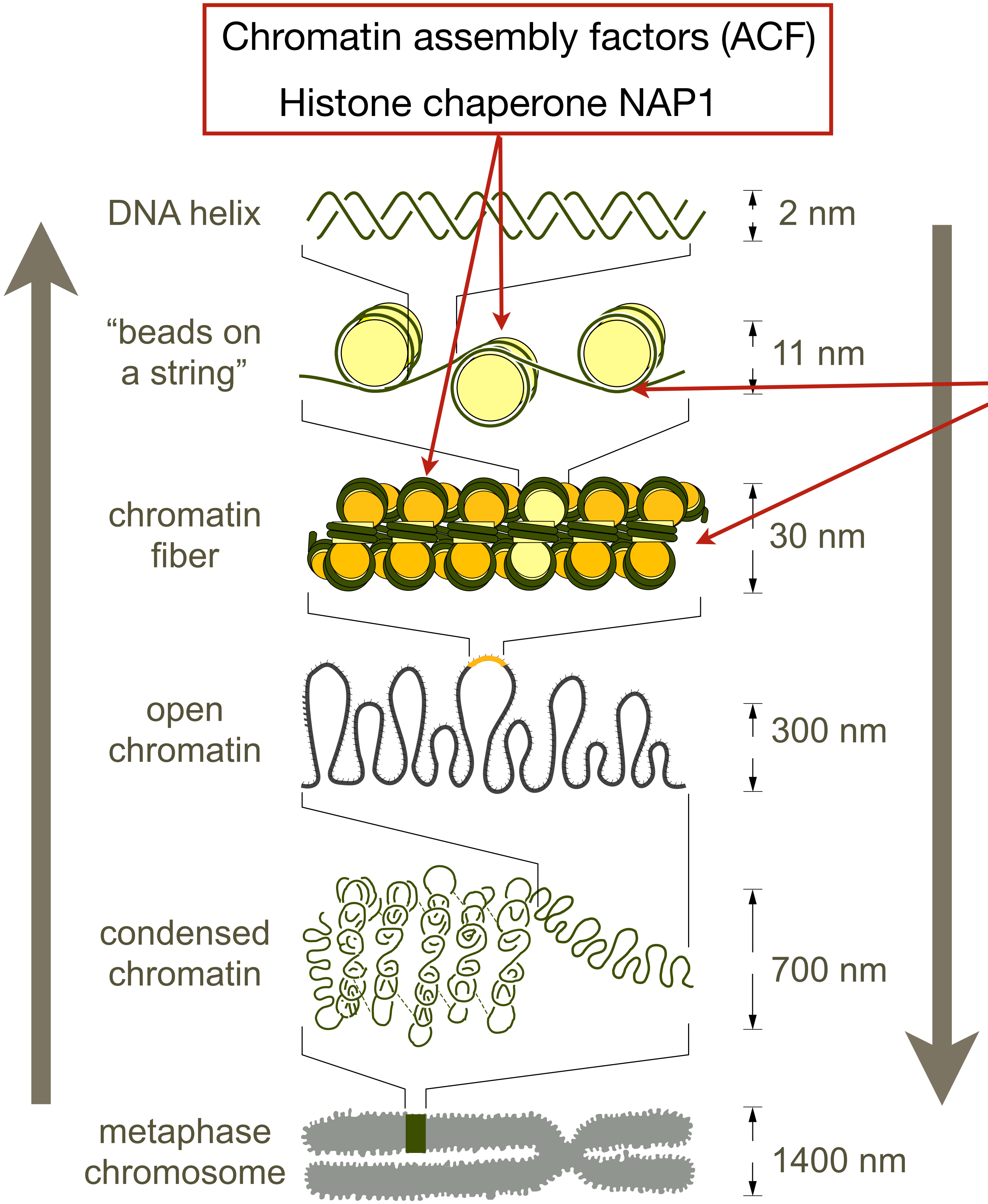

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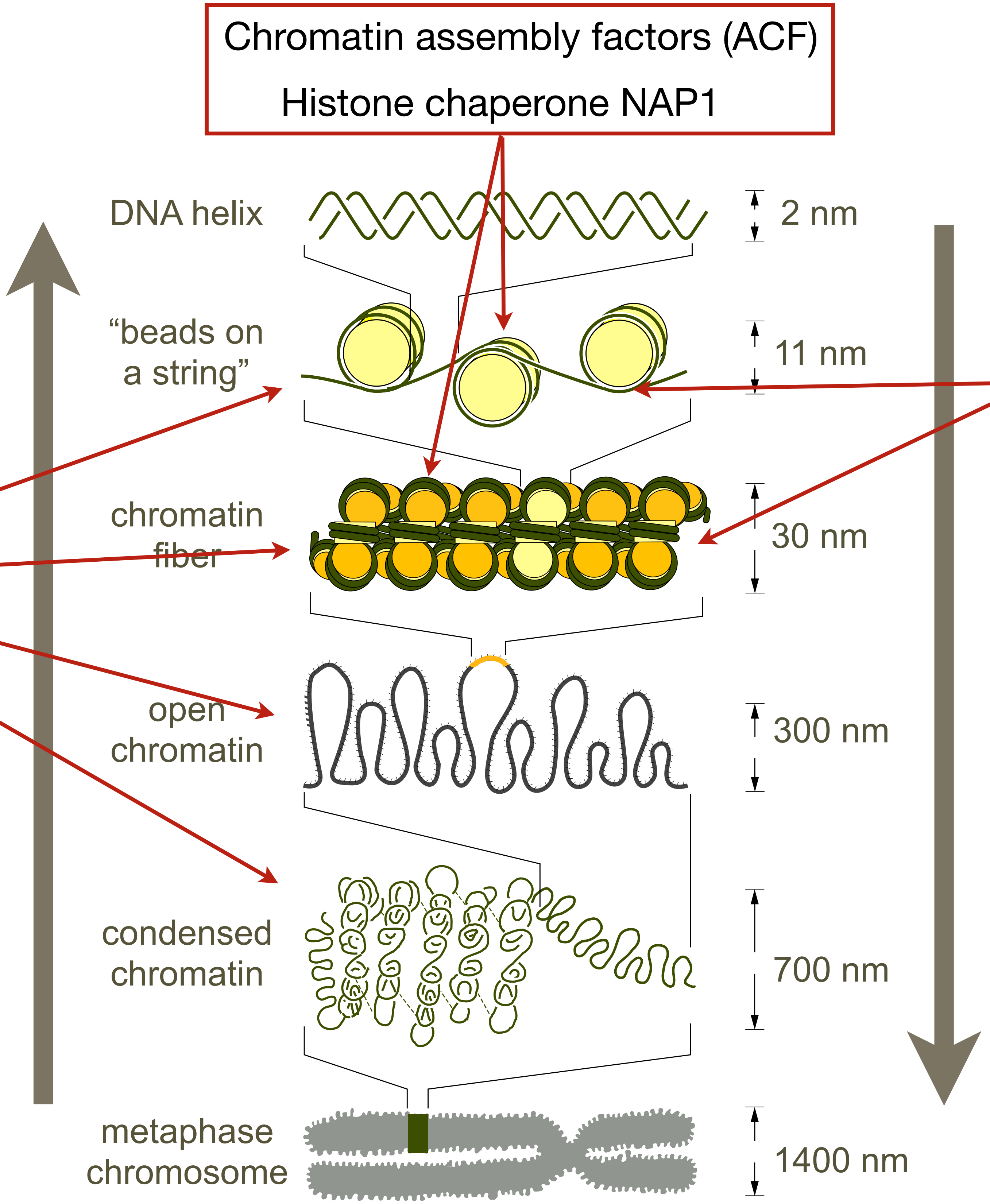

Chromatin assembly factors (ACF)

Histone chaperone NAP1

DNA helix — 2 nm

“beads on a string” — 11 nm

chromatin fiber — 30 nm

open chromatin — 300 nm

condensed chromatin — 700 nm

metaphase chromosome — 1400 nm

inactive, compact conformation

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DNA methylation

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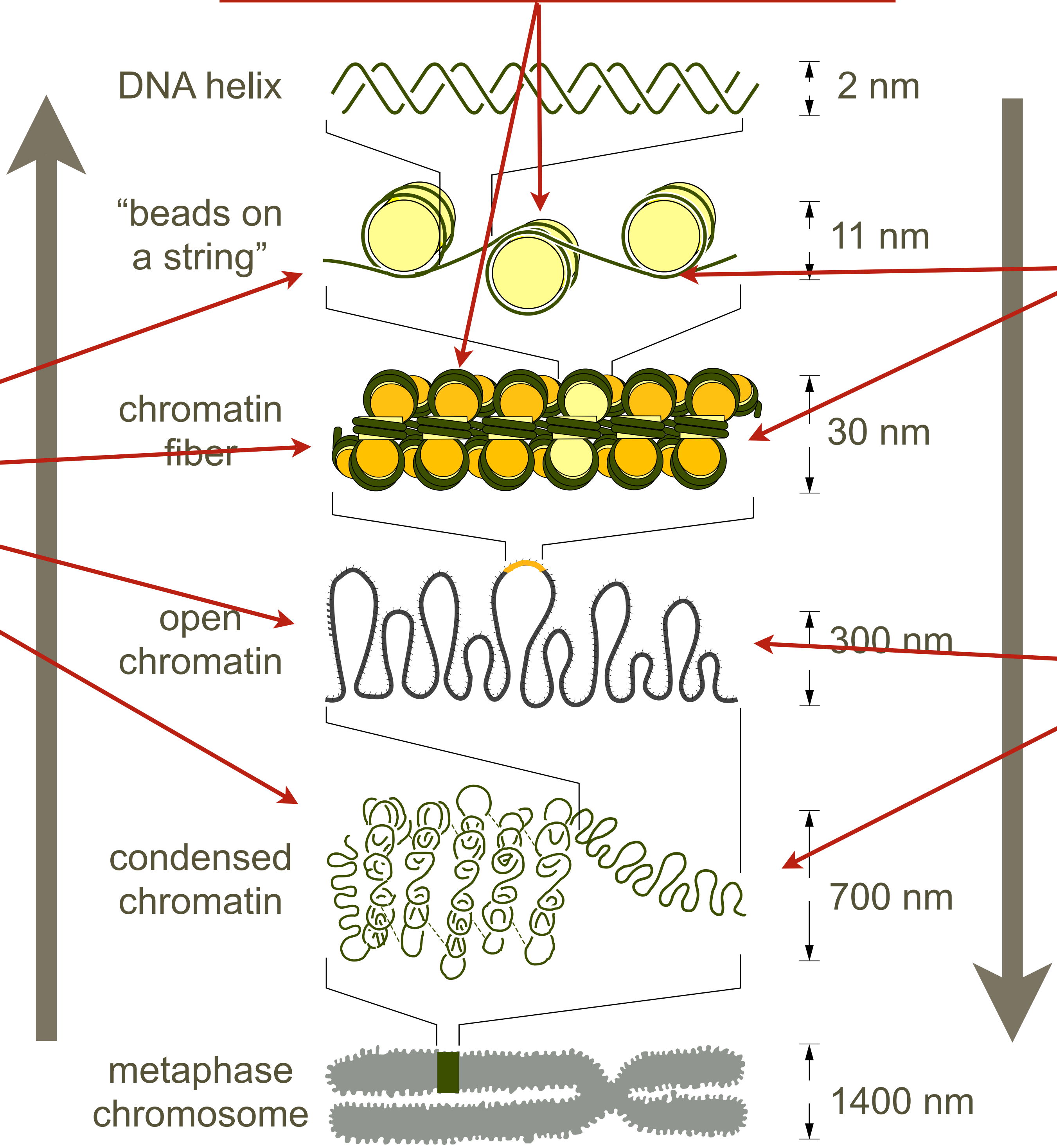

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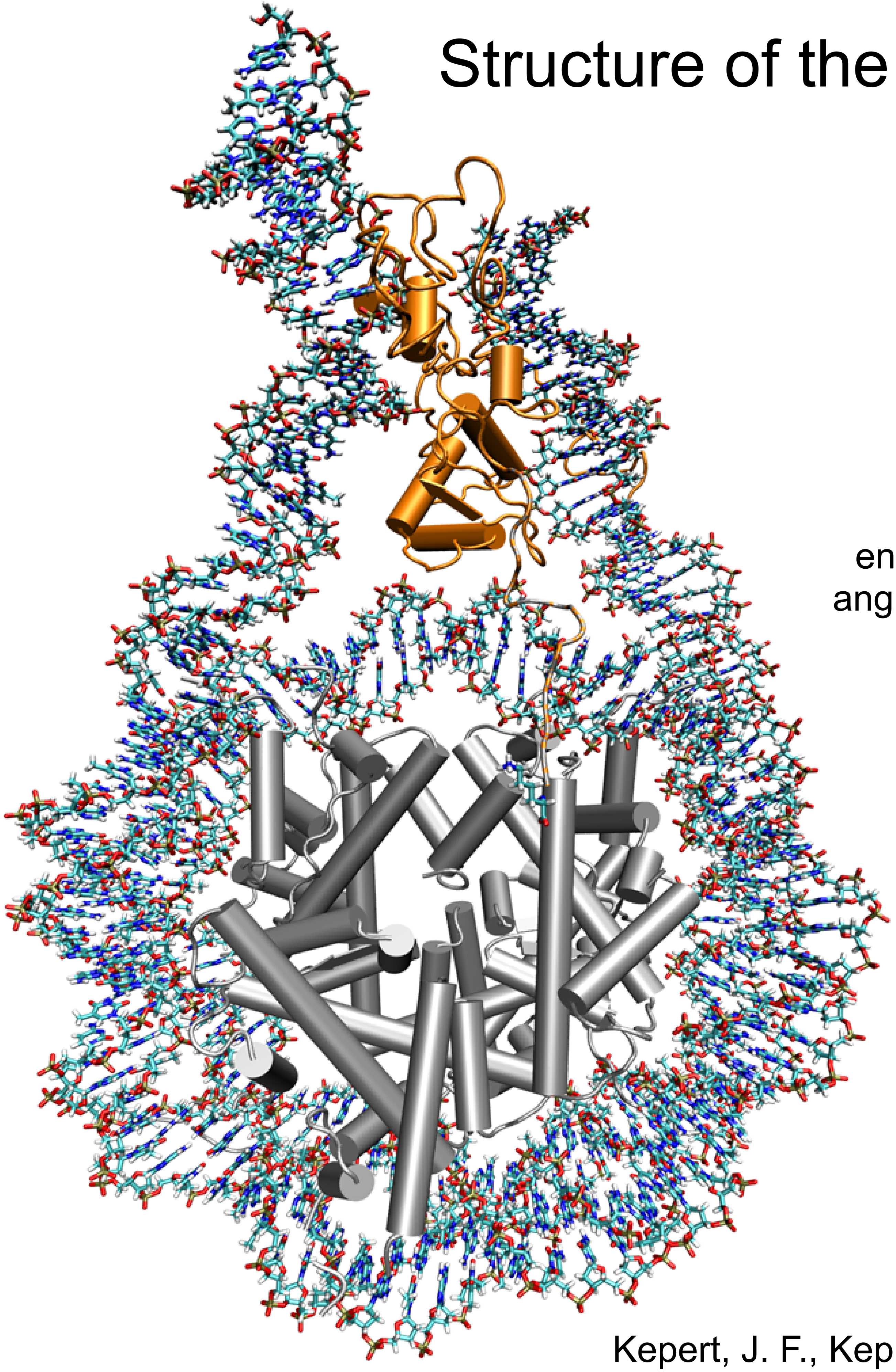
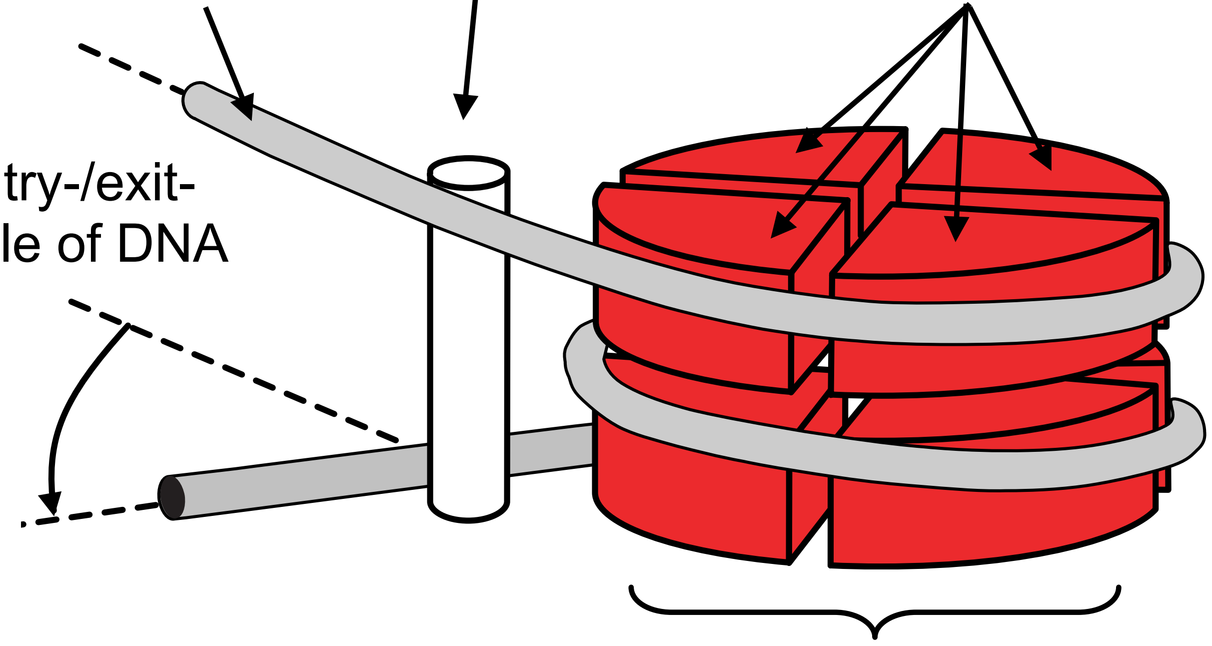
Structure of the nucleosome with H1





linker histone H1

core histones H2A, H2B, H3, H4 (each 2 x)

DNA

entry-/exit-angle of DNA

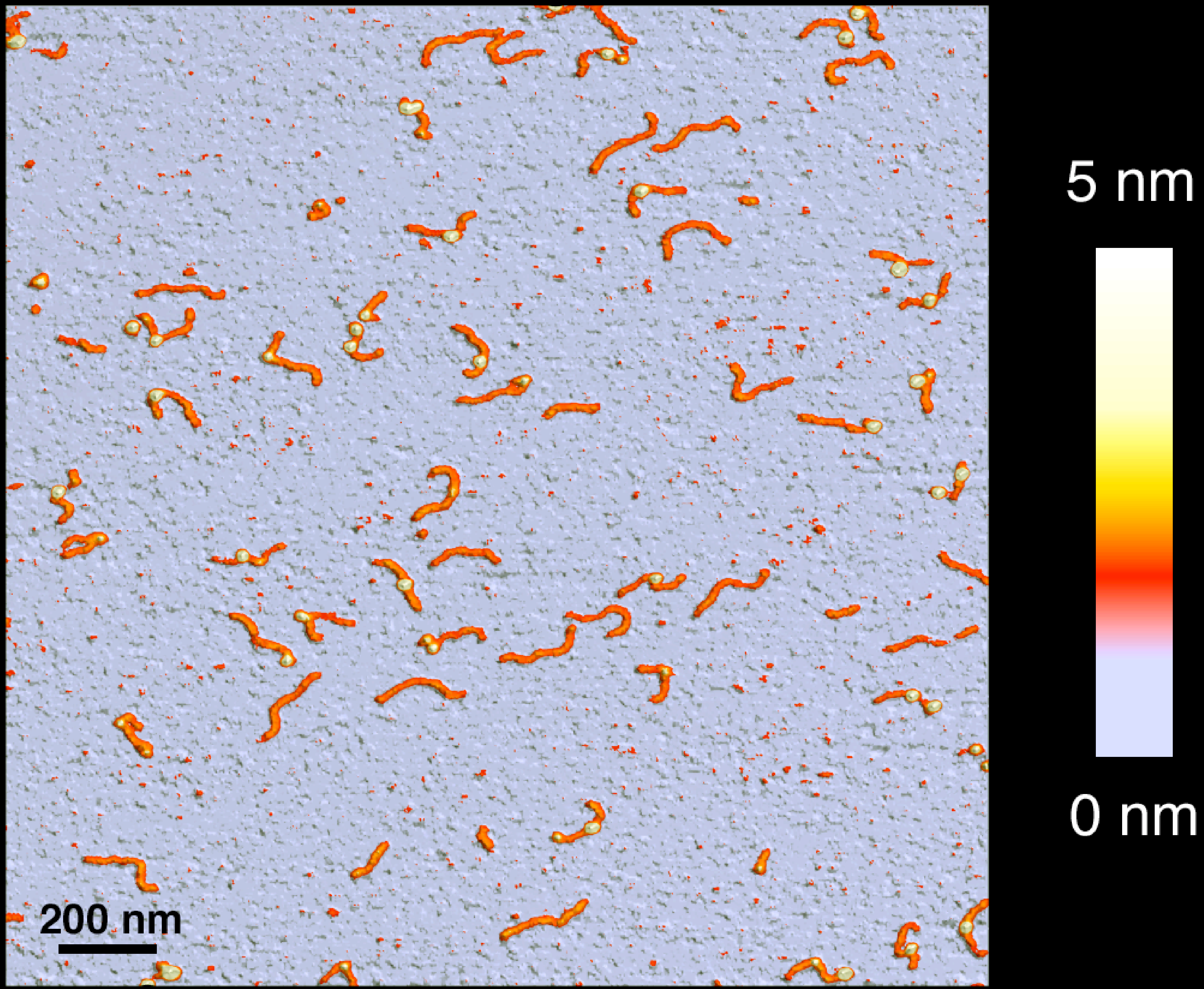
histone octamer

nucleosome core particle

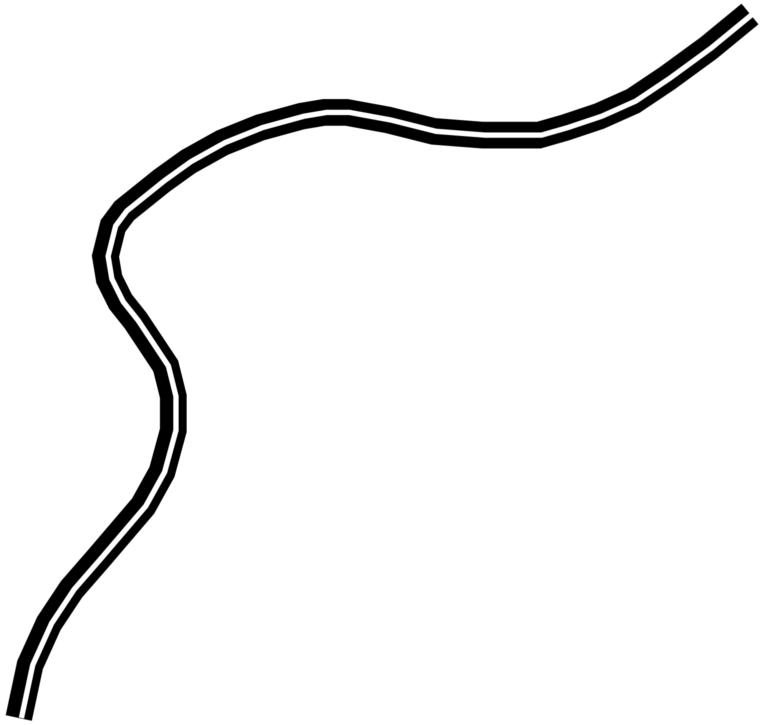
chromatosome (= nucleosome + H1)

Kepert, J. F., Kepper, N., Klement, R. and Rippe, K. (2006), submitted

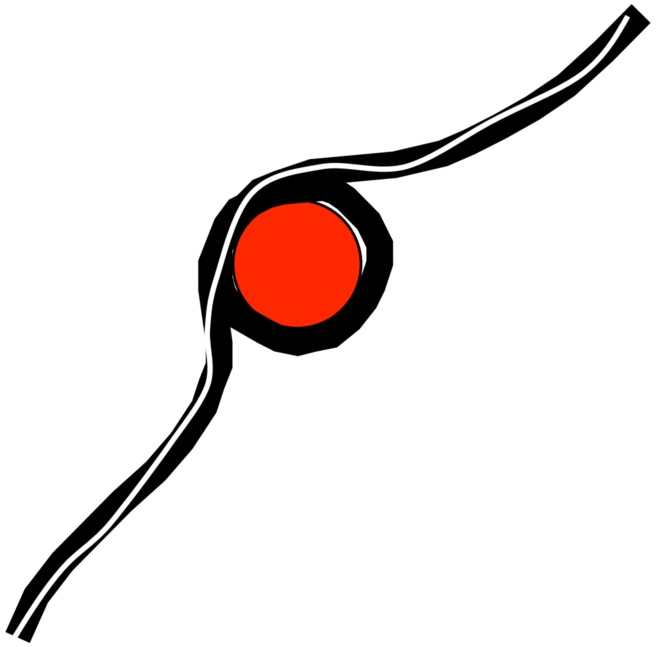
SFM image of dried mononucleosomes on a 614 base pair DNA with a central 5 S rRNA positioning sequence



Wrapping of the DNA around a protein leads to an apparent shortening of the DNA contour length

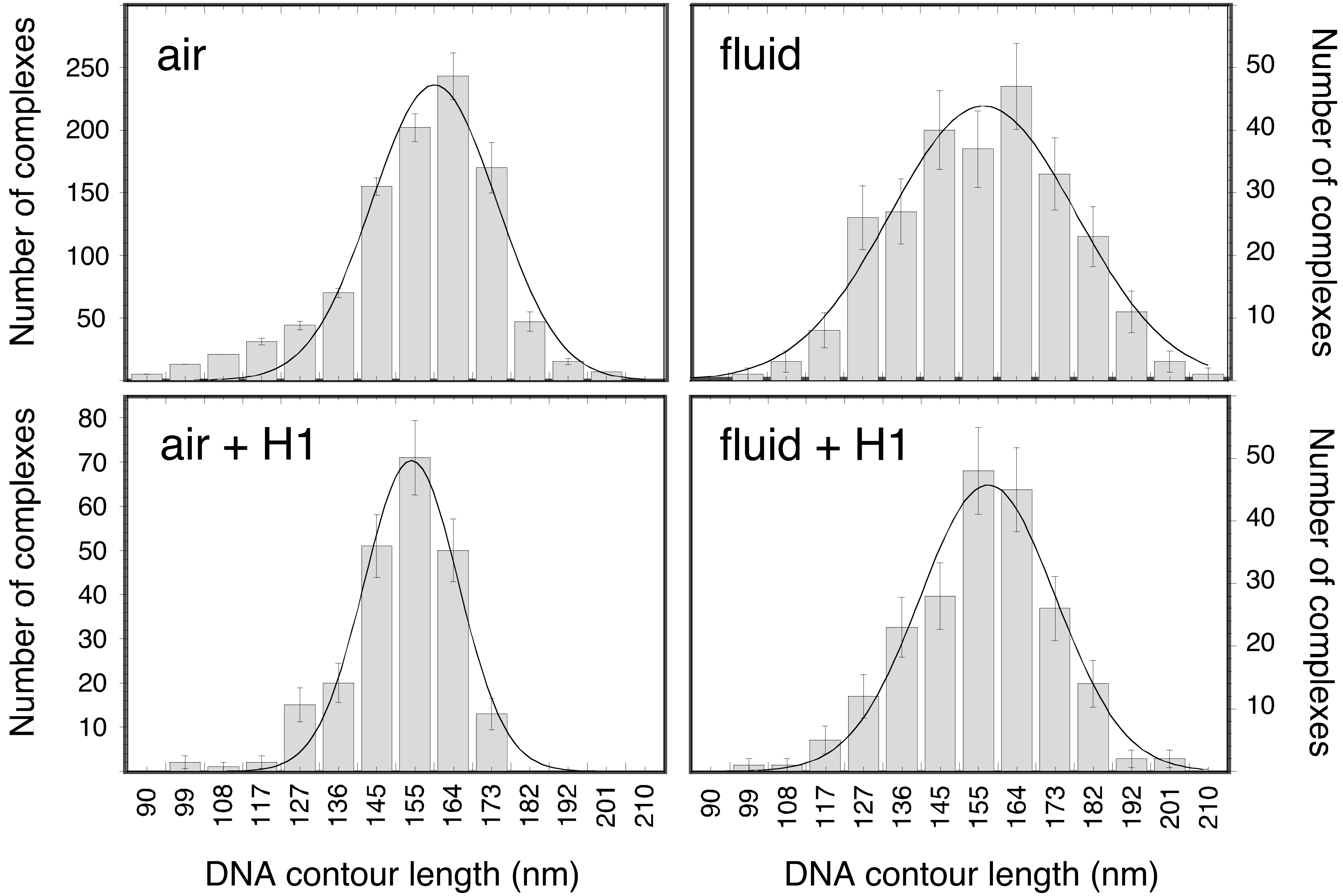


free DNA

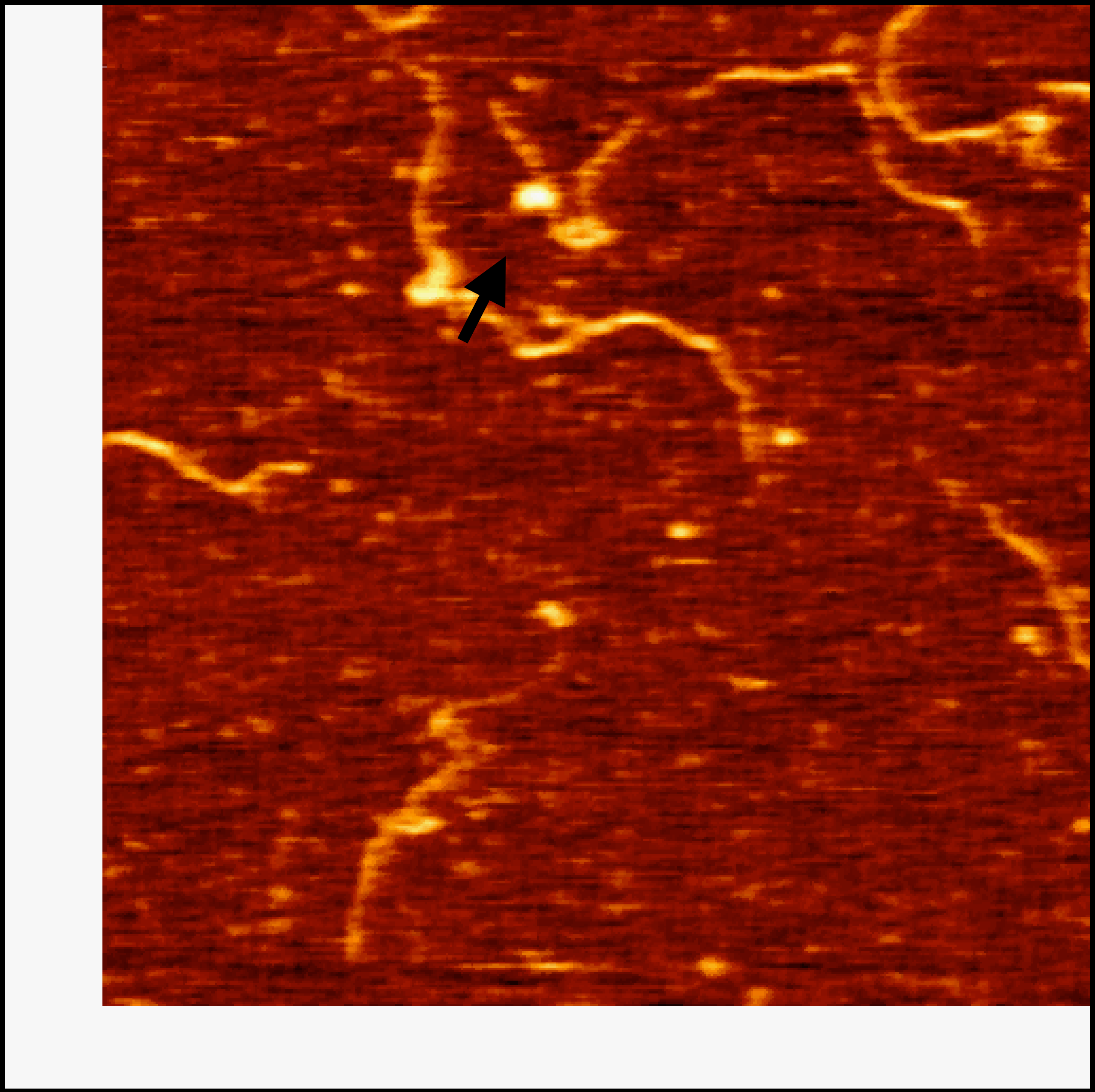


DNA wrapped
around protein

Contour length measurements indicate that 140±34 base pairs are wrapped around the histone octamer

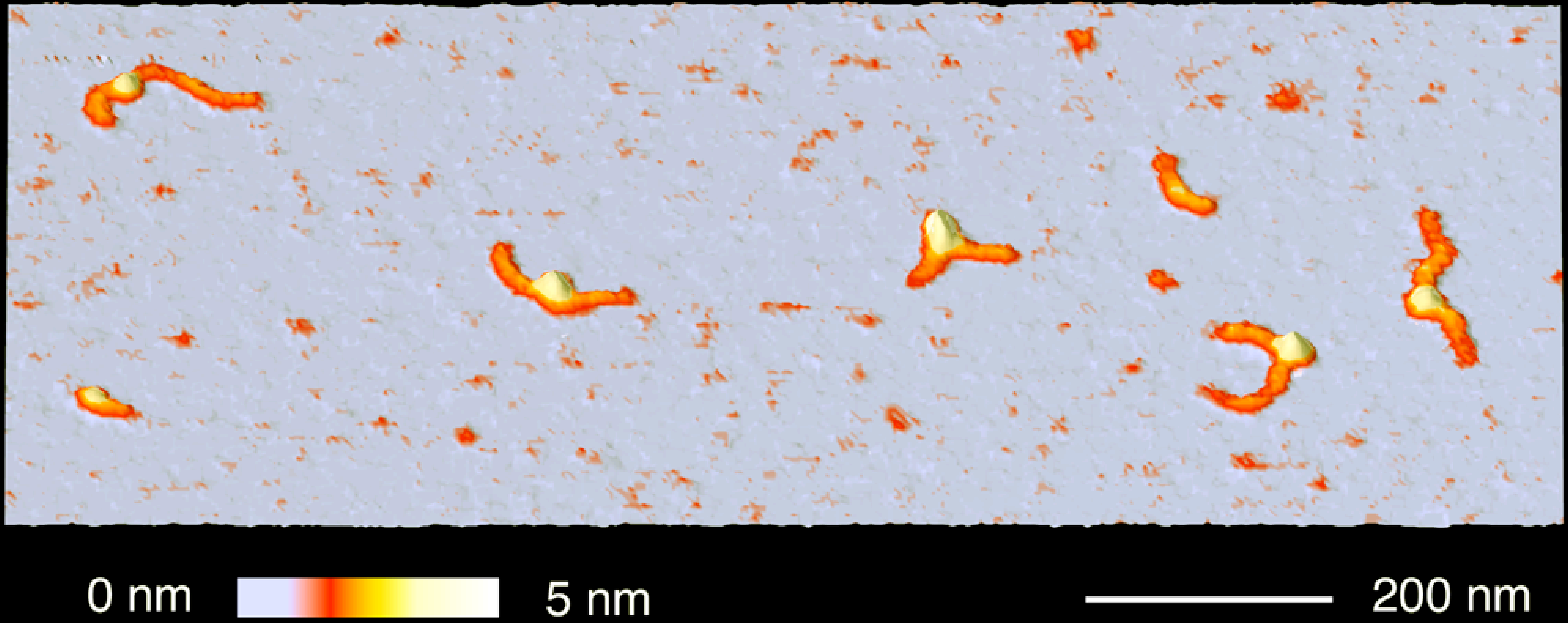


Dissociation of a nucleosome from the DNA



DNA contour length increases as the DNA unwraps from the histone core

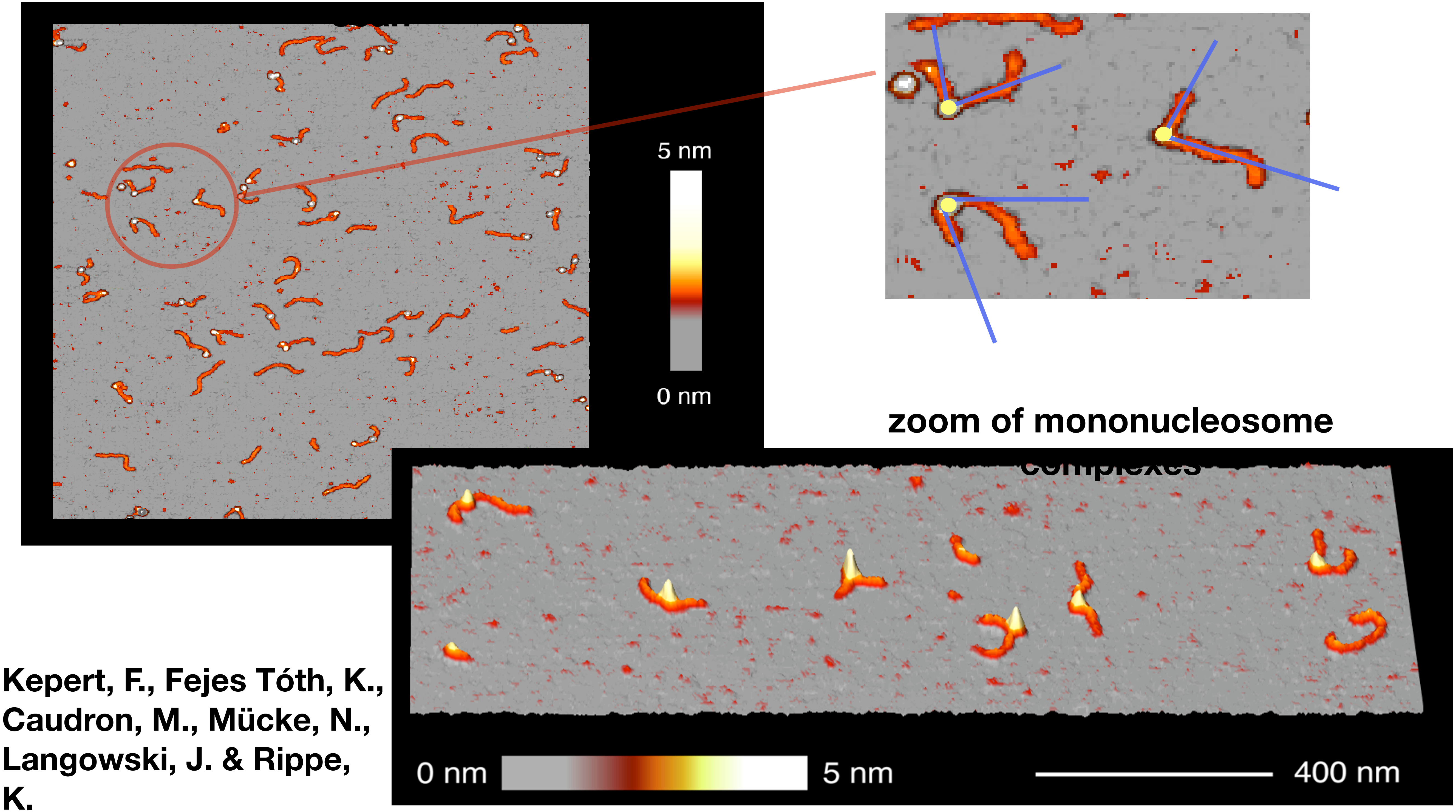
SFM image of single nucleosomes on a 200 nm DNA fragment



J. Kepert, K. Fejes Tóth, M. Caudron, N. Mücke, J. Langowski and K. Rippe (2003), Biophys. J. **85**, 4012-4022

SFM image of a nucleosome on a 614 base pair DNA

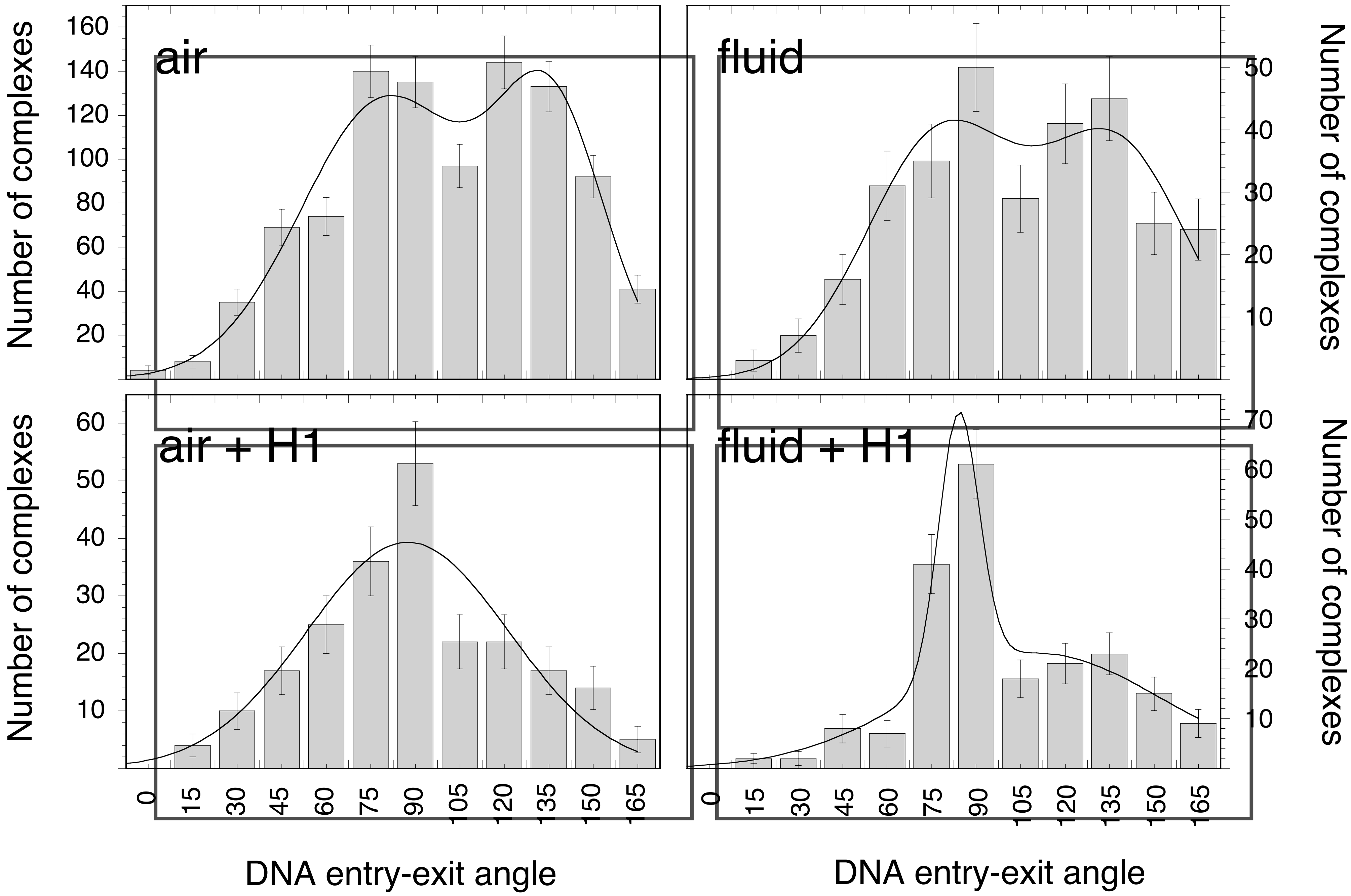
2 µm x 2 µm overview



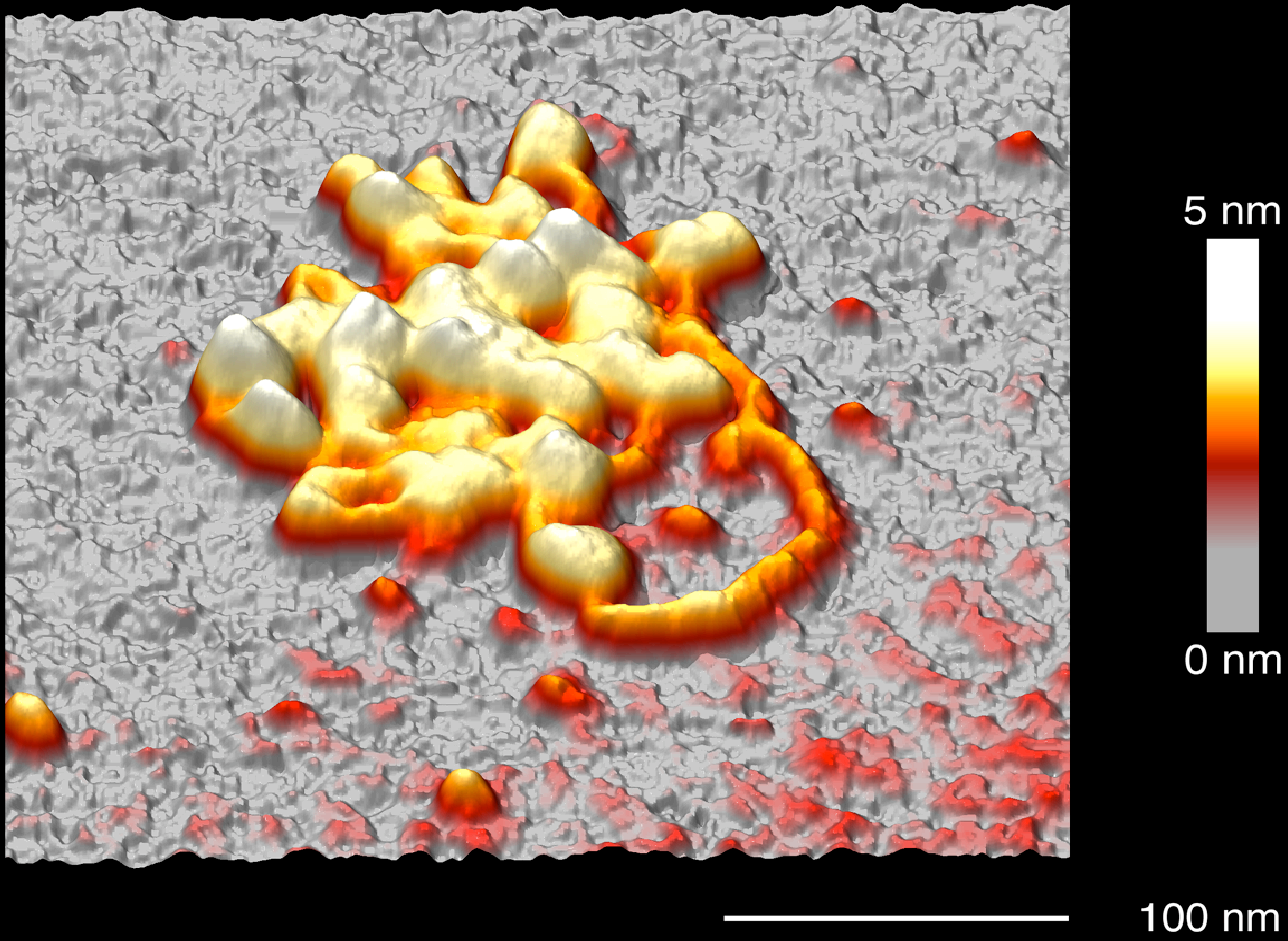
zoom of mononucleosome complexes

Kepert, F., Fejes Tóth, K., Caudron, M., Mücke, N., Langowski, J. & Rippe, K.

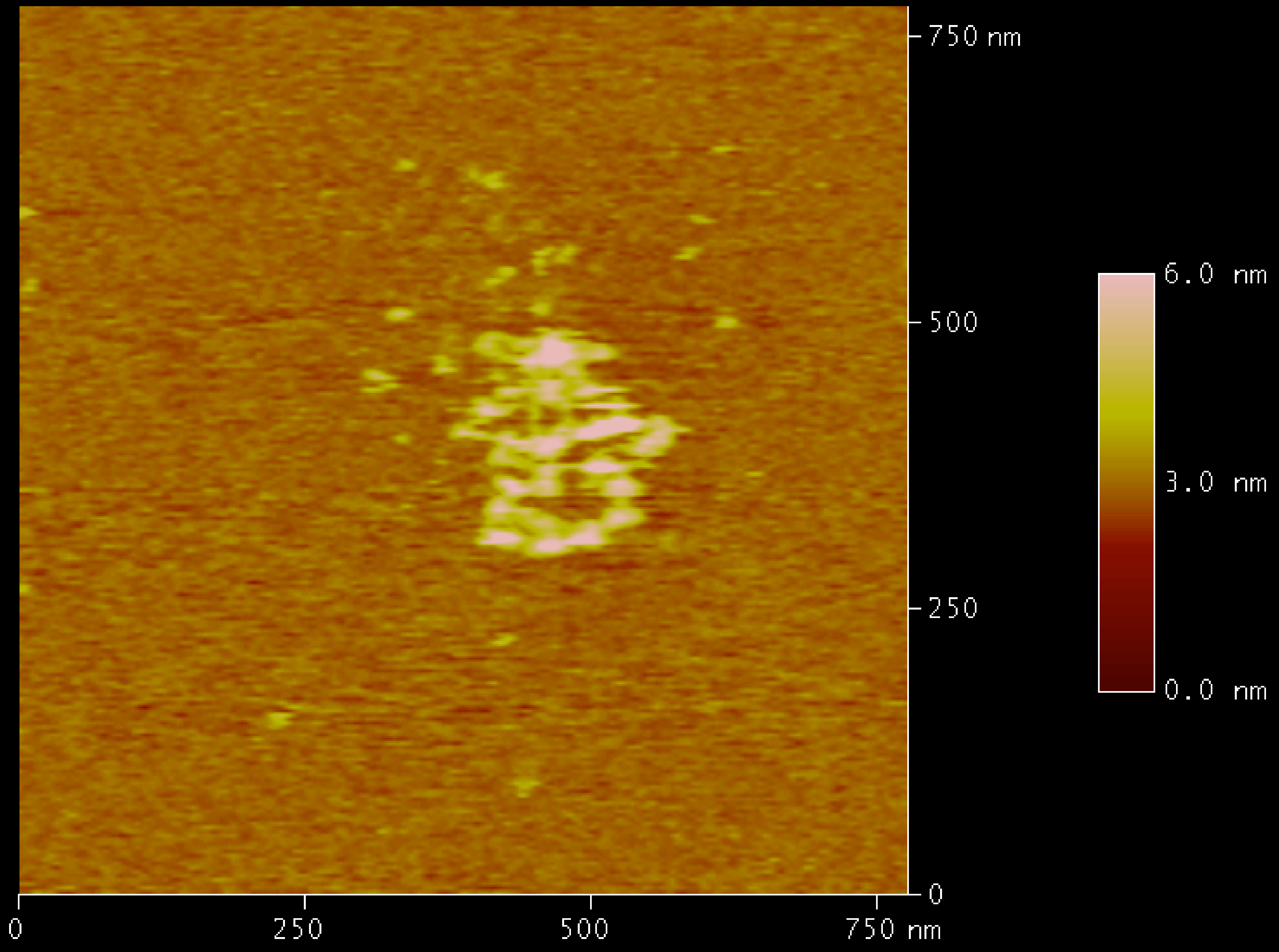
Linker histone H1 stabilizes a smaller entry-exit angle



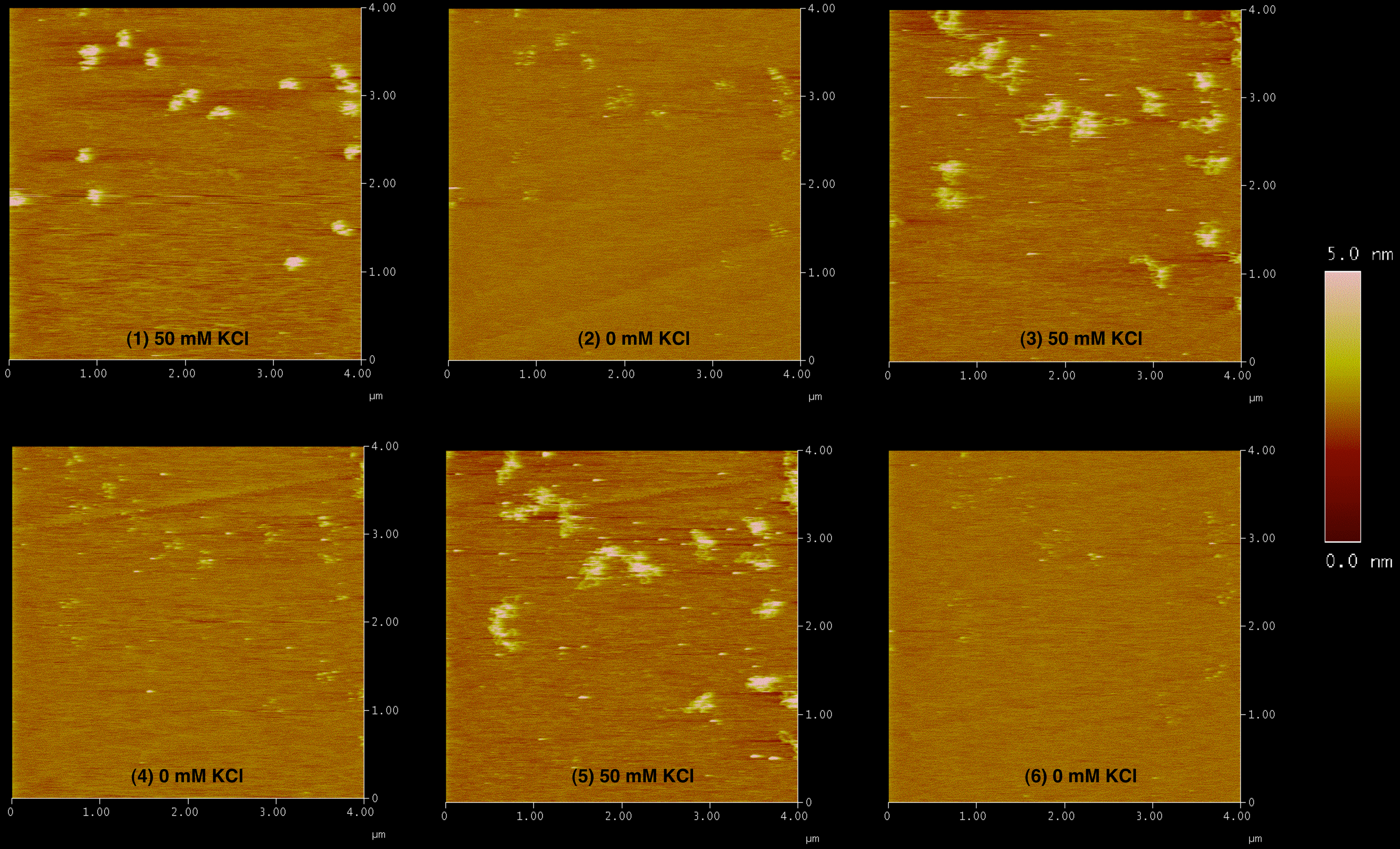
SV 40 minichromosome



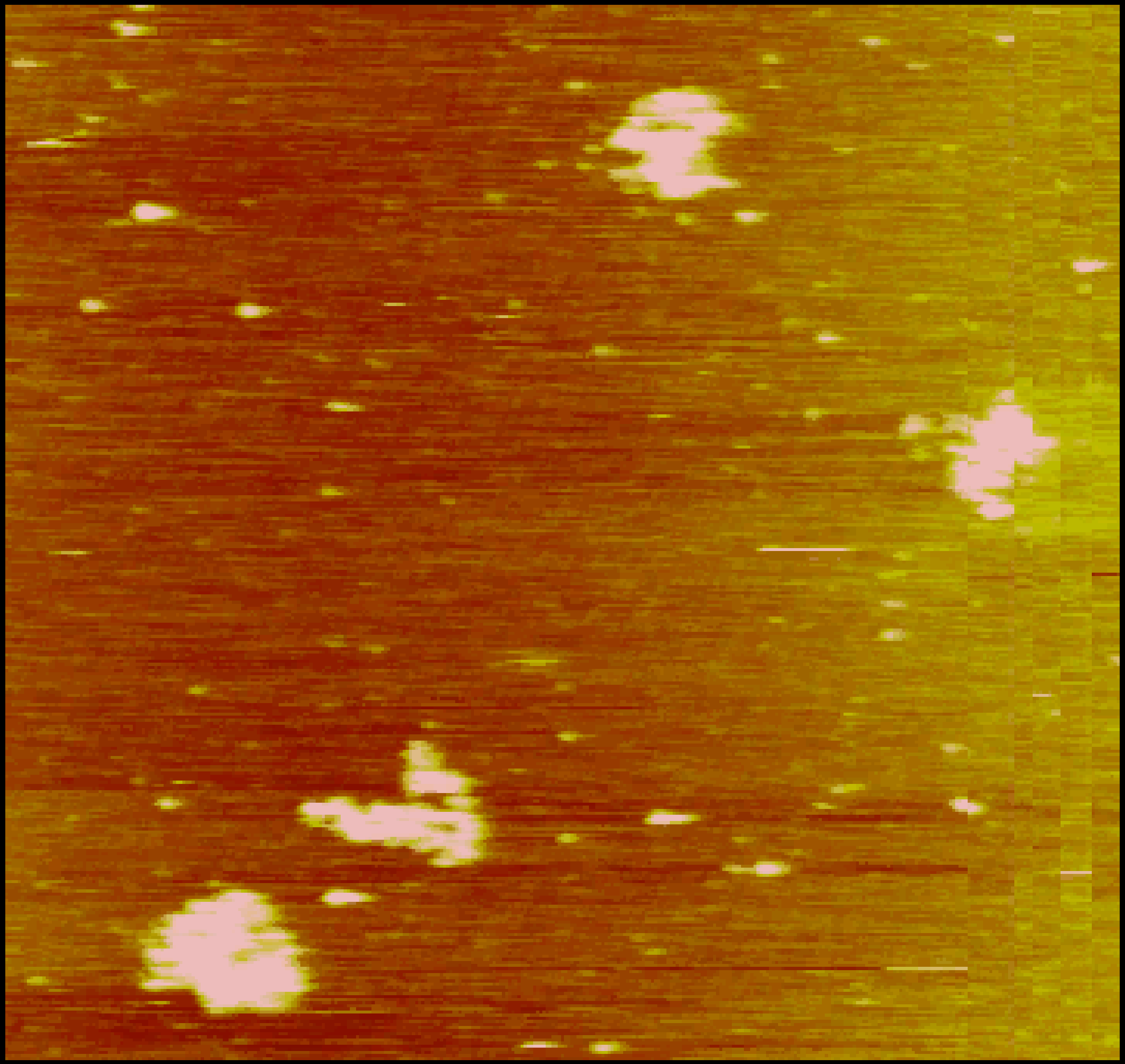
Nucleosomes reconstituted on a plasmid imaged by SFM in buffer



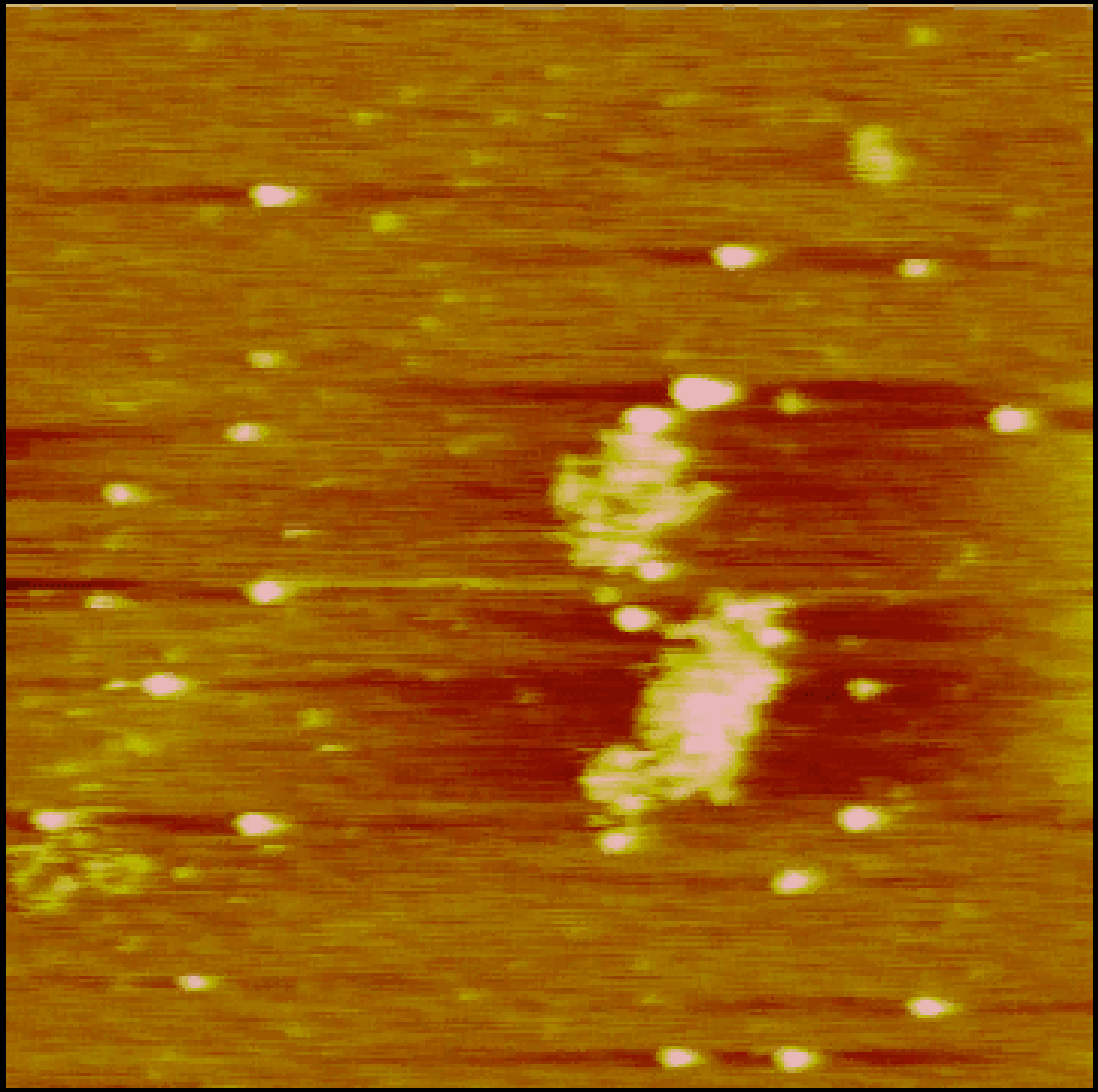
(De)condensation of nucleosome chains by changes of the salt concentration



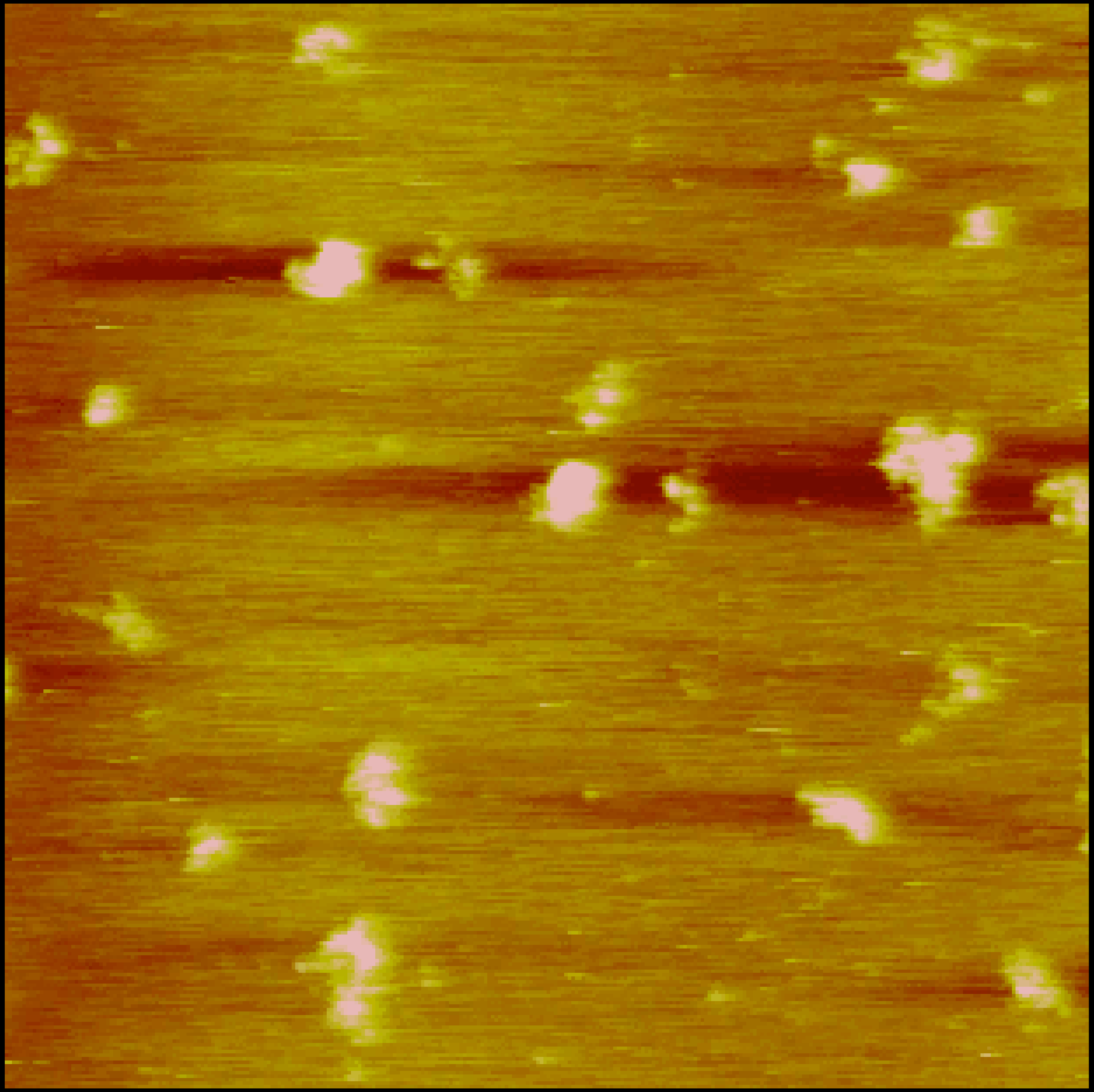
Changing salt from 50 to 0 to 50 mM KCl to induce (de) condensation of a nucleosomal array (2 x 2 µm scan)



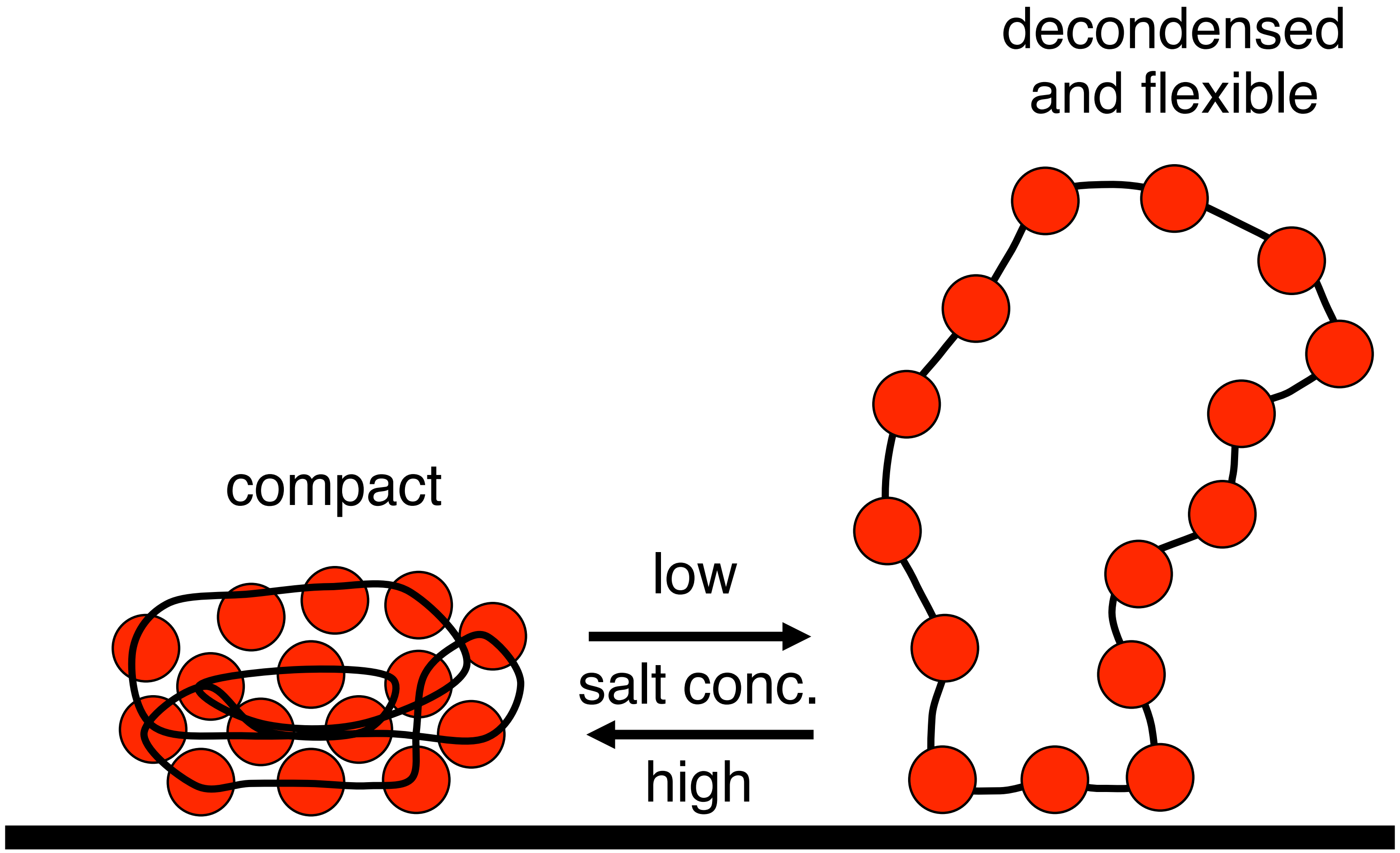
Salt induced (50 to 0 to 50 mM KCl) (de)condensation of a nucleosomal array (1 x 1 µm scan)



Changing the salt concentration has little effect on a glutaraldehyde fixed nucleosomal array (4 x 4 µm scan)



Model for salt induced changes of the nucleosome array conformation



decondensed
and flexible

compact

low

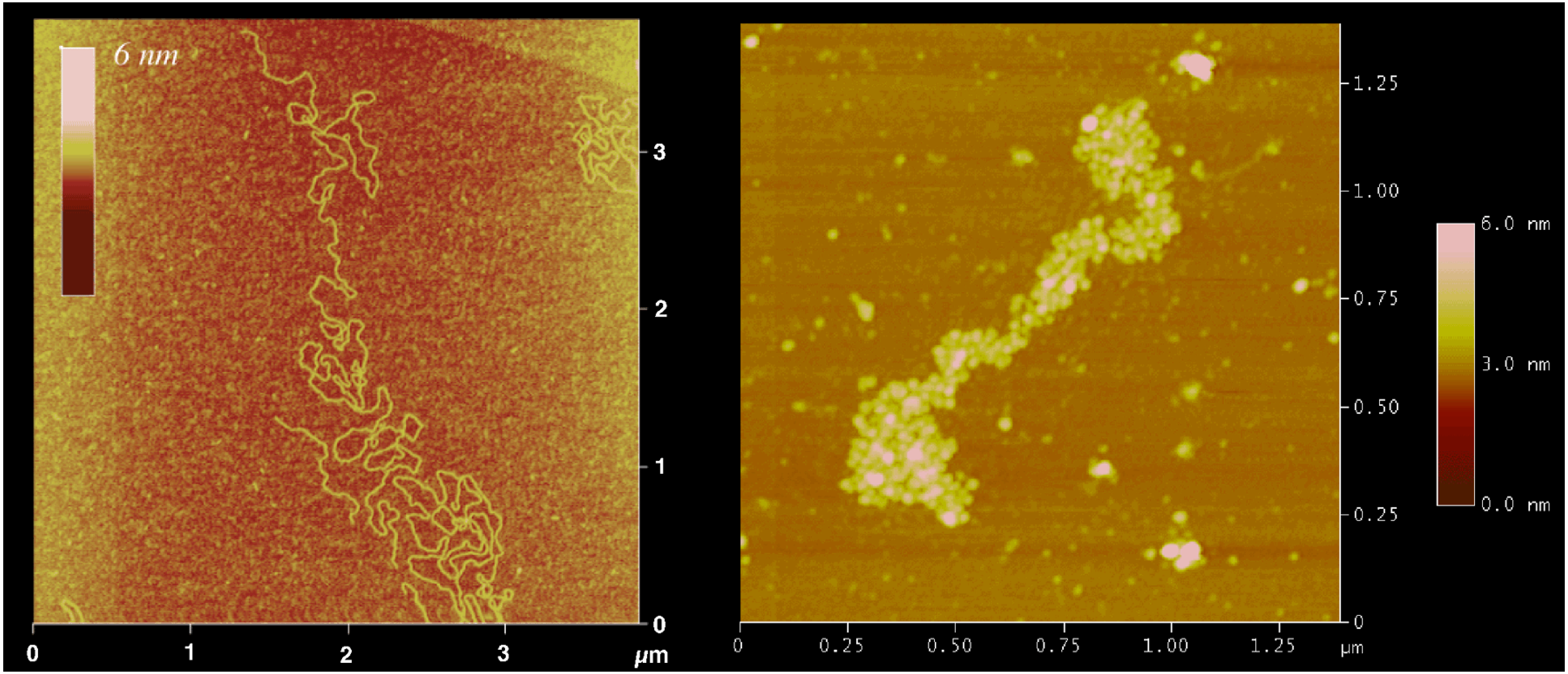
salt conc.

high

Compaction of DNA into the 30 nm chromtin fiber visualized by SFM imaging

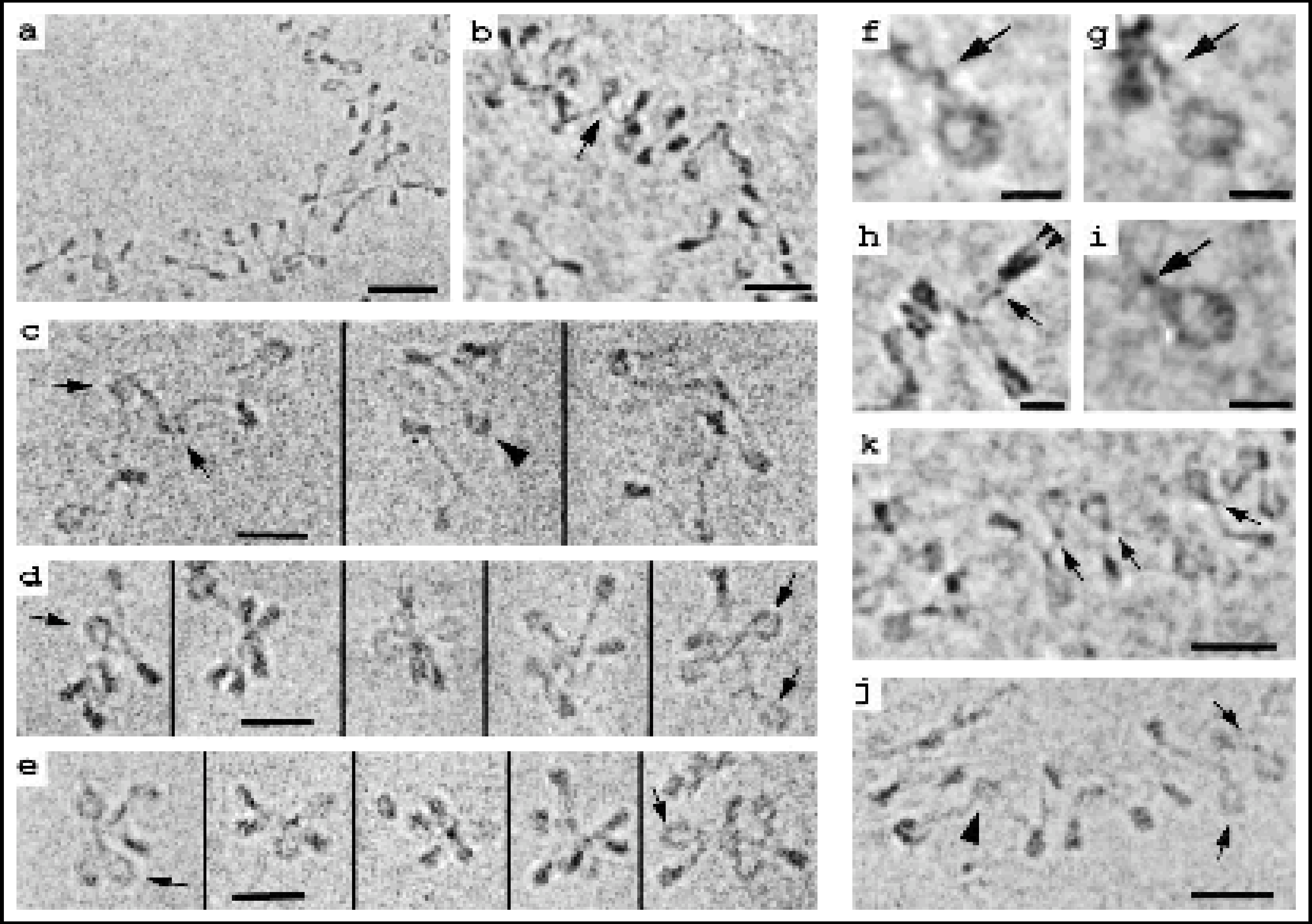
free DNA

chromatin fiber

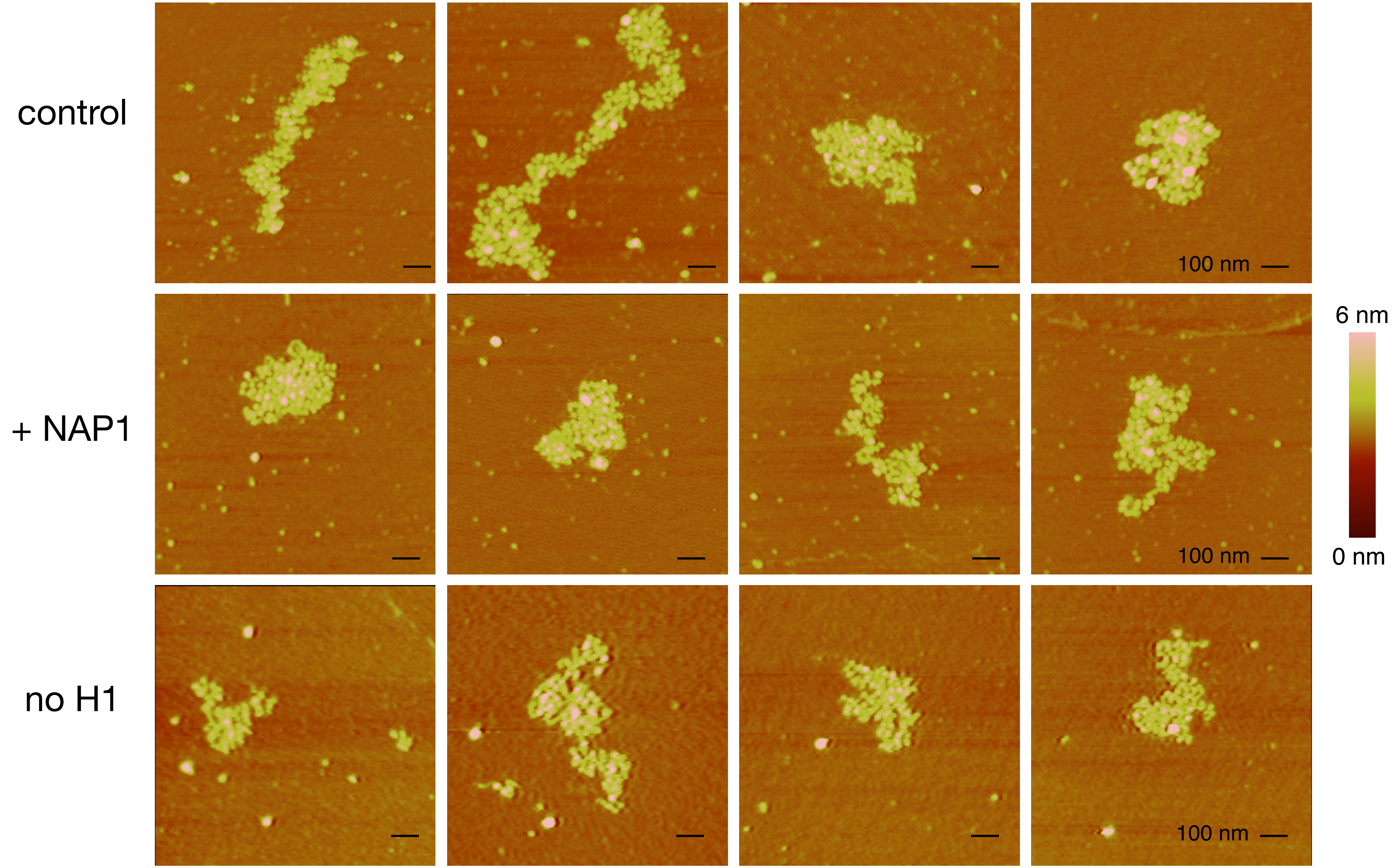


≈ 30 fold compaction contour length

Bednar et al. PNAS (1998) 95, 14175

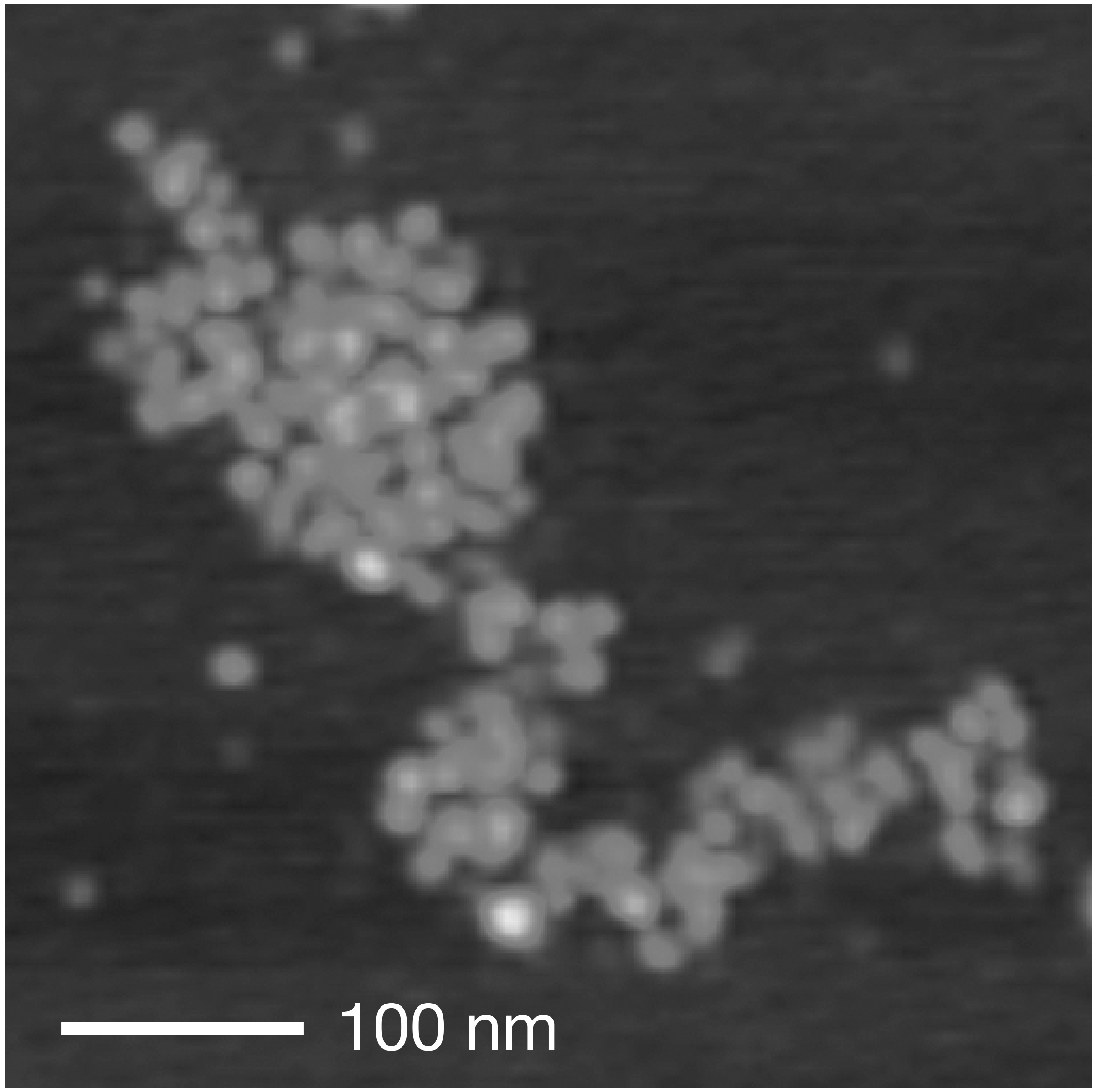


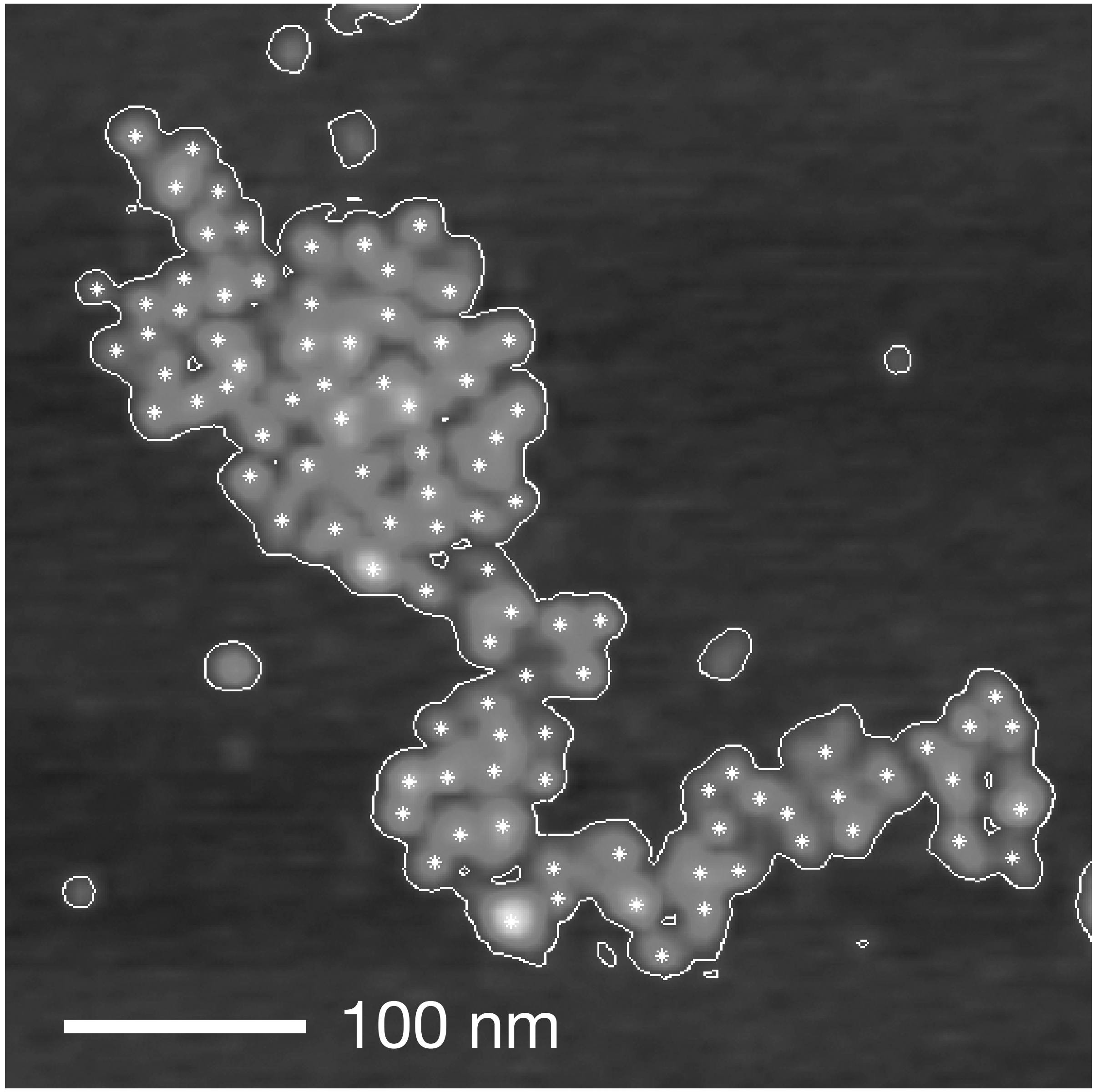
SFM images of chromatin fibers from HeLa cells



Kepert, J. F., Mazurkiewicz, J., Heuvelman, G., Fejes Tóth, K. and Rippe, K. (2005). *J. Biol. Chem.* **50**, 34063.

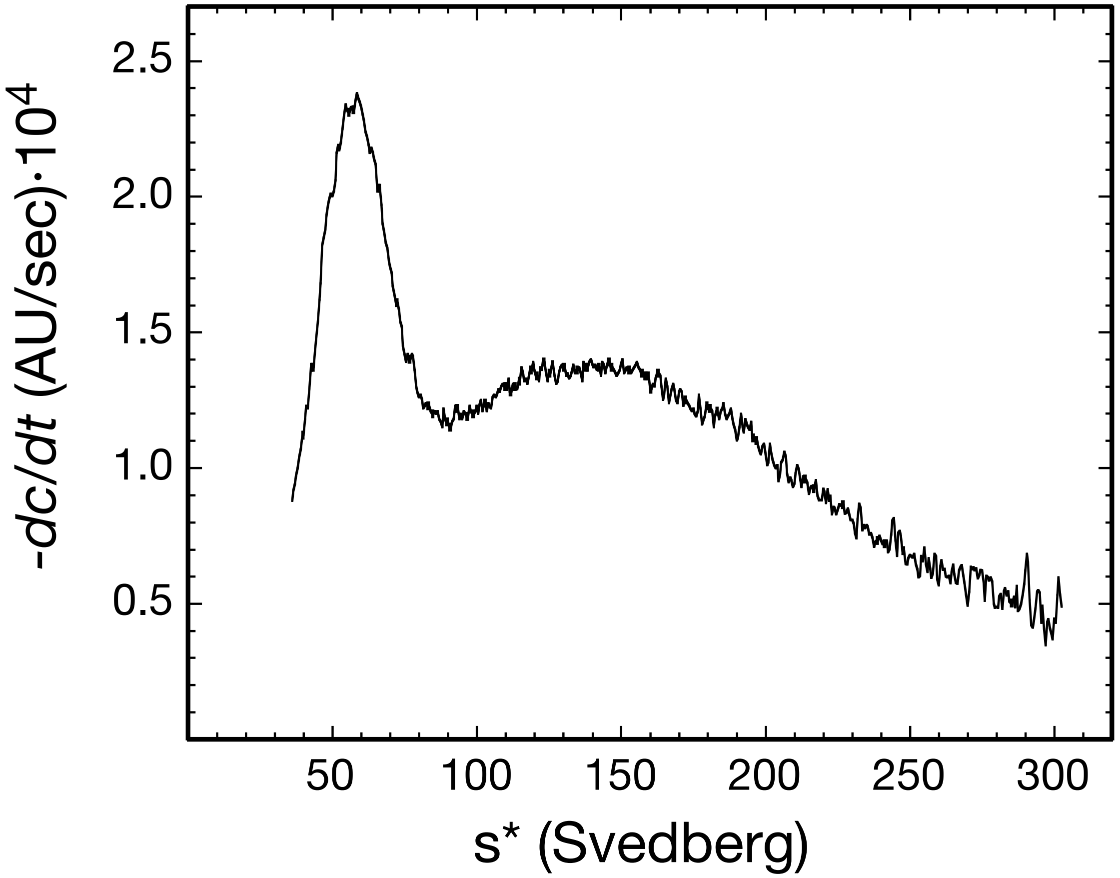
Evaluating chromatin fibers on SFM images



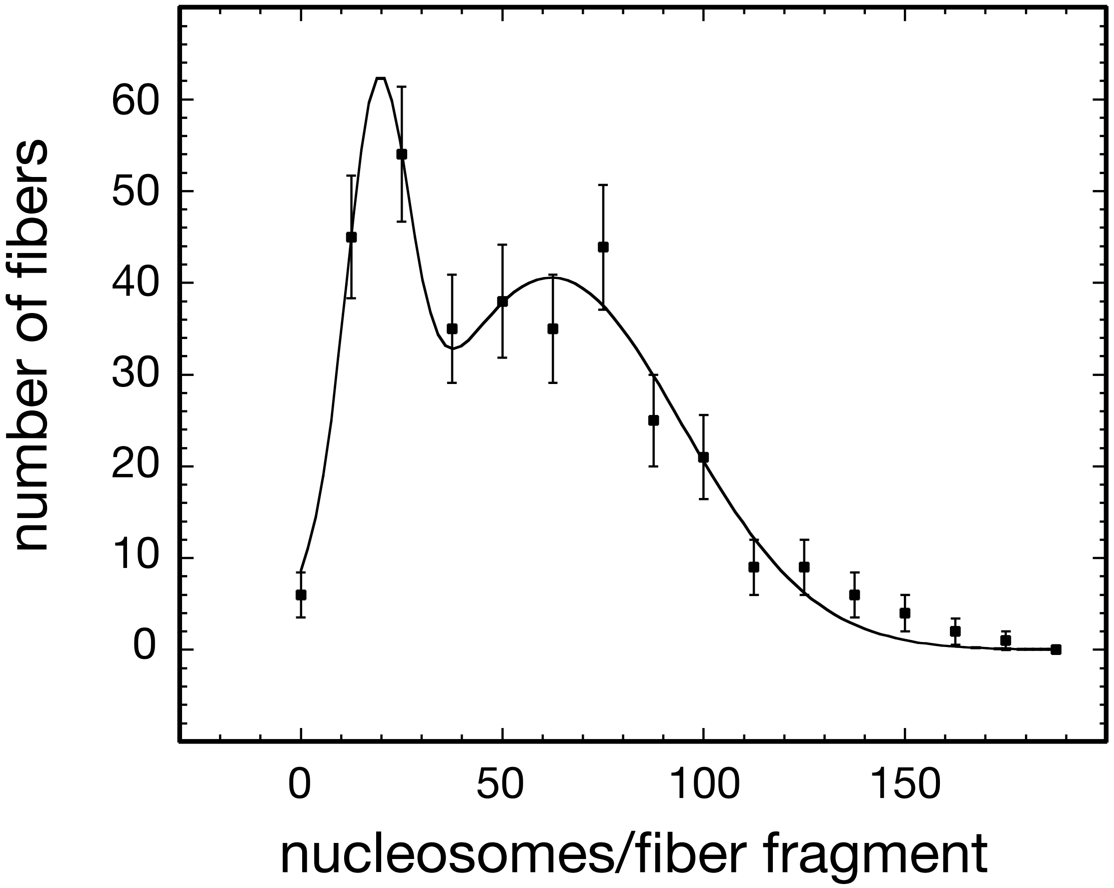


Characterisation of chromatin fiber fragments by analytical ultracentrifguation (AUC) and SFM

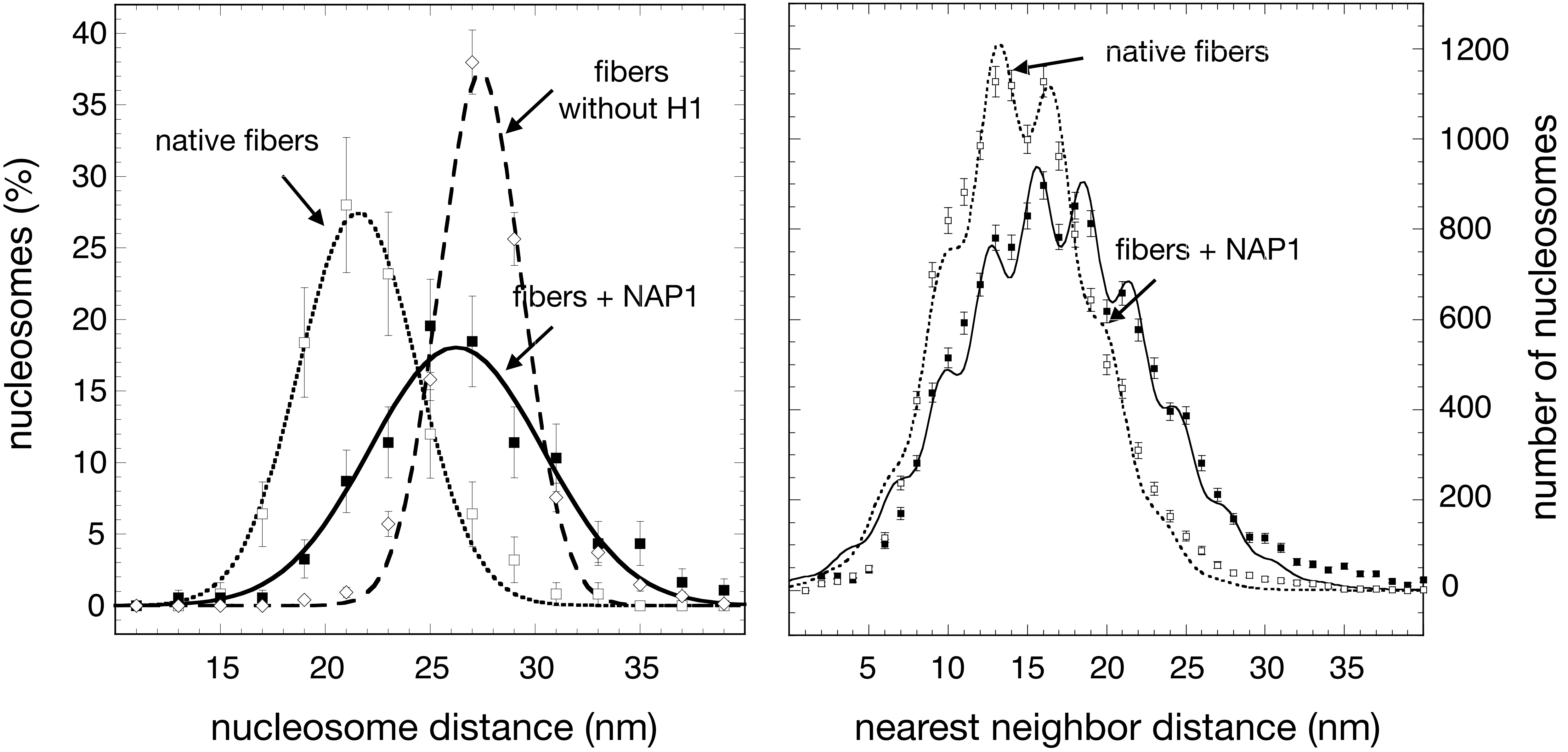
AUC sedimentation coefficient distribution



number of nucleosomes per fiber fragment determined by SFM

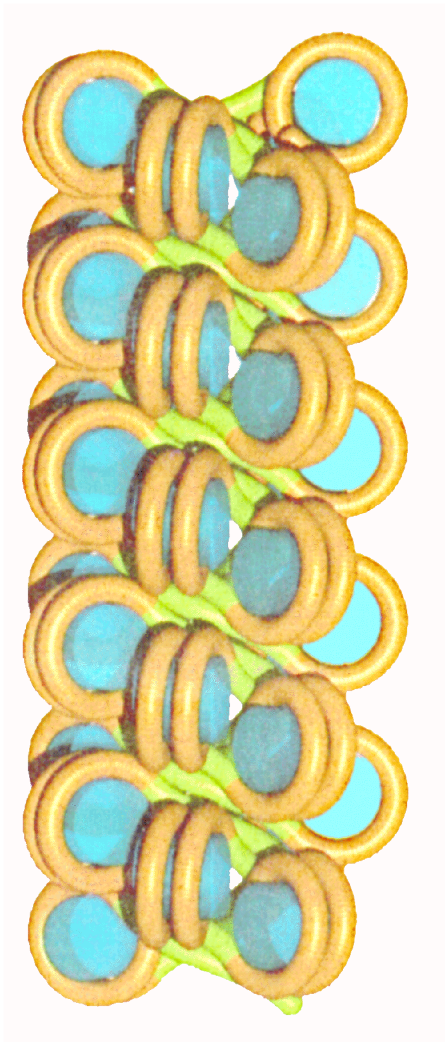


NAP1 reduces the average nucleosome distance in chromatin fiber by 5.3 ± 0.7 nm or 19 base pairs

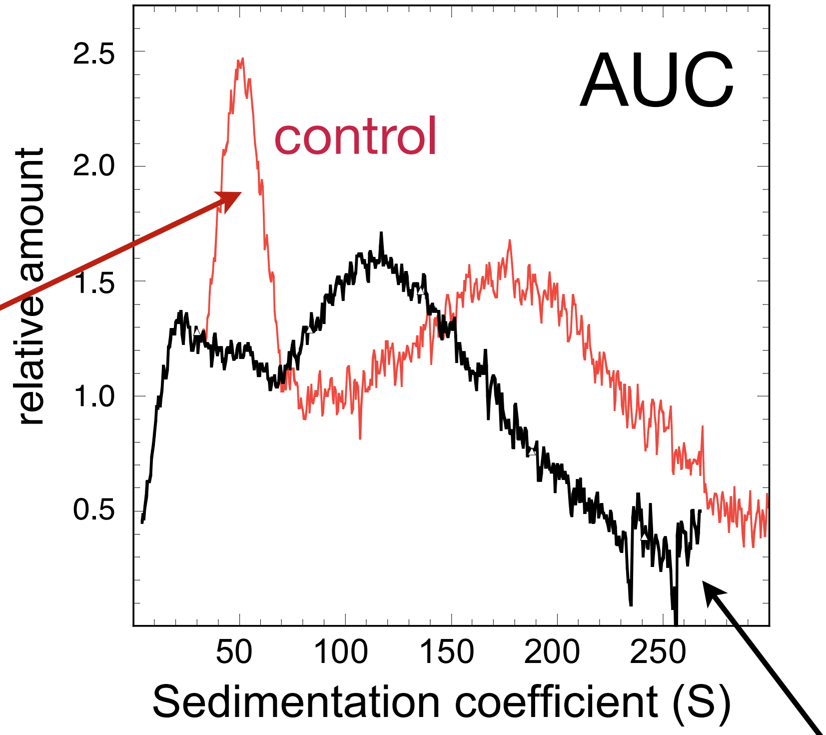


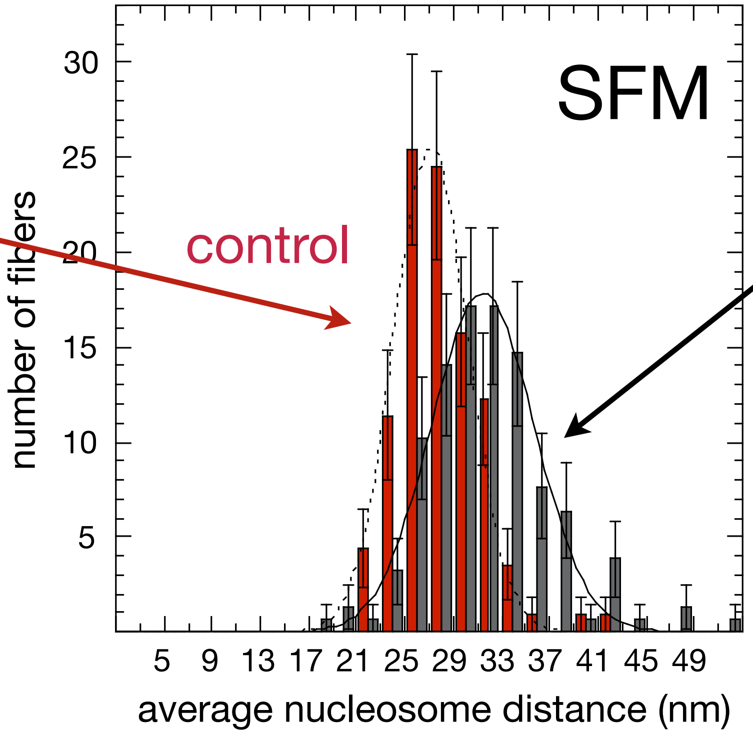
Combined AUC and SFM *in vitro* analysis of conformation changes of the chromatin fiber

condensed conformation
with high s-value



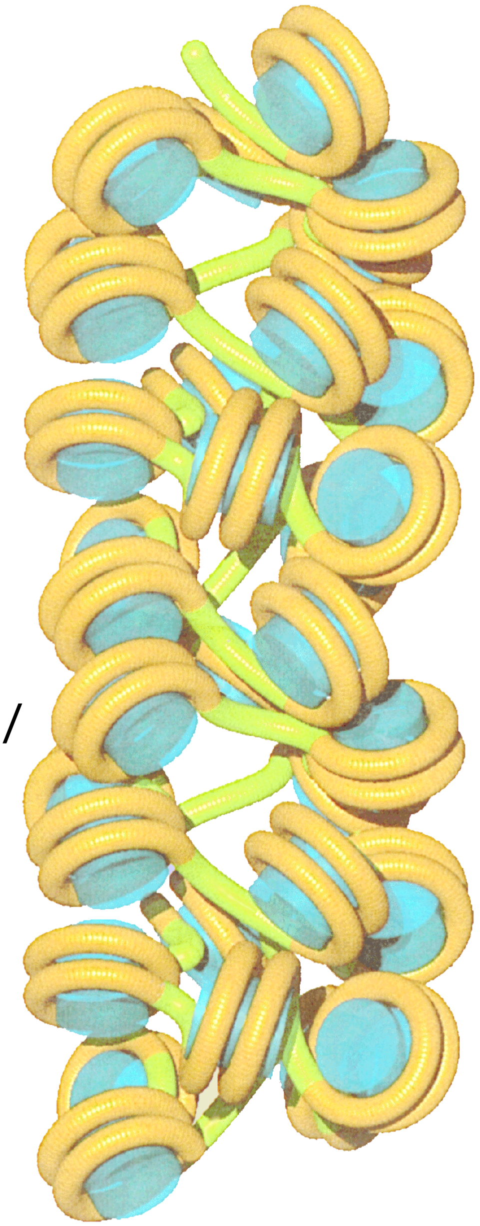
lower distance
between nucleosomes





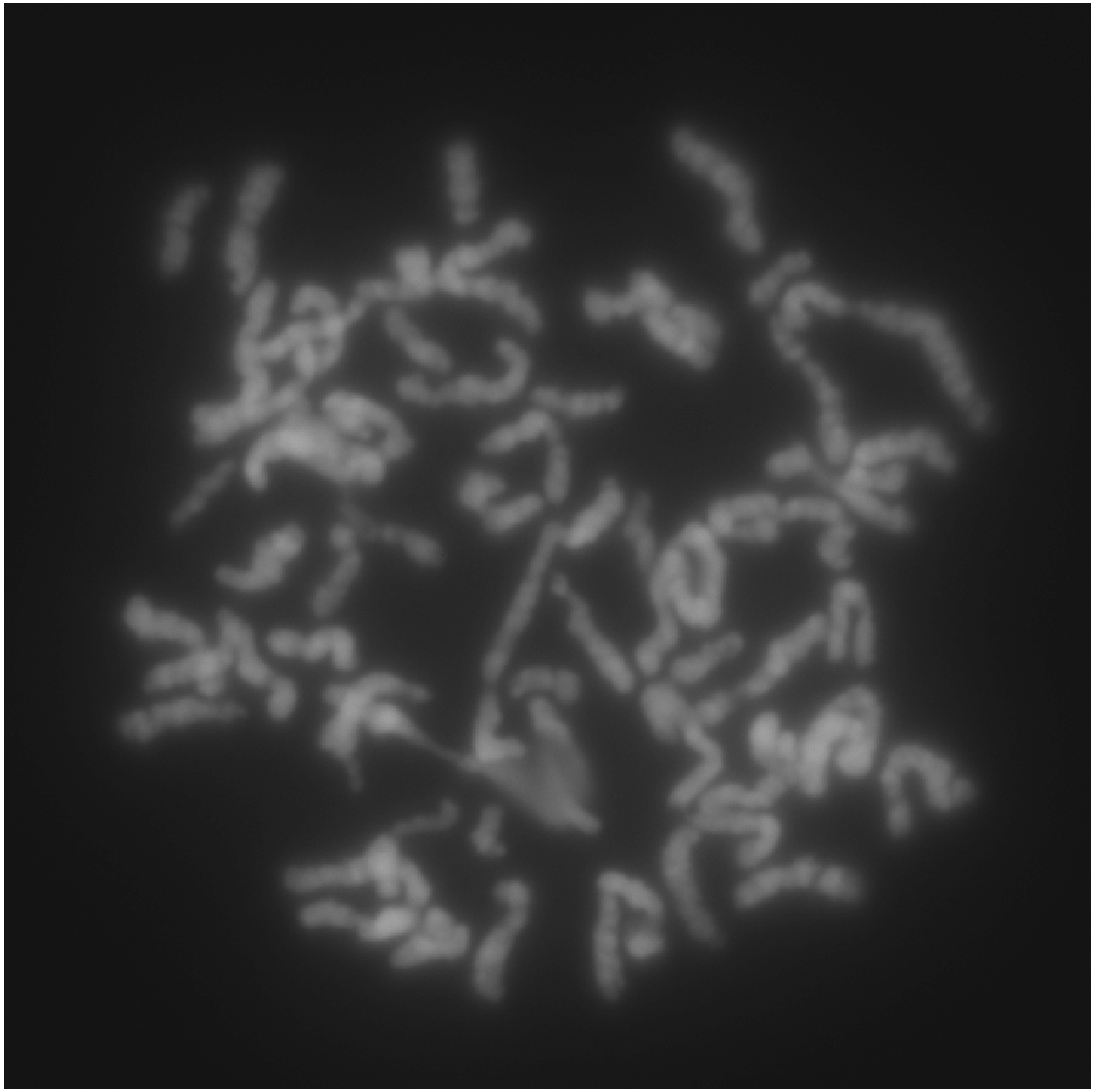
H1 removal/
acetylation

extended conformation
-> reduced s-value

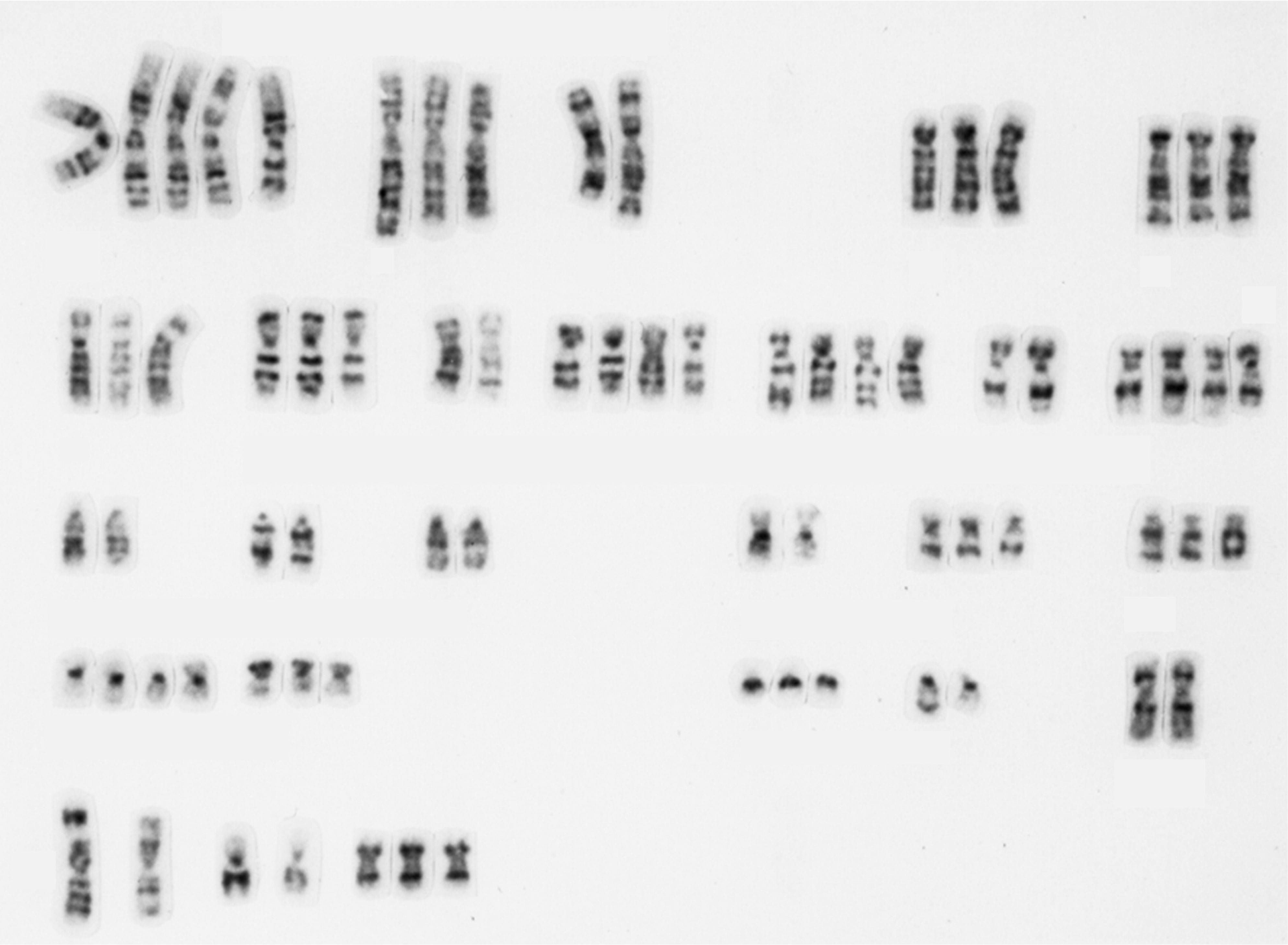


increased distance
between nucleosome

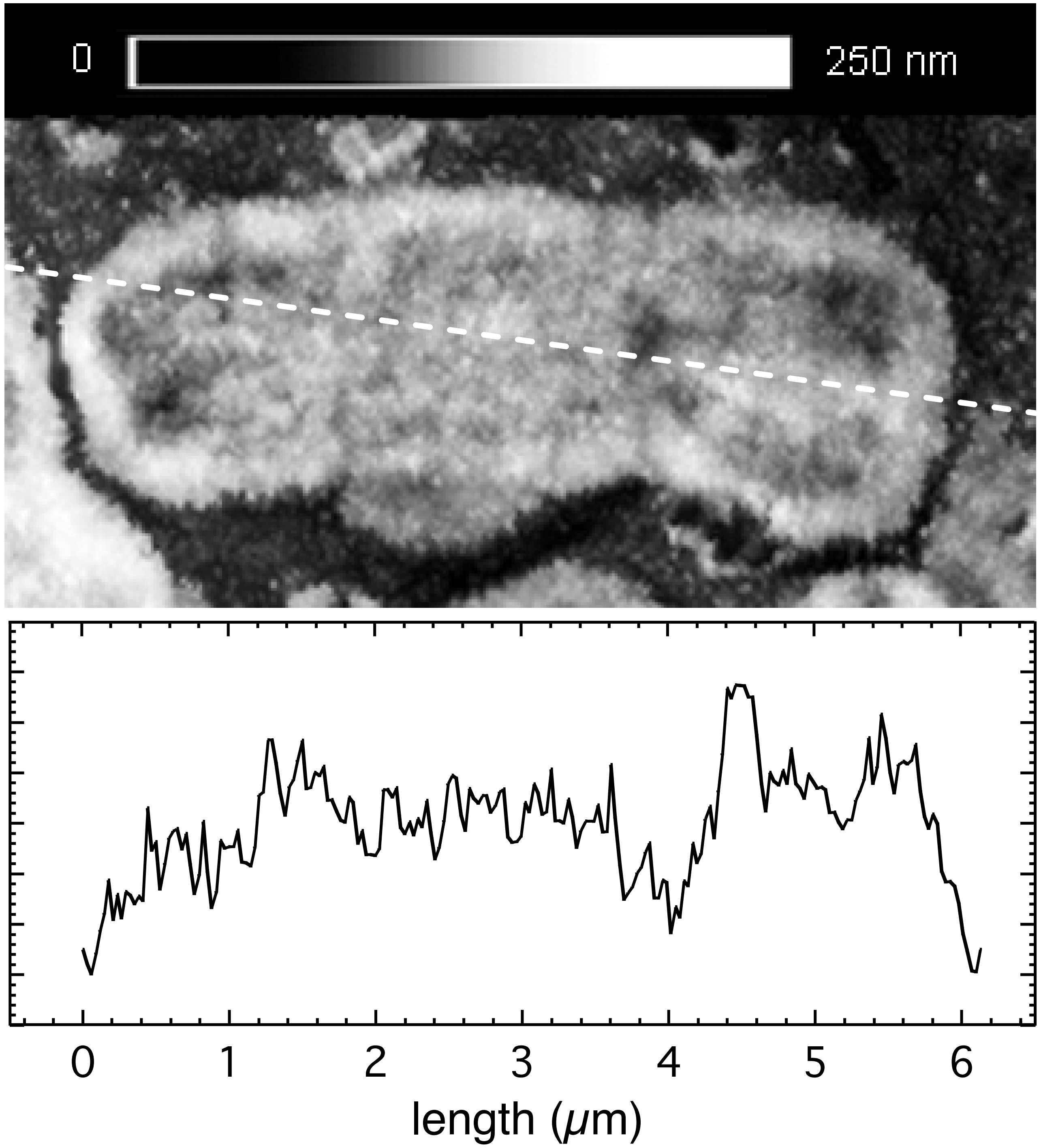
Fluorescence microscopy image (100 µm x 100 µm) of unfixed condensed chromosomes during cell division



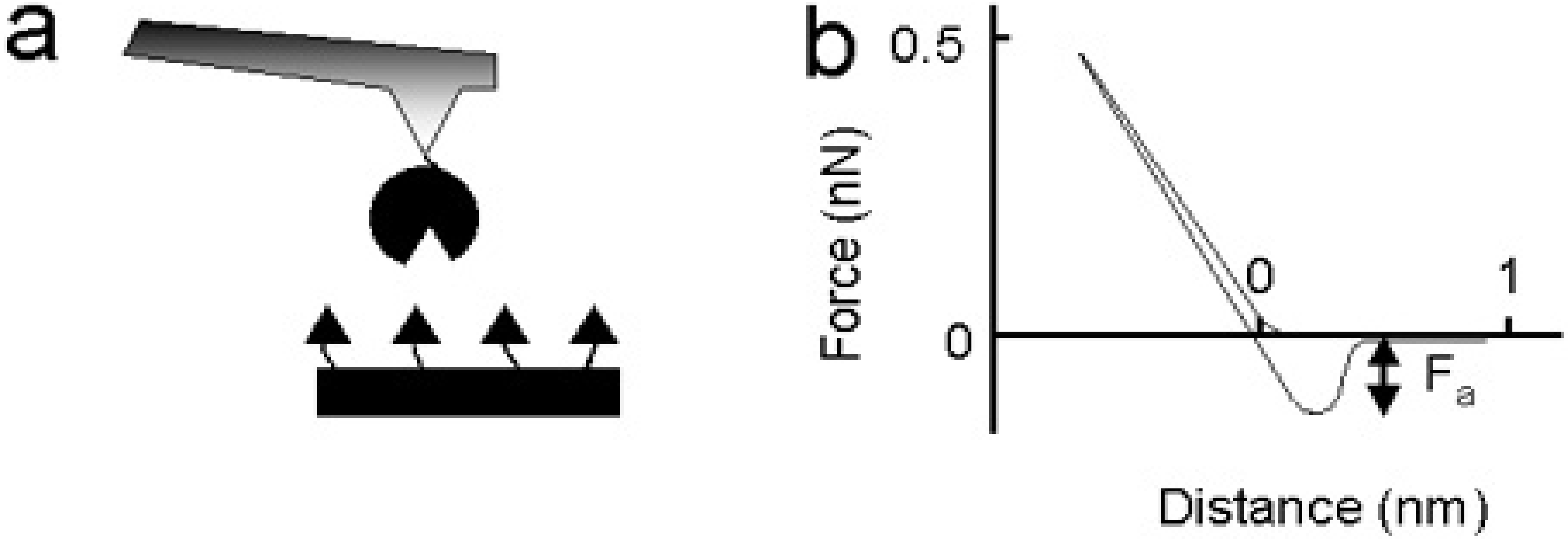
Chromosome banding pattern after fixation and staining



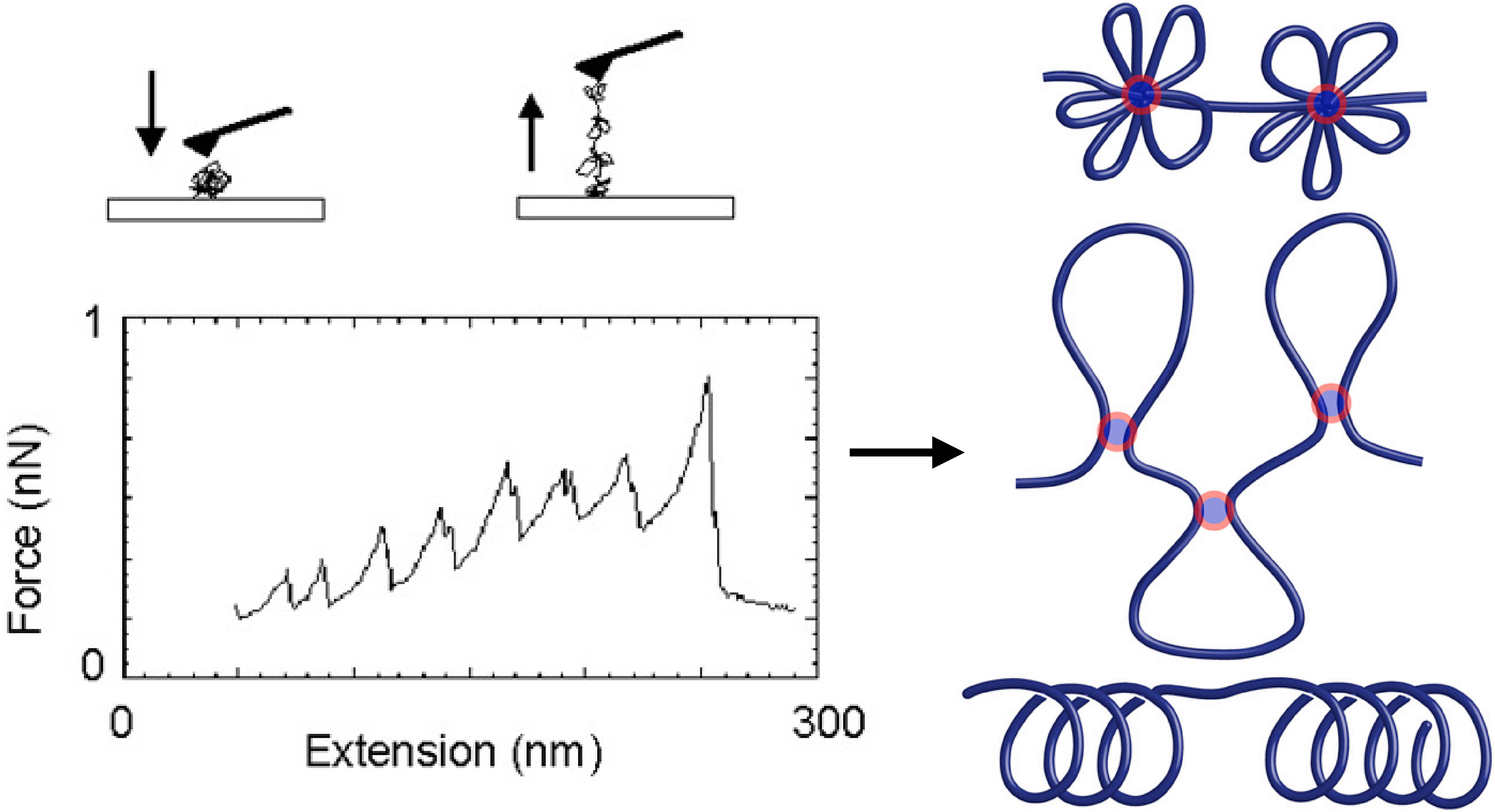
SFM topography analysis of the banding pattern of a single metaphase chromosome



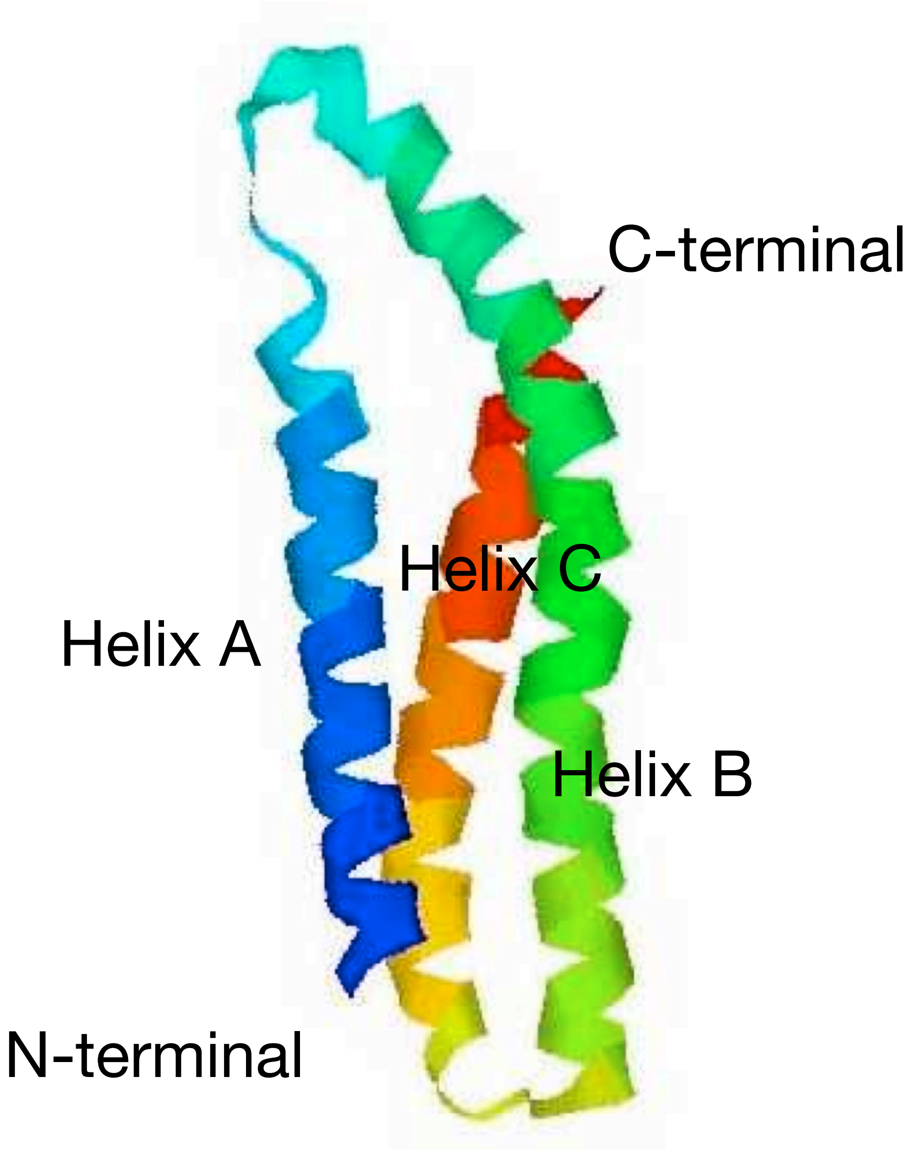
Force spectroscopy



Outlook: Force spectroscopy of chromosomes to analyze higher order folding of chromatin



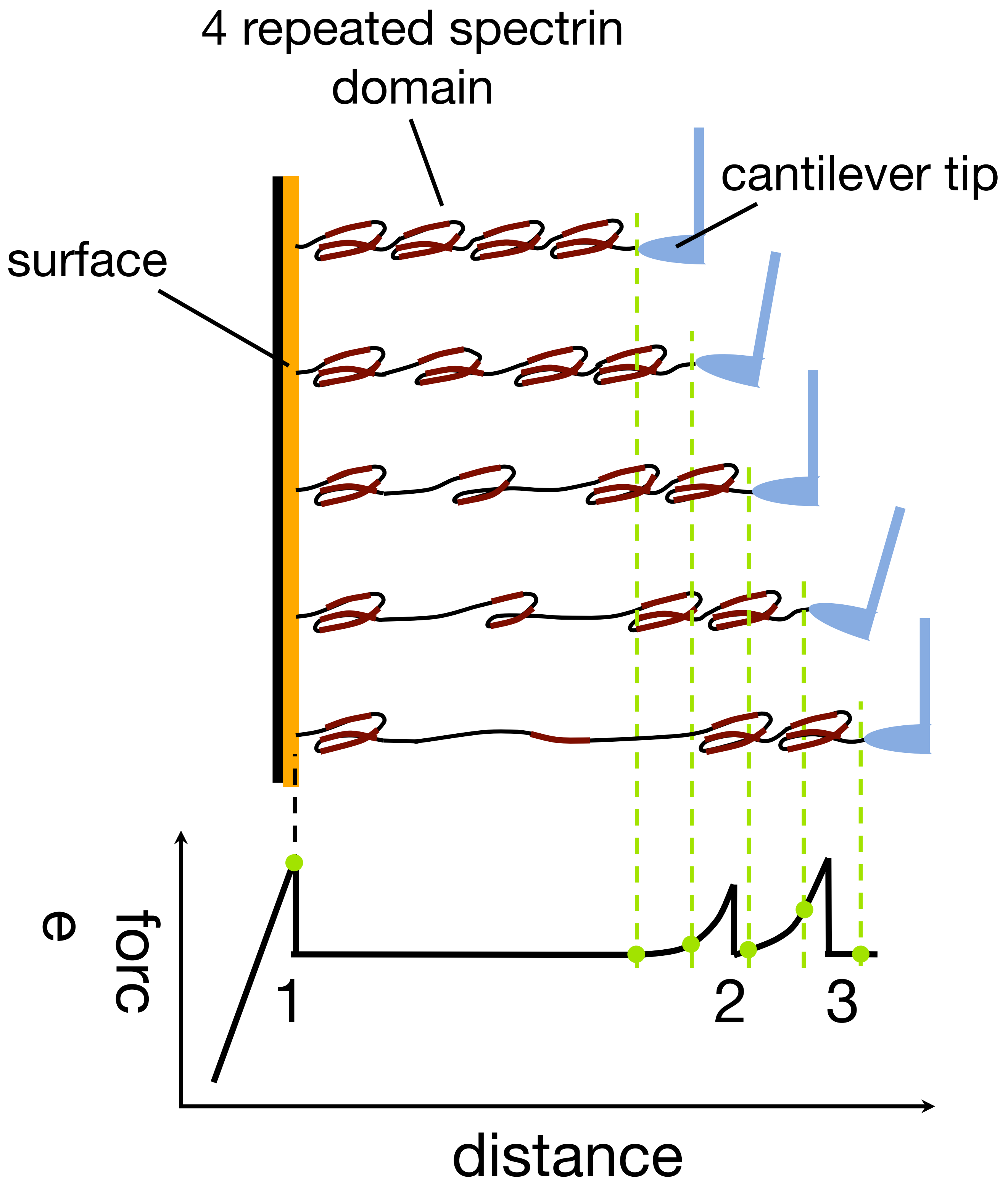
Spectrin



molecule that contributes to the mechanical properties, especially the elasticity of the cells

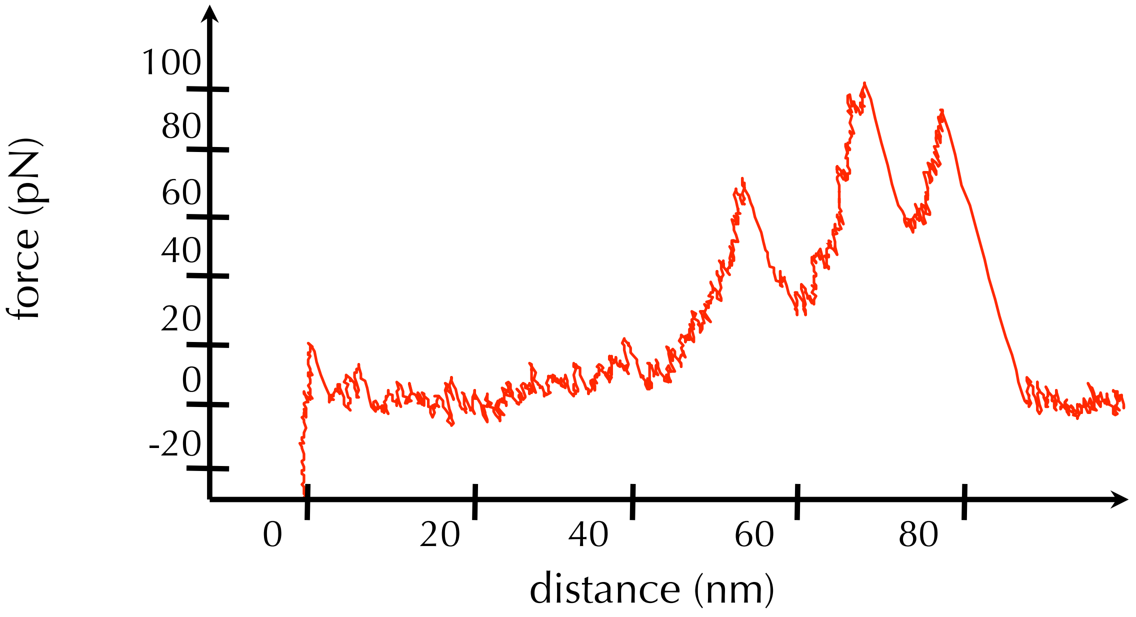
measurement of its mechanical stability provides information about the physiological function

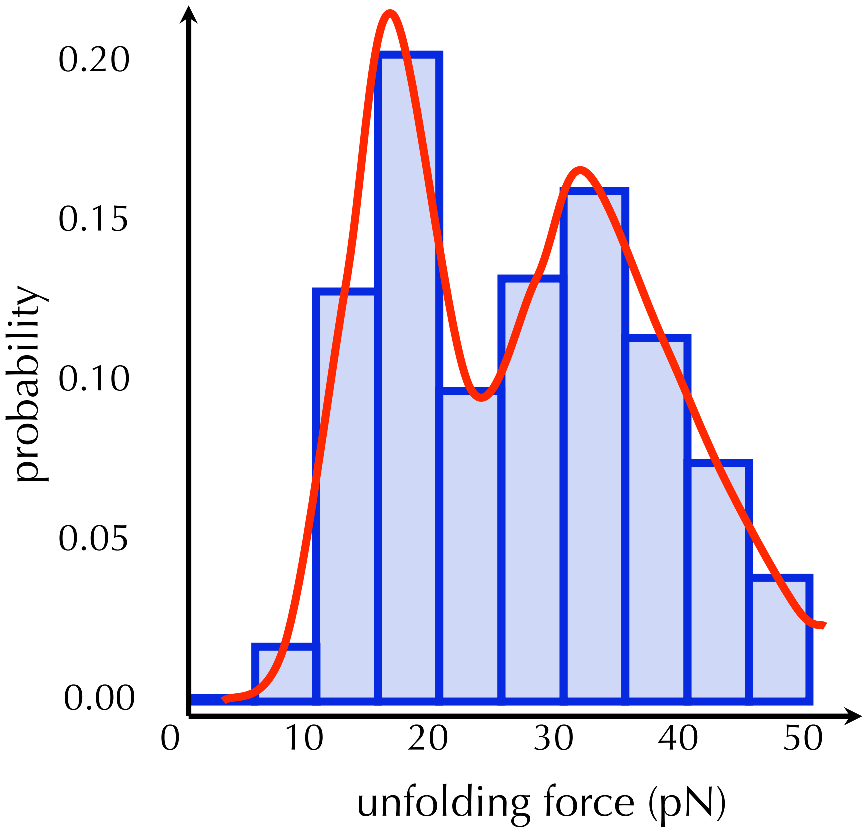
Stretching spectrin with an SFM



1. adhesion force between cantilever tip and surface
2. dissociation from the folded state to the intermediate unfolded state
3. dissociation from the intermediate to the total unfolding state

Stretching spectrin with an SFM





Summary on SFM and its applications to chromatin

- Molecular resolution (typivally 5-10 nm lateral, several Å height), sufficient to measure DNA bending angle or protein binding position on DNA but no atomic resolution (protein subunits, DNA double helix etc.), good contrast
- 3D information but requires binding of sample to a surface
- Sample preparation and imaging is “simple” and fast
- Conformation changes of chromatin like salt dependent (de)condensation in physiological buffer solutions can be directly visualized by SFM
- Important parameters that determine chromatin organisation (single nucleosome, array of nucleosomes, chromatin fiber, chromosomes) can be identified by SFM from the nm to µm length scale
- Force spectroscopy experiments can be conducted with an SFM setup to measure force in supramolecular complexes and to derive information on their higher order structure, e. g. folding of chromatin fiber