Goal

The AFM images used for in this project were obtained in the Langowski Lab of the DKFZ. The group is interested in nucleosome dynamics. To further investigate the dynamics of nucleosomes, K.Tegeler cloned histone H2A mutants. Two arginines (81 and 88), located in the docking domain of the H2A, were replaced by either alanine or glutamic acid. The group worked on *in vitro* reconstituted mutant and wild-type nucleosomes. The nucleosomes were reconstituted on DNA-strands, which contain the Widom 601 Sequence. This 170 bp DNA-Sequence is a strong positioning sequence for histone octamers (Quelle\_New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning). The H2A R 81, 88 mutants were proved to be good tools to analyze the stability and the opening behavior of nucleosomes on the/a single molecule level. In that context, *Single particle Förster resonance energy transfer* (spFRET) studies demonstrated that the mutants are significantly destabilized compared to wild-type nucleosomes (unpublished data).

For the AFM experiments wild-type and H2A R 81, 88 mutant nucleosomes were reconstituted on a 660 bp DNA fragment containing the Widom 601 positioning Sequence 199 bp apart from one end of the fragment. The DNA and the different nucleosomes were measured in air on a poly-L-lysine coated mica with different nm to pixel ratios.

The aim of our project was to implement a fully automatic algorithm to analyze the features of DNA and mono-nucleosomes from AFM-images. We work on images converted into TIFF Format to cover a broad range of possible applications for the algorithm.