P12 – Tip-enhanced Raman Spectroscopy on DNA

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Recent developments in sequencing methods aim towards single-molecule sequencing techniques, but most of those methods require some kind of labeling which might influence the biochemical behavior of the sample. Our approach applies tip-enhanced Raman spectroscopy (TERS), a method that combines a conventional Raman setup with an atomic force microscope (AFM), so that vibrational spectroscopic measurements with a high lateral resolution can be conducted.

For TERS experiments the AFM tip is coated with silver to enhance the intrinsically weak Raman signal. This tip is then positioned in the focus of the laser and only the sample moved underneath. TERS spectra were collected on several points on single strands of calf thymus DNA. Figure 1 shows a typical example for a TERS spectrum of DNA and a reference spectrum on a sample free spot, which has been recorded to prove that the probe itself is not contaminated.

Due to the size of the interaction area of the TERS tip and the DNA strand, the respective TERS spectra contain spectral contributions of approximately 30-60 bases. Our results show that although the Raman scattering cross section of the four nucleobases differs remarkably, specific bands of all bases can be determined in the spectra.

This is a further step towards a direct and label-free Raman sequencing method.

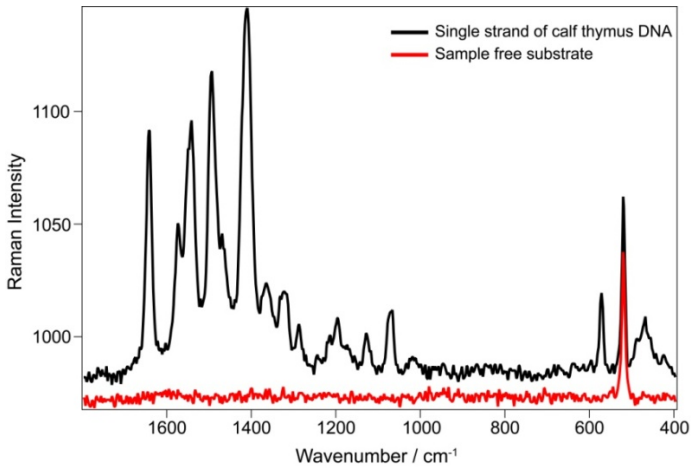
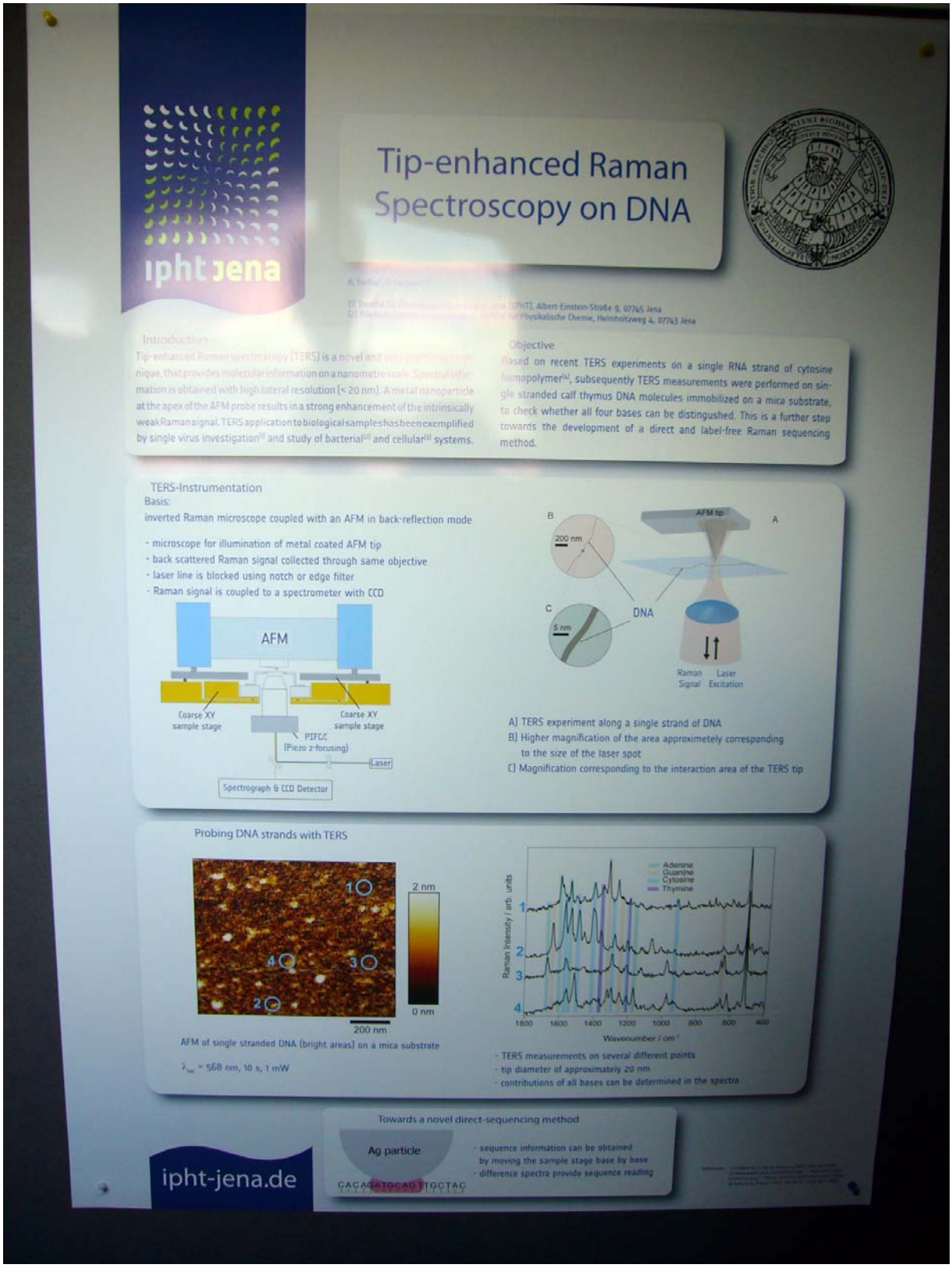


Fig. 1: TERS spectrum of a single strand of calf thymus DNA (black) and reference spectrum (red).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Tip-enhanced Raman Spectroscopy on DNA
Introduction
Tip-enhanced Raman spectroscopy (TERS) is a novel and very promising technique, that provides molecular information on a nanometre-scale. Spectral information is obtained with high lateral resolution (< 20 nm). A metal nanoparticle at the apex of the AFM probe results in a strong enhancement of the intrinsically weak Raman signal. TERS application to biological samples has been exemplified by single virus investigation[b] and study of bacterial[c] and cellular[d] systems.
Objective
Based on recent TERS experiments on a single RNA strand of cytosine homopolymer[a], subsequently TERS measurements were performed on single stranded calf thymus DNA molecules immobilized on a mica substrate, to check whether all four bases can be distingushed. This is a further step towards the development of a direct and label-free Raman sequencing method.
TERS-Instrumentation
Basis:
inverted Raman microscope coupled with an AFM in back-reflection mode
- microscope for illumination of metal coated AFM tip
- back scattered Raman signal collected through same objective
- laser line is blocked using notch or edge filter
- Raman signal is coupled to a spectrometer with CCD
AFM
Coarse XY sample stage
Coarse XY sample stage
PIFOC (Piezo z-focusing)
Laser
Spectrograph & CCD Detector
B
AFM tip
A
200 nm
C
5 nm
DNA
Raman Signal
Laser Excitation
A) TERS experiment along a single strand of DNA
B) Higher magnification of the area approximetely corresponding to the size of the laser spot
C) Magnification corresponding to the interaction area of the TERS tip
Probing DNA strands with TERS
1
2
3
4
2 nm
0 nm
200 nm
AFM of single stranded DNA (bright areas) on a mica substrate
λ_{ex} = 568 nm, 10 s, 1 mW
Adenine
Guanine
Cytosine
Thymine
Raman Intensity / arb. units
1800 1600 1400 1200 1000 800 600 400
Wavenumber / cm^{-1}
- TERS measurements on several different points
- tip diameter of approximately 20 nm
- contributions of all bases can be determined in the spectra
Towards a novel direct-sequencing method
Ag particle
CACAGATGCAGTTGCTAC
- sequence information can be obtained by moving the sample stage base by base
- difference spectra provide sequence reading
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