Diamond in Nanoscale Biosensing

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Outline

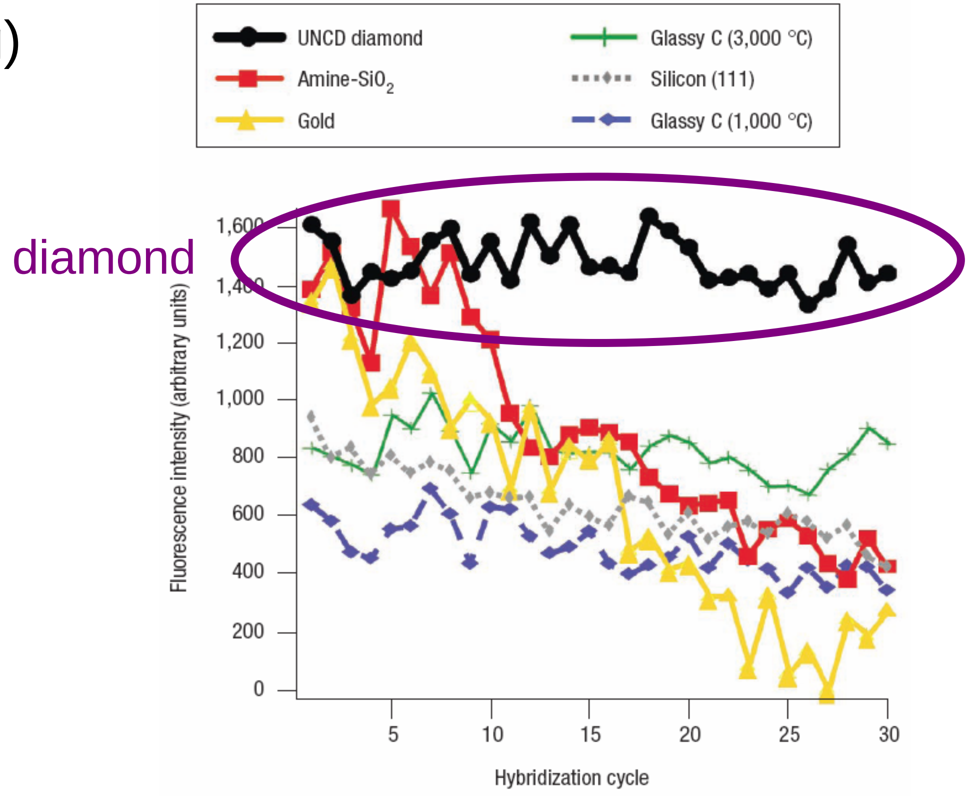
- Why nanoscale biosensing?
- Why diamond?
  - Hydrogen-terminated and oxidized diamond surfaces
- Attachment of DNA to diamond
  - Photo- and electrochemical methods
  - Fluorescence microscopy
- Structural and mechanical properties of DNA
  - Atomic force microscopy in liquids
  - Optimized detection of DNA thickness (phase shift)
  - Detailed DNA morphology
  - Geometric model $\rightarrow$ DNA orientation and density
  - Mechanical stability of DNA bonding
- Comparison with other substrate materials
- Conclusion

Biosensing

- **Crucial** for health care, medical treatment, drug development, …
- Typical: recognition of DNA sequences
  - Encoded genetic information and functions
  - Unique matching of base pairs (A-T,C-G)
- Big machines can do it well, but…
- Nanoscale biosensing
  - higher sensitivity (<fM), lower cost
  - portable and remote diagnostics (aging society!)
  - Needs <u>substrate</u> to carry DNA
  - Needs new ways of detection

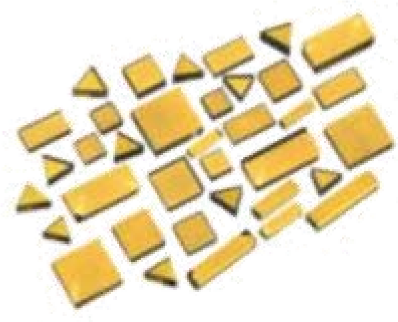
Diamond and biosensing

- Diamond is very interesting for bio-sensors
  - semiconductor (wide band gap)
  - considered highly biocompatible
  - transparent (optical sensing)
  - hard, durable, and stable…
  - well accepted by public



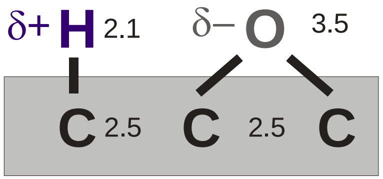
[W.Yang et al., Nature Mat. 1 (2002) 254]

Diamond surface functionalization



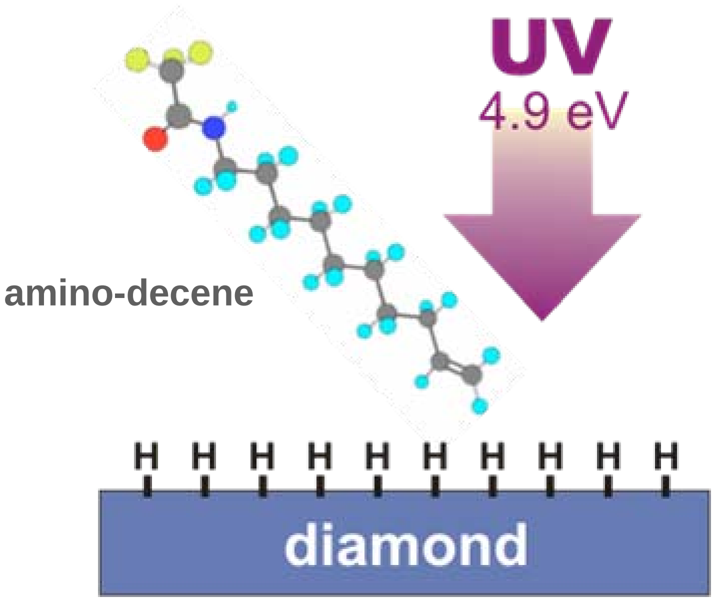
- How to make diamond?
  - from methane using plasma assisted chemical vapor deposition (CVD)
  - polycrystalline: on silicon or glass
  - **monocrystalline:** on diamonds (homoepitaxy), advantageous for research

- How to attach molecules to “inert” diamond?
- Surface can be functionalized by atoms
  - plasma techniques, wet chemical techniques
  - we use H-terminated and oxidized surfaces
- Atoms can be replaced by organic molecules
  - Photochemical reactions
  - Electrochemical reactions



Attachment of linker molecules to diamond

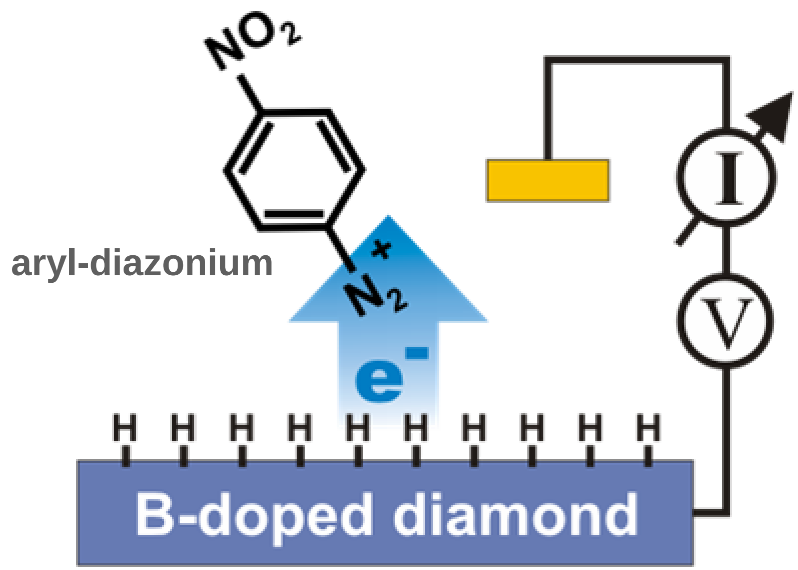
Photochemical



5-7 hours

reaction with hydrogen atoms

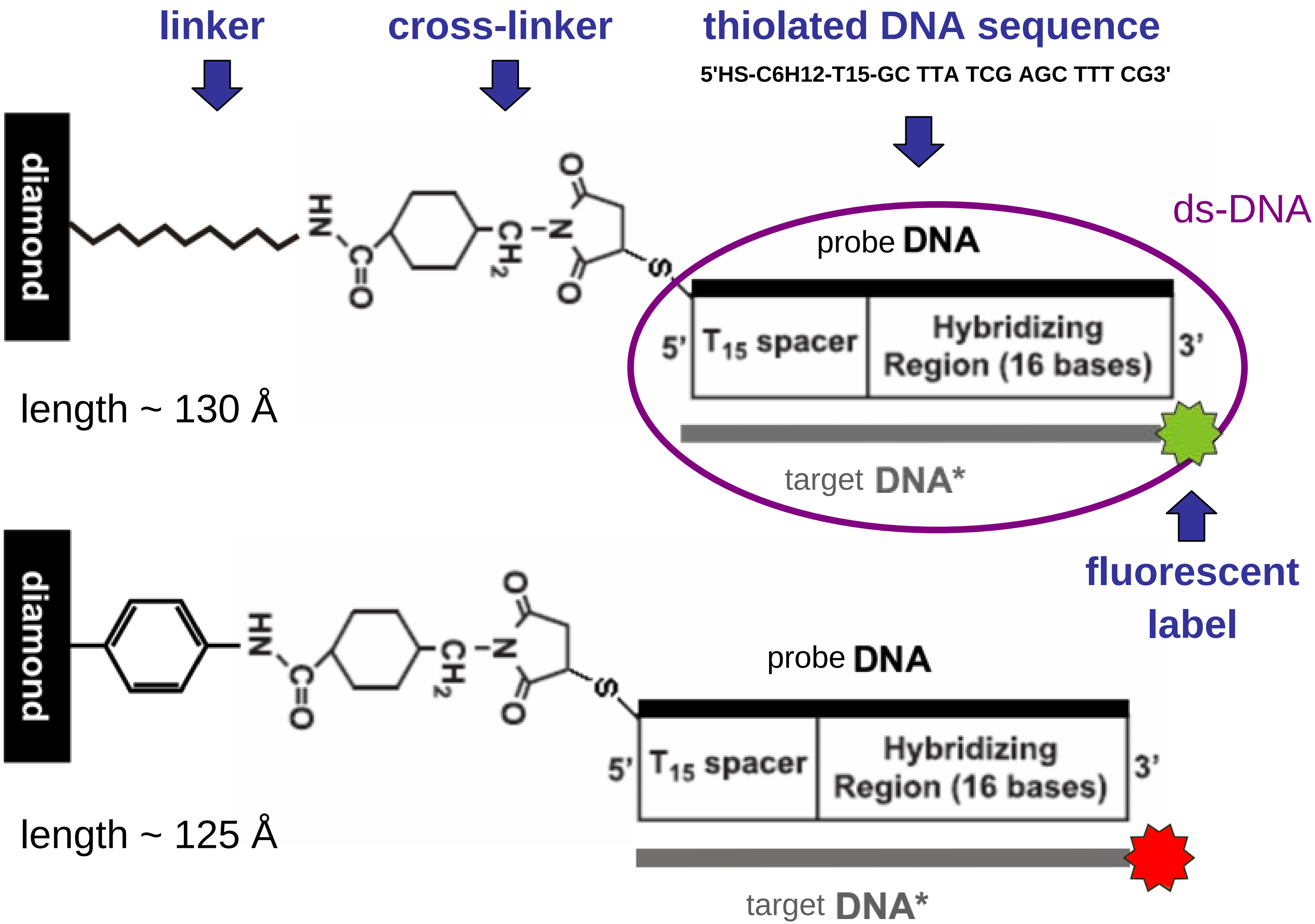
Electrochemical



1 minute

conductive substrate required

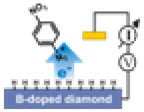
Linking of DNA molecules



Probing DNA by Fluorescence Microscopy

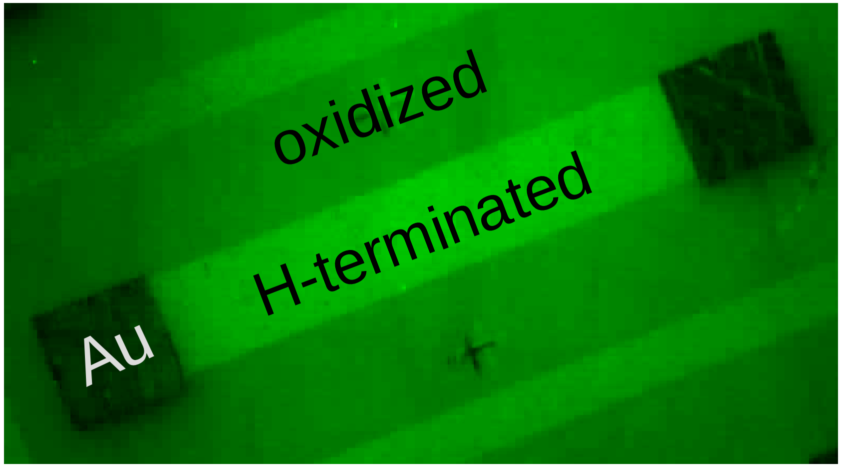
common technique

Photochemical



Electrochemical

fluorescence (FAM)



100 μm

➔ **DNA present on H-terminated!**

(oxidized areas not fully dark)

fluorescence (Cy5)

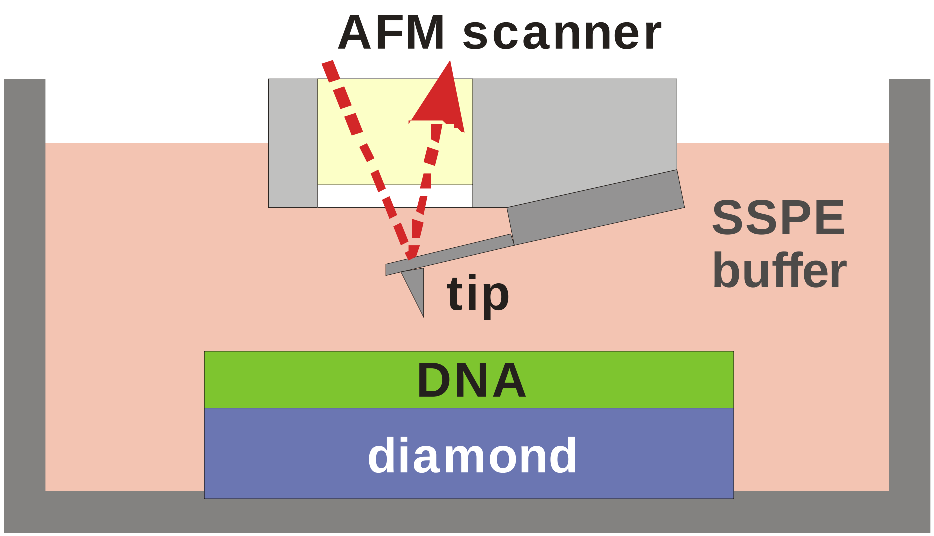
100 μm

➔ **DNA present!**

Crucial for bio-sensor functionality:
morphology, arrangement, and stability of DNA

beyond abilities of fluorescence

AFM in buffer solutions



BUFFERS

SSPE/SDS buffer
2x SSPE/ 0.2% SDS buffer, pH=7.4 by NaOH

advantages: bio-environment, no meniscus at tip

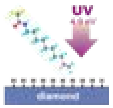
AFM TIPS

silicon cantilevers (~75kHz in air, ~29kHz in liquid)
force calibration: 56 nN/V

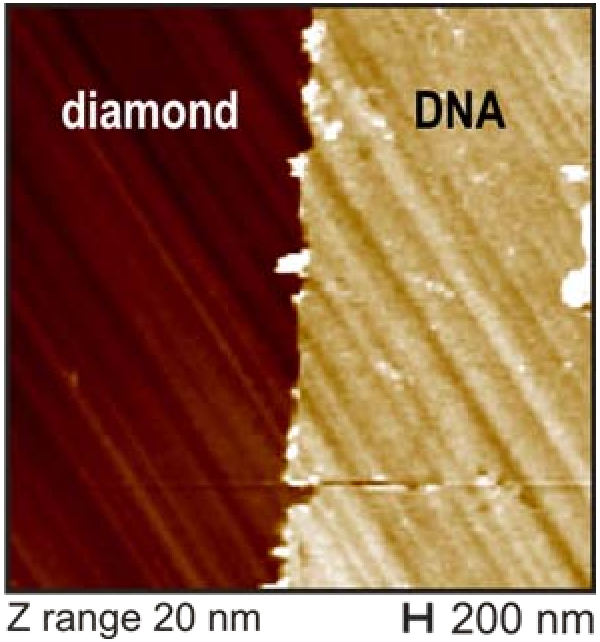
REGIMES

contact (CM-AFM), oscillatory (OM-AFM),
phase detection

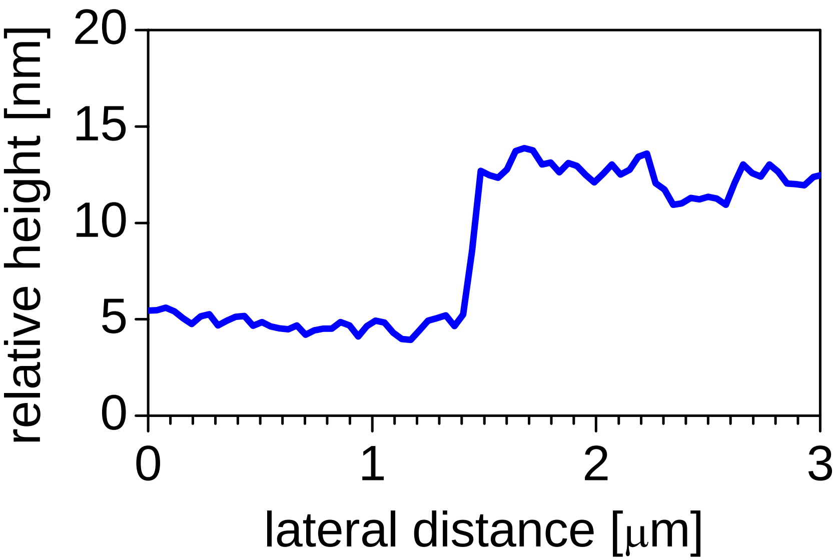
Thickness of DNA layers



OM-AFM image in liquid



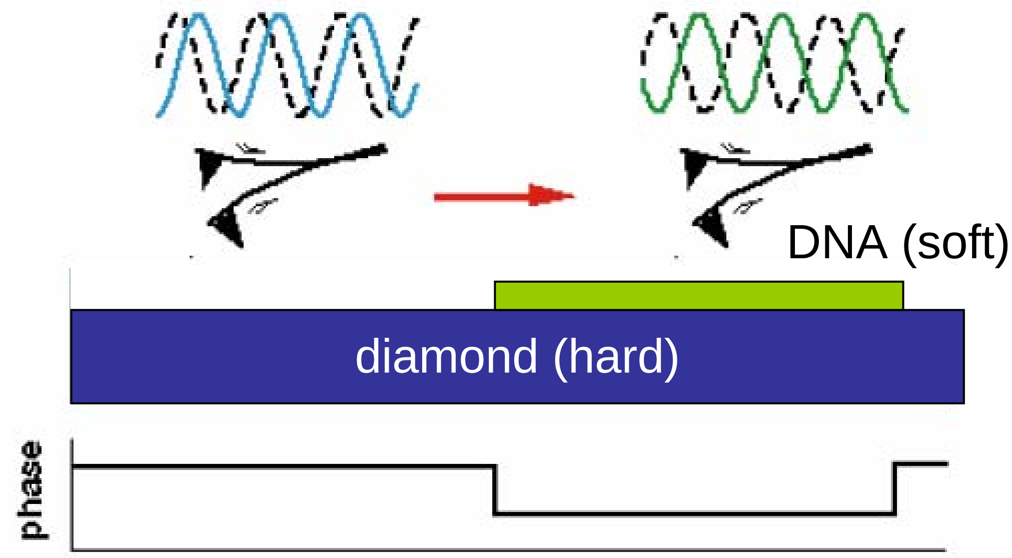
height profile



- step in height ~ 70-80 Å resolved
- but DNA is soft matter → true DNA layer thickness?

➔ AFM measurement optimized by monitoring phase contrast

Phase contrast in OM-AFM



diamond-DNA: phase contrast ~ difference in elastic properties

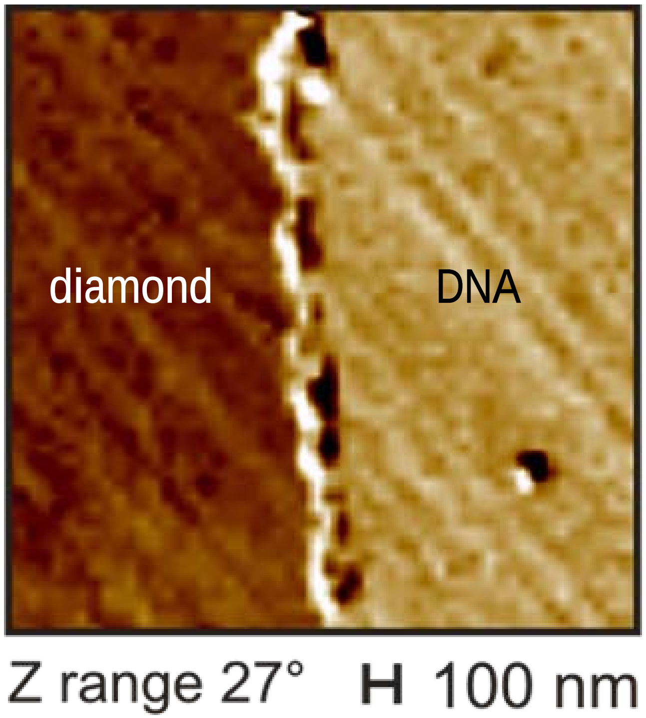
phase contrast influenced by strength of tip-surface interaction

➔ adjusted by **AFM setpoint ratio** (A_{SP}/A_0)

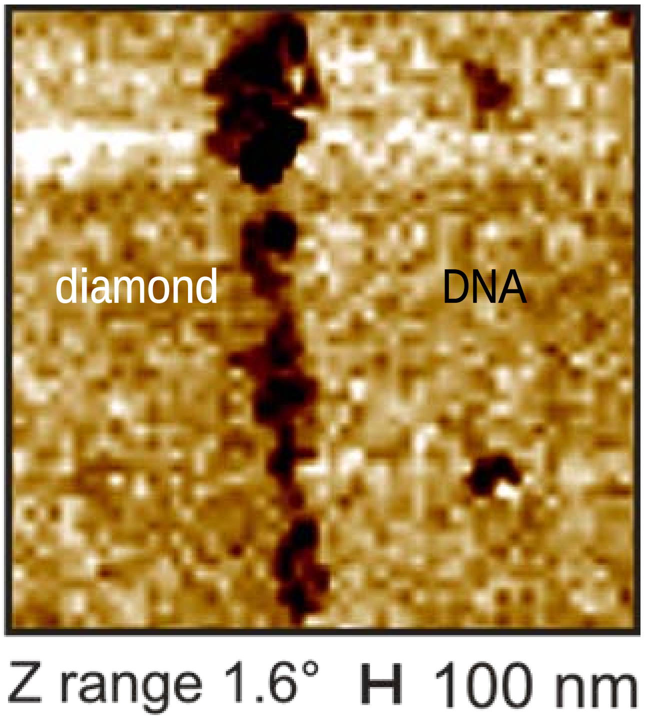
AFM phase images

= 1 for free cantilever

A_{SP}/A_0 = **0.40**



A_{SP}/A_0 = **0.95**



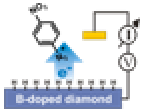
material contrast diamond – DNA

no contrast, DNA not affected by tip

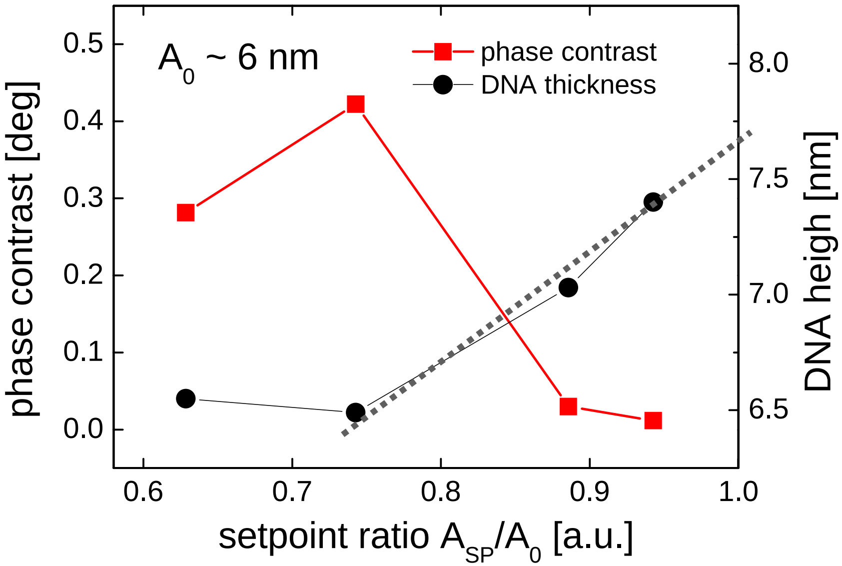
$\rightarrow$ thickness?

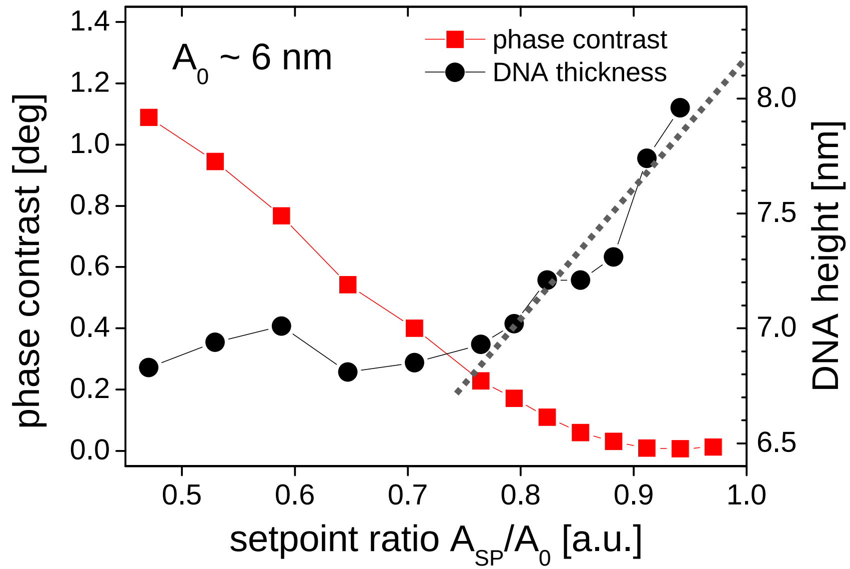
Extrapolated DNA thickness

Photochemical



Electrochemical





thickness ➔ (76 ± 8) Å

thickness ➔ (82 ± 5) Å

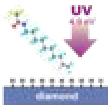
As tip-surface distance increases (setpoint ratio → 1)…

➔ DNA/diamond phase contrast decreases (less tip-DNA interaction)

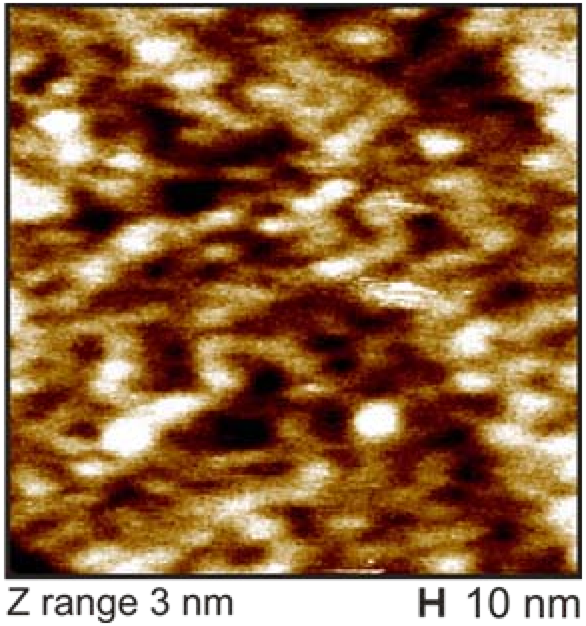
➔ thickness increases → **extrapolated DNA thickness**

(error bar ~ RMS surface roughness)

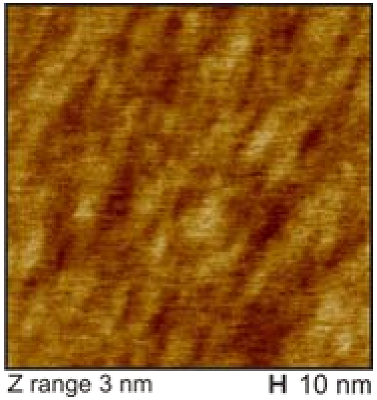
DNA layer morphology



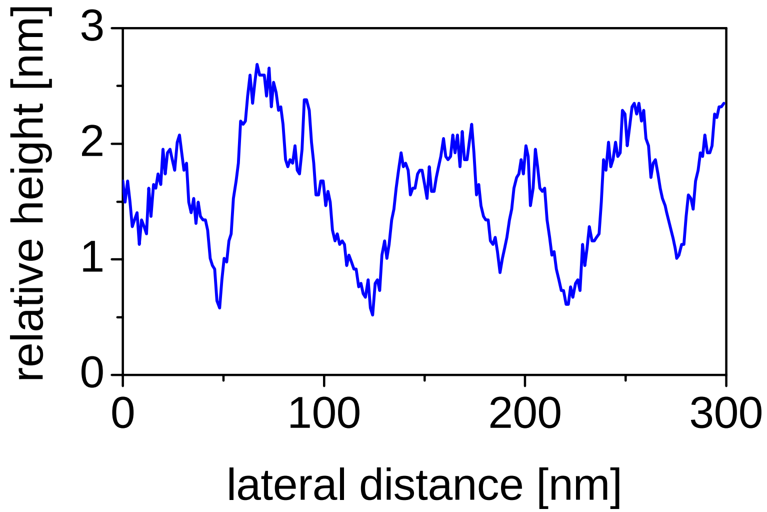
OM-AFM image in liquid



substrate ➔



height profile



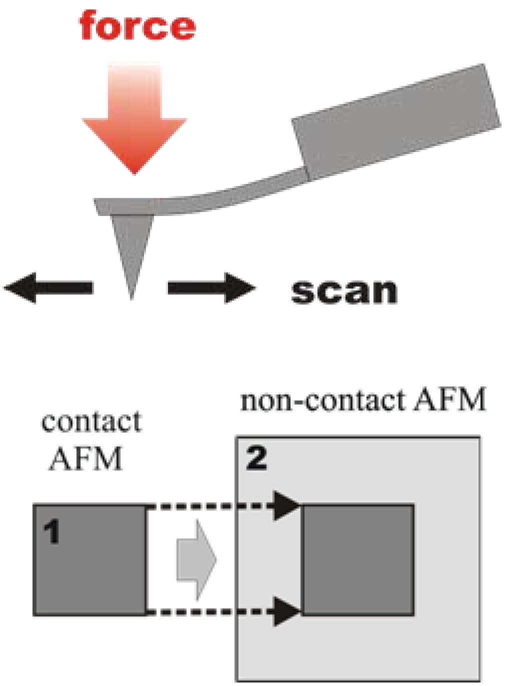
Features

- surface modulations ~ 30 nm width (typical for closely packed DNA)
- roughness RMS ~ 6-8 Å << thickness

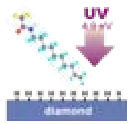
closely packed layer, no pinholes

- fine structure ~ DNA?

Mechanical stability of DNA on diamond

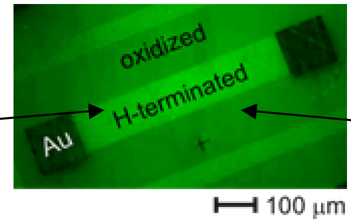


Mechanical stability of DNA on diamond

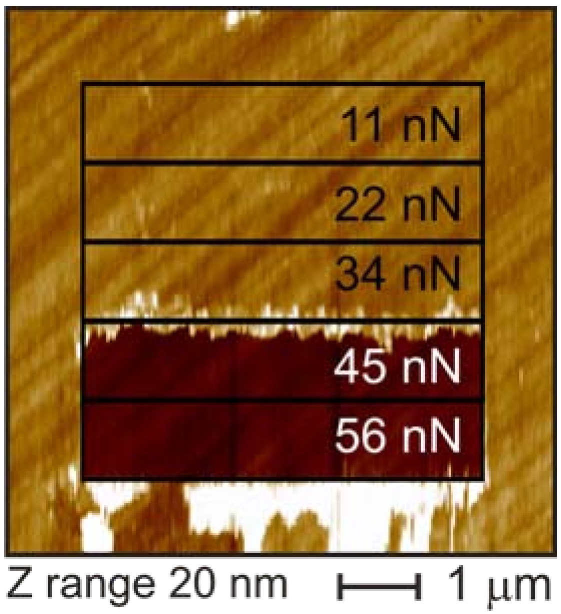


Photochemical

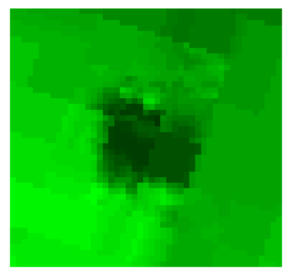
H-terminated

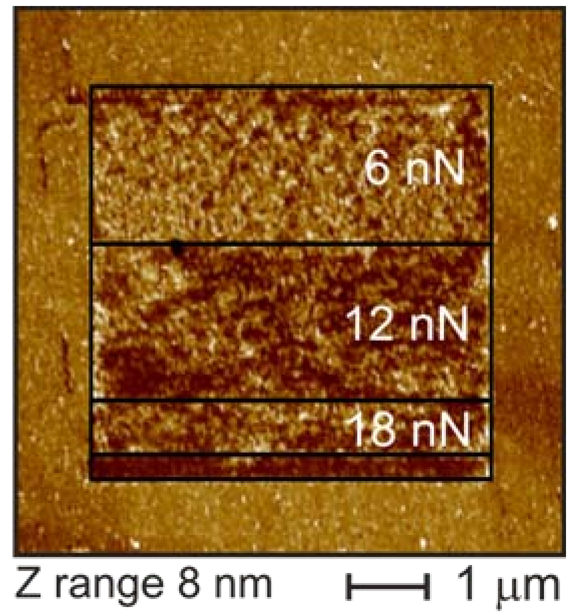


oxidized



fluorescence:



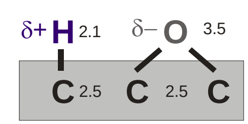


threshold (45 ± 12) nN
➔ covalent bonding

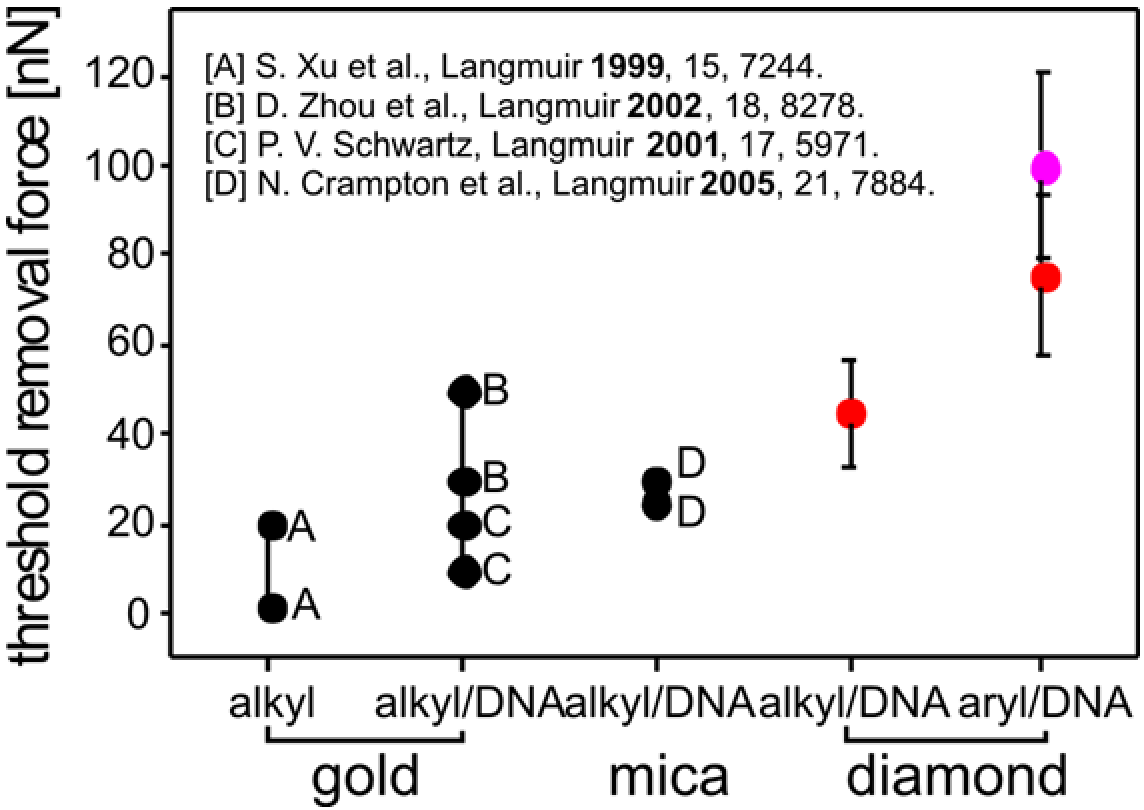
DNA removed

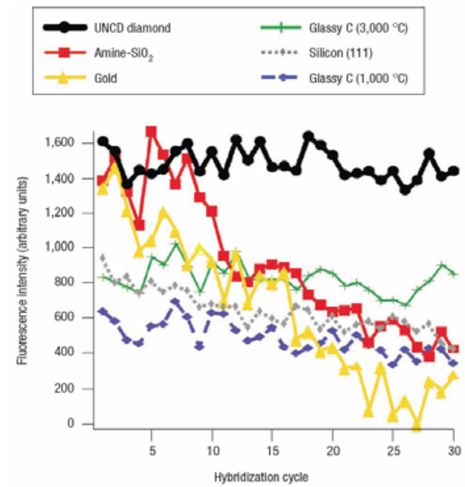
threshold < (6 ± 4) nN
➔ non-covalent bonding (electrostatic?)

<u>error:</u> interval, spring constant, statistics



Comparison of DNA removal forces





[W.Yang et al., Nature Mat. 1 (2002) 254]

⇨ diamond superior with regard to DNA bonding stability, important for bio-sensor reproducibility

<u>note:</u> only approximate comparison, because the parameters and threshold values not clearly specified in the literature

Conclusions

- DNA attached to mono-crystalline diamond
  – by photochemical and electrochemical methods
- Functionality as DNA-sensor demonstrated
  – by fluorescence images of complementary DNA
- Properties of DNA layers on diamond resolved by AFM in liquids:
  – **closely packed**, no pinholes, RMS roughness < 1 nm
  – DNA molecules **inclined**, under angle of 29-36°
  – mechanically **stable** for forces up to 76 nN (good!), indicates **covalent bonding**

	threshold force	DNA thickness	angle	DNA bonding
photochemical process				
H-terminated surface	(45 ± 12) nN	(76 ± 8) Å	31°	covalent
oxidized surface	< (6 ± 4) nN	(20 ± 4) Å	n/a	non-covalent
electrochemical process				
H-terminated surface	(76 ± 18) nN	(82 ± 5) Å	36°	covalent
oxidized surface	(34 ± 9) nN	(69 ± 5) Å	29°	covalent

*[B. Rezek et al., J.Am.Chem.Soc. **128** (2006) 3884]*