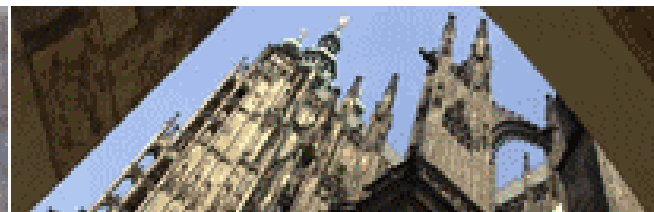
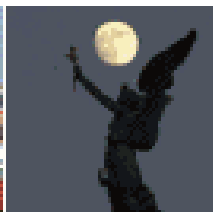
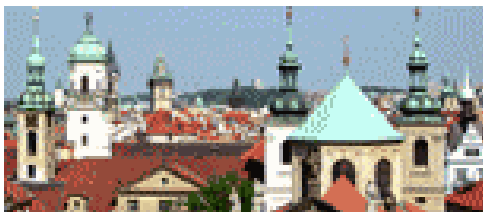


Nové chemické a elektronické funkce diamantu pro biologická rozhraní

Bohuslav Rezek



Fyzikální ústav
Akademie věd ČR, v. v. i.



Obsah přednášky

- **Koncept projektu a historie vzniku**
- **Mezioborový řešitelský tým**
 - Akademie věd, univerzitní fakulty, průmysl
- **Vybrané významné výsledky**
 - **proč diamant** a organické materiály
 - funkce povrchových atomů → bio-rozhraní a bio-elektronika
 - **uspořádání buněk** do struktur pomocí diamantu
 - vliv vodivosti, smáčivosti, adsorbce a adheze proteinů, etc.
 - vliv buněčného prostředí na elektrické vlastnosti diamantu:
Cell-FET senzor, role hranic zrn, proteinů a buněk
- **Závěry a výhledy do budoucna**
 - shrnutí, návazný výzkum a aplikace
 - významné výstupy a přínos pro ČR: patenty, publikace, funkční vzorky, vliv na ekonomiku

Historie vzniku projektu

- **Zkušenosti a myšlenky** z mé práce v zahraničí (5 let)
 - Německo, Švýcarsko, Japonsko
- **Návrat díky podpoře Fellowship J.E.Purkyně AVČR (2006-11)**
 - podařilo se vybudovat úspěšný výzkumný tým v ČR
 - velmi děkuji!
- **Formování projektu** pro 2. výzvu programu Nanotechnologie pro společnost (NpS)
- **Výhody programu NpS**
 - trvání výzkumného projektu 5 let
 - možné významné přístrojové investice (řádově 10 MKč)
 - jednoduchá administrativa (finance, reporty)
 - *to vše chybí v současné VaV a je přitom zásadní!*



Formování projektu, výzkumný tým

FZÚ AVČR (B.Rezek et al.) - počátek a koordinace

- nano-materiály, hybridní organicko-anorganické systémy, mikroskopické metody, výpočty

1.LF UK (S. Kmoch et al.)

- buňky a biomolekuly, detekce pro medicínu

MFF UK (P. Malý et al.)

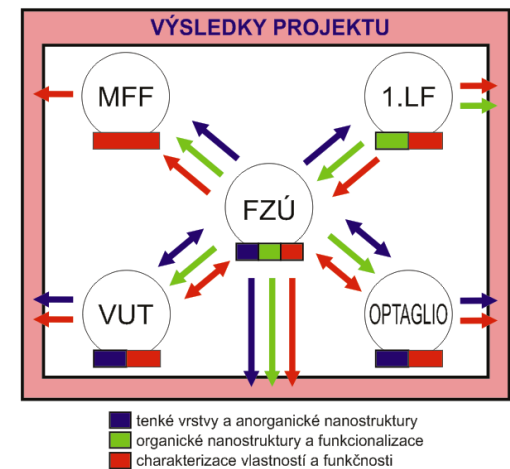
- optické spektroskopie pro studium materiálů a dynamiky elektronických procesů

FSI VUT Brno (T.Šikola et al.)

- vytváření nanostruktur, plazmonika

Optaglio, s.r.o. (L. Kotačka et al.)

- průmyslový partner, vytváření funkčních nanostruktur



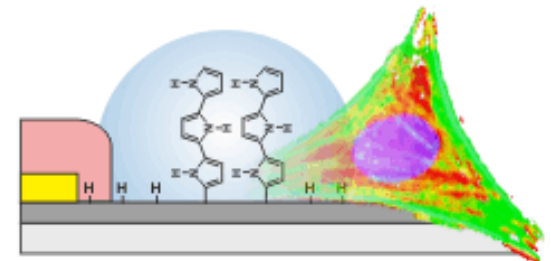
Představení vybraných výsledků

- **Celá řada výsledků v při řešení projektu**
 - v oblasti vytváření nano-materiálů a nano-struktur
 - v oblasti opto-elektroniky a přeměny energie
 - v oblasti bio-elektroniky a bio-medicíny
 - přehled např. <http://funs.fzu.cz>
- **Představíme vybrané významné výsledky z oblasti bio-elektroniky a bio-medicíny**
 - **nové chemické a elektronické funkce diamantu pro biologická rozhraní**

Nanotechnologie pro společnost

FUNS

funkční hybridní nanosystémy
polovodičů a kovů
s organickými látkami



Diamond as gemstone

from the ancient Greek αδάμας – adámas "unbreakable"



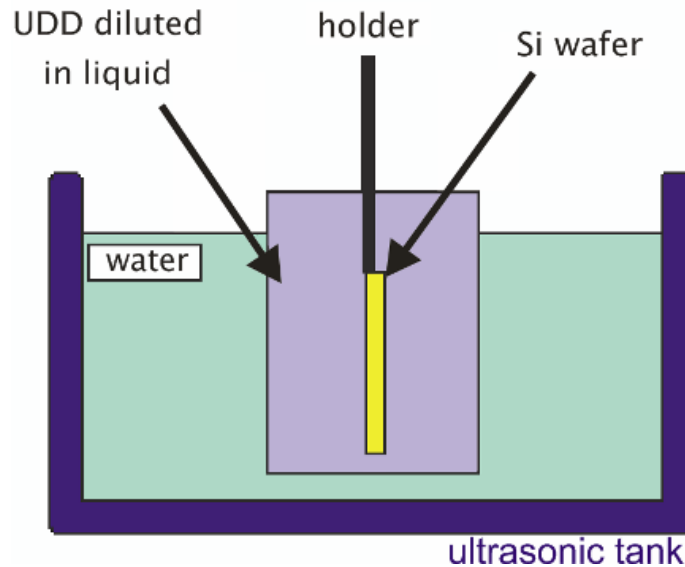
traditional applications: jewelry, polishing pastes, cutting tools

Diamond as novel electronic material



we see diamond as a class of materials with properties and functions tailorable on demand: morphological, chemical, electronic, optical

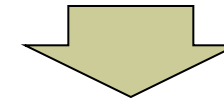
Diamond synthesis on any surface



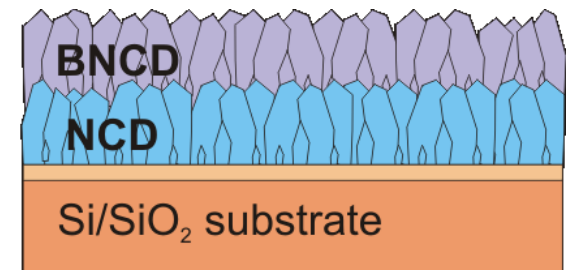
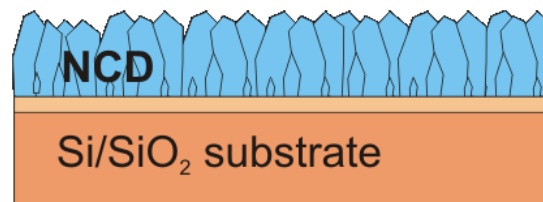
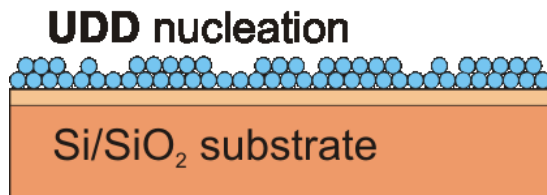
nucleation process

UDD diamond powder 5-10 nm dispersed in aqueous solution

UDD = ultradisperse diamond, produced by detonation (research of its own, including cells)



growth on “foreign” substrates (Si, glass, metals, polymers!)

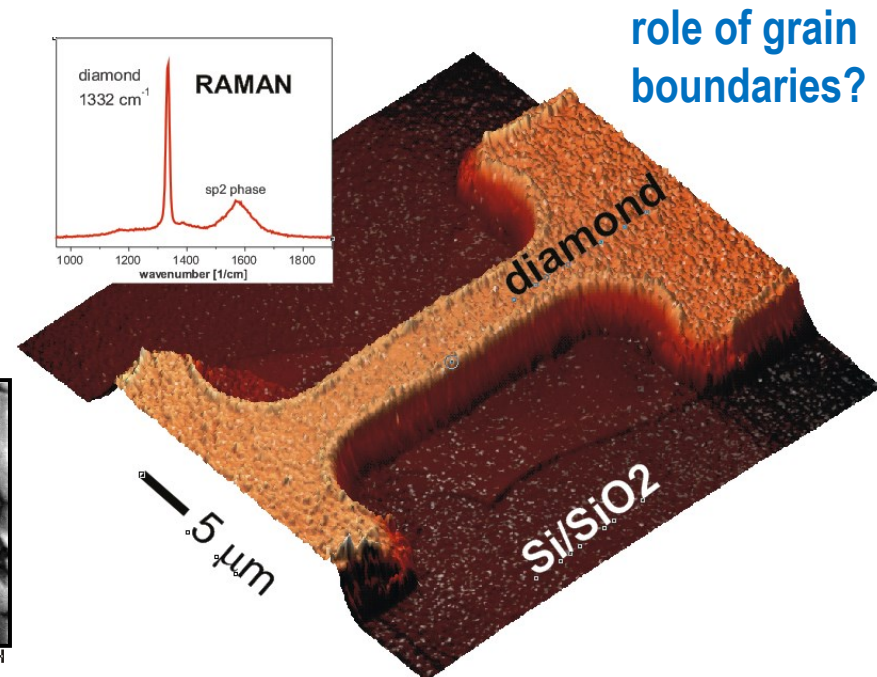
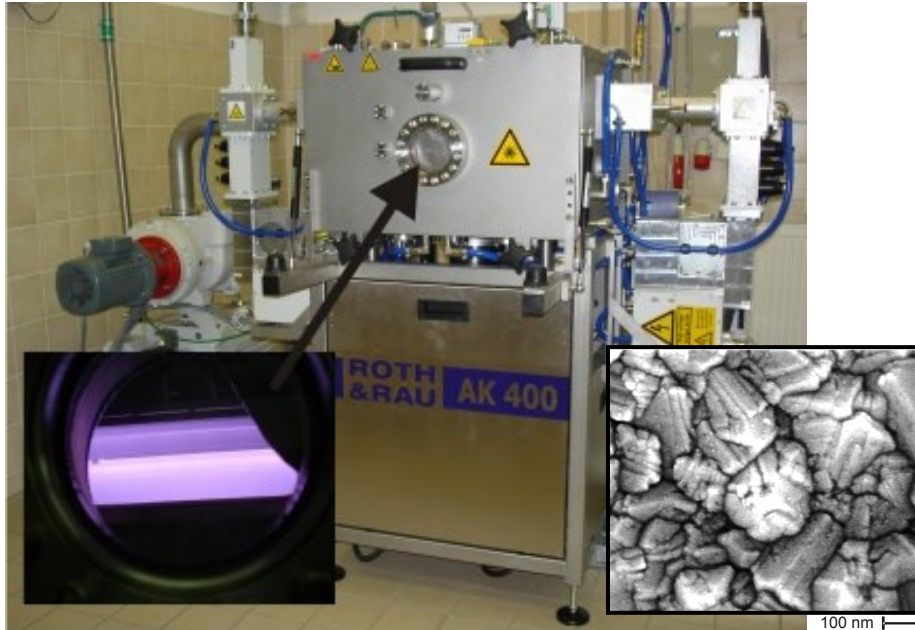


“templated” growth

[Kromka et al., Chem. Vap. Deposition 14 (2008) 181]

Nanocrystalline diamond (NCD)

- **We employ mostly nanocrystalline diamond (NCD)**
 - fabrication **easy, inexpensive, tailorable** (compared to monocrystals) from methane in MW plasma [*Kromka et al., CVD 14 (2008) 181*]
 - can be deposited on **various substrates** (silicon, glass, metals, plastic)
 - **large areas / numbers** possible (linear antenna systems)
 - **direct growth of microstructures** possible (via patterning nucleation)



Diamond - material features

Diamond is fascinating material for bio-interfaces, with unique set of properties:

- **carbon purely**

- non-toxic, biocompatible, environmentally friendly


- **transparent to visible light (~90%)**

- **semiconductor (wide band gap 5.5 eV):**

- devices and sensors (FET, UV, p-i-n)
[Rezek et al., Sens. Act. B 122 (2007) 596]

- **chemically inert and stable**

- good for electrochemical electrodes, harsh environments
- **yet surface can be changed and functionalized!**
[Nebel et al., J. Phys. D: Appl. Phys. 40 (2007) 6443]
- most prominent surface moieties: H, O, N, Cl, F



plasma discharge,
ozone, photo- or
electro-chemistry,...

Diamond – functional interface

Functionality of H/O surface atoms

- opposite dipoles, wetting, el. affinity
- **surf. conductivity** of intrinsic H-diamond
 - planar field-effect transistors (FET) without gate oxide
 - in solution sensitive to pH, molecules

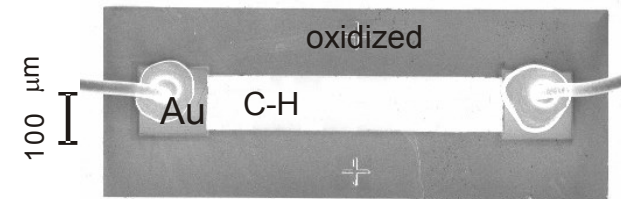
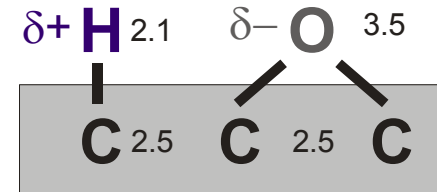
[Rezek et al., Sens.Act. B 122 (2007) 596]

[Rezek et al., Thin Solid Films 517 (2009) 3738]

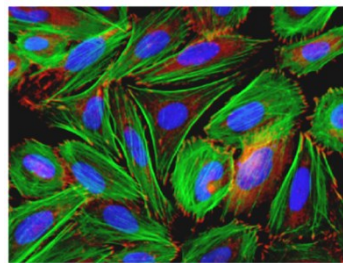
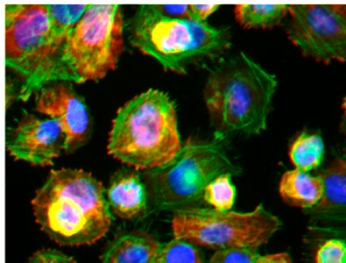
- **influence on cell growth**

[Kalbacova et al., phys. stat. sol. (b) 245 (2008) 2124]

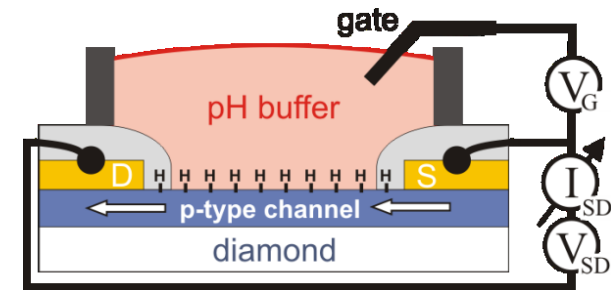
[Rezek et al., Sensors 9 (2009) 3549]



H-diam:
rounded
cells



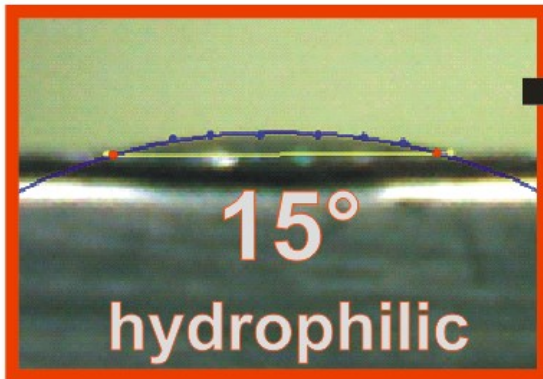
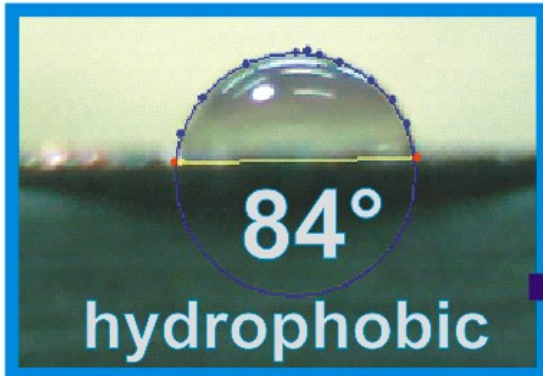
O-diam:
spreaded
cells



merging → bio-electronic applications

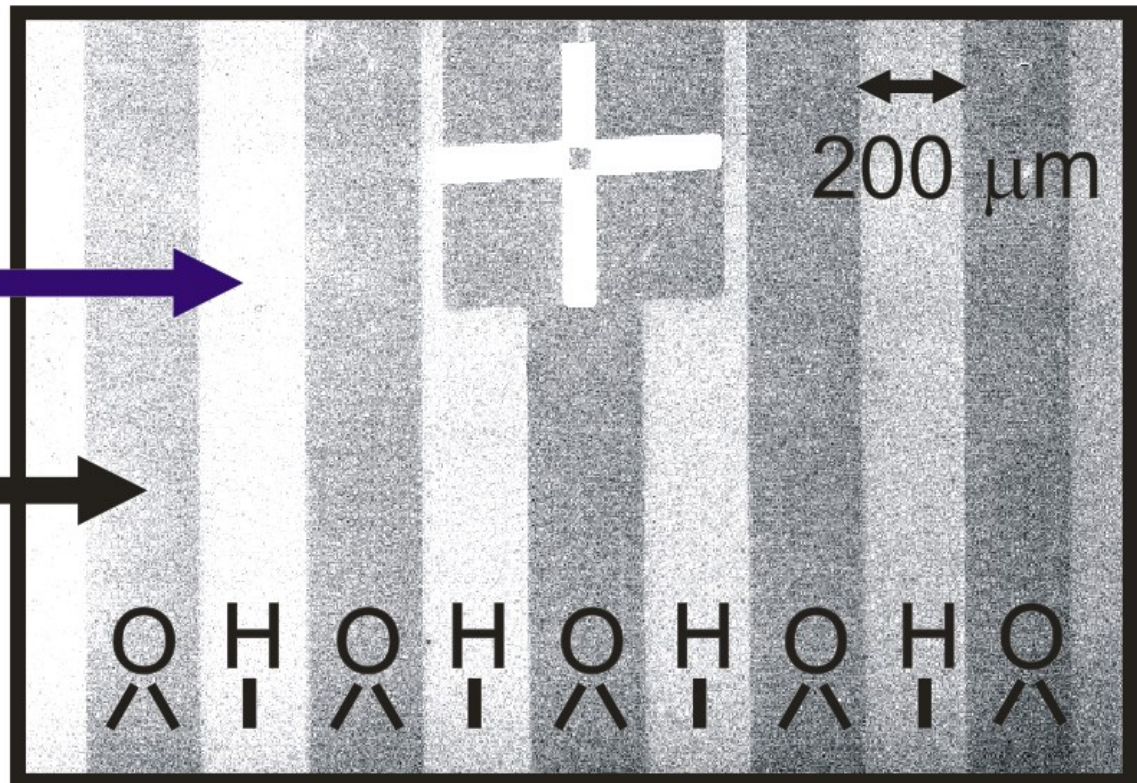
Properties of H/O-micropatterns (basic device)

electrically conductive



electrically resistive

SEM micrograph of H/O micropattern



optically fully transparent!

B. Rezek et al.: *Diamond as functional material for bioelectronics and biotechnology*
In: "New Perspectives in Biosensors Technology and Applications"
Intech 2011, pp. 177-196, ISBN 978-953-307-448-1

Plating cells on diamond

■ medium for cell plating and growth

■ McCoy's 5A medium + fetal bovine serum (FBS)

- McCoy's 5A: inorganic salts, aminoacids, vitamins, glucose, etc.
- FBS: cell growth factors (proteins), heat inactivated, conc. 0-15%
- penicillin, streptomycin

■ cell plating

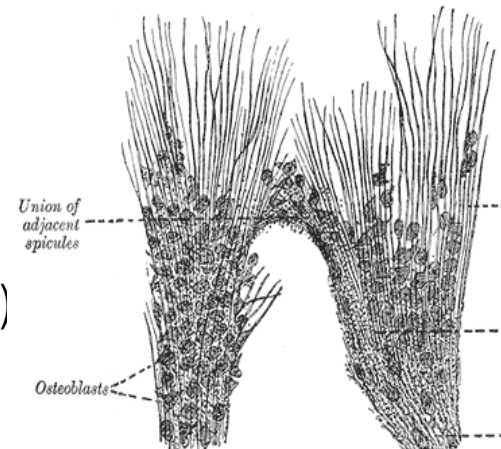
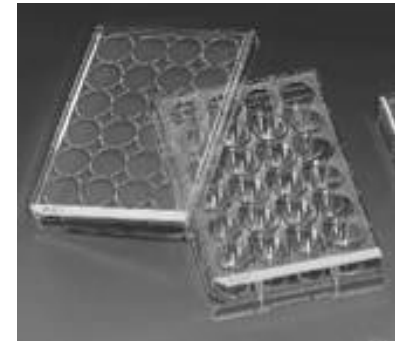
- droplet with cell solution spread on sample
- concentration: **2.500 and 10.000 cells/cm²**
- McCoy's medium added

■ cell cultivation

- tissue culture plates
- 37°C, 5% CO₂, **2 days**

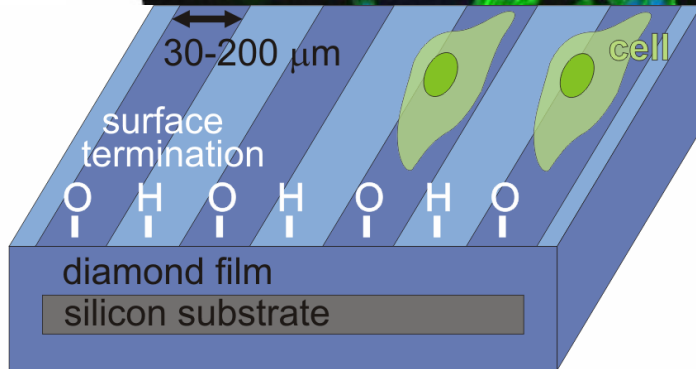
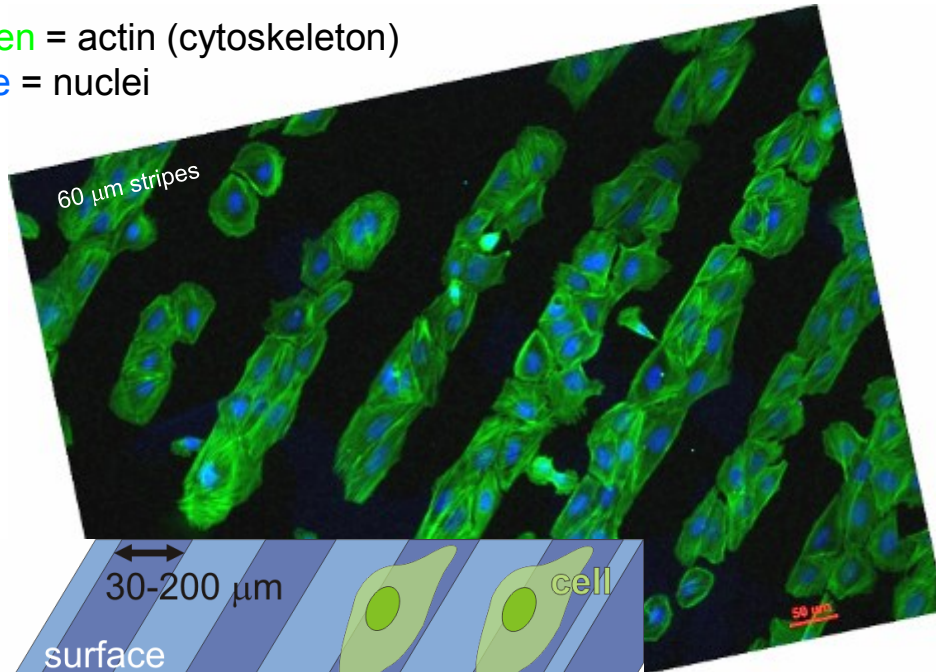
■ cell types

- **osteoblastic cells SAOS-2** (standard, well defined line)
 - cells that form bone tissue and later mineralize it
- other cell types (fibroblasts, HeLaG, neurons)

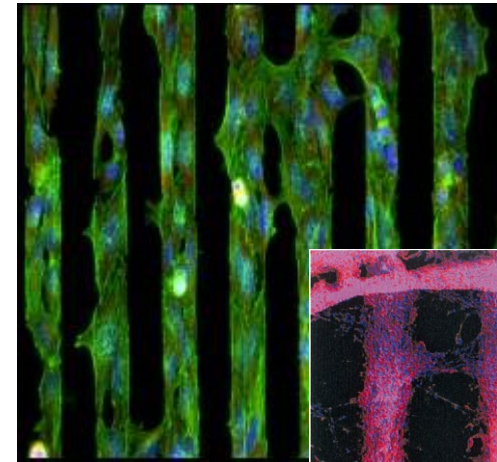


Diamond assembles cells into micro-arrays

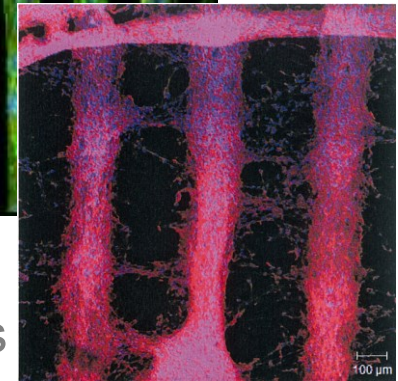
green = actin (cytoskeleton)
blue = nuclei



osteoblasts



HeLaG



neurons

➔ preferential arrangement of cells into micro-arrays

Cells strongly prefer diamond surfaces with O-termination, with sharp boundaries and cell stretching on narrow lines.

[Rezek et al., Sensors 9 (2009) 3549]

Cells on B-doped diamond (BNCD)

BNCD from K.Haenen,
University Hasselt, IMO

100 μm H/O-termination stripes, SAOS-2

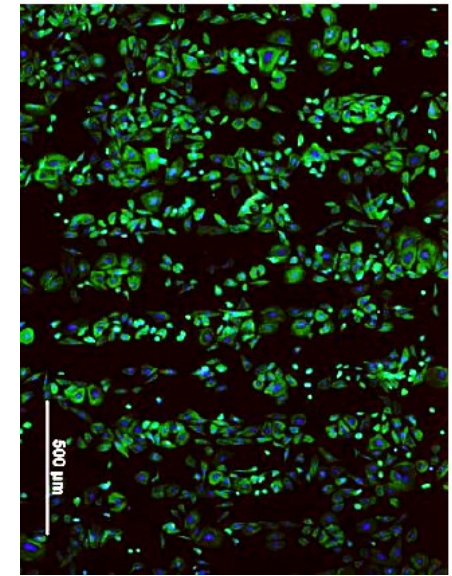
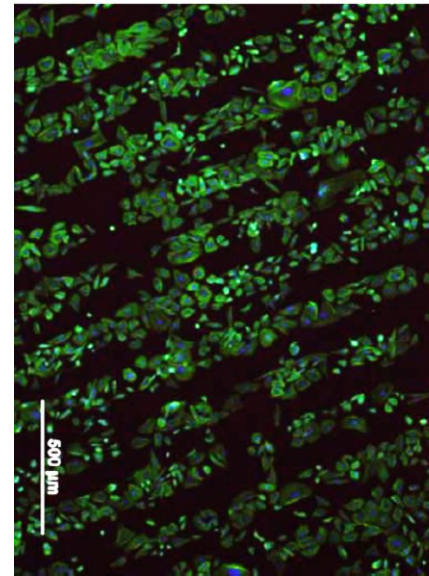
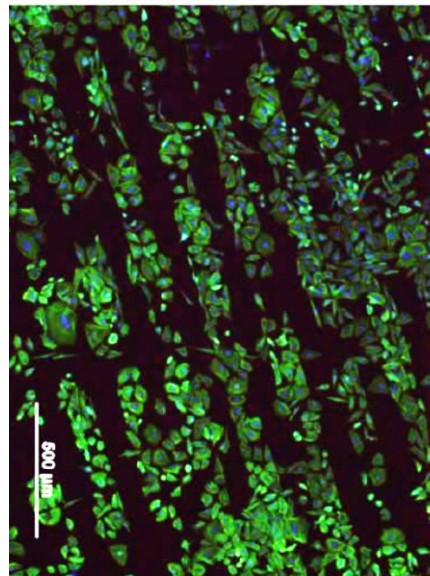
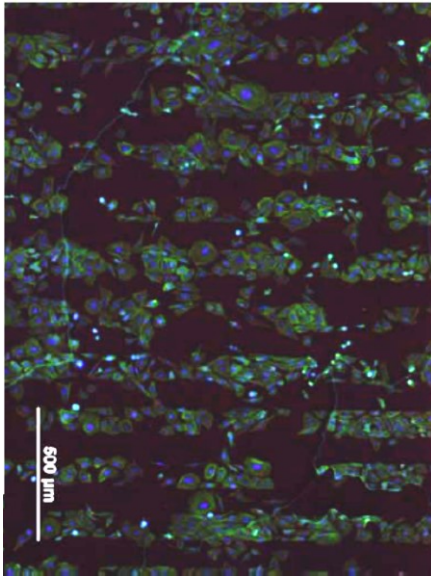
McCoy's, 15% FBS

0 ppm (undoped)

500 ppm

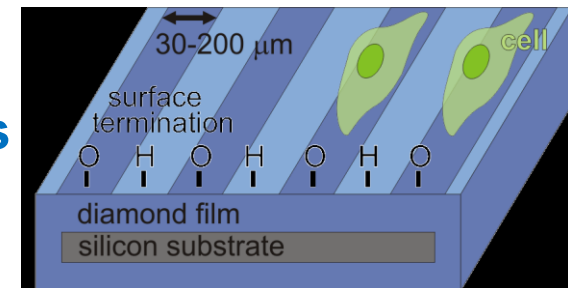
1500 ppm

6000 ppm



B-doped diamond doping nominal = 500-6000 ppm, B-concentration $\sim 1e19-1e20 \text{ cm}^{-3}$

- cell assembly independent of conductivity
- determined only by different wetting properties of H- and O-terminated diamond

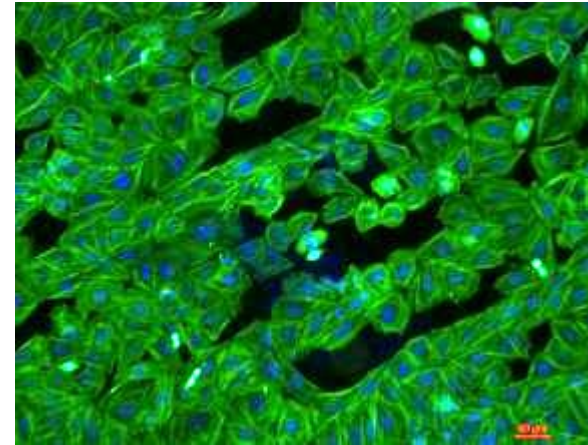
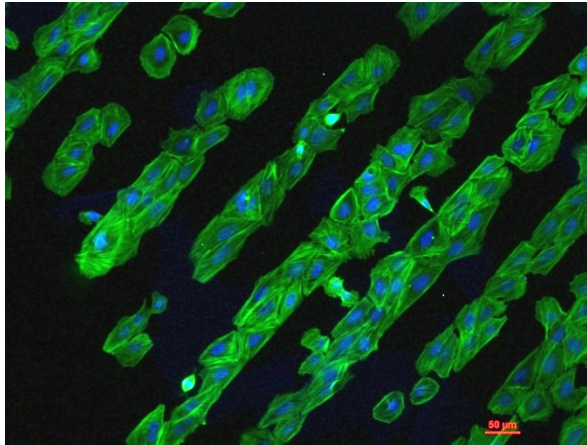


Influence of cell concentration on selectivity

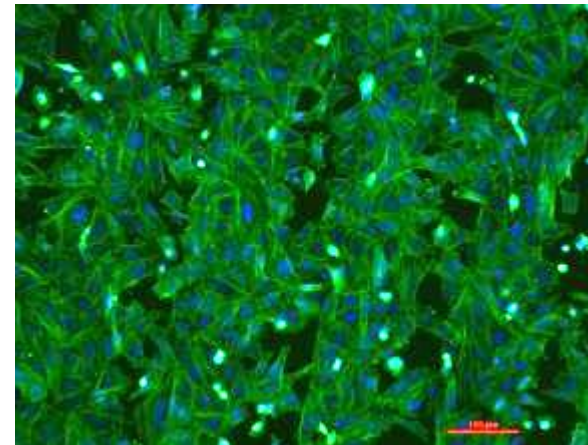
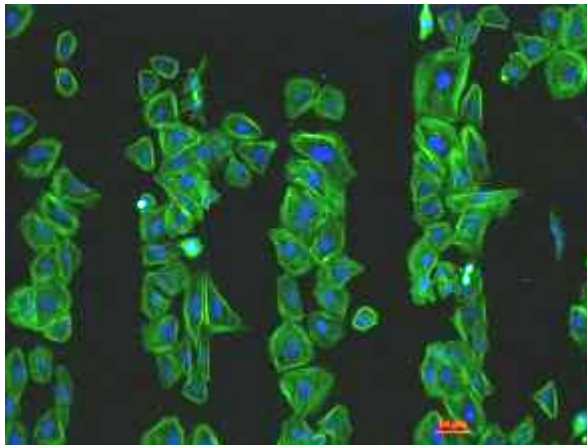
2.500 cells/cm²

10.000 cells/cm²

60 μ m
stripes



100 μ m
stripes

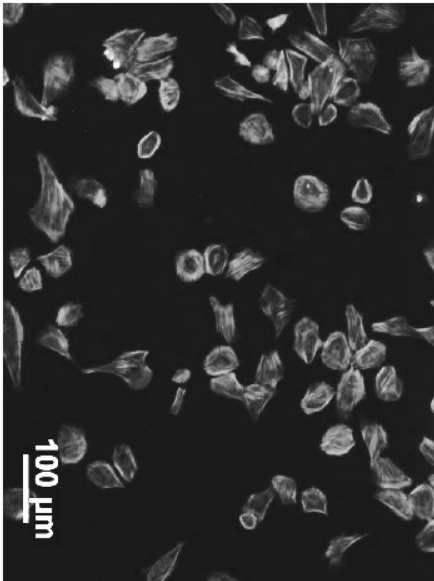


→ initial cell concentration is crucial factor for selectivity,
if too large, cells colonize all surface

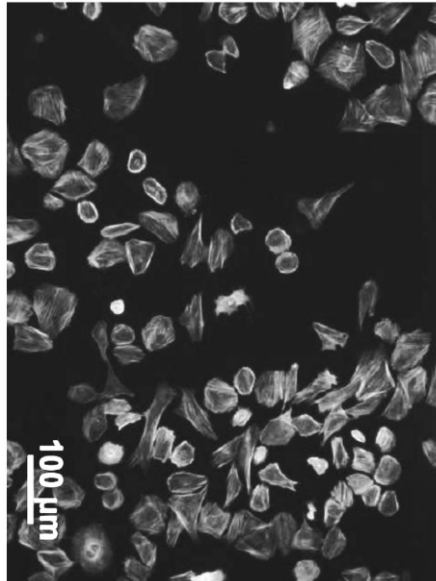
Influence of protein presence

*SAOS-2 cells seeded in FBS-free medium, FBS added later for cultivation,
100 μm H/O-termination stripes*

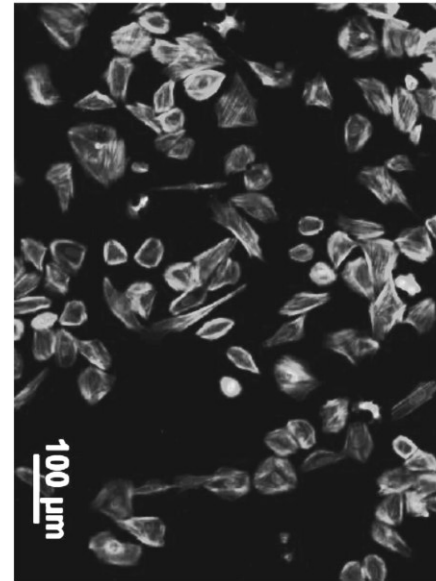
0 ppm (undoped)



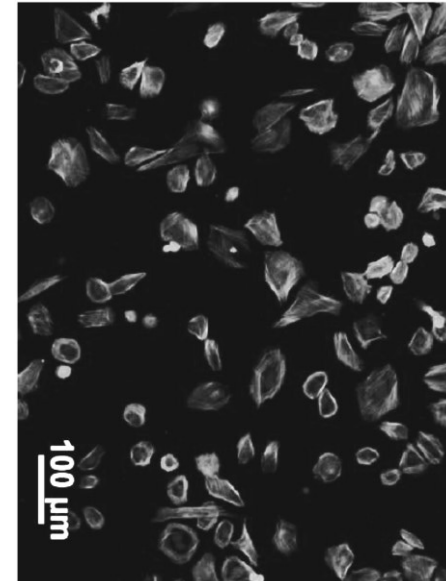
500 ppm



1500 ppm



6000 ppm

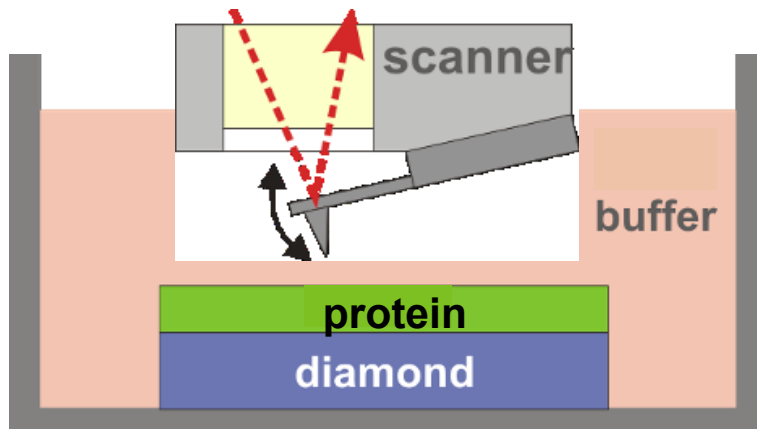


- no cell selectivity on bare H/O-diamond w/o FBS, independent of doping
- direct effect of diamond surf. dipoles excluded, proteins play crucial role
- what are properties of FBS proteins on H/O-diamond?

Protein adsorption on diamond

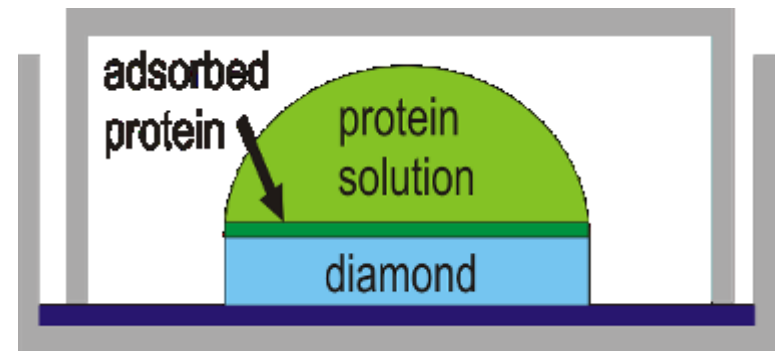
AFM in solution

- advantages: bio-environment, no meniscus at the tip
- silicon cantilevers, $\sim 75\text{kHz}$ in air, $\sim 29\text{kHz}$ in liquid, $A_0 \sim 60\text{ nm}$



protein adsorption

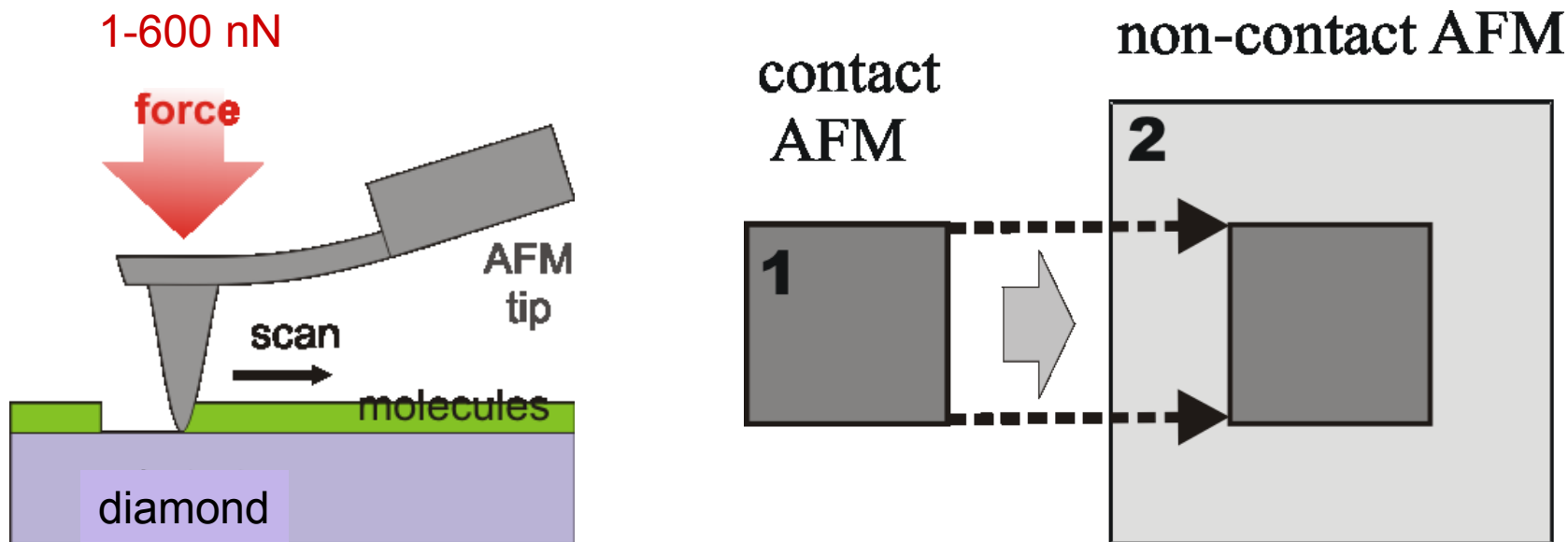
- diamond immersed in McCoy's 5A medium +15 % FBS, 10 min
- rinsed by: a) McCoy's b) water
- measured by AFM: a) in air, b) McCoy's, c) in-situ w/o rinsing



[B. Rezek et al., pss(a) 204 (2007) 2888; Langmuir 23 (2007) 7626; JACS 128 (2006) 3884]

AFM nanoshaving

- combined contact and non-contact regime to learn more



[B. Rezek et al., *pss(a)* 204 (2007) 2888 Editor's Choice]

[B. Rezek et al., *Langmuir* 23 (2007) 7626]

[B. Rezek et al., *JACS* 128 (2006) 3884]

FBS layers on H/O-diamond – AFM analysis

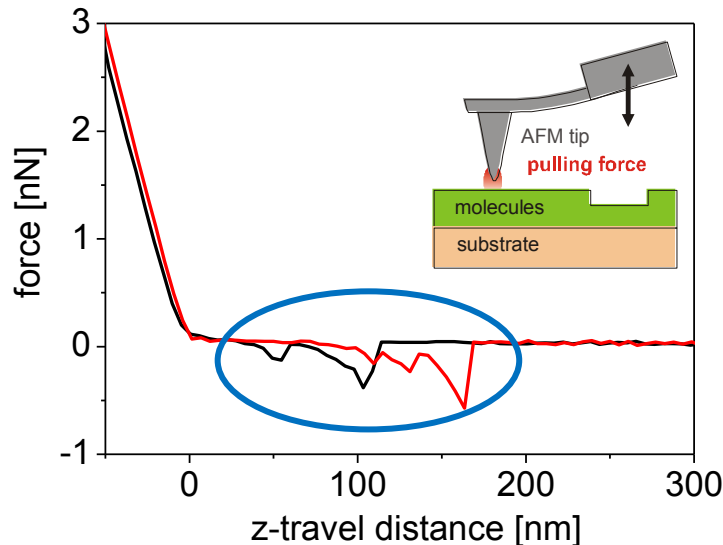
→ FBS layer adsorbed on both H/O-diamond, in similar thickness (2-4 nm)

- **rinsing** by water or McCoy's → similar results → no influence of McCoy's composition
- **in-situ** AFM in McCoy's (no rinsing) → similar results
[Ukrainsev et al., pss(b) 246 (2009) 2832]

→ typical protein “fingerprint” on both surfaces

[A.V.Kransnoslobodtsev, Nanomedicine 1 (2005) 300]

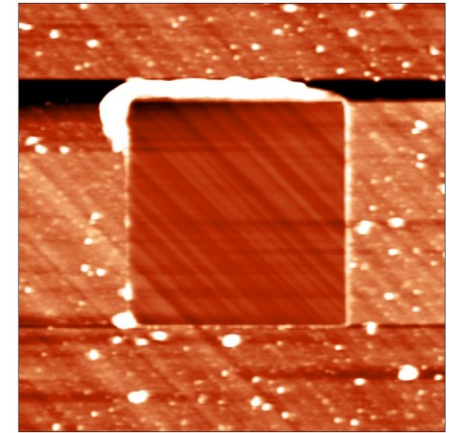
[C. Popov, Diam.Relat.Mater. 16 (2007) 735]



Force spectroscopy:
cantilever tip pressed to the surface and pulled back, force monitored.

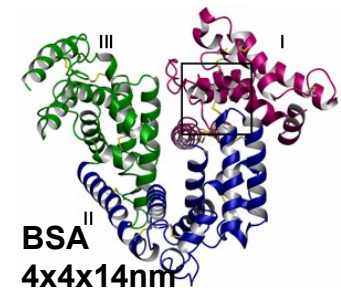
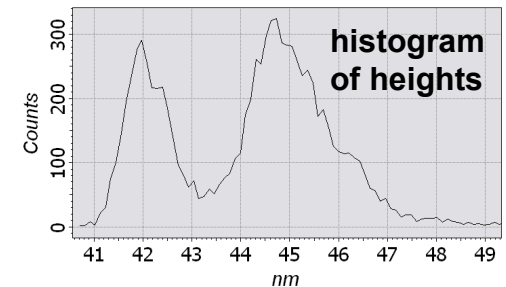
statistics: 10%

measured in liquid



1 μ m

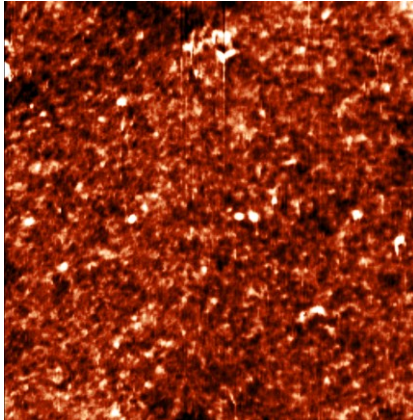
Z scale=20nm



FBS protein layers on diamond - AFM

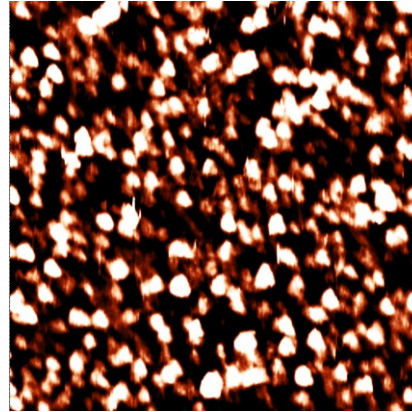
TOPO

H-diamond



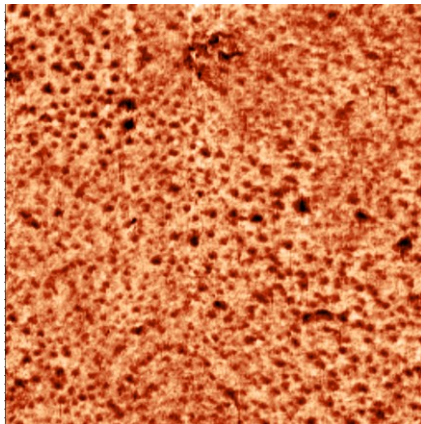
200 nm Z scale=3nm
RMS=0.6nm, Lx=12nm

O-diamond

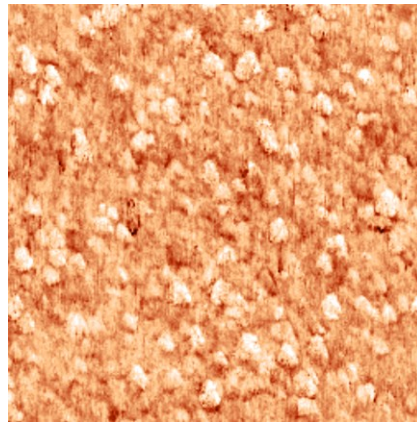


200 nm Z scale=3nm
RMS=1.7nm, Lx=18nm

PHASE



200 nm Z scale=10°
RMS=1.4°, Lx=10nm



200 nm Z scale=10°
RMS=1.0°, Lx=22nm

in McCoy's
cell medium

Different:

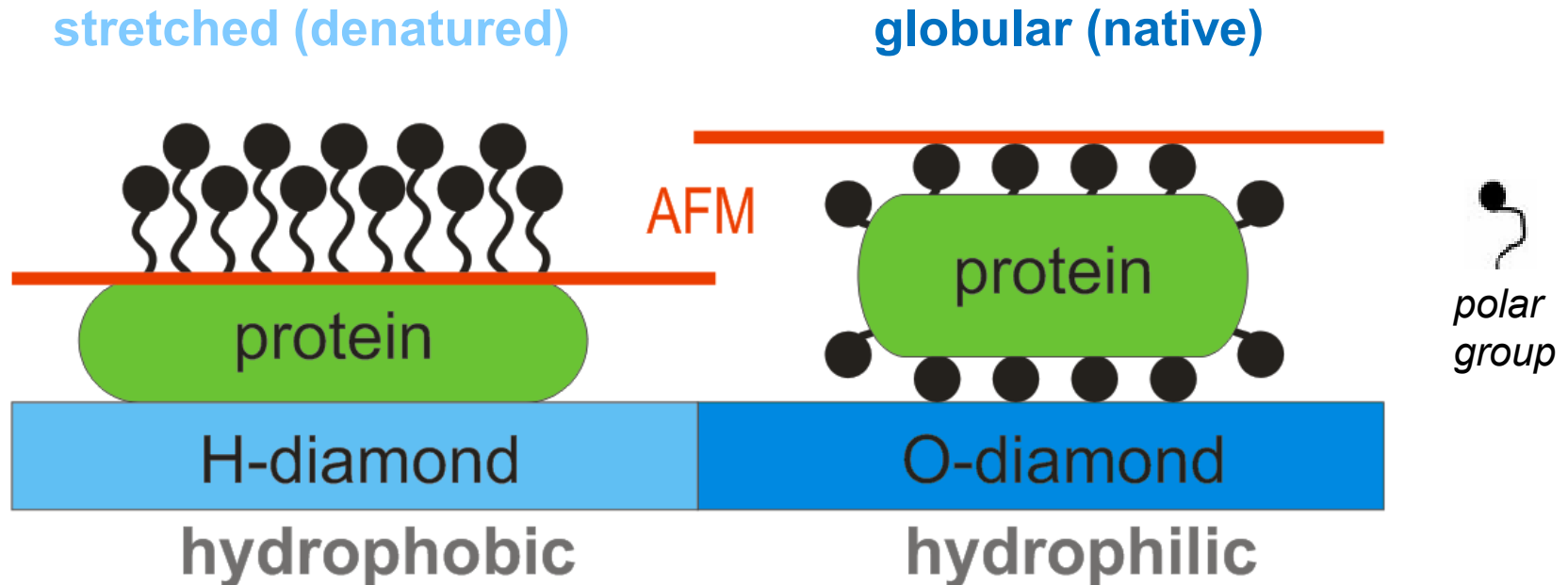
- thickness (2 - 4 nm)
- topography feature shape and size (Lx-autocorrelation)
- surface roughness
- **AFM phase features**

→ diamond surface atoms control protein conformations via wetting properties

[Rezek et al., *Sensors* 9 (2009) 3549]
[Rezek et al., *DRM* 18 (2009) 918]

measured using same tip, same AFM parameters, on various spots

Model: FBS on diamond in solution



- model in agreement with **general effect of hydrophobic/-philic** surfaces on proteins [Browne et al., Surf.Sci. 553 (2004) 155]
- independent of conductivity (good for electronic sensors)
- but wetting range of polymers 85° - 125° vs. diamond 15° - 85° ???

[Rezek et al., Sensors 9 (2009) 3549]

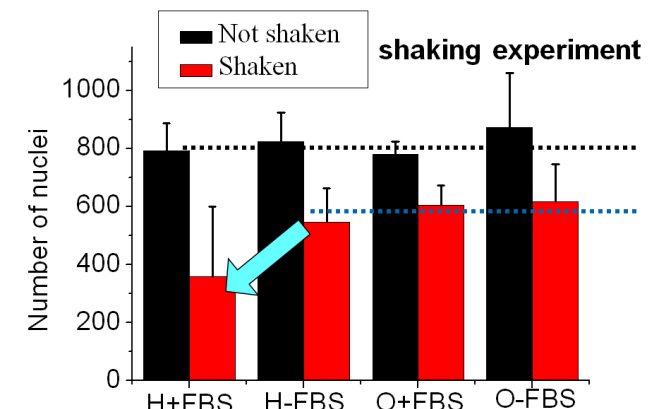
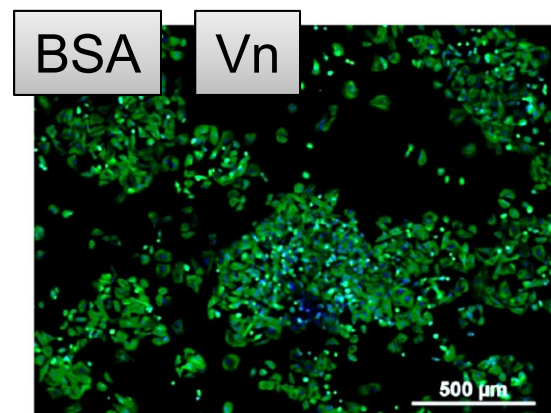
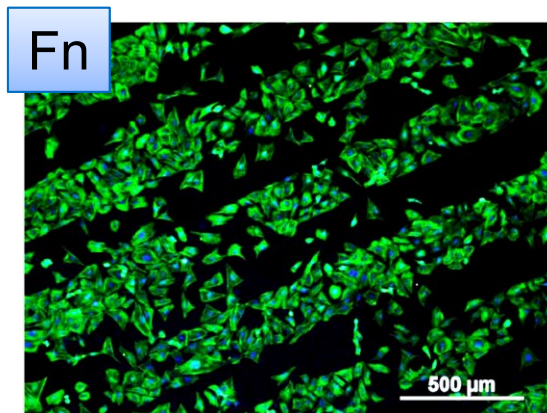
Proteins on diamond driving cell selectivity

→ the cell selectivity is driven by different conformation of proteins on H/O-diamond in the cell medium

recall: not by a direct effect of H/O dipoles on cells

Yet other factors to be considered:

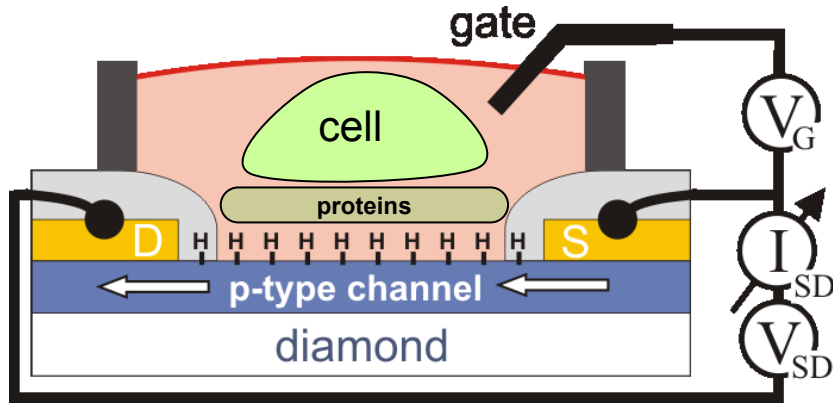
- different **adhesion** of proteins and cells to H/O diamond?
 - role of FBS inter-layer? cell movements (passive, active)?
 - **adhesion of cells reduced by FBS on H-diamond (not on O-diamond)**
- different FBS layer **composition** on H/O diamond?
 - what protein from FBS is responsible for the selective growth?
 - **selective cell growth induced by Fn!**



Bio-electronická funkce diamantu

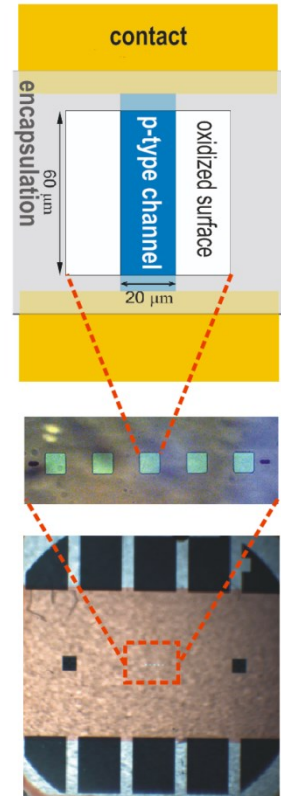
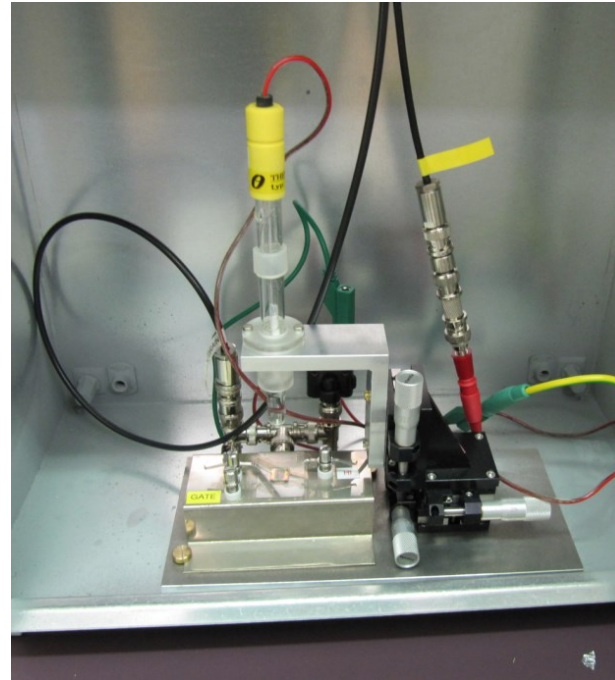
Diamond in-plane SG-FET scheme

side view scheme



solution-gated field-effect transistor (SG-FET)
based on surface conductivity of H-diamond
gate electrode: *Ag/AgCl reference electrode*

setup and sample view

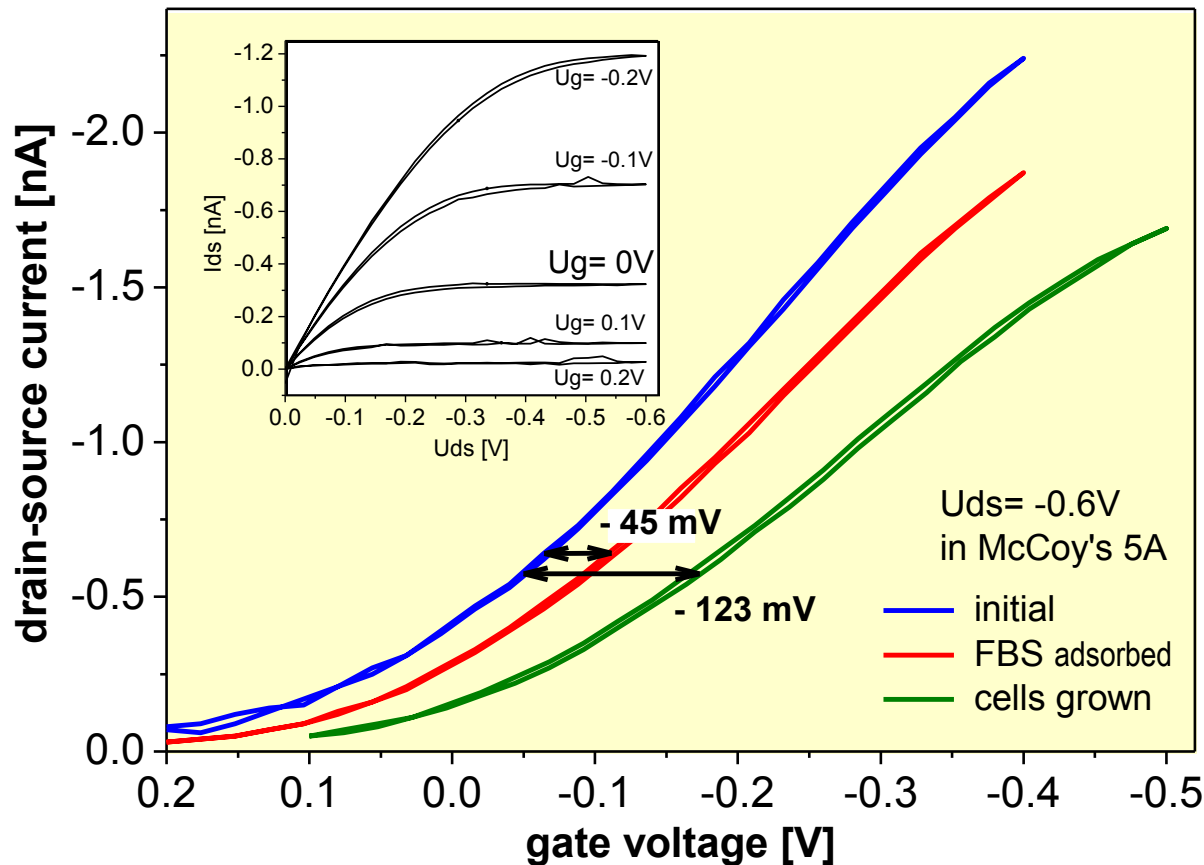


- to characterize (and amplify) electronic effects at diamond interfaces
- **no gate oxide**, direct contact between molecules and channel surface

[Rezek et al., *Sens.Act. B* 122 (2007) 596]

[Rezek et al, *Biosens. Bioelectron.* 26 (2010) 1307]

Effects of protein layer on diamond SG-FET



Specific effects on H-diamond

- protein adsorption: transfer characteristics **shift negative (not a field effect!)**
- decreased slope, i.e. **lowered transconductance** (~30 nS, gain ~ 0.9)
- remains **persistent** after washing/rinsing

Model of diamond-protein-cell interface

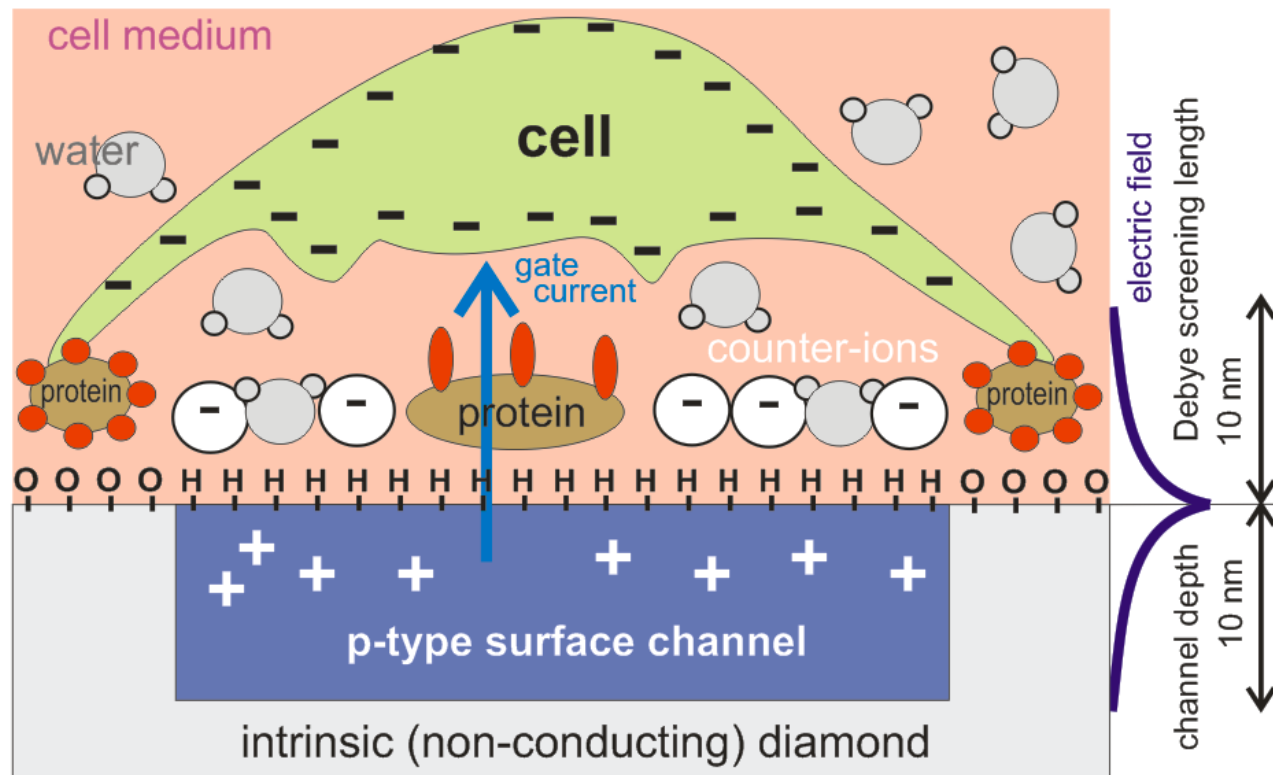
SPECIFIC MODEL FOR H-DIAMOND

recall AFM data

proteins modify original equilibrium of the surface conductivity system

(they replace ions in the very vicinity of the diamond surface)

→ negative shift of transfer char. and change of transconductance



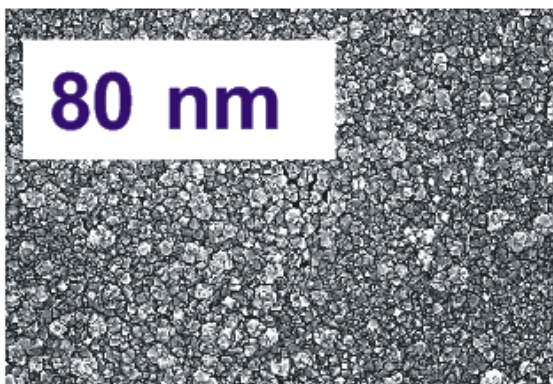
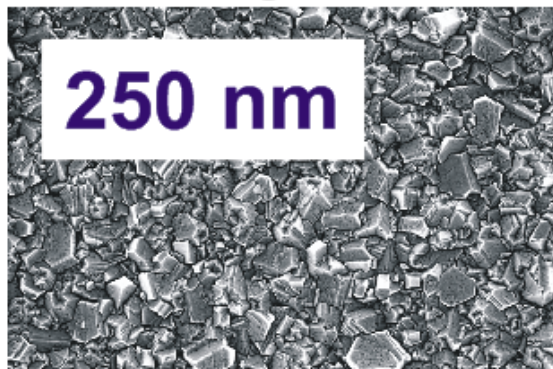
[Rezek et al., *Biosens.Bioelectr.*26 (2010) 1307]

Role of grain boundaries in diamond function

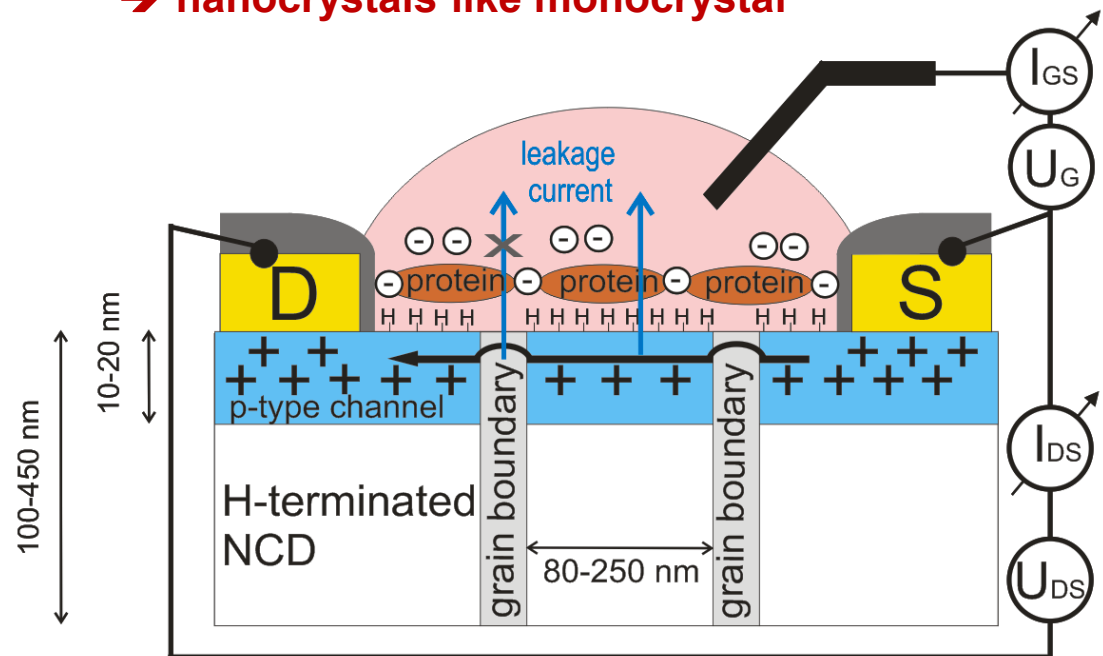
Time [h]	Temper. [°C]	Grain size [nm]	Thickness [nm]	Roughness [nm]
4.3	550 - 600	250 ± 50	445	27
1	550 - 600	80 ± 50	108	19

- various grain sizes investigated (50-500 nm)
- even 100 nm thin transistor fully operational
- **function controlled by C-H surface of grains, not by grain boundaries** (they limit mobility)

→ nanocrystals like monocrystal



1 μm

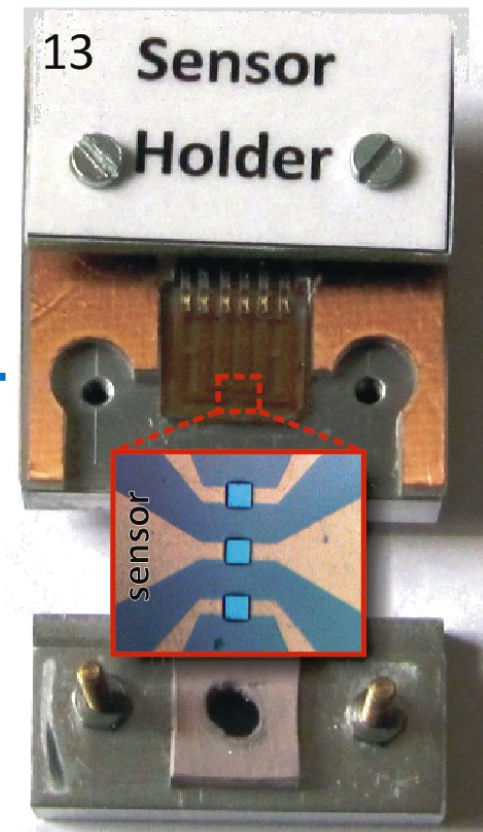
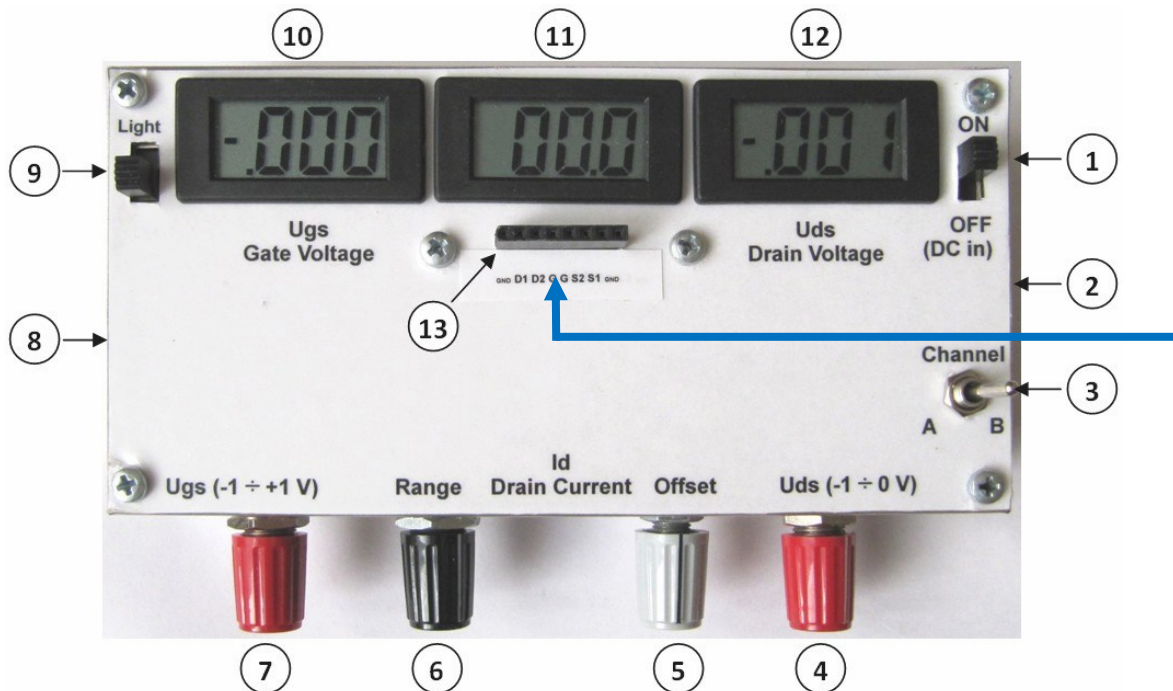


[Krátká et al., *Sens. Actuators B* 20 (2012) 239]

[Hubík et al., *Diam. Relat. Mater.* 24 (2012) 63]

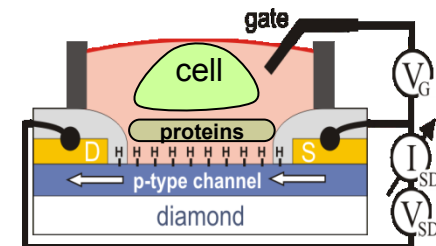
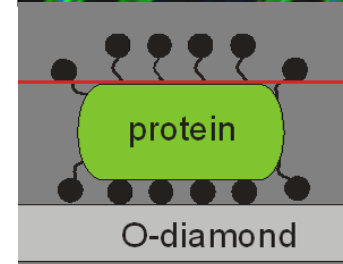
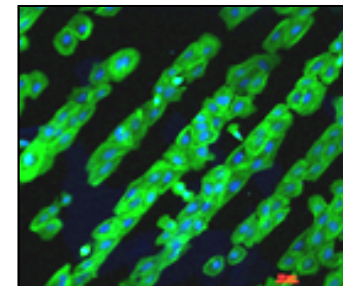
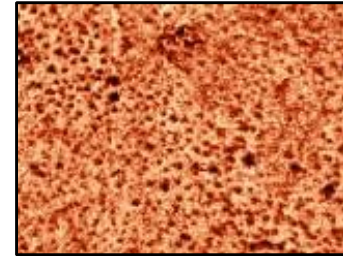
Funkční vzorek – přenosný senzor

- zařízení pro výzkum v naší laboratoři ~ 10 MKč
- znalosti a zkušenosti transformovány do funkčního vzorku: **“DEMONSTRÁTOR SENZORU NA BÁZI NANO-DIAMANTU”**
(náklady ~10 tis.Kč, včetně diamantového senzoru)



Shrnutí a přínosy

- **Dosáhli jsme uspořádaného růstu buněk pomocí povrchových atomů na nanokrystalickém diamantu**
 - **rozdíl ve smáčivost** vede k různé konformaci proteinů, nezávisle na elektrické vodivosti a hrubosti diamantu
 - **obecný jev** pro různé typy buněk (osteoblasty, karcinom, neurony)
- **Uspořádávání buněk můžeme řídit:**
 - **mikro-strukturováním** H/O atomů (30-200 μ m vs. uniformní)
 - **ne/přítomností proteinů** během nanášení buněk (FBS, Fn)
 - **počáteční koncentrací** buněk při nanášení
- **Ukázali jsme, že diamantové tranzistory jsou vysoce citlivé na biologické prostředí (proteiny, buňky)**
 - díky AFM v roztoku byl **objasněn mechanismus** citlivosti, který je specifický pro diamant (změna rovnováhy rozhraní)
 - **hranice zrn a malá zrna (< 100 nm) nevadí**, funkce nanokrystalických senzorů je stejná jako u monokrystalu

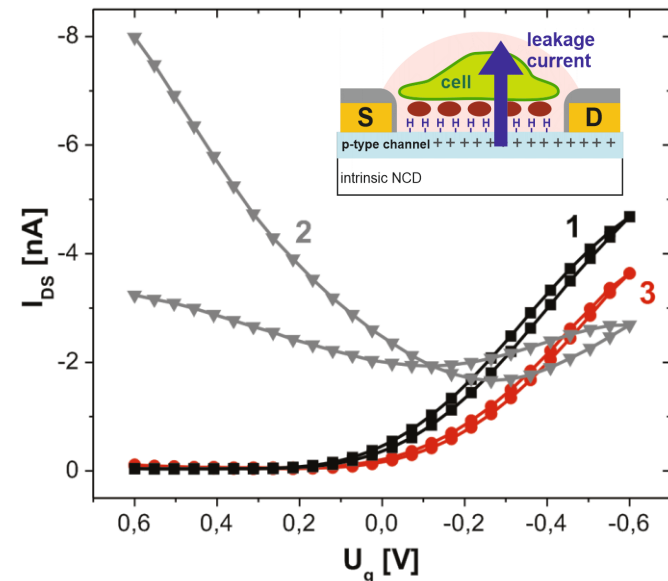
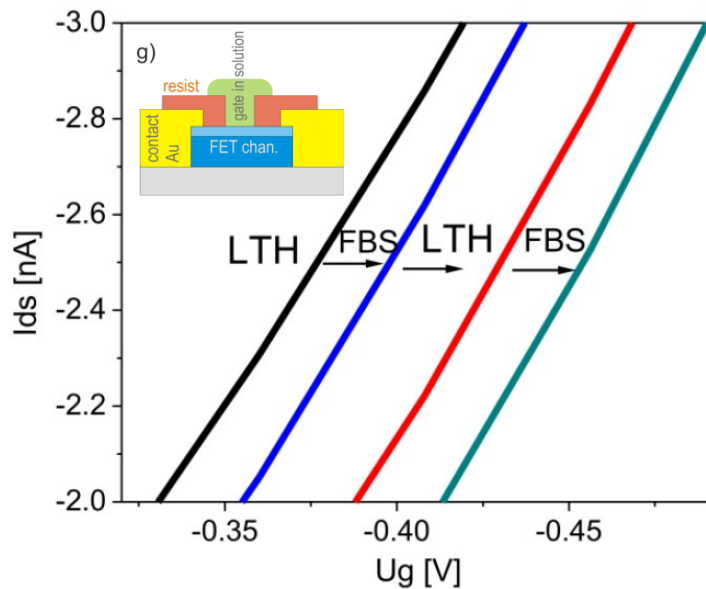


→ tkáňové inženýrství, bio-senzory a bio-elektronika

Návazný výzkum

- **podařilo se** vyvinout plazmatickou technologii pro jemné odstranění proteinů a tím **obnovení citlivosti Cell-FET** na proteiny [*Int. J. Electrochem. Sci. 2013*]
- **podařilo se** objasnit spínání proudů buňkami na hradle z diamantu (K^+ ionty) a **navrhnout senzor** buněčných kultur [*Appl. Phys. Lett., v recenzi*]

a mnoho dalšího řešíme...



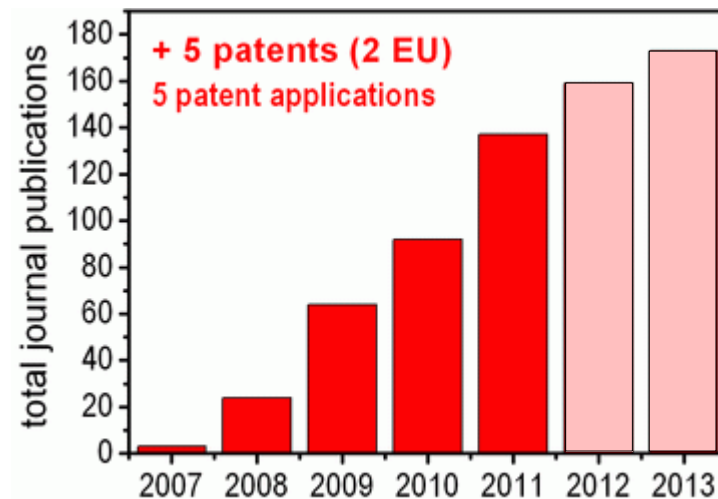
Významné výstupy a přínos ČR

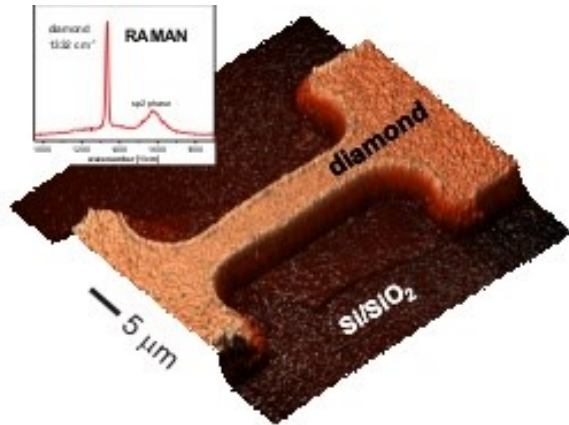
Významné výstupy k tomuto tématu

- **EU patent** (a další), **funkční vzorky**
- **publikace v renomovaných časopisech**: *Biosensors and Bioelectronics*, *Sensors and Actuators*, *Langmuir*, etc.
- **dílčí ocenění**: *L'Oreal for Woman in Science* 2010, *Česká hlava Doktorandus* 2012

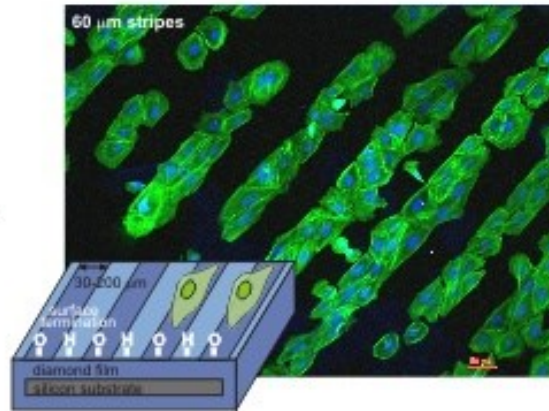
Přínos pro ekonomiku ČR

- **zvýšený obrat** firmy ~20 MKč/rok
- **pracovní místa**, kvalifikovaná (~10)
- **prodané licence** průmyslu
- **investice průmyslu do VaV**, tento a nové společné projekty
- **mezinárodní prestiž**

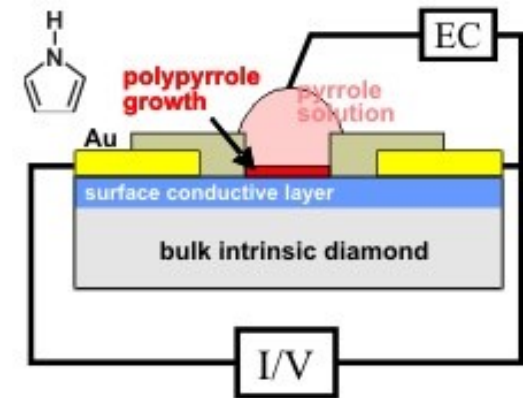




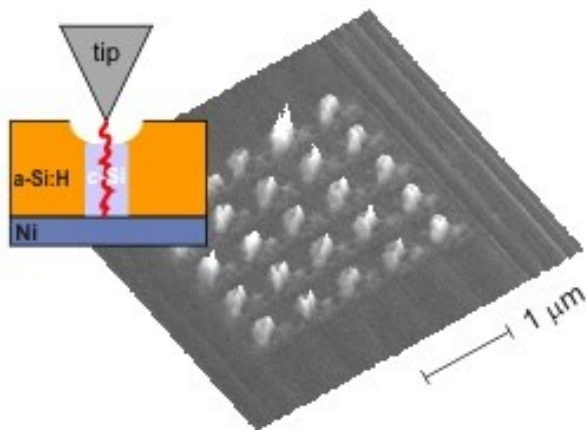
direct growth of diamond micro-devices



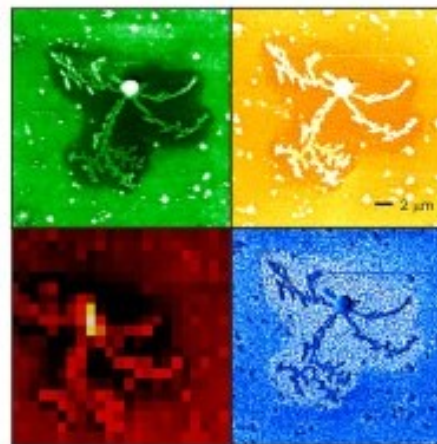
guided-assembly of cells on diamond



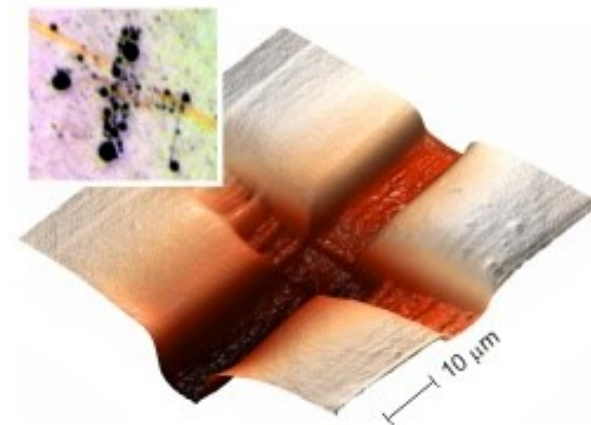
polypyrrole-diamond nanosystems



conductivity map of Si nanocrystal array prepared by AFM electrical crystallization



multidimensional microscopic map of polymer blend for PV



local electrostatic charging of nanocrystalline diamond films