# Nanotechnologies and selfassemblages for the bio-inorganic interface

Piero Morales
piero.morales@enea.it

The NAST-ENEA collaboration on

1) **Lasers+SPMs for the bio-inorganic interface**
2) **Instrument development**
3) **The biological route: a) ready made structures**
   **b) selfassemblage**

# Both life and electronics involve nano sciences

- **Life is complex, slow and cheap (in spite of your complaints!)**
- **Electronics is simple, fast and expensive**

But we want to make our life quicker and (disgracefully!) more complex

so:

We need technologies based on electronics, to be quick (e.g. computers, cellphones, sensors…..)

We need technologies based on biology to account for the inevitable complexities (e.g. medical / pharmaceutical technologies, very specific sensors, parallel processing devices etc.)

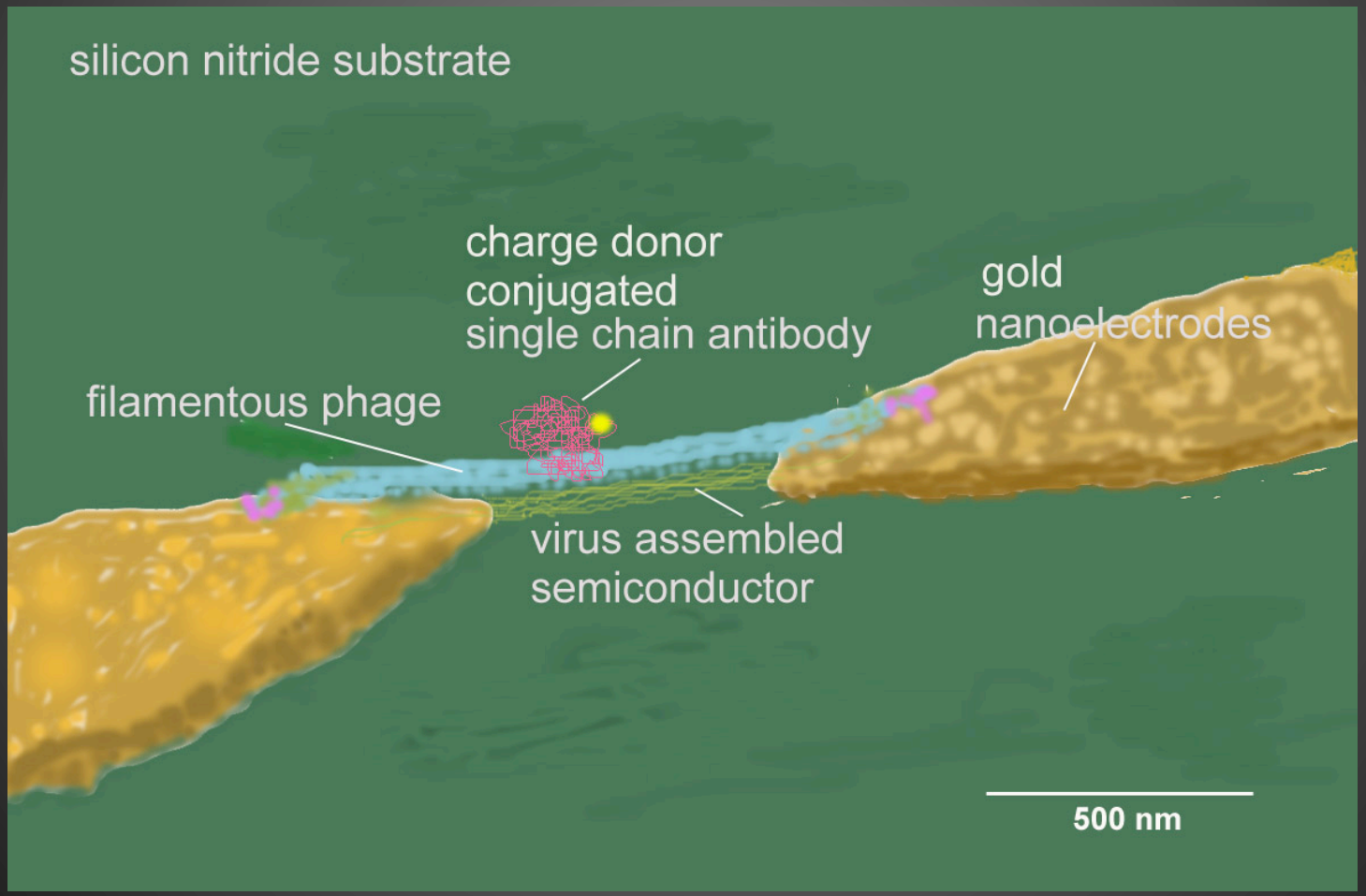
**We actually want both together!!**

## We want both together?
## BUT…, to do this

- We must use and enhance artificial nanofabrication
- We must understand the interface
- We must exploit selfassemblage, both bio and inorganic

# Brainstorming: a hypothetic, virus based, hybrid electronic device

(Angela Belcher 2000/2002)



# Is it just science fiction?

- Can we contribute to get closer to such “devices”?
- What technology would we need?
- What skills shall we develop?

# 1) Lasers and SPMs to couple “smart” molecules to silicon based nanodevices

- Inorganic: Nanoelectrodes; nanotubes, carbon nanostructures

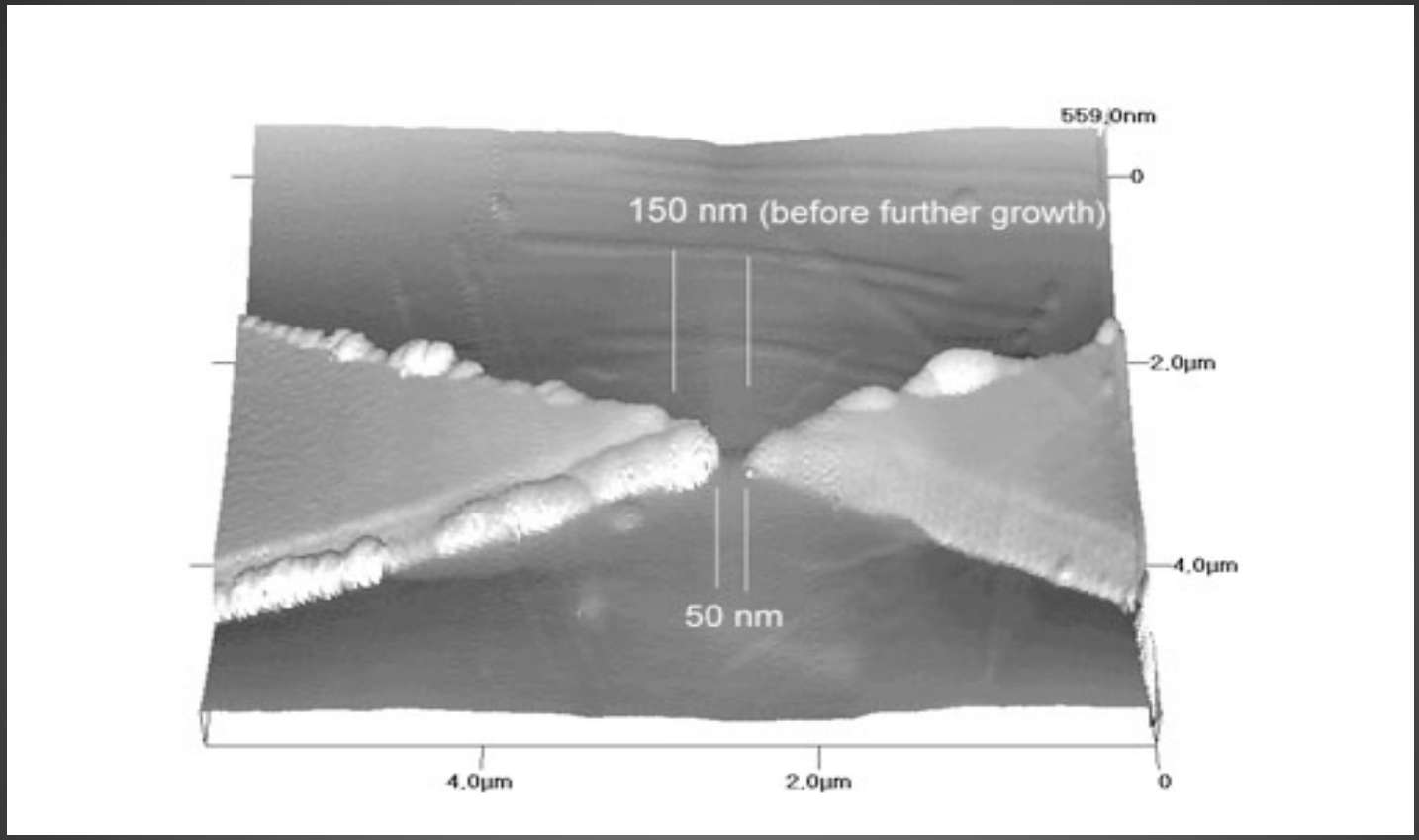
- Bio-Organic: Smart (functional) molecules: biomolecules (antibodies, enzymes), aromatic and conjugated molecules (dyes, charge donors-acceptors, molecular rectifiers)

**One idea common to both:**

**Assembling at the molecular scale, or Nanofabrication:**

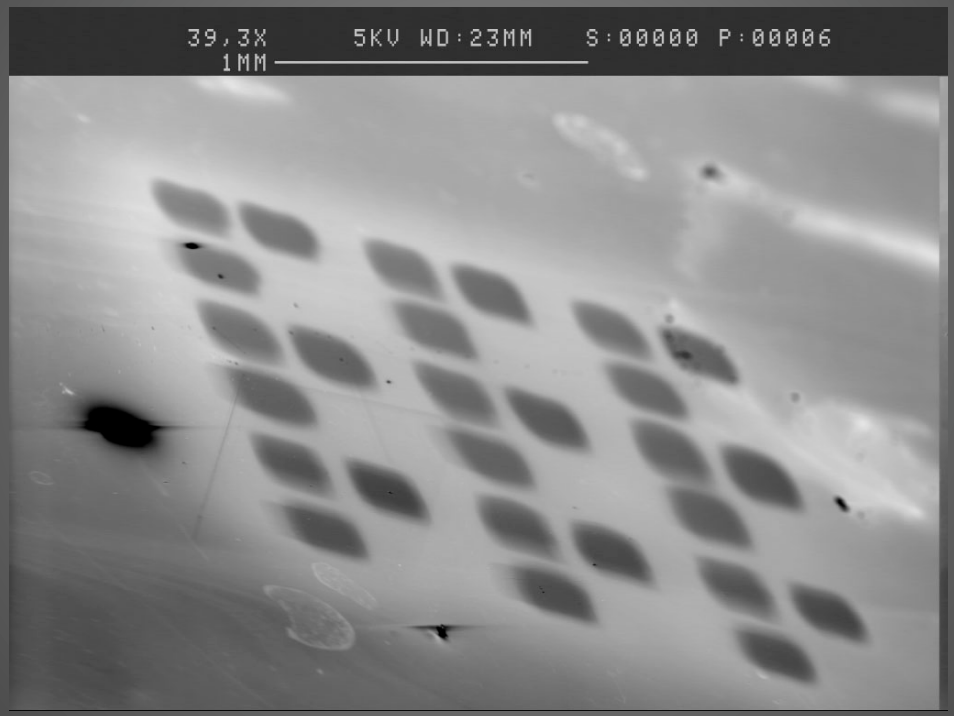
**e.m.radiation and SPMs, near field techniques**

# Artificial nanofabrication: nanoelectrodes




**If brought to 2 nm size, the gap would look more like a crack; facing surfaces would be large:**
**quantum tunnelling >> conduction through molecular elctronic components → need for thin uniform metal layers**

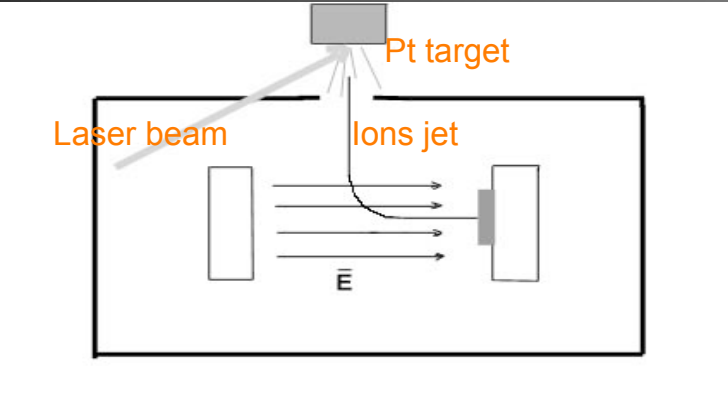
# Nanoelectrodes: Pulsed Laser Deposition (PLD) is a suitable method to obtain thin controllable metal layers

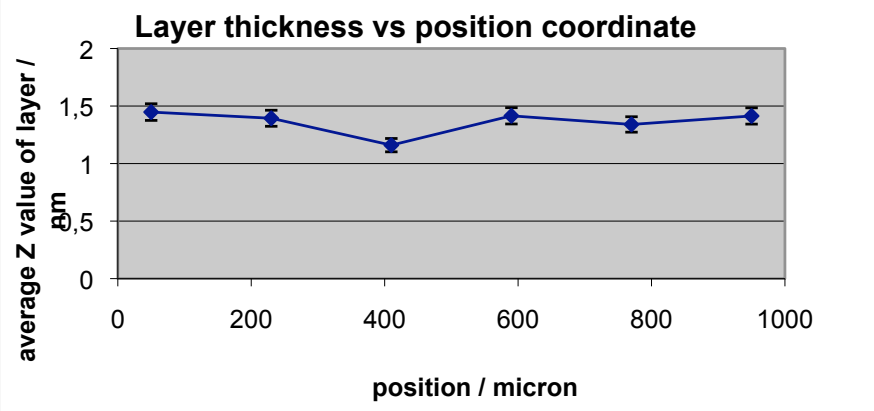


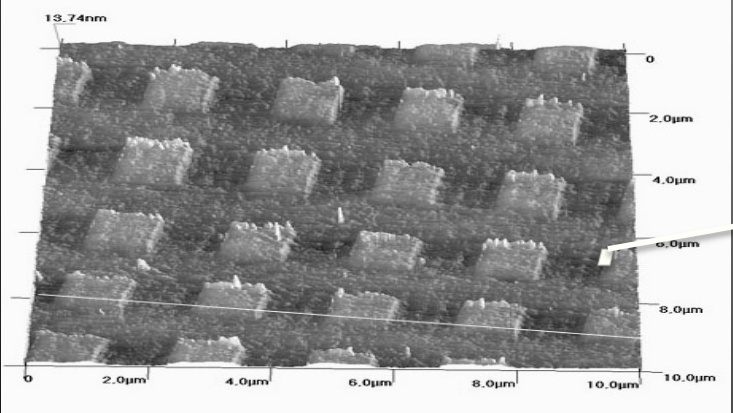

Micropositioning of shadow mask yields Pt thin film patterns. Layer thickness approx 5-10 nm

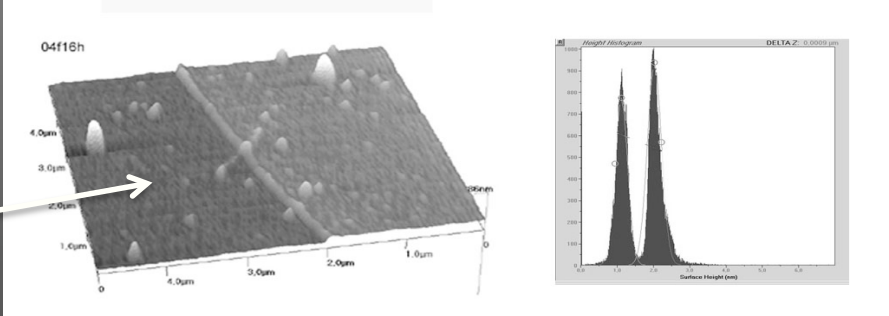
# How to obtain ultrathin, uniform and controllable nanoelectrodes by PLD

**To produce uniform and thin layers, we use pulsed laser deposition and electric field assisted ion steering. Growth rate is extremely low (1 nm/h). E-beam lithography supplies sharp, easily measurable, layer step boundaries**



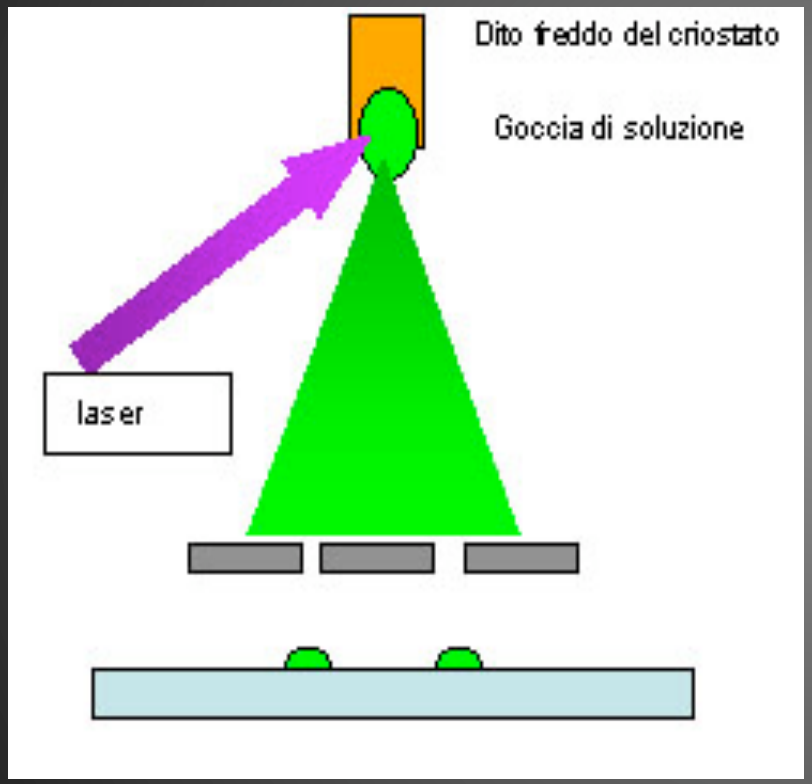



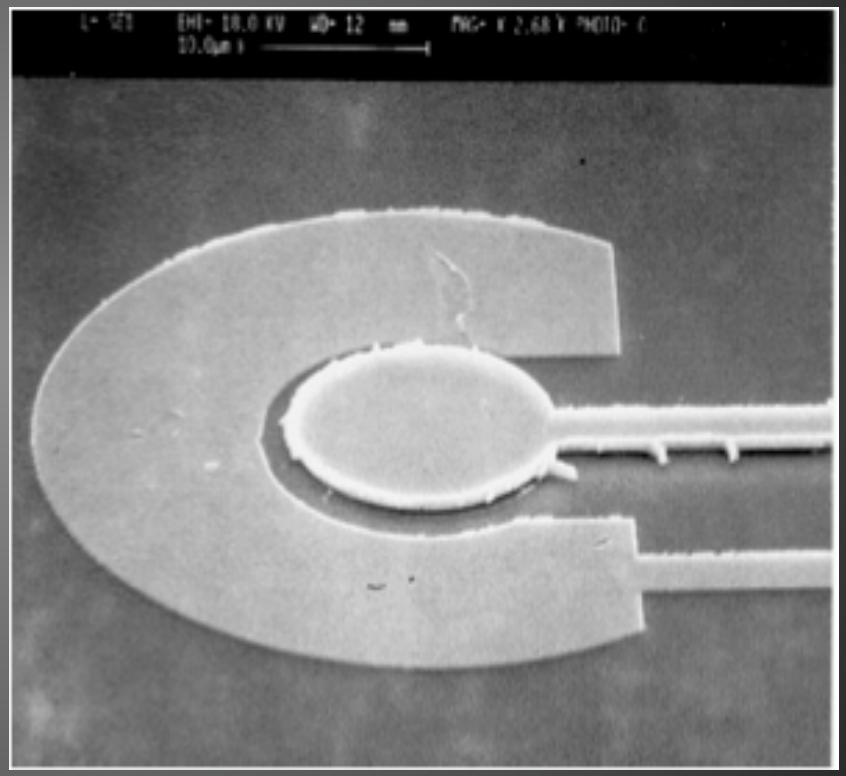




**<u>14 or even 9 Angstrom (!!)</u> thick Pt layers, atomically uniform**

# Organic and biomolecule PLD patterning



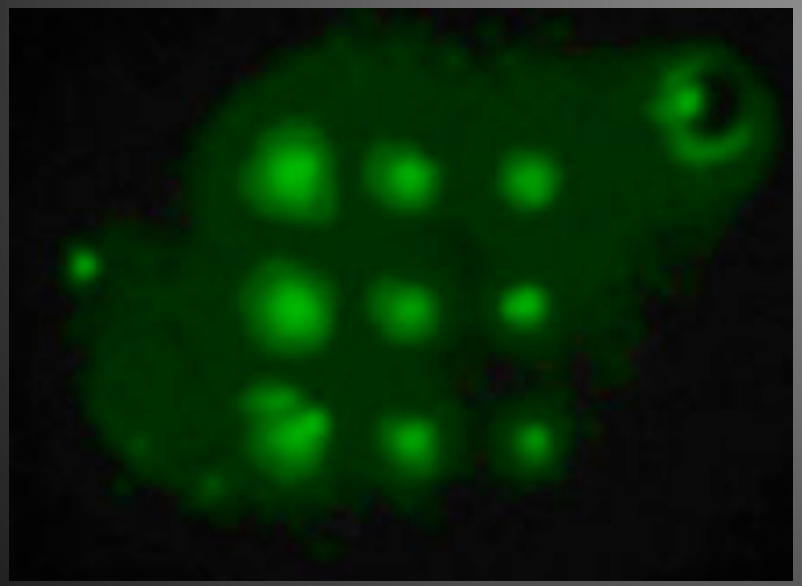

schematic

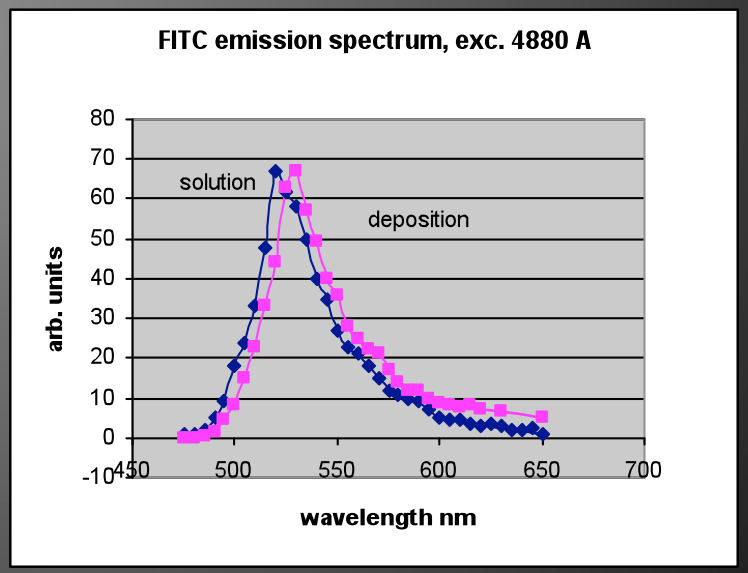


the smallest biosensor
(Glucose Oxidase, 1995)

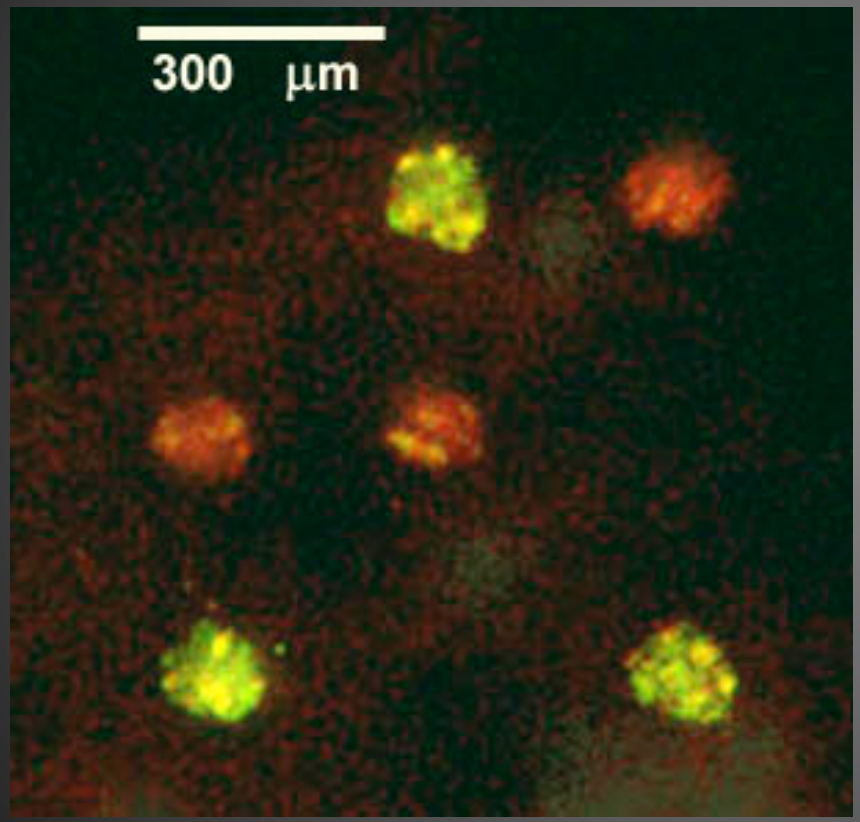
# Pulsed Laser Deposition maintains spectroscopic properties of aromatic molecules

- Fluorescence from laser deposited aromatic dye molecules: Fluorescein.
- Emission spectrum from PLdeposition almost identical to that of solution





# *Microdevices*: biosensing microarrays by pulsed laser deposition

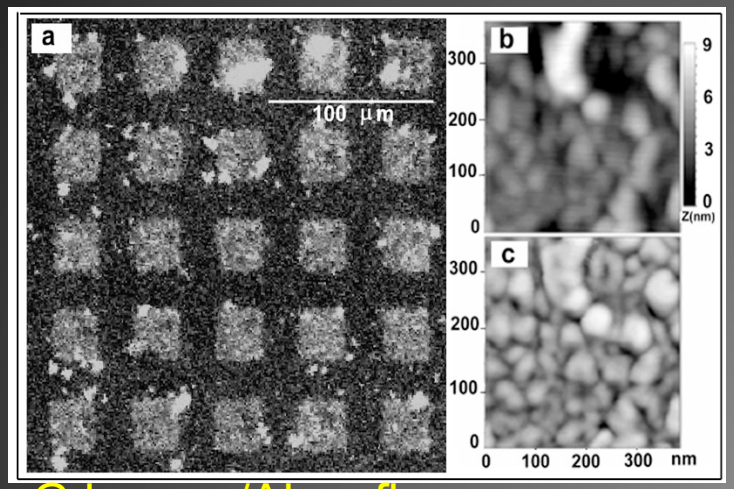



Biomolecular monolayers, 5-10 nm thick

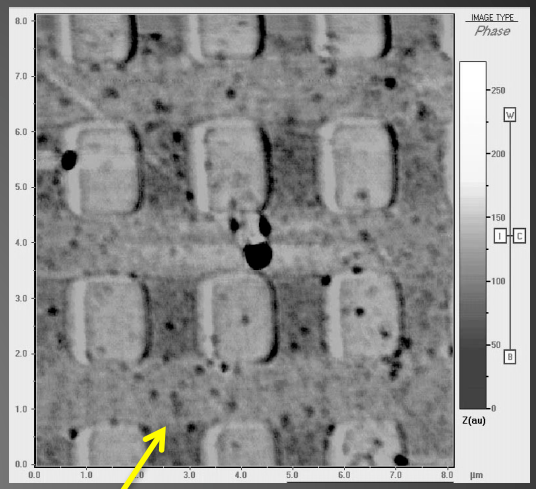
Two different antigens have been deposited in monolayers on six arbitrary locations of a 3x3 array. The remaining three locations have been coated by platinum layers for control. The device is incubated for 20 min. in a solution containing the antibodies specific for the two deposited antigens. The antibodies are tagged with fluorescent molecules of different colours, which make the antigens visible when they bind their antibodies

**S.Gagliardi et al. IEEE Trans. Nanobioscience 6, 242, 2007**

# Improving the quality of biomolecular films




Micron scale

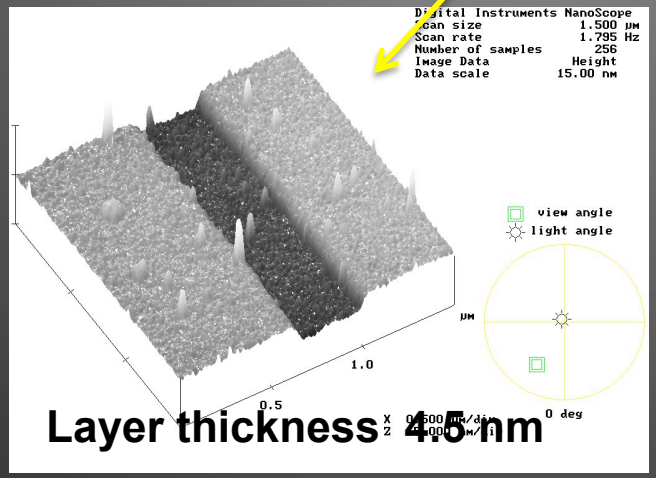



Nano scale

IgG human/Alexafluor
Confocal microscopy

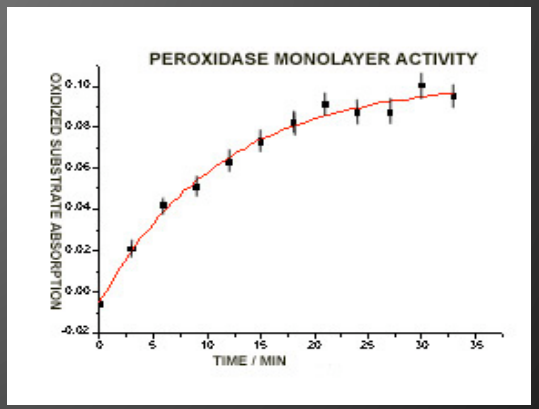
AFM

Horseradish peroxidase, AFM




**Layer thickness 4-5 nm**

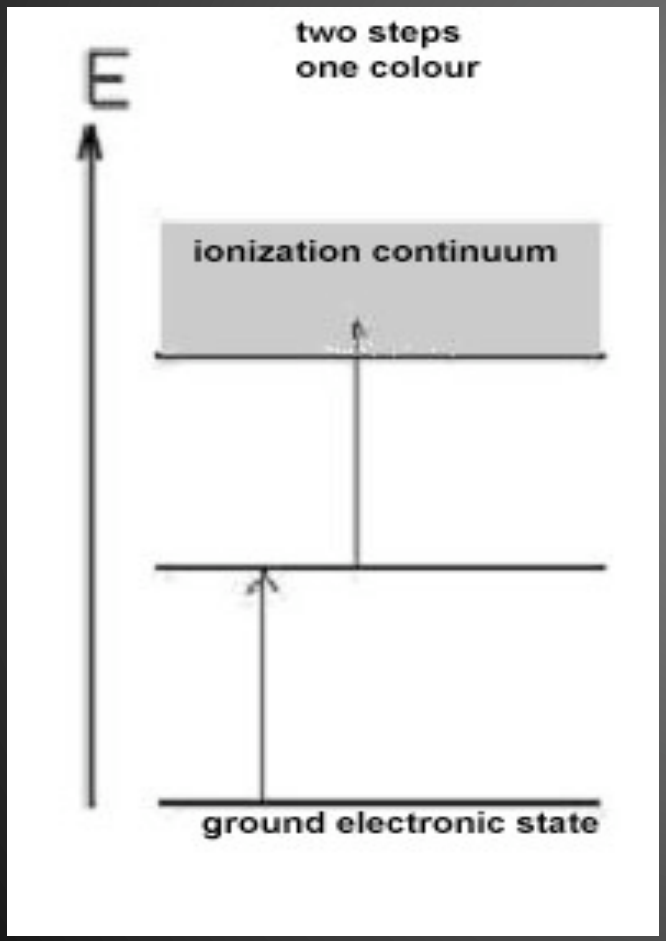
**Molecular size 2-3.5 nm**

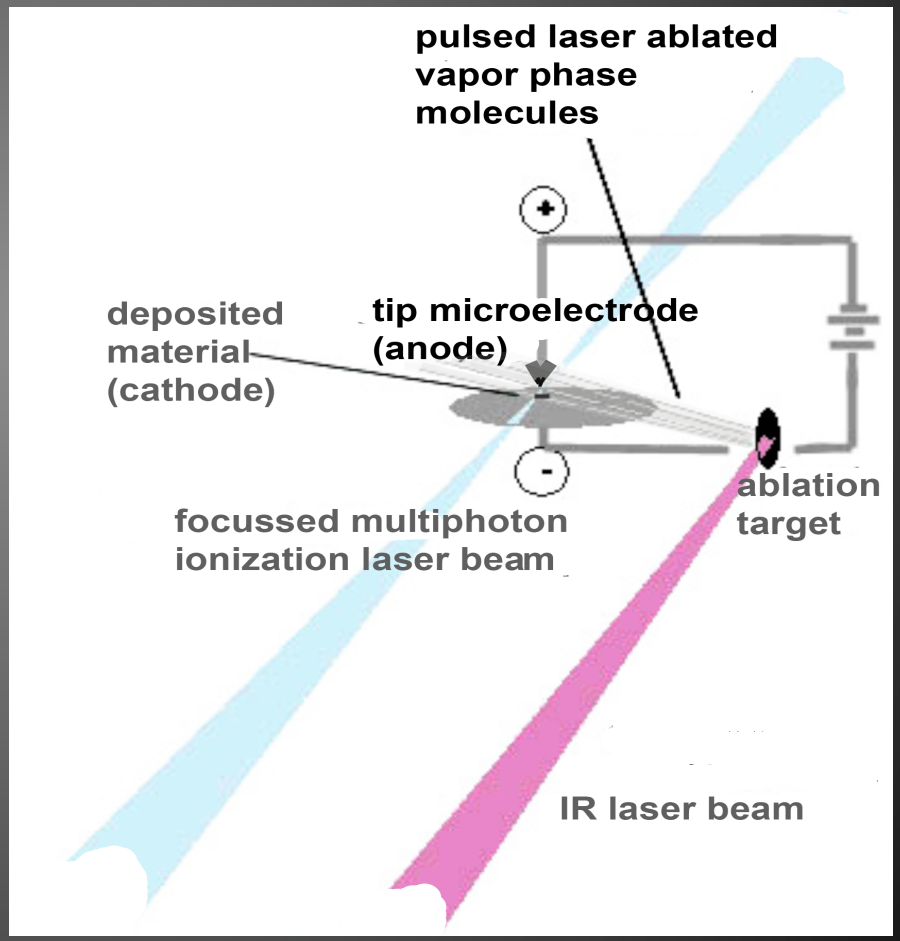



Reaction kinetics curve

# Can we improve our direct writing?
# Ablation / 2photon ionization

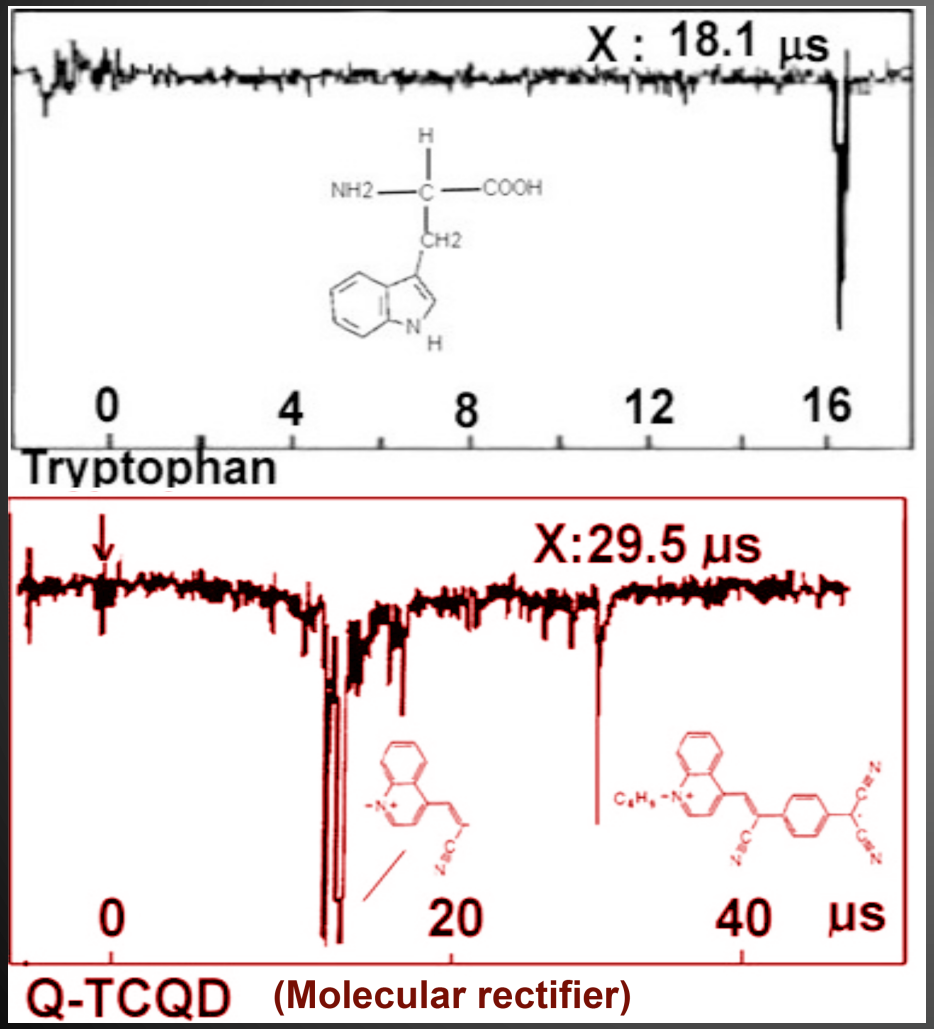
**REMPI: Resonance Enhanced Multiple Photon Ionization**

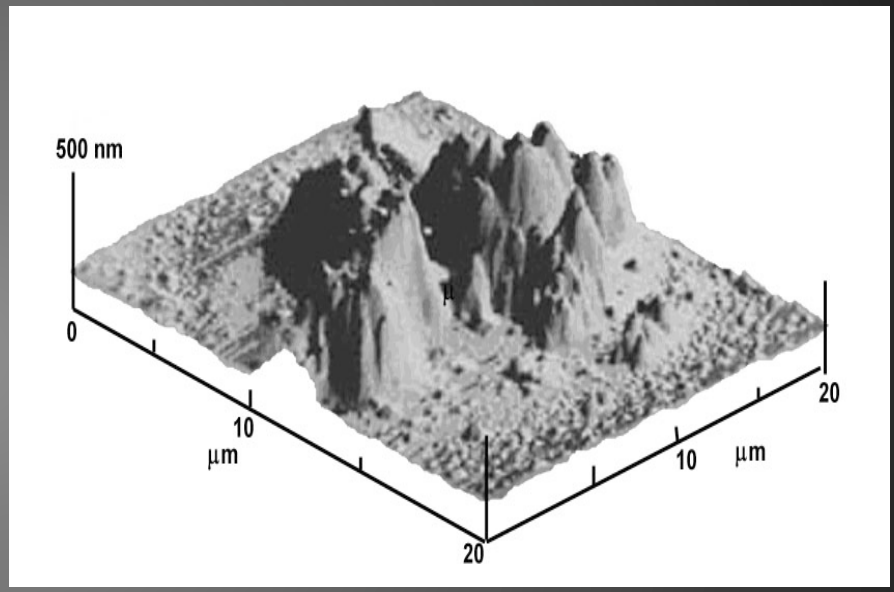


# Early demonstrator: direct writing of unfragmented tryptophan

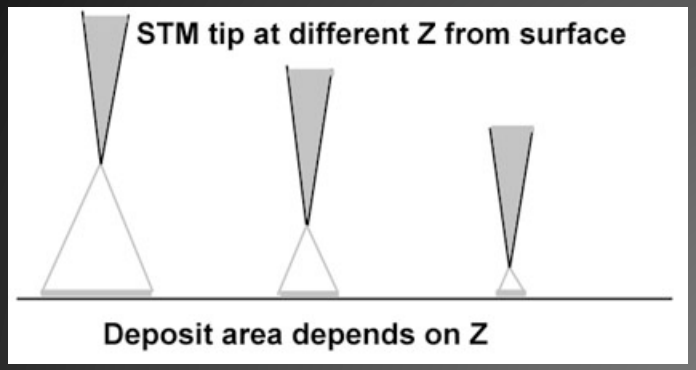
**Time of Flight mass spectra:**

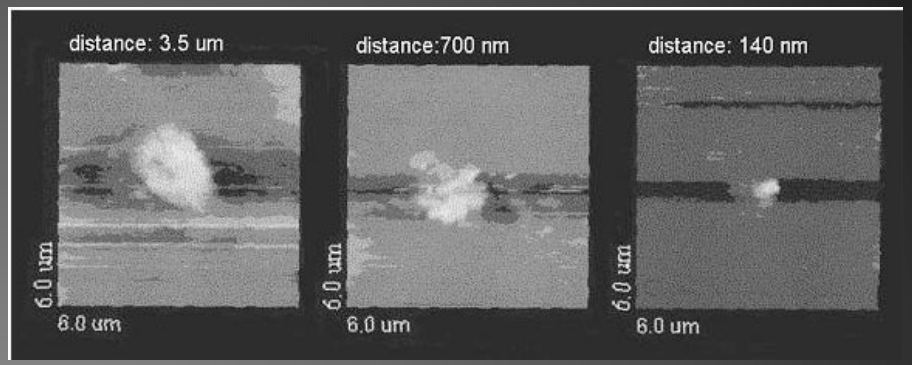


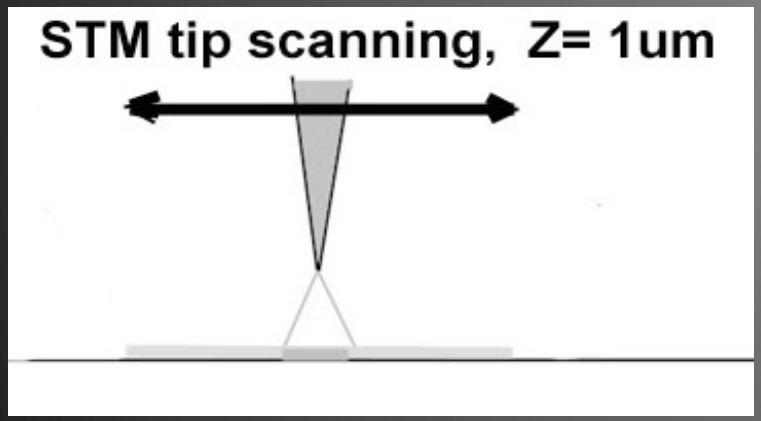




Cannot be done satisfactorily with rectifier quinolinium-tricyanoquinodimethane due to C-C bond breakage

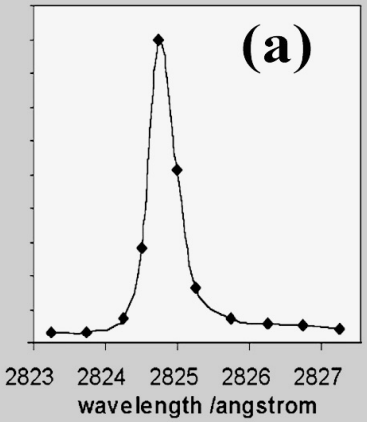
# STM direct writing using MPI of metals

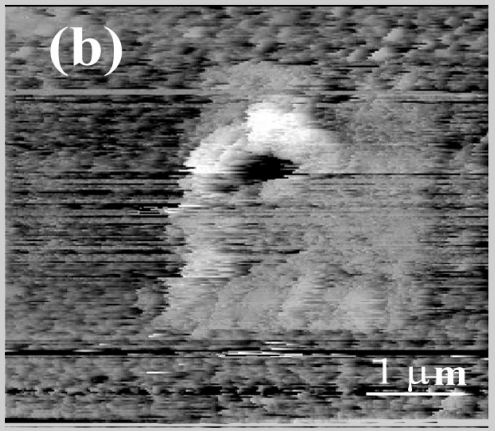


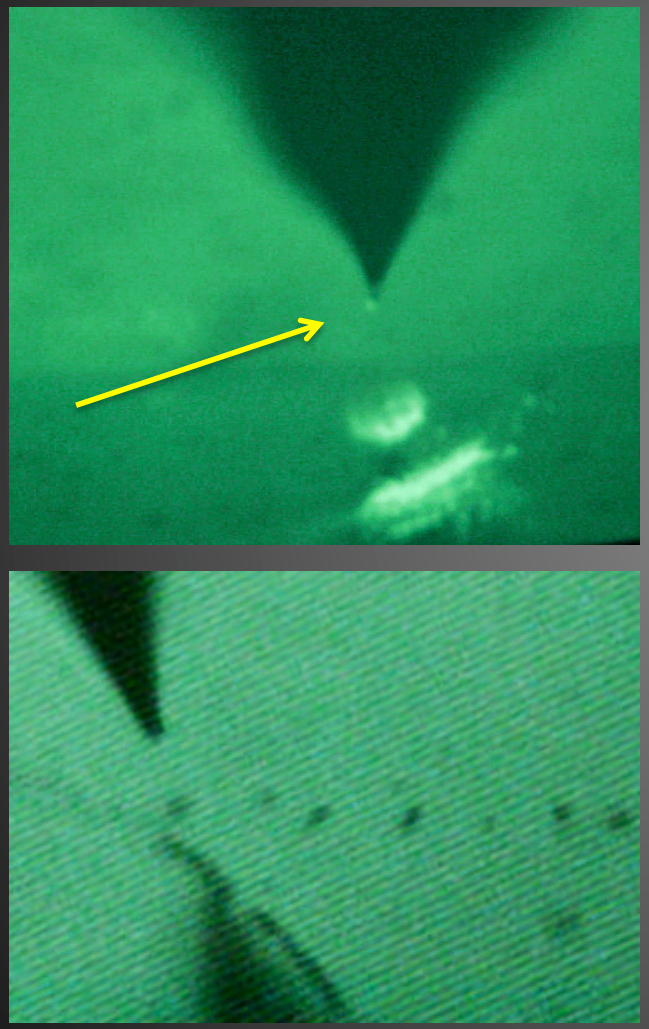

S. Gagliardi et al, SPIE 2005




**Note: laser beam size is 200 µm, structures are µm to nm! Why? Next slide.**

# Light Scattering from nanoantennas !



Optical microscopy

$$\underline{\mathbf{P}}(t) = \alpha\, \underline{\mathbf{E}^{\circ}} \cos \omega t$$

$\underline{\mathbf{P}}$ is the electric polarization of the antenna; $\alpha$ is the (generally anisotropic) polarizability tensor

$\alpha = 3/4p(\varepsilon-1)/(\varepsilon+2)$ $\quad \varepsilon(\omega_n) = -(n+1)/n$

Resonances at n=1 and n=-2

**Temperature rise on surface:**

$$\Delta T_0 = \pi^{1/2} I_0 (1-r) g w_0 / 8\lambda$$

where

$g = I_{loc}/I_0$ = field enhancement

$w0$ = near field spot size (at 1/e of the maximum)

r = sample reflectivity at normal incidence

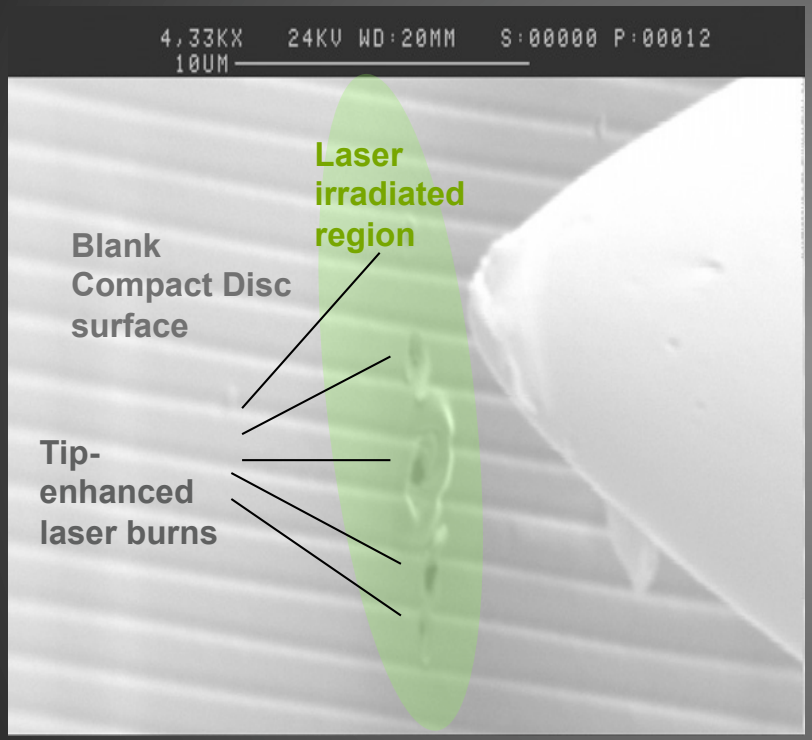
$\lambda$ = thermal conductivity of the sample

# Applications

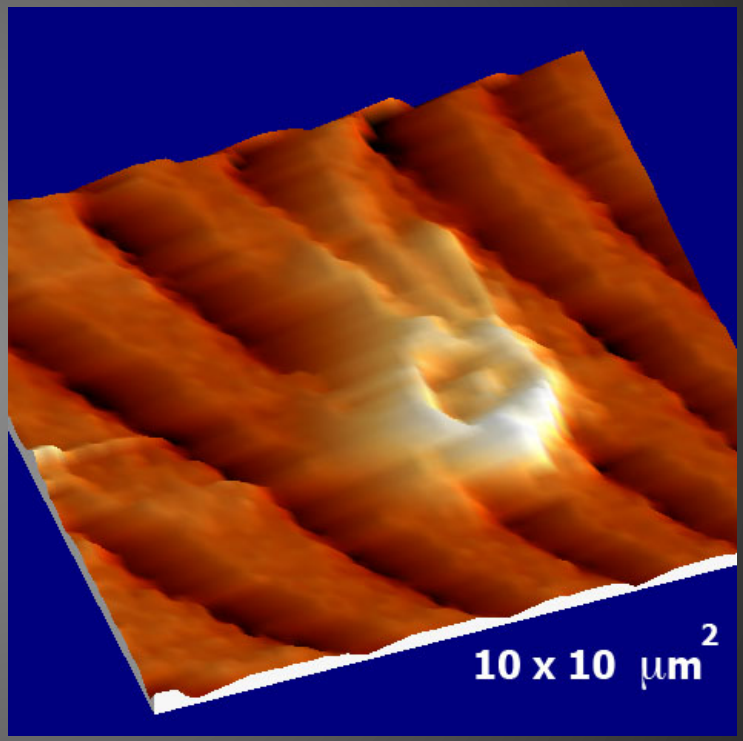
- Direct writing for nanoelectronics and nanobiodevices
- Photoinduced surface chemistry (e.g. lithography)
- Ion implantation
- Cutting and welding of micro and nano wires

**All below the diffraction limit (or below the practical beam size)**

# Example: “tip enhanced” burning of nanobits in CDs with unfocused laser beams




SEM

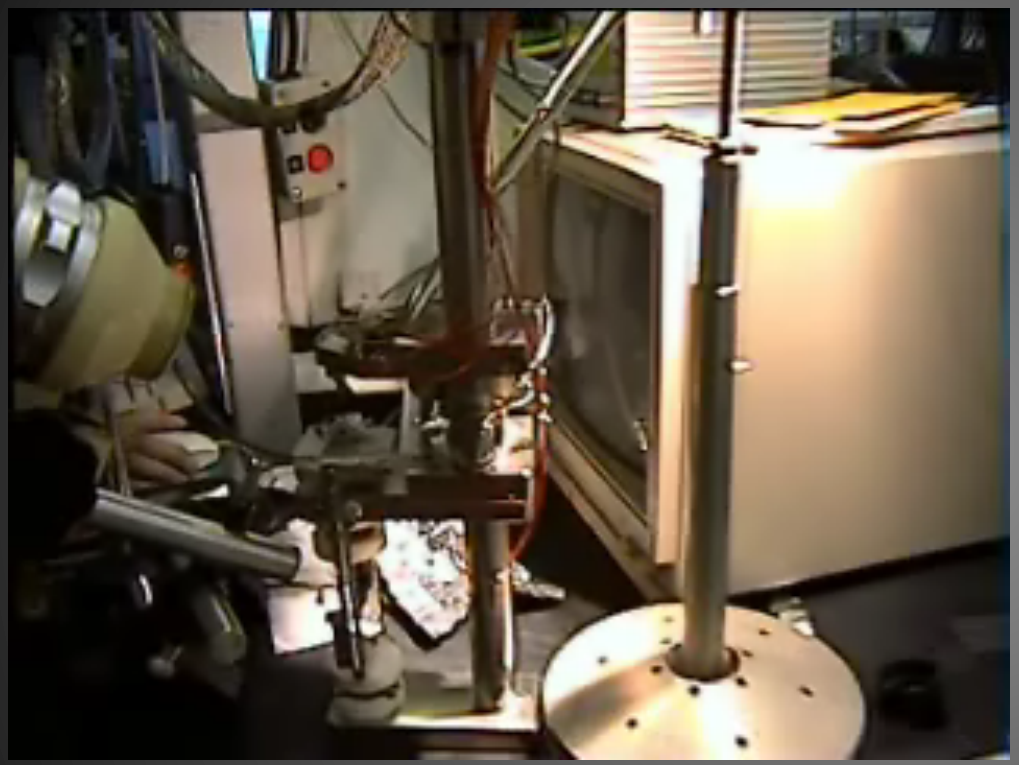



STM

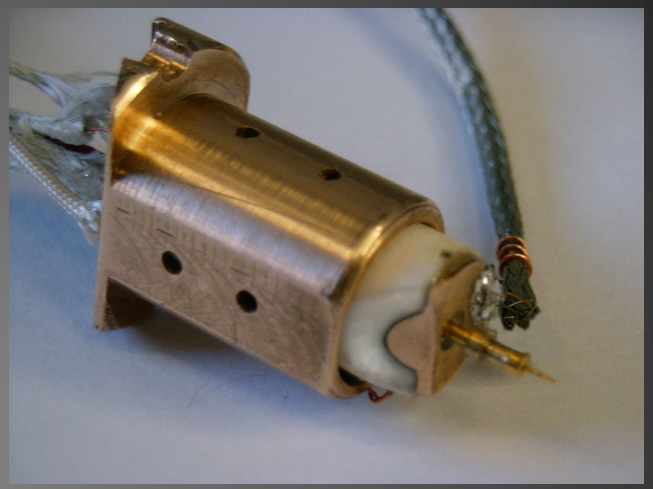
# 2) Instrument development

- SPMs
- Nanomanipulators
- Piezo inertial sliders
- Piezo dynamometers
- SPMs in SEM

# Instruments I: Our home made STM

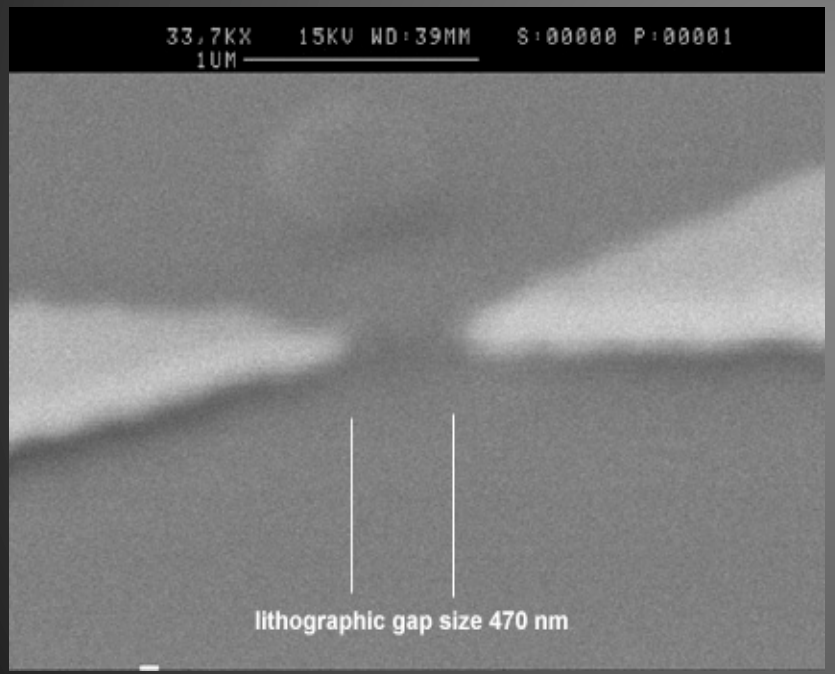


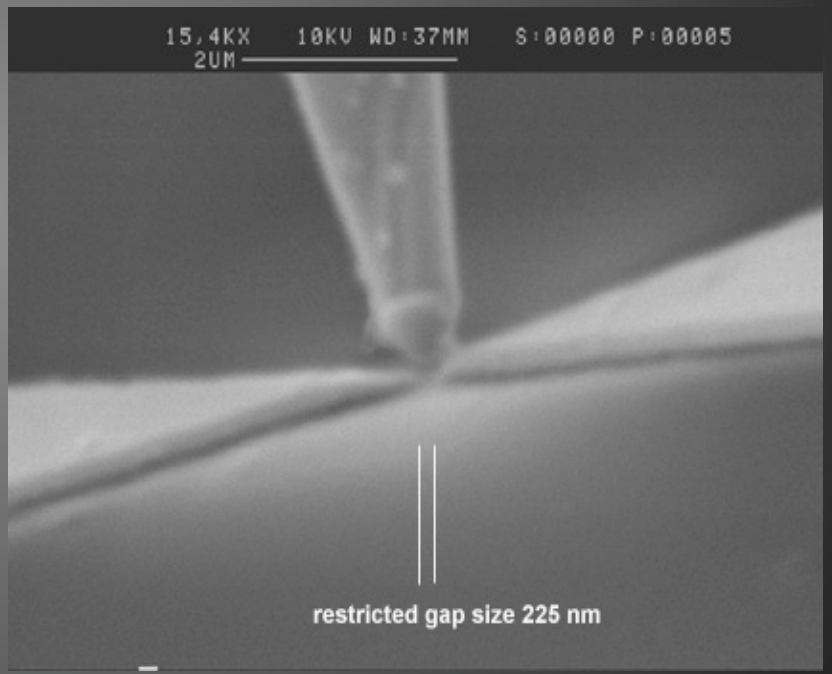
Electronics and software by ElbaTech srl



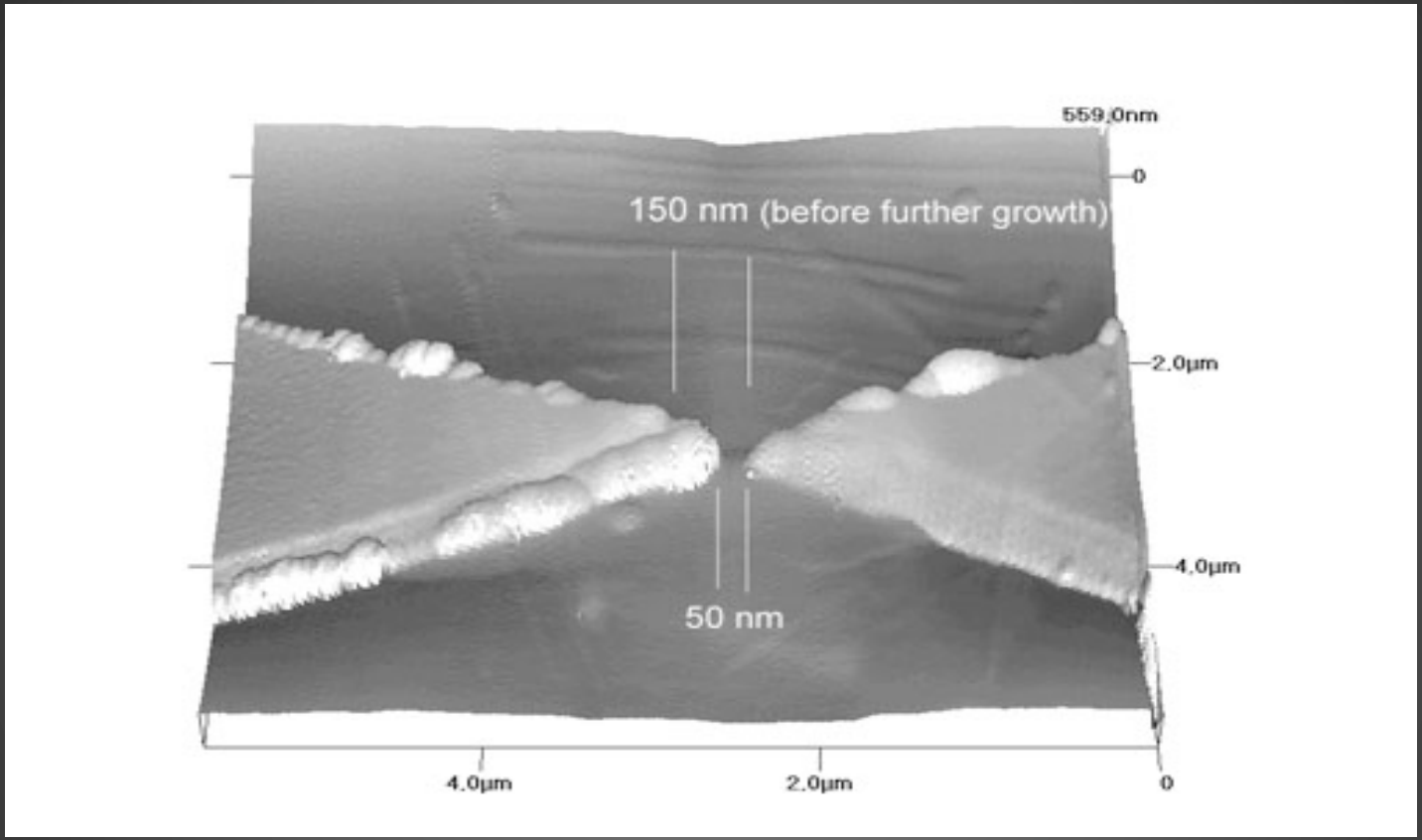
Piezo scanner with probe

# STM in SEM accessory: observing the tip while depositing material to restrict the gap between nanoelectrodes



# Compare to the AFM imaging of PLD gap narrowing



# 3) The biological route

a) Exploiting biological structures as moulds or stencils

b) Exploiting the selfassembling properties of biomolecules

- Spidersilks
- Viruses and their engineering
- DNA grids and other architectures
- ….more?

# Spider silks

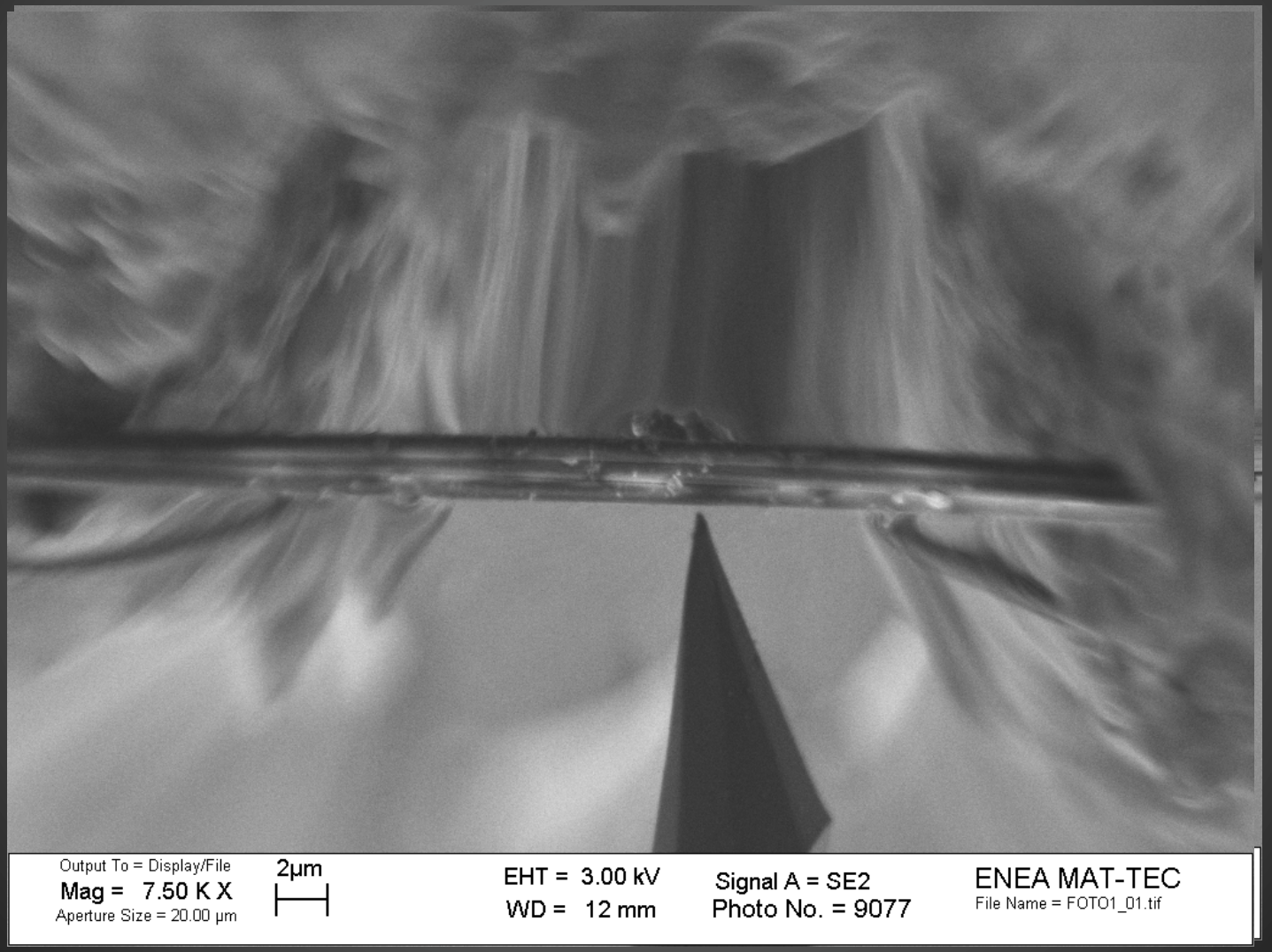
- Spider silks are extraordinary proteic fibers: stronger than steel, extremely elastic, extremely durable
- They are cm or m in one dimension, nm or micrometer in the other two.
- They are easy to manipulate, they cost nothing
- They are biocompatible, they can be used for medical applications

**BUT:**

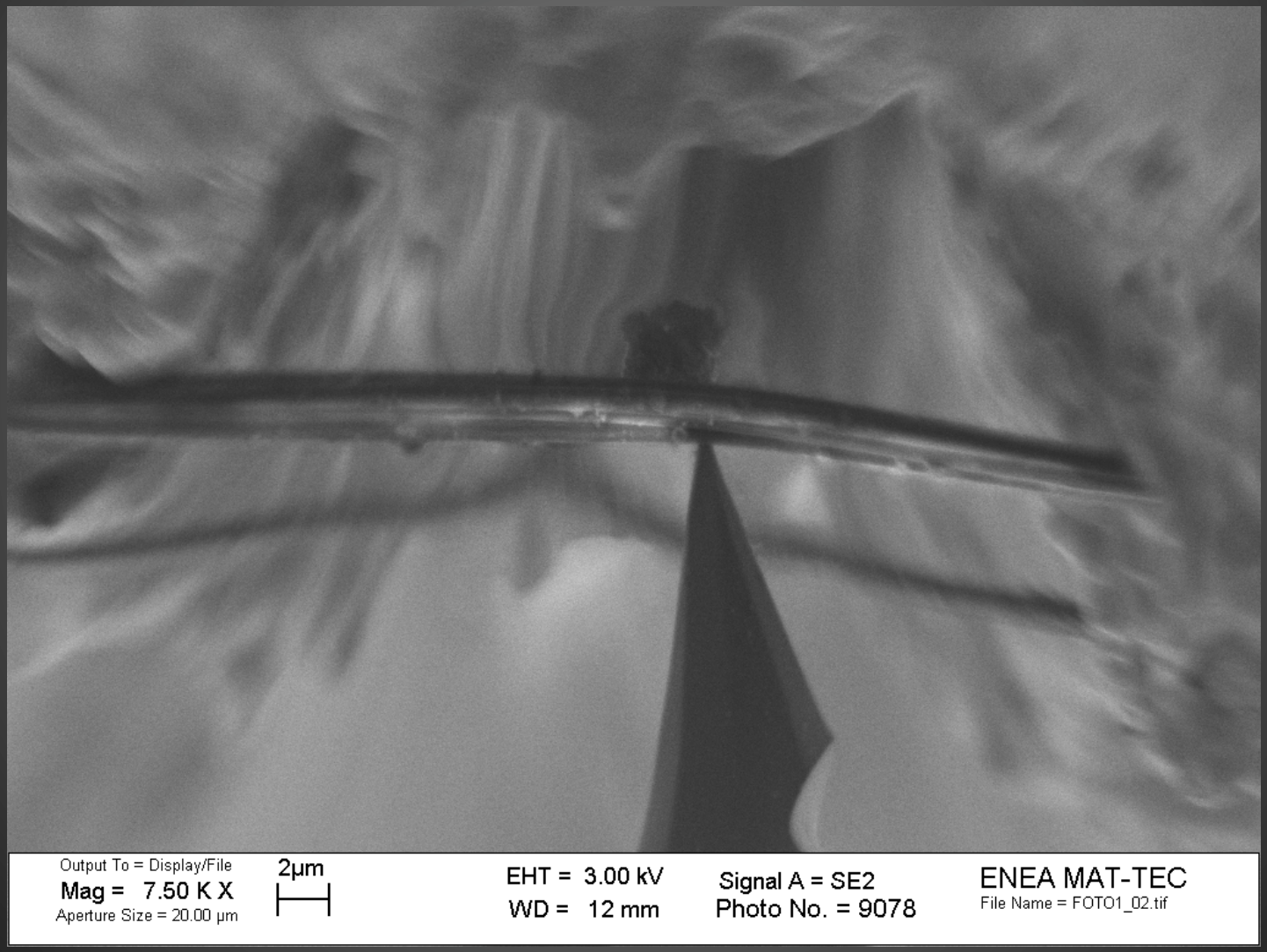
**spiders kill each other and cannot be bred like silkworms**

**We learned how to manipulate natural harvested spider silks and obtained a few applications**

# … measuring their mechanical properties



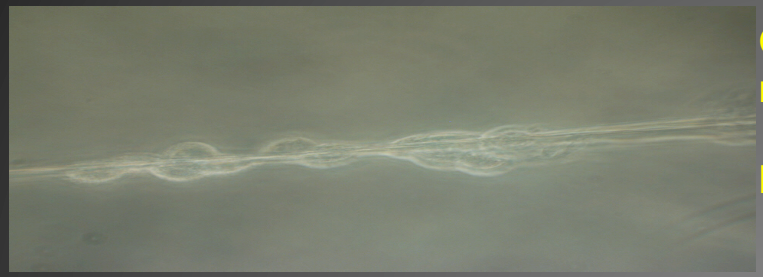
# … measuring their mechanical properties



# More about spiderwebs

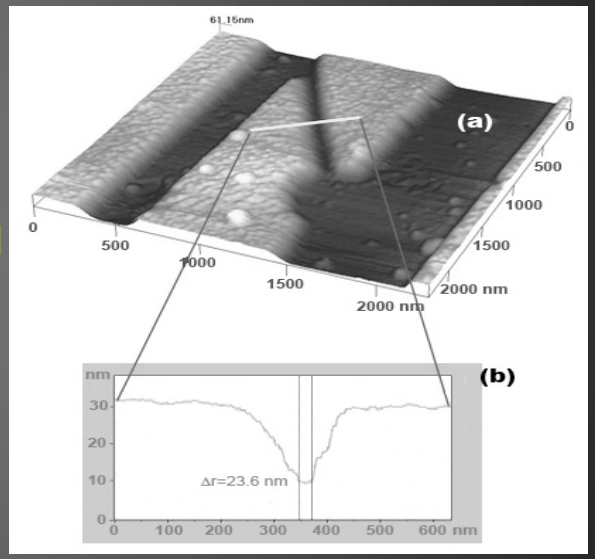
## Aim: exploiting selected spider silks for:

## a) Spatial organization of nervous cells and tissues



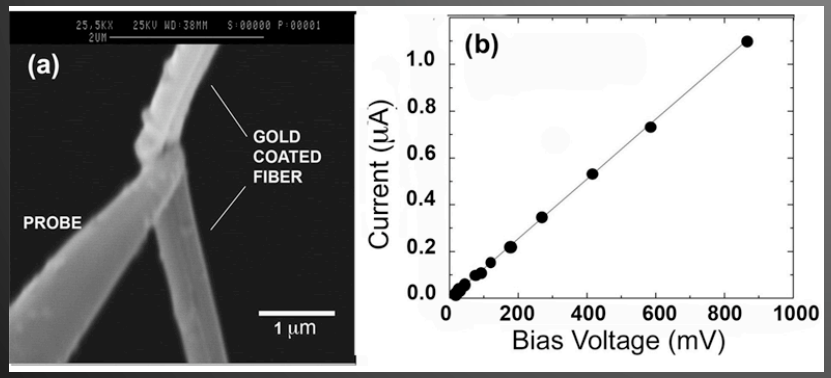
Growth of LAN5 neuroblastome cells on Nephila Aurata fibers

## b) Lithographic fabrications (nanogaps)




20 nm (!!!) channel in gold/mica

## c) Electrical micro- nanoconductors

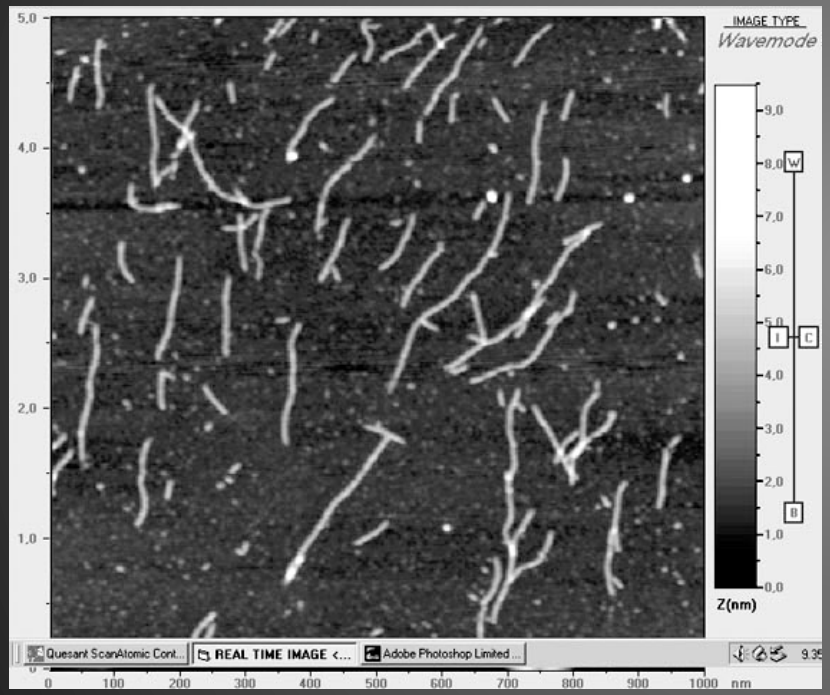


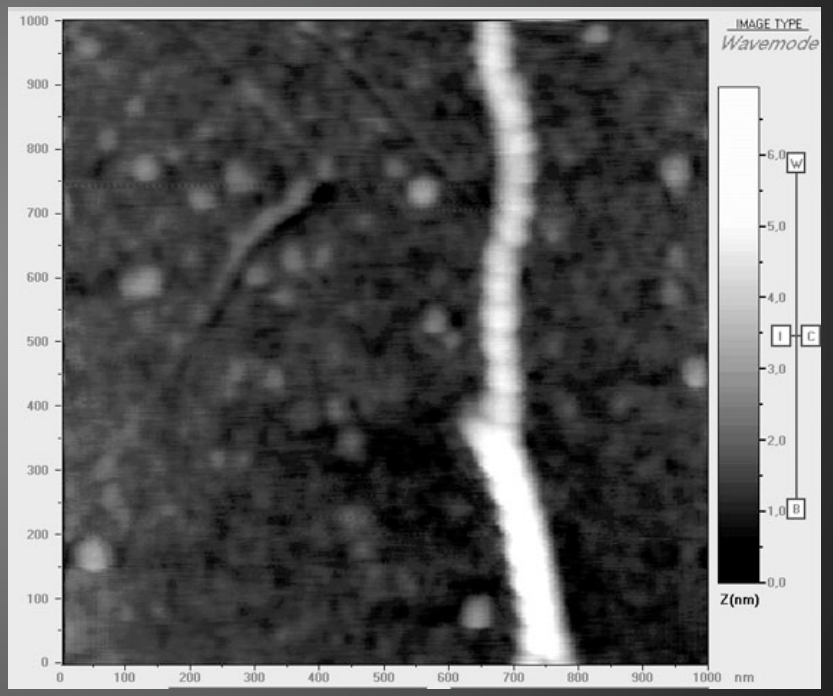

Fiber based gold nanowires probed by an STM tip

**Negligible costs of fabrication !!!!**

# Smaller nanofibers? Vegetable viruses!

13 nm thick a few hundred nm long

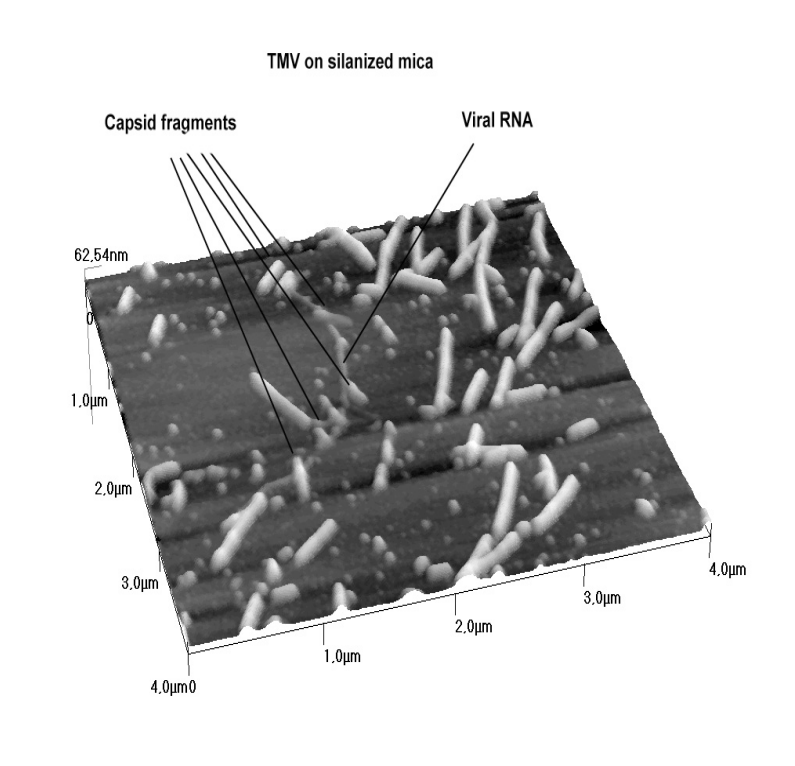


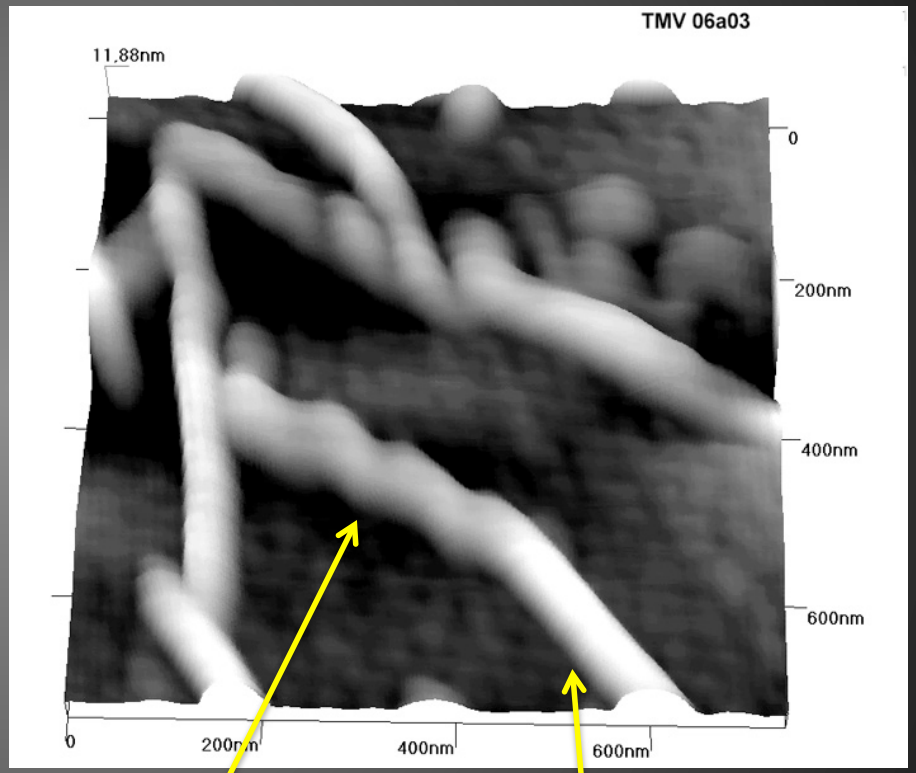




**Potato Virus X on silanized mica, fragmentation of capside proteic coat (48 nm period) due to interaction with the silanized mica. Why periodic? What is the interaction?**

# Tobacco Mosaic Virus

18 nm thick, a few hundred nm long




Stressed region

Unperturbed capside

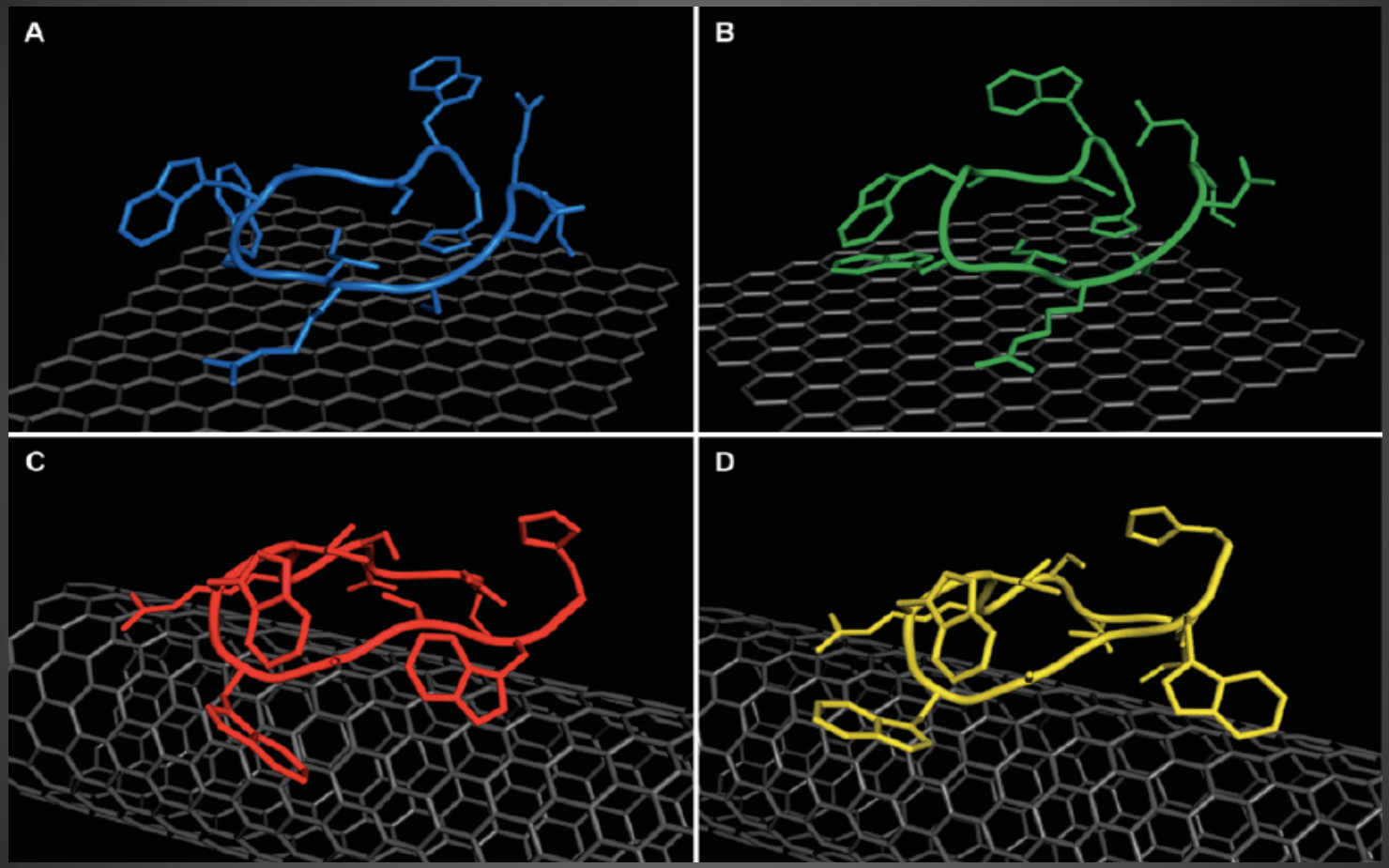
# Bacteriophage viruses can attach selectively to inorganic surfaces by peptides hosted in their terminal proteins

Surface selective peptides can thus be a clue to the safe docking of biomolecules onto semiconductors and conductors

Investigated surface selective peptides are large enough to reach a fairly stable conformation, yet small enough to allow for reasonably long computer simulations

Surface selective peptides can be used to dock biomolecules onto a suitably engineered inorganic surface or viceversa to assemble inorganic nanostructures into a complex biomolecular scaffold

# Computer simulation of surface selective adhesive peptides




Classical molecular dynamics simulation of the conformation induced adhesion of isolated dodecapetide on graphitic carbon

# A different view : DNA self-assemblage of plug-in components

- Significant example (not the only one): DNA assemblage of scaffold structures

- DNA sequence is programmable;
- Its basic units are subnanometric;
- It can bind protein molecules at specific programmed locations
- The designed structures can be produced at very low cost in billion replicas

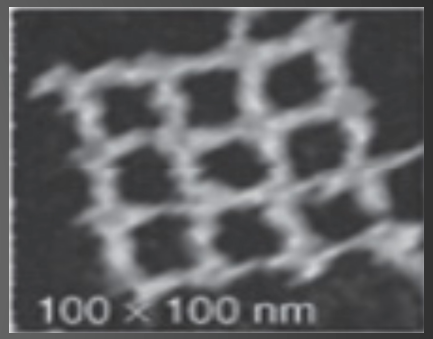
**We thus want to use DNA as the building material for spatial organization of proteic molecular components**

# The "META" project: DNA "motherboards"

**(ENEA, the NAST Center of the Tor Vergata University, Oak Ridge Natnl. Labs, Comenius Univ. Bratislava)**

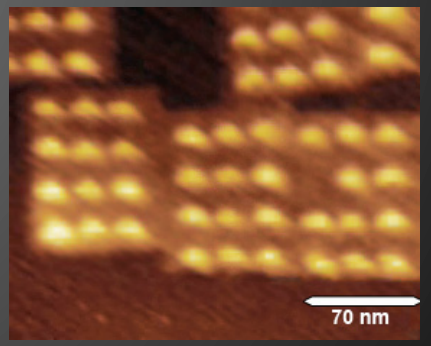
***Aim: produce a planar ordered structure to selfassemble smart molecular plug-in components in predesigned nanometric locations.***

**The simplest DNA geometries that we can use as "motherboards" are grids having 20 nm pitch, or phage genome based "origamis" of regular shape.**



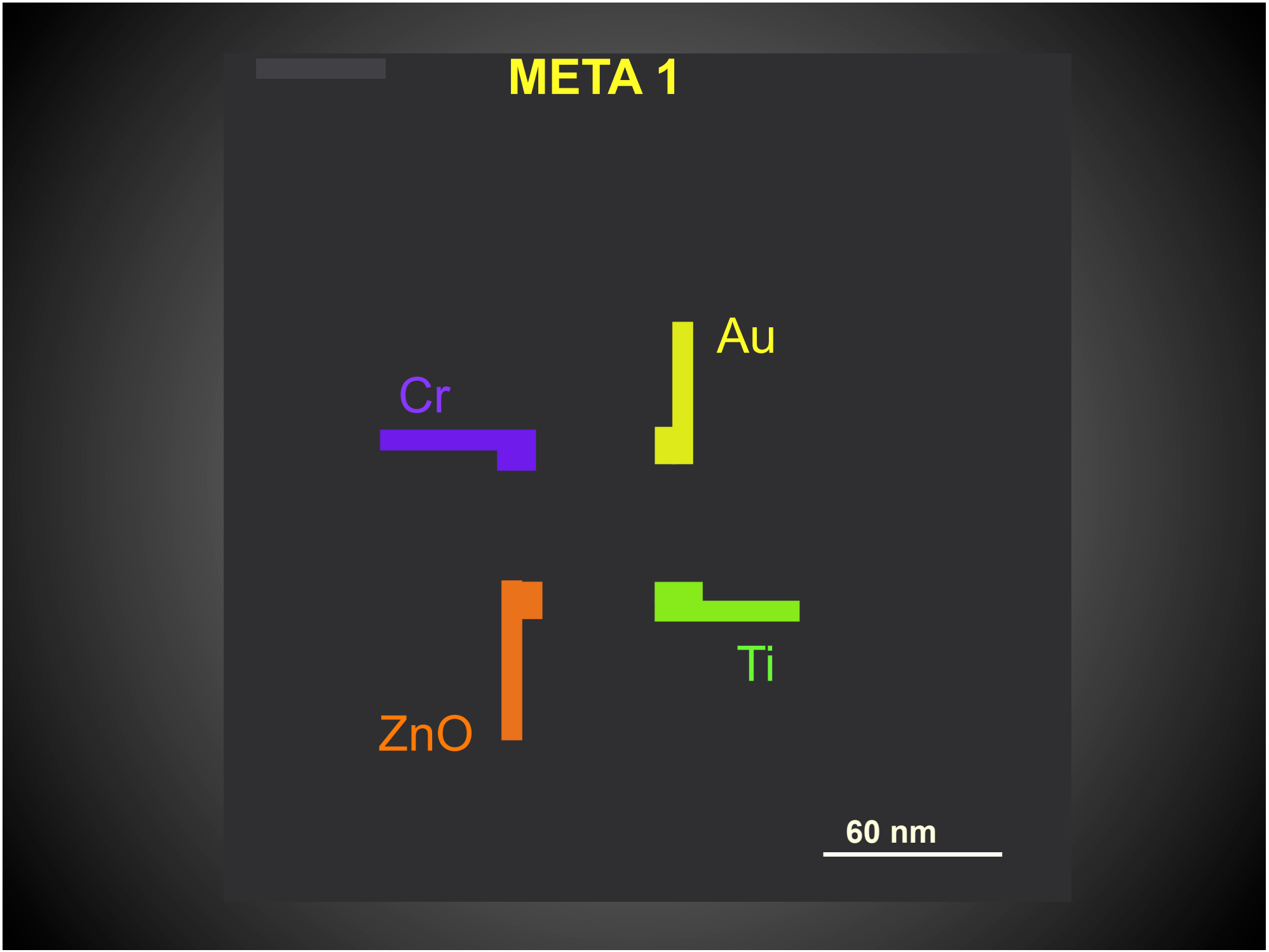

T. LaBean et al. 2006

**DNA grids and origamis can be immobilized at specified locations on the surface by advanced lithography and covalent sulphur-metal bonds or material selective adhesive peptides**

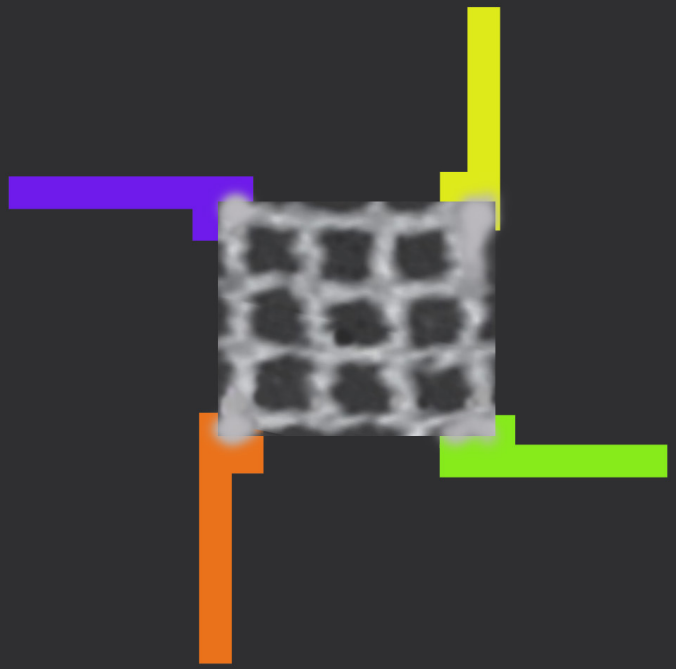



K.V. Gothelf et al. 2010

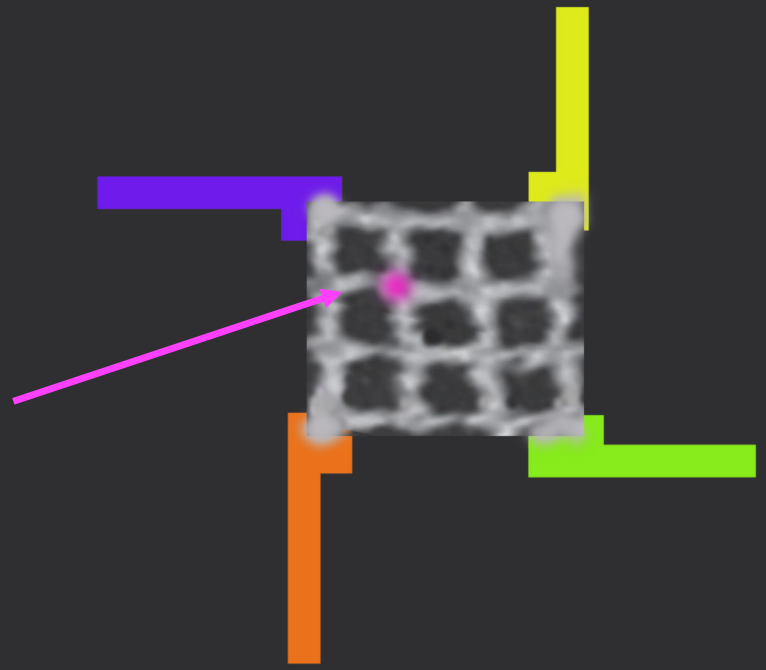
**"Plug-in" proteic components can then be selfassembled at programmed sites on these nanostructures by means of aptameric DNA "connectors"**


META 1
Au
Cr
Ti
ZnO
60 nm

# META 2



# META 3



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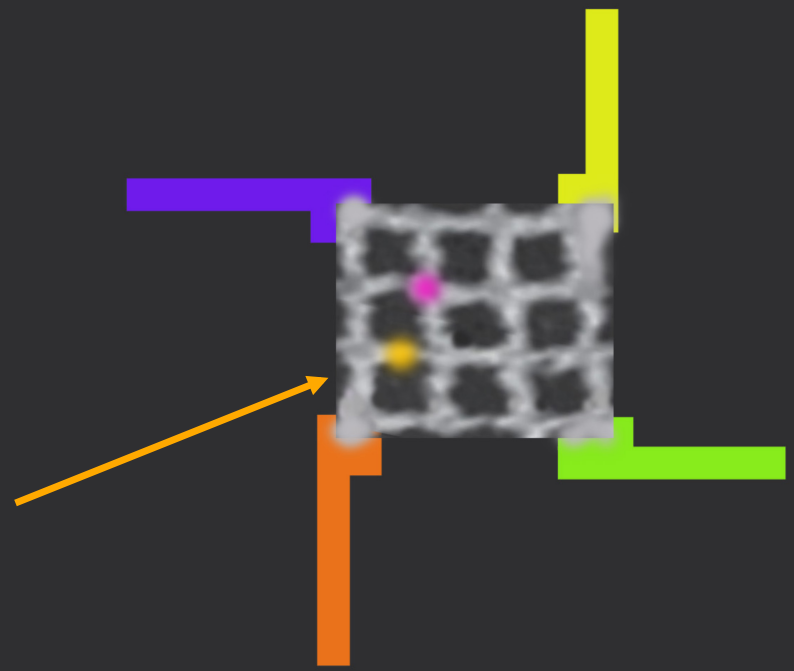
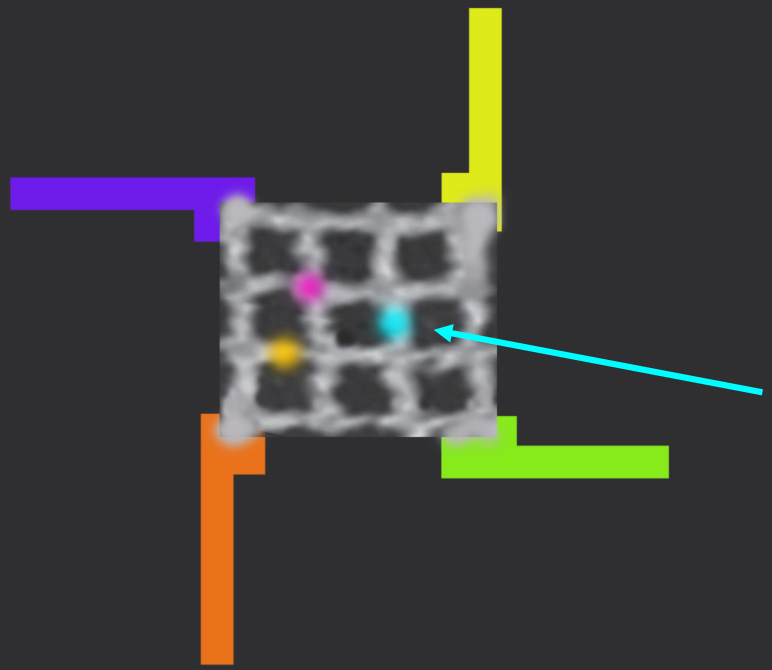
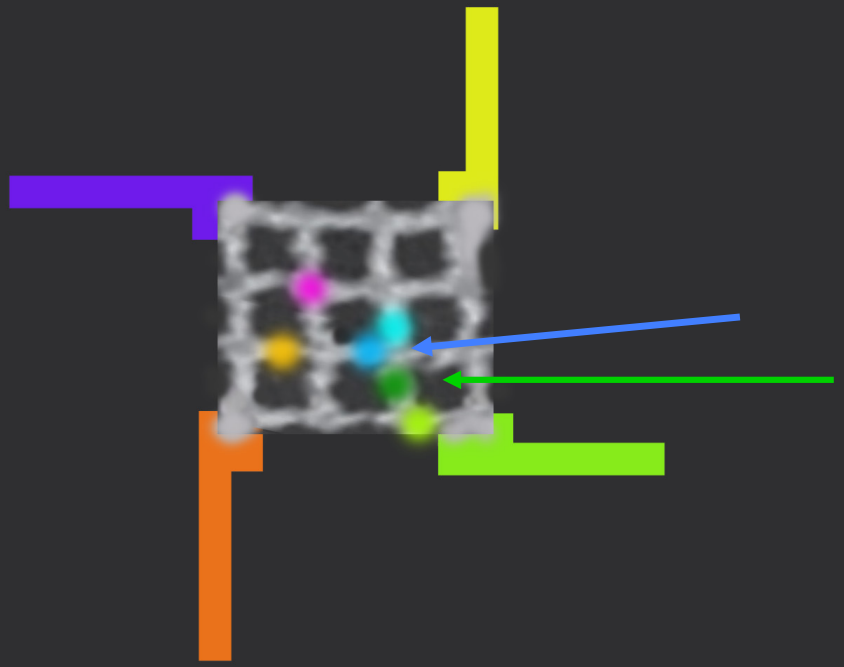


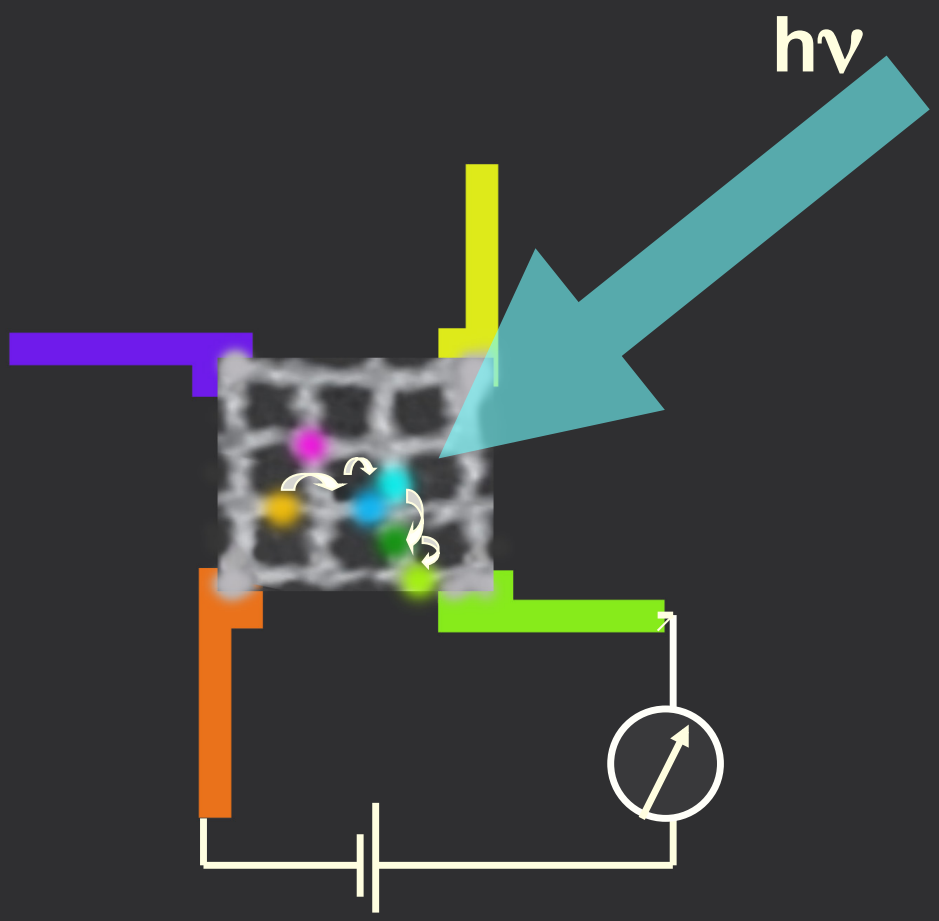
# META 5



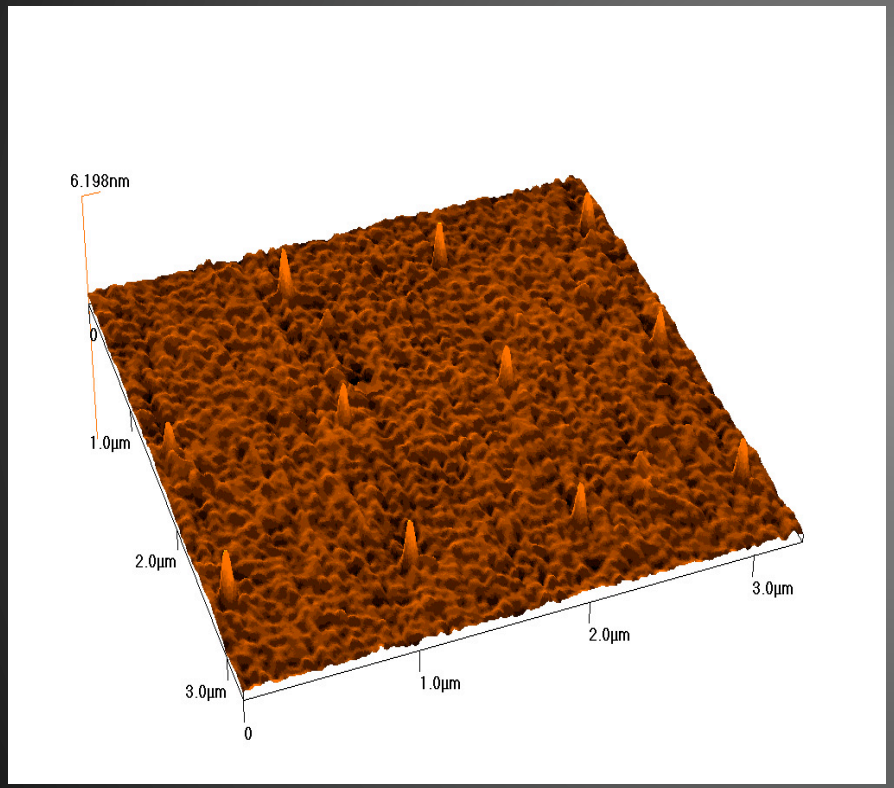
# META 6

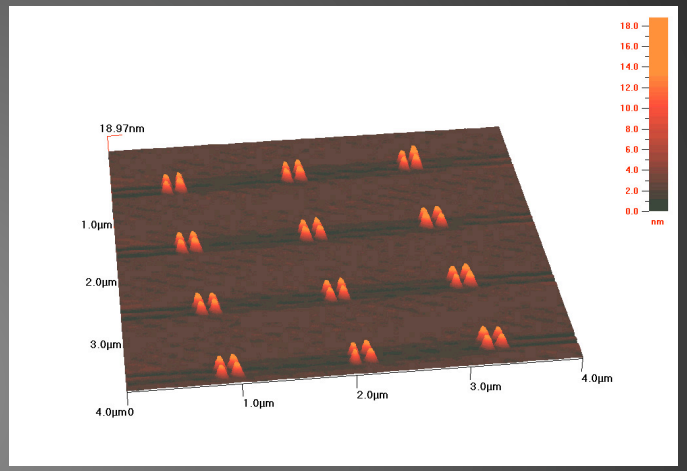


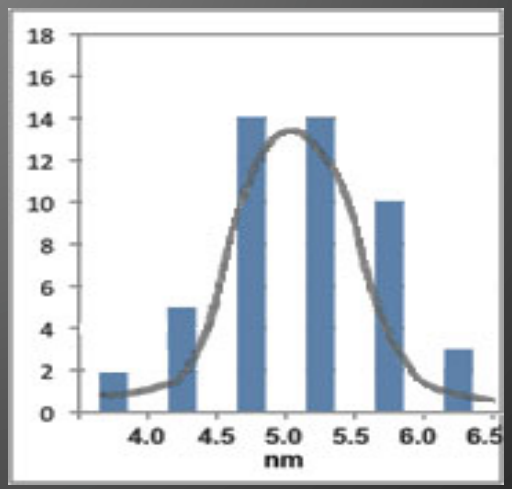
# META 6



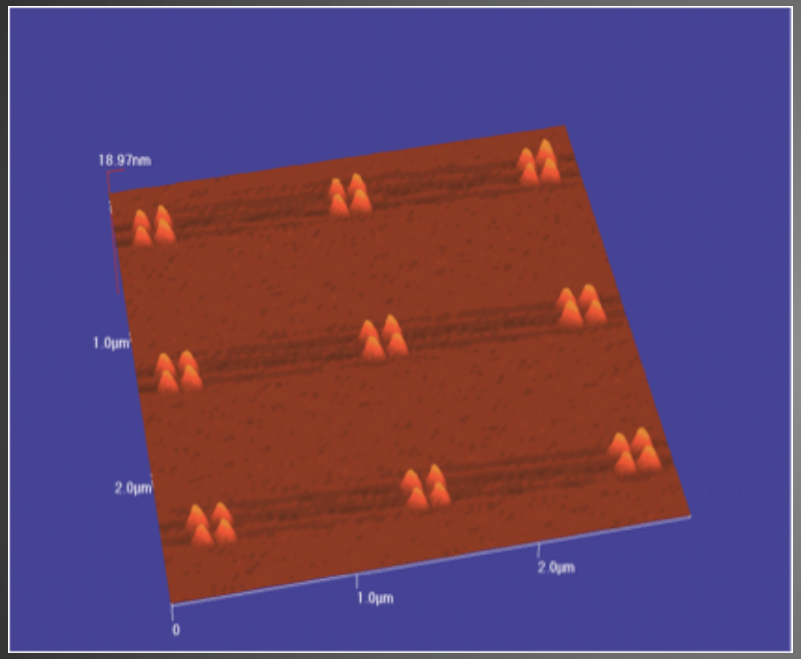
# Preliminary work: high quality docking nanopillars for DNA nanostructures organization



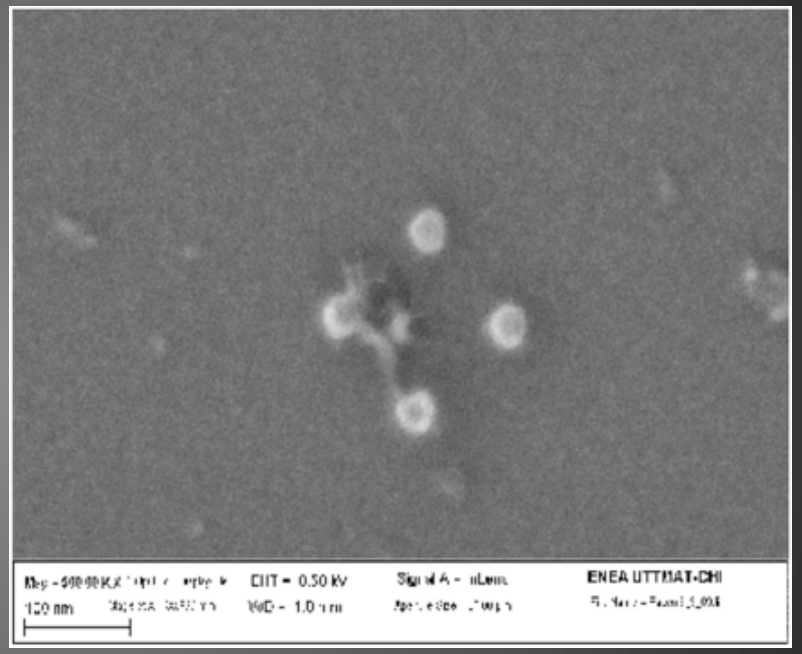



# META first results: positioning DNA filaments accross regular gold nanopads arrays

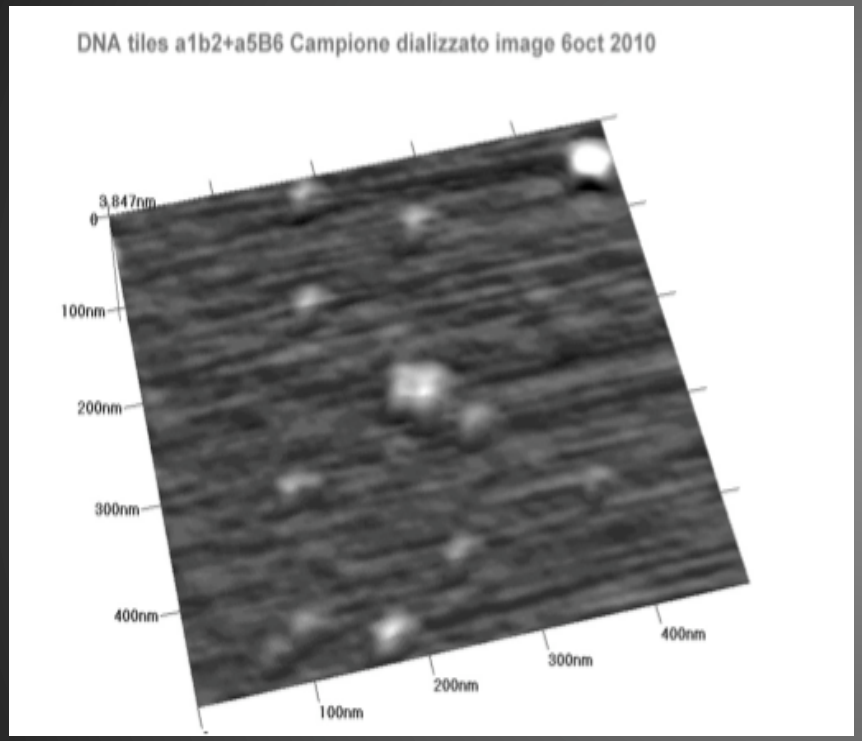



AFM topographic image of 30 nm wide 115 nm spaced gold nanopads

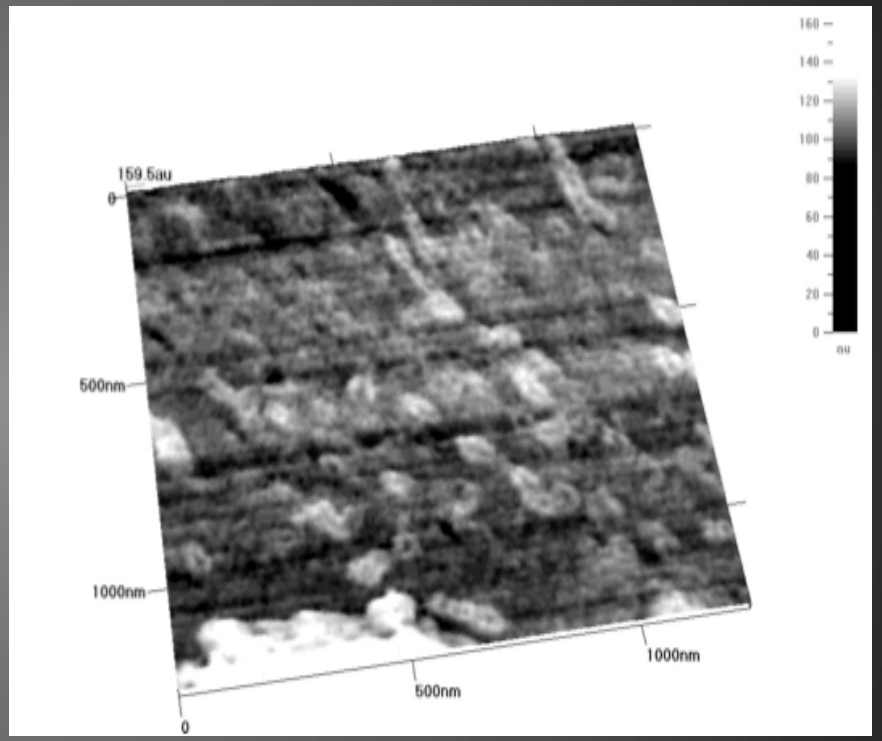



SEM image of 115 nm long, thiolated DNA double strand accross gold spots

# META first results: nanosquares and nanoladders at random on a mica surface




AFM topography of a 20 nm side DNA square on mica




AFM phase contrast image of partially and completely assembled DNA nanoladders (20x60 nm)

## Contributing to this work:

- Caterina Arcangeli, Francesco Buonocore, Massimo Celino, Serena Gagliardi, Bruno Rapone, Antonio Rinaldi, Luciano Pilloni (ENEA UTTMAT)
- Selene Baschieri, Cristina Cantale, Claudia Dalmastri, Patrizia Galeffi, Chiara Lico, Lucia Mosiello (ENEA UTAGRI and UTMED)
- Mario Caruso, Davide Flammini, Stefano Vespucci, Liqian Wang, Katia Spinella (Centro NAST, Università di Tor Vergata)
- Annamaria Gerardino, Roberto Pilloton (CNR - IFN)
- Scott Retterer, Mike Simonson, (CNMS, Oak Ridge)
- Tibor Hianik (Comenius University of Bratislava)
- Kurt V.Gothelf and Abhichart Krissanaprasit (Univ. of Aarhus)
- Fabrizio Cleri (Univ. of  Lille)
- Giulio Gianese, Vittorio Rosato (Ylichron srl)
- Manuela Adami, Antonio Sardi, Marco Sartore (Elbatech srl)

**Thank you for your patience**