Nanofabrication for optical biosensors

M. Liley

Swiss Centre for Electronics and Microtechnology, CSEM SA

Biosensors

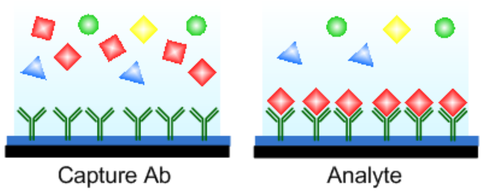
- "… a device for the detection of an analyte that combines a biological component with a physicochemical detector component."

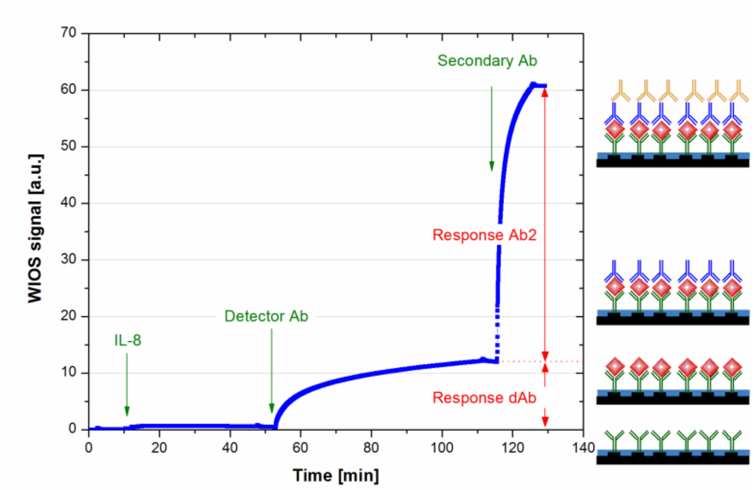
  (*Compendium of Chemical Terminology*)

- Self-contained integrated device, three parts:
  - Sensitive biological element
  - Physicochemical transducer (detection element)
  - Associated electronics or signal processors

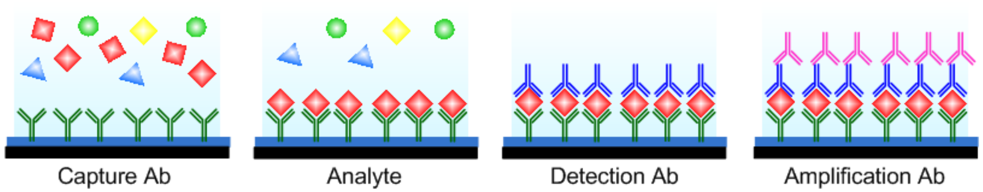
Solid-phase immunoassays

- Direct detection (MW > 10 kD)

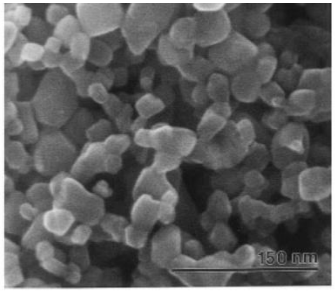




- Sandwich immunoassay (MW 1-10 kD)

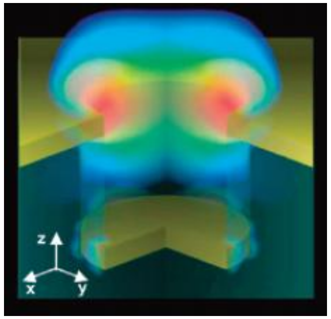


Why nano for biosensors?

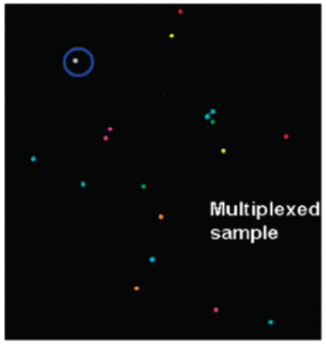


- ~~Enhanced selectivity~~

- Enhanced sensitivity
  - High surface area (catalytic properties)
  - Electromagnetic field enhancement (optical properties)
  - Efficient electron transfer (electrochemical properties)



- Multiplexing
  - Very high density arrays

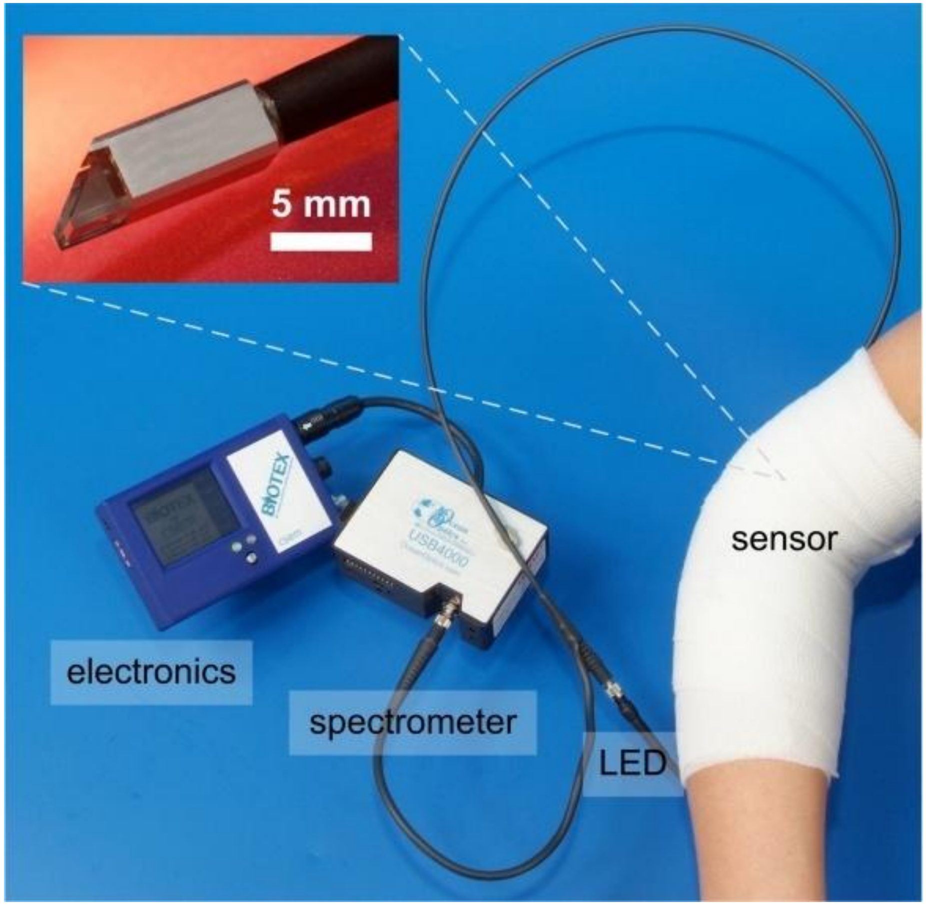


Optical biosensors:

- Direct, label-free detection – usually based on refractive index
  - Waveguide, surface plasmon resonance, ellipsometric sensors
  - $n_{protein}$ ~ 1.45; n_{water} ~ 1.33
  - Detection limits ~ pg on classical waveguides and similar
  - Continuous measurements

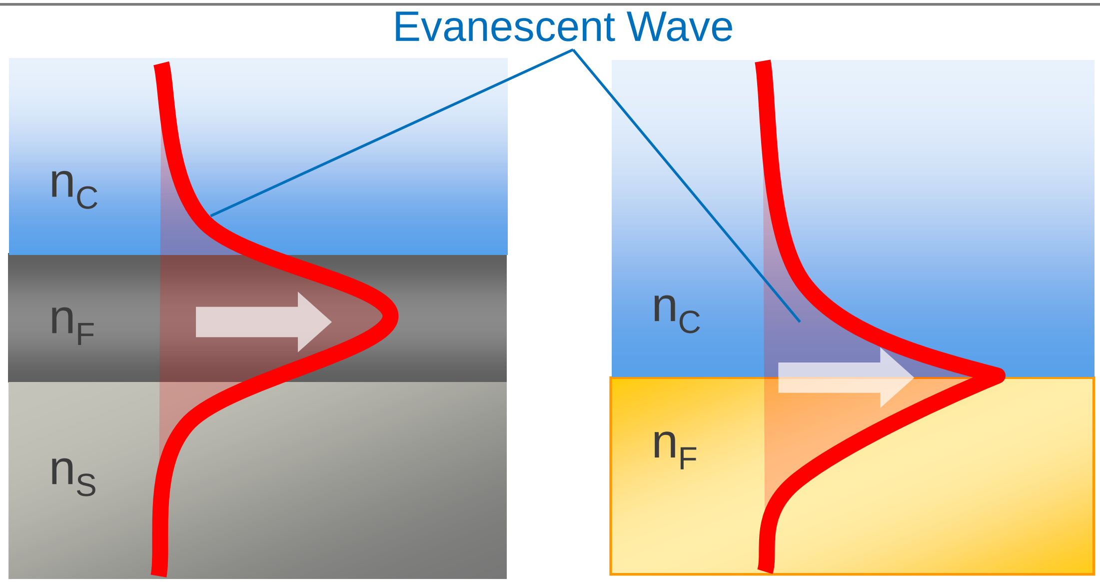
- Fluorescence detection
  - Need to label at least one species and add to sample
  - Extreme sensitivity and specificity
  - Fluorescence intensity – difficulties due to bleaching and background fluorescence

A biosensor for wound dressings



S. Pasche et al
*Adv. Science & Tech.**57** (2008) 80*

Evanescent wave sensing



Dielectric Slab Waveguide

Concentration of wave energy in core layer. Exponential decay in cover/substrate layers. TE, TM, mono- / multi-mode

Surface Plasmon Interface

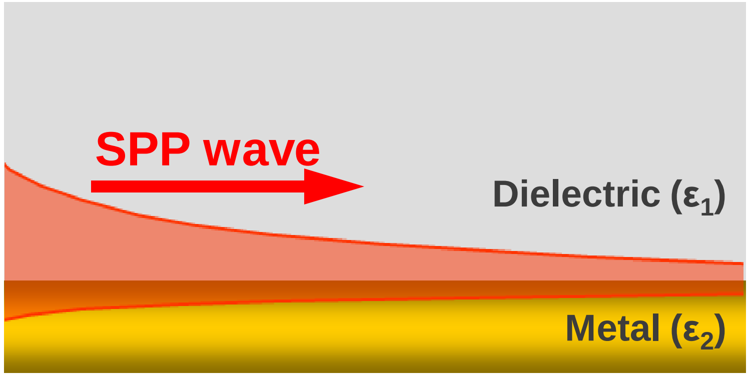
Special electromagnetic mode propagating at a metal/dielectric interface with exponential decays. Single propagating TM mode

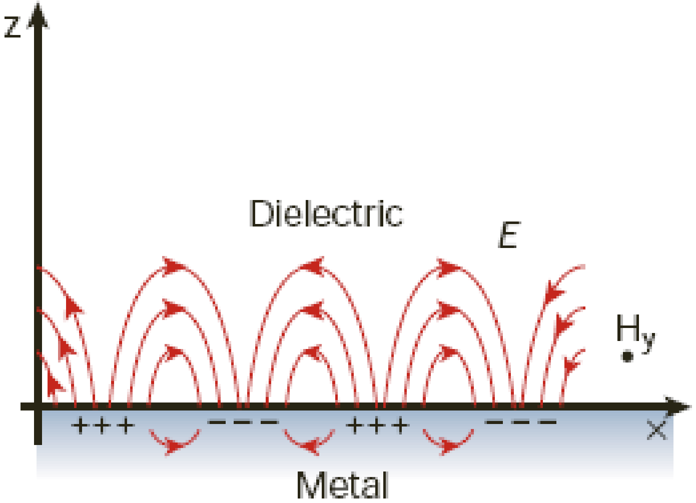
Surface Plasmon Polariton (SPP) waves

- SPP wave:
  - EM wave confined at metal/dielectric interface
  - EM wave and charge oscillations interaction

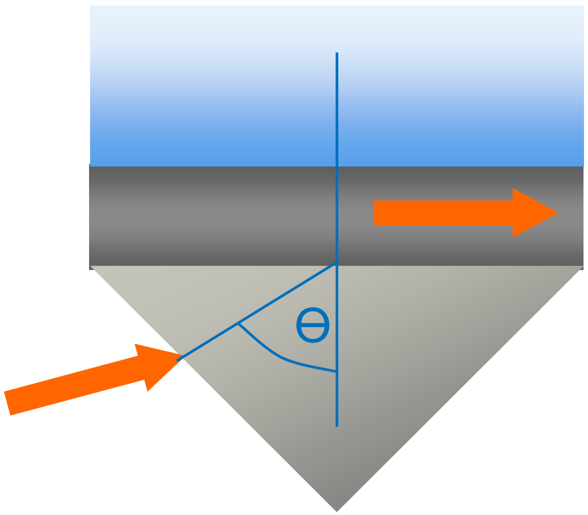
  Dispersion relation from Maxwell's equations

$$k_{SPP} = \frac{\omega}{c}\sqrt{\frac{\varepsilon_1 \varepsilon_2}{\varepsilon_1 + \varepsilon_2}}$$



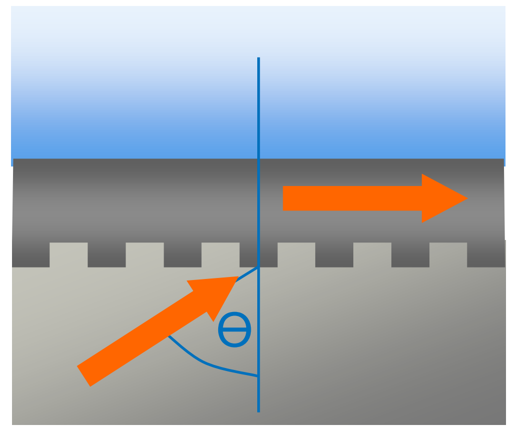


Coupling into confined modes



Prism coupling

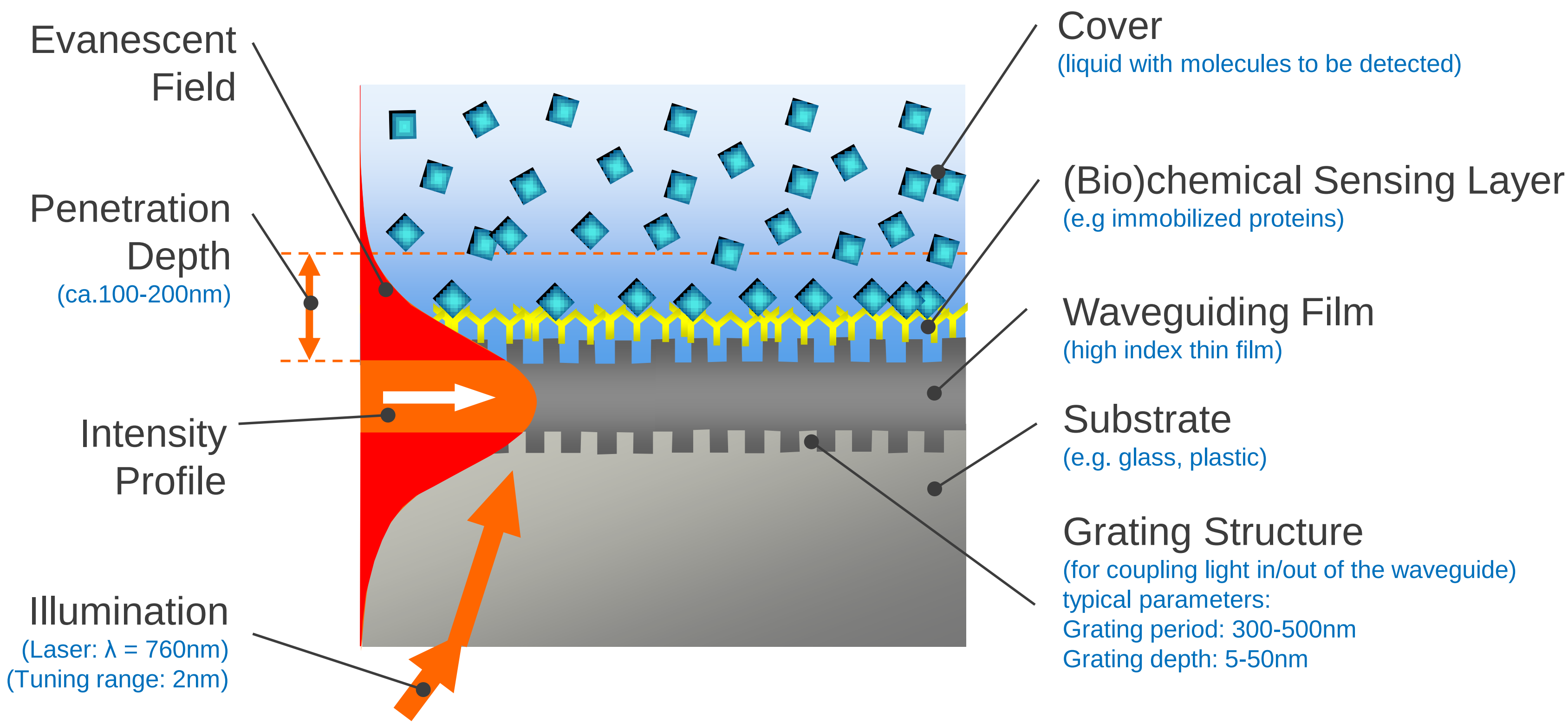
Applied for:
- Waveguide
- Surface plasmon resonance



Grating coupling

Applied for:
- Waveguide
- Surface plasmon resonance

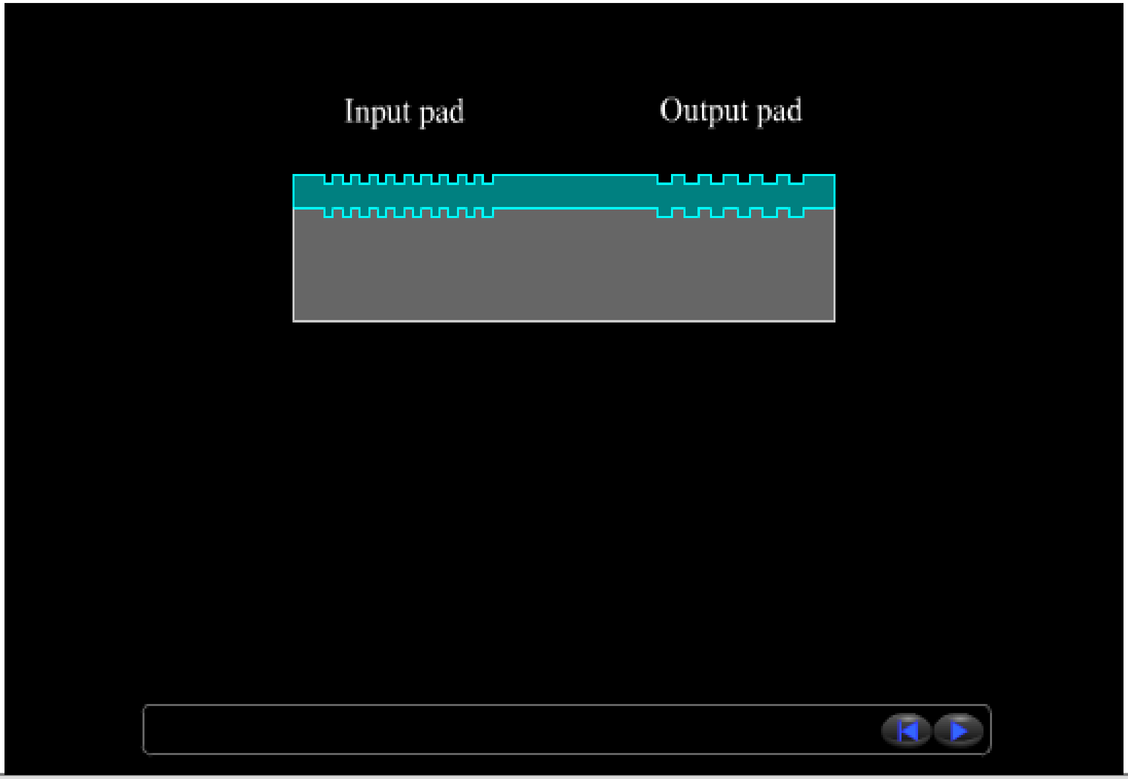
Evanescent sensors



Evanescent field sensors are highly sensitive to any changes close to the sensor surface (e.g. such as binding of molecules to an immobilized sensing layer)

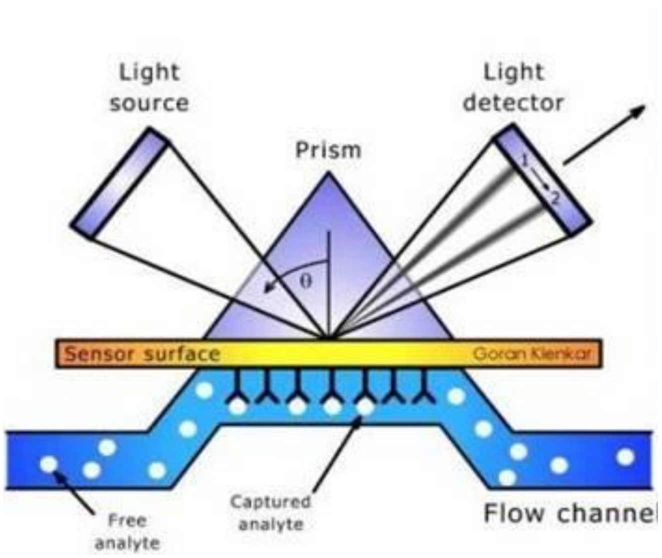
Evanescent sensing by wavelength tuning:

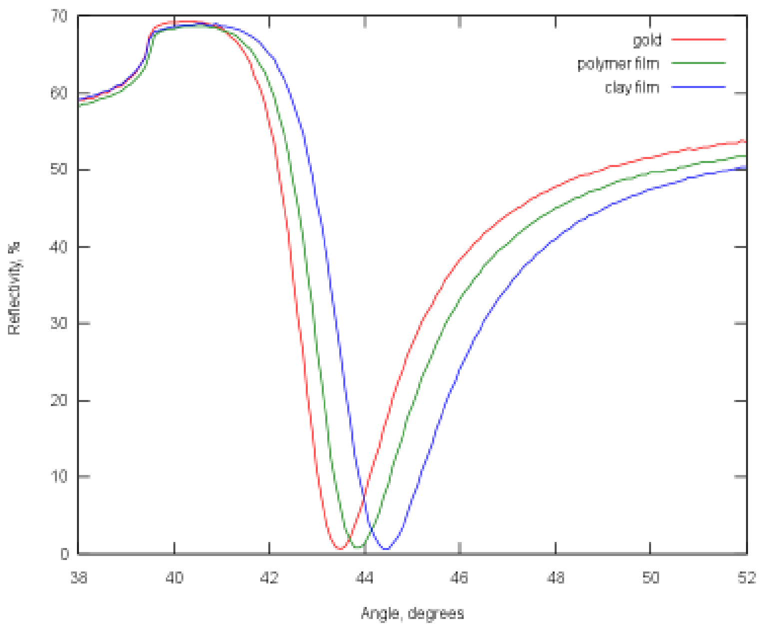
- Tunable laser light (laser diode, $\Delta\lambda \sim 2$ nm)
- Sensitivity: $\Delta n < 10^{-5}$ (< 1 ng/cm^2)



Refractive index sensors: surface plasmon polaritons

- Operating principle of SPR sensors is similar to waveguides
- Sensitivity is similar too
- Commercially available (e.g. www.biacore.com)

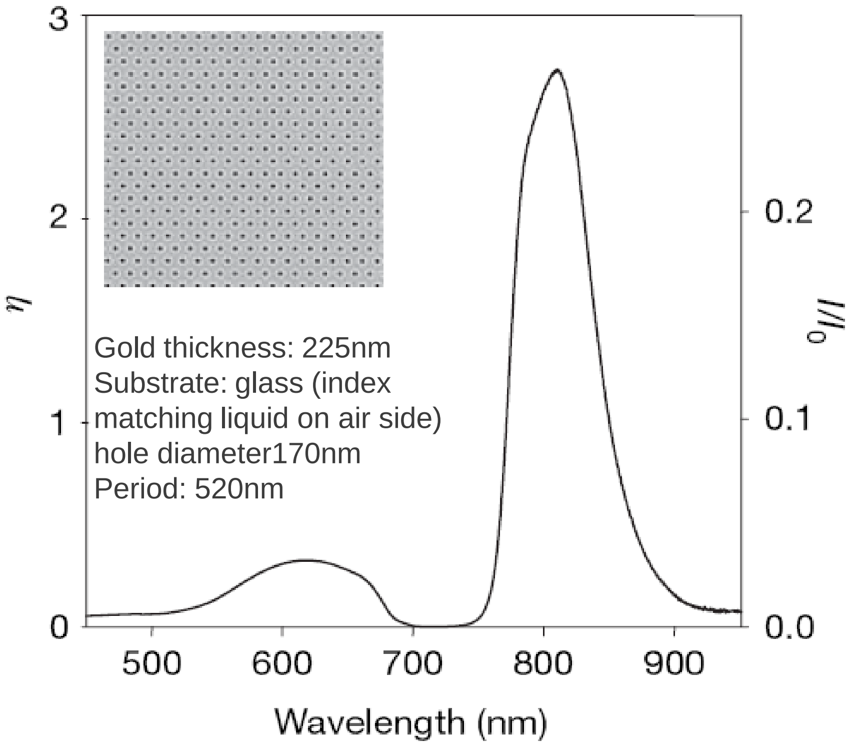




Extraordinary optical transmission

- Gold film with a periodic array of holes
- Measure the amount of light passing through the gold film:

$$\eta = \frac{P}{P_0} * f$$ where f is the filling fraction of the holes



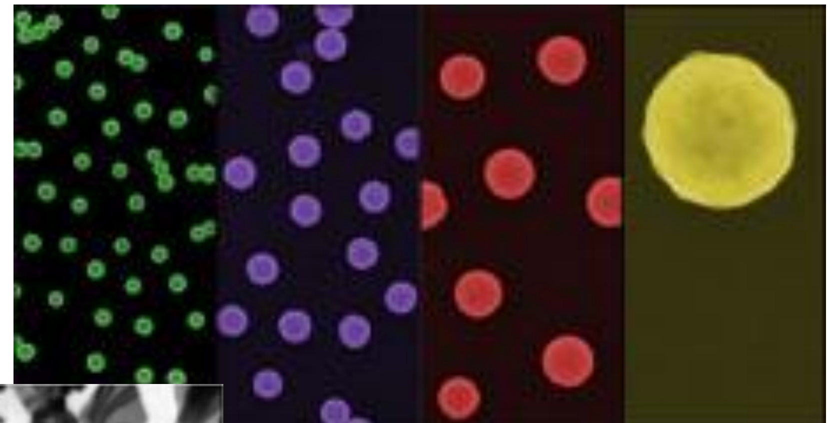
$\eta > 1$ means that the photon flux transmitted by each hole is higher than the incident one!

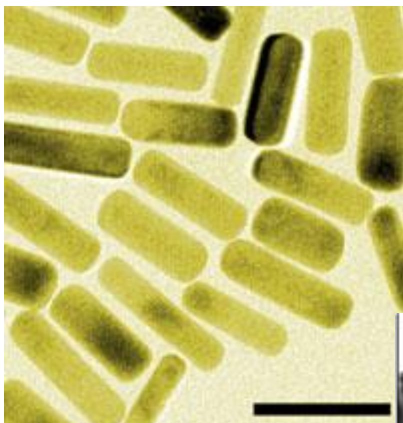
Extraordinary Optical Transmission (EOT)

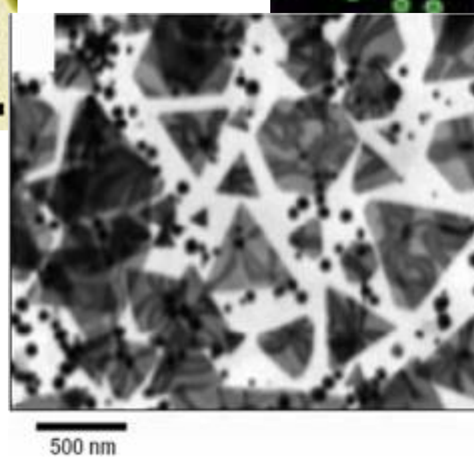
*T. W. Ebbesen et al., Nature **391**, 667–669 (1998)*
*C. Genet et al., "Light in tiny holes", Nature **445**, 39-46 (2007)*

Metal nanoparticles:

- New synthetic methods allow the production of quantities of metal particles of all shapes and sizes







Local surface plasmon polaritons:

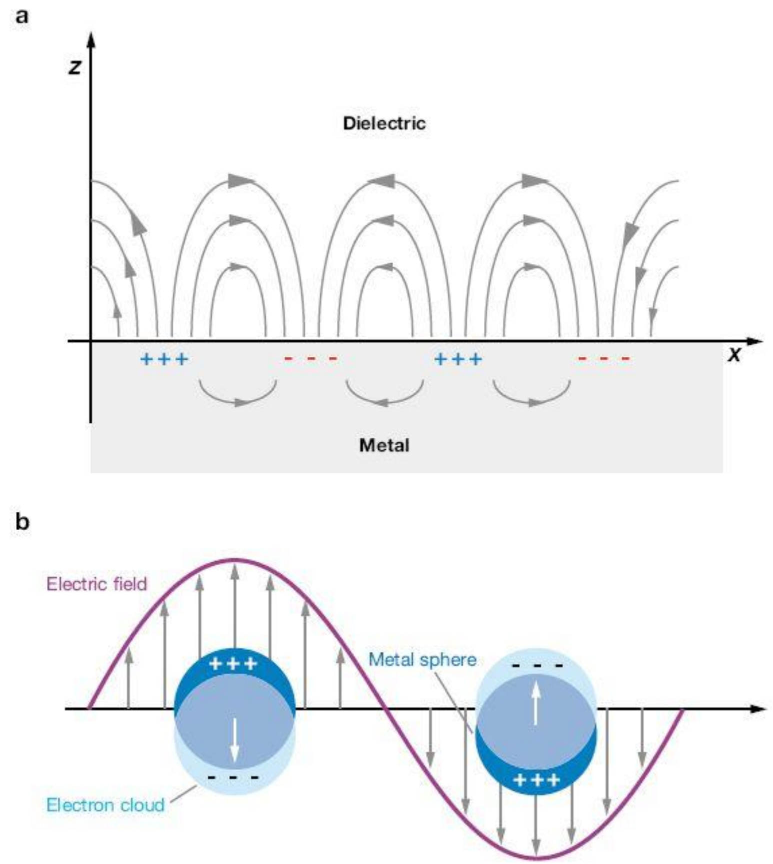
- Surface plasmons exist on metal nanostructures
- Resonance is determined using extinction spectrum (absorption + scattering)

Real and imaginary parts of metal dielectric constant

$$E(\lambda) \propto \frac{a^3 \varepsilon_{out}^{3/2}}{\lambda} \left[\frac{\varepsilon_i(\lambda)}{(\varepsilon_r(\lambda) + \chi\varepsilon_{out})^2 + \varepsilon_i(\lambda)^2} \right]$$

geometric factor ($2 < \chi < 20$)

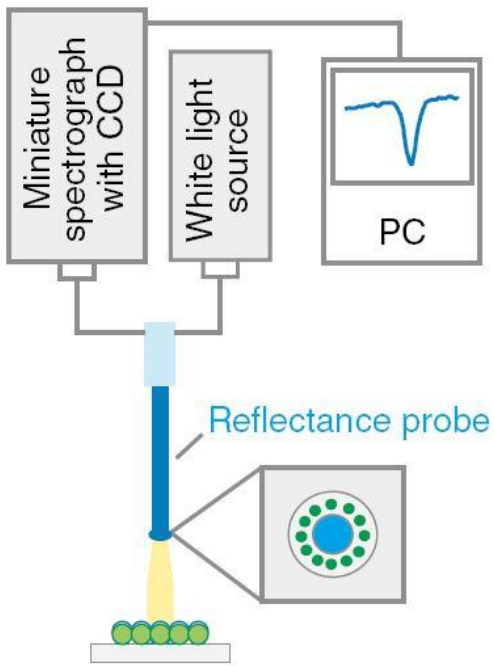
dielectric constant of external medium

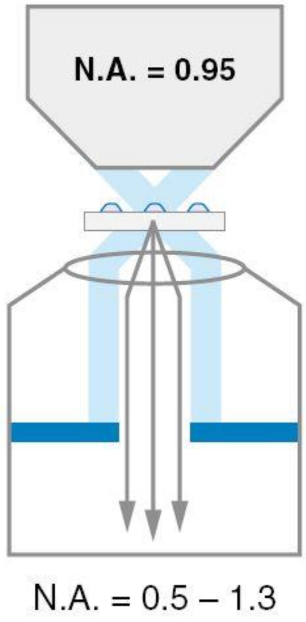


K.A. Willets & R.P. Van Duyne, Annu. Rev. Phys. Chem. 2007. ***58****:267*

Measuring extinction/scattering spectra:

- Spectra can be acquired in transmission or reflection
- Single particles can be interrogated using dark field microscopy

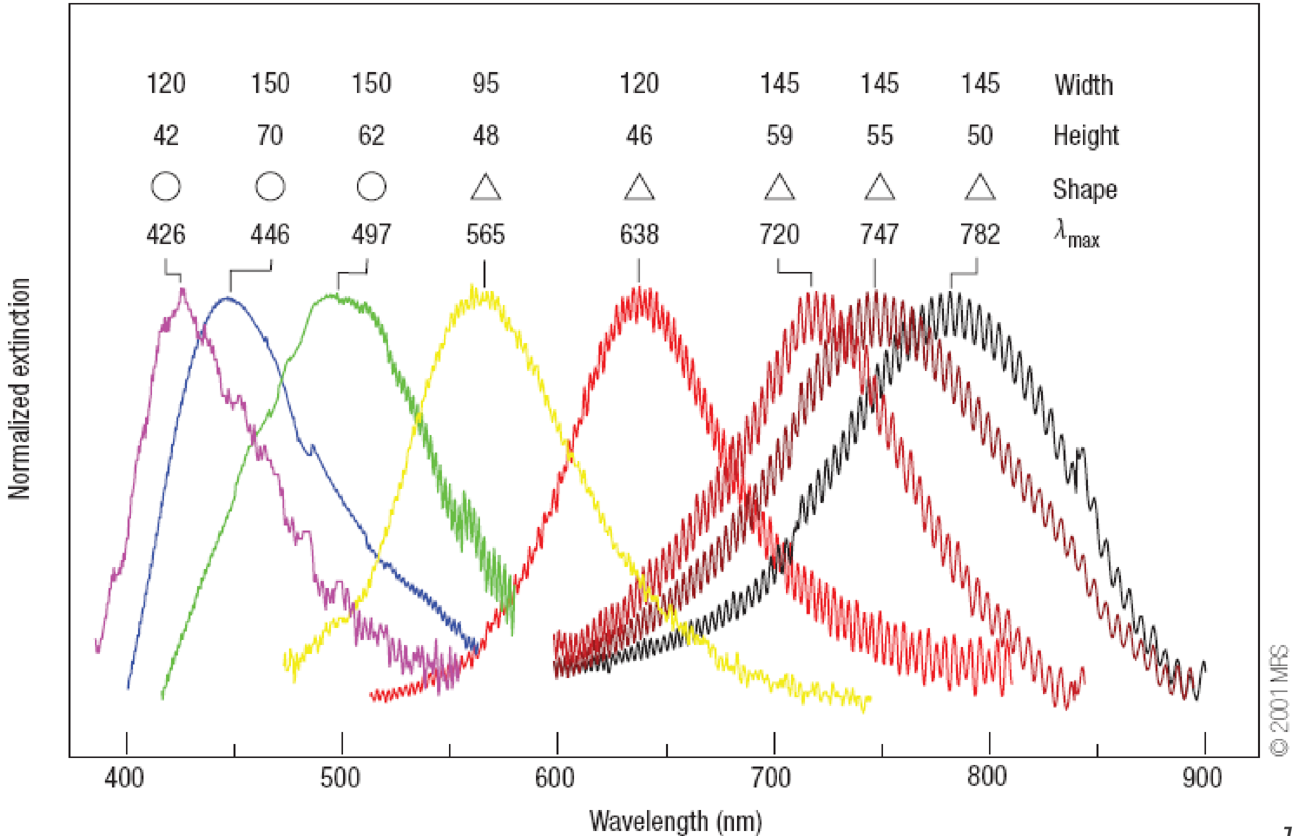




K.A. Willets & R.P. Van Duyne,
Annu. Rev. Phys. Chem. 2007. ***58****:267*

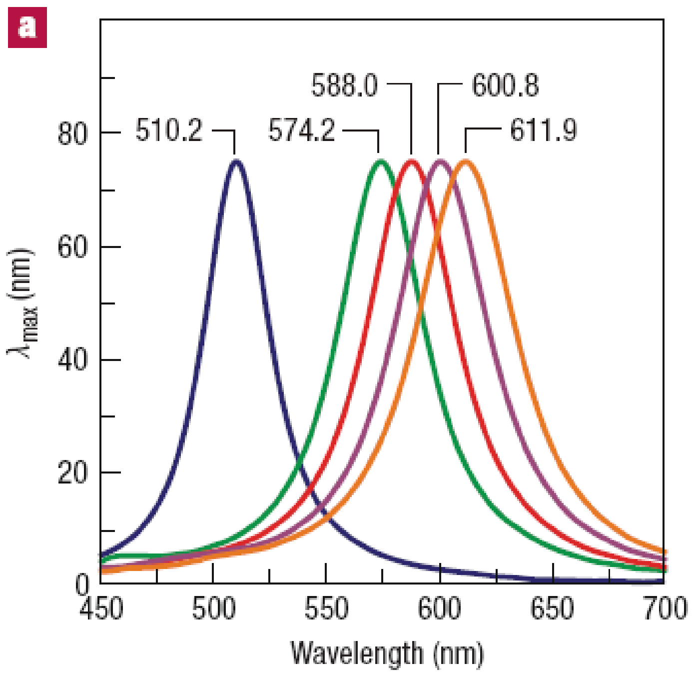
Optical properties depend on size, shape and material

- Extinction spectra from silver structures on mica

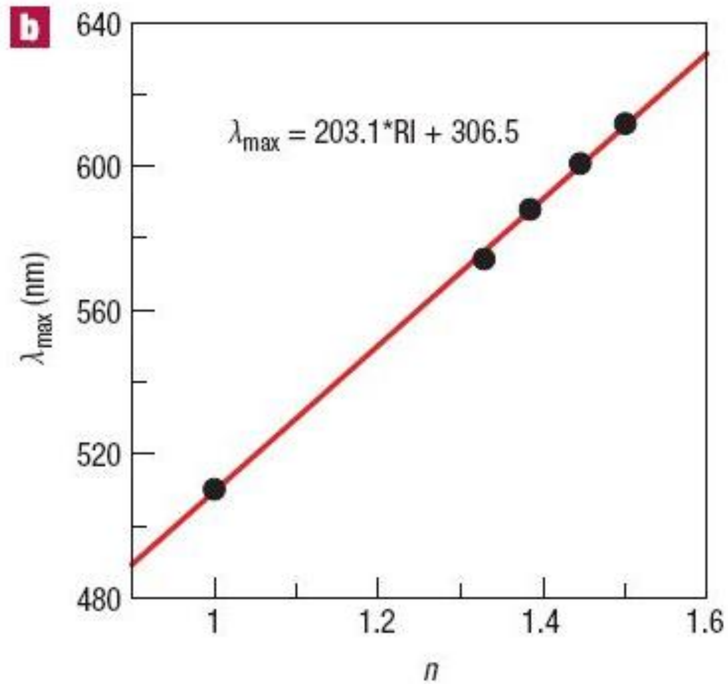


J.N. Anker et al
Nature Materials 7 (2008) 442

Single nanoprisms can be used for refractometry



Scattering spectra from a single silver nanoprism in different environments: nitrogen, methanol, propan-1-ol, chloroform and benzene



Extinction peak wavelength vs solvent refractive index

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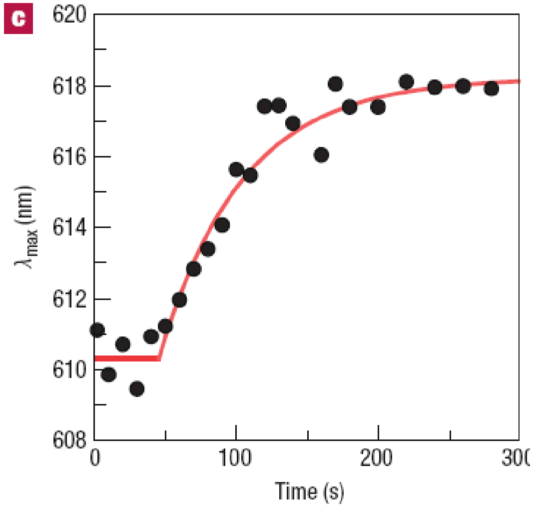
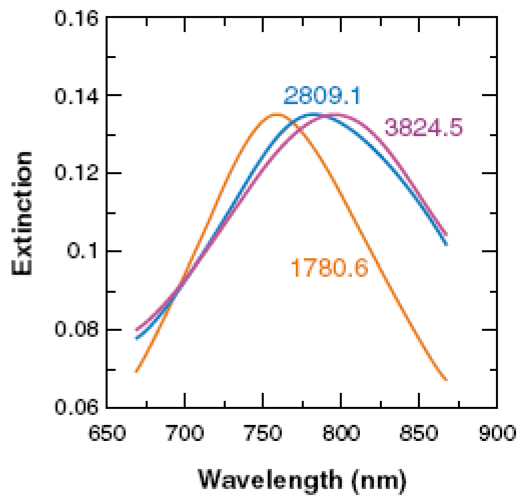
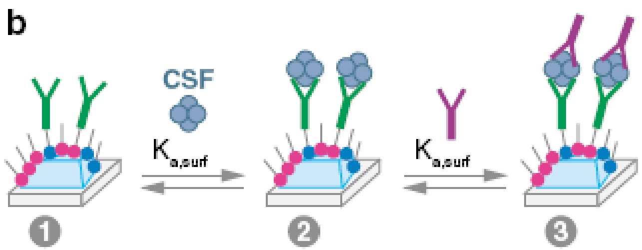
Silver prisms can be used for biosensing:

- Adsorption of molecules to the prisms is observed as a refractive index change
- Biosensing has been demonstrated

Adsorption of octanethiol onto single nanoparticle







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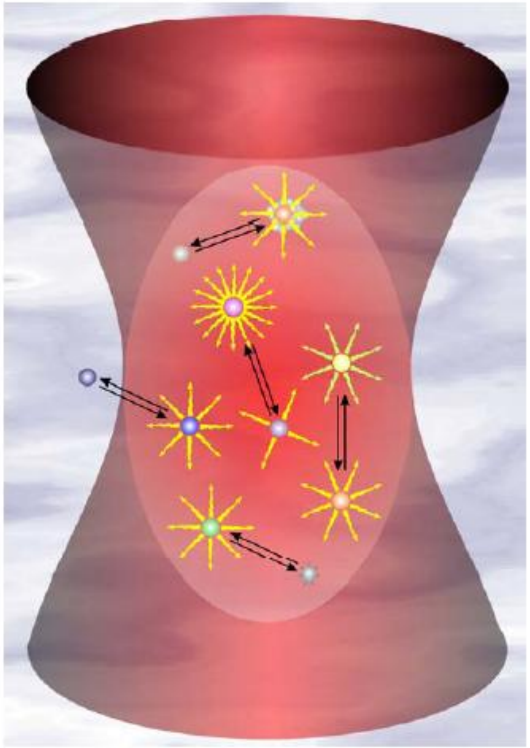
Fluorescence measurement of single molecules

- Fluorescence correlation spectroscopy (FCS)
- Use a small sample volume (fL)
- Measure fluorescence intensity variations caused by single molecules diffusing across the volume
- Concentrations in pM-nM range



P. Schwille and E. Haustein, Biophysics Textbook Online

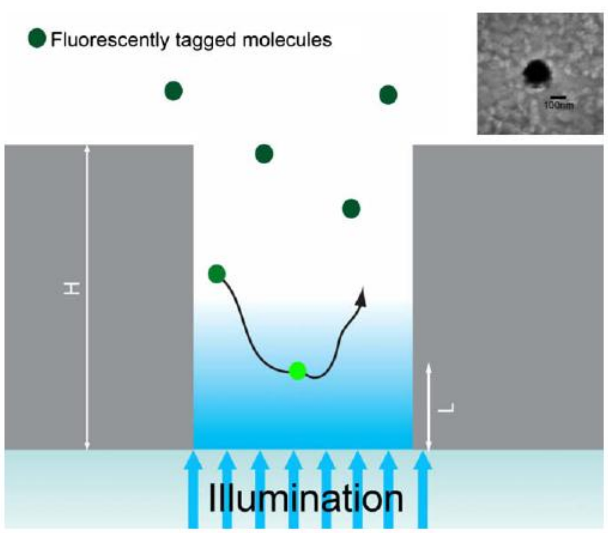
Fluorescence measurement of single molecules

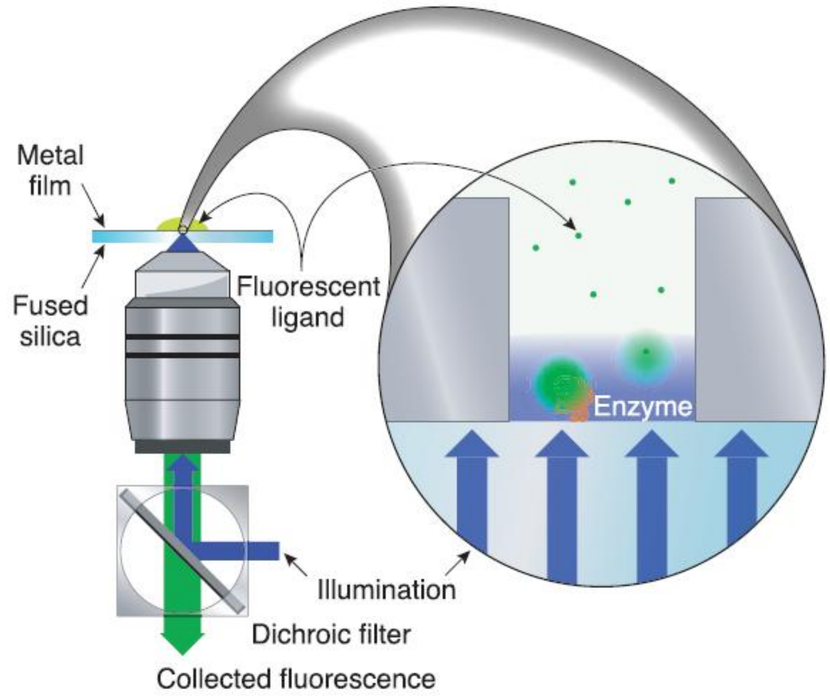
- Number (concentration) of fluorescent species can be determined
- Diffusion constant gives us dimension of molecule
  - $R_H \alpha 1/D$
- Binding of small fluorescent ligands to large receptors can be measured (but only if increase in mass > 5)
- BUT max concentration of ligand 10^{-9} – binding constant must be higher!

- Enzymatic reactions can also be studied – but same problem with concentrations.

Zero order waveguides:

- Define FCS volume using sub-wavelength holes in metal film
- Evanescent field within hole
- Excitation volumes: $10^{-18} – 10^{-21}$
- Easier alignment

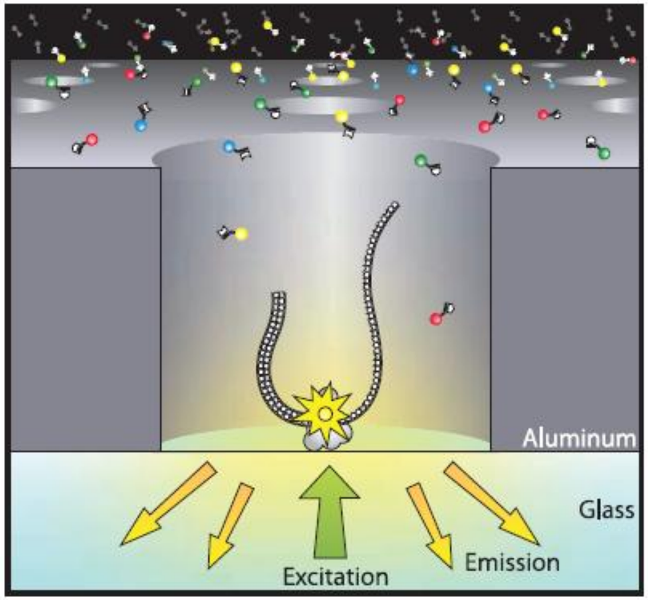


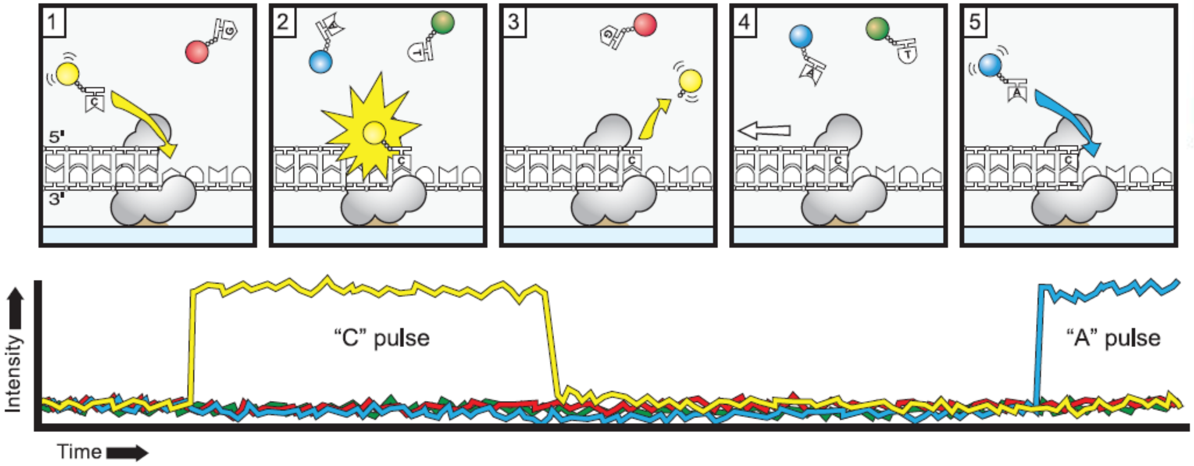


M. J. Levene, *et al*
*Science **299,** 682 (2003);*

Zero order waveguides: DNA sequencing

- DNA polymerase in wells
- Fluorescently labelled nucleotides
- Base incorporation gives fluorescent pulse

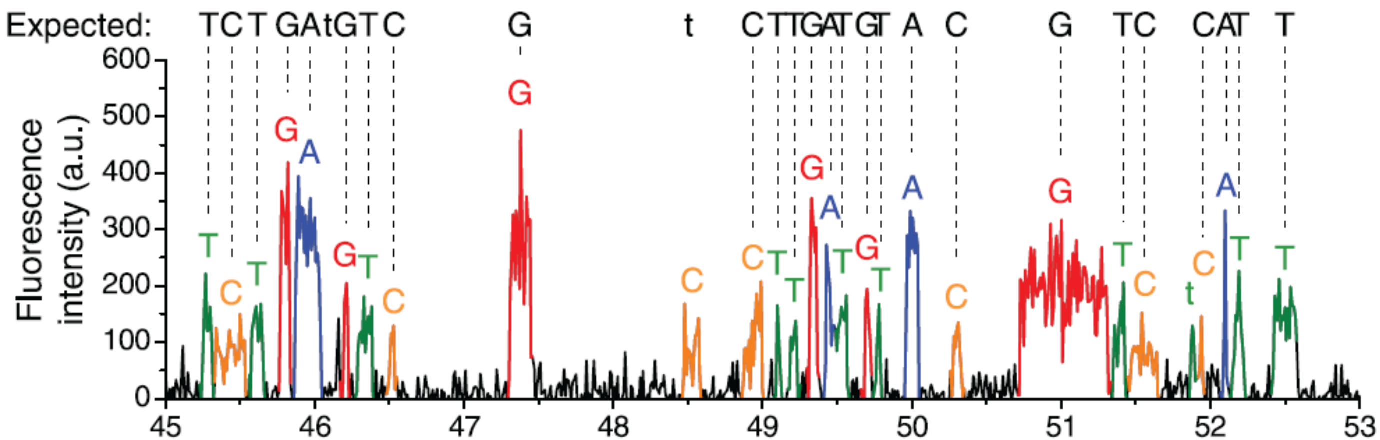




John Eid, *et al.*
Science ***323,*** *133 (2009);*

Zero order waveguides: DNA sequencing

- Errors in sequencing corrected by repeat sequences
- Arrays of 3000 wells increase data acquisition speed



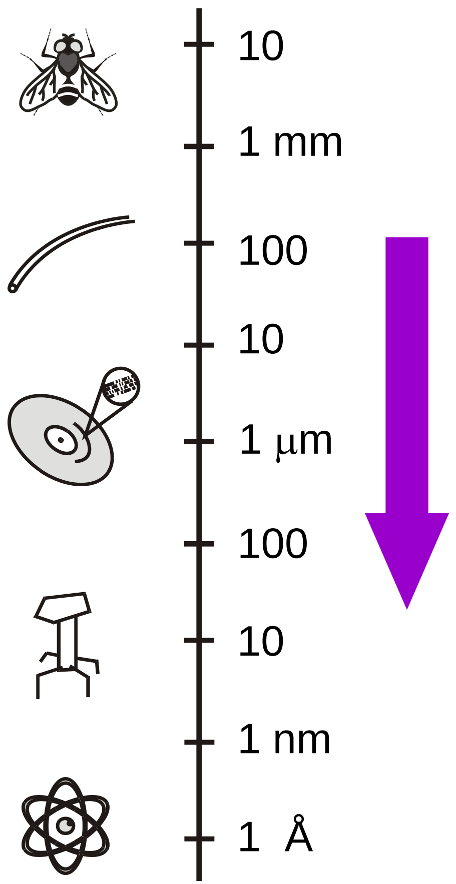
John Eid, *et al.*
Science ***323,*** *133 (2009);*

Plasmonic structures for nanobiosensing

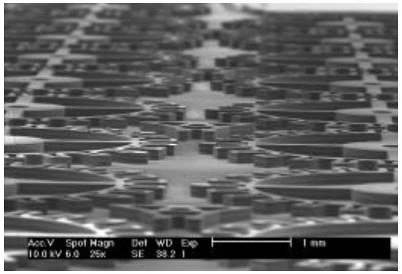
- Fabrication of plasmonic nanostructures often based on focussed ion beam milling or e-beam lithography
- Versatile, serial method.
- Top-down methods: the best structures but expensive and slow

- Methods for mass production are necessary
- Bottom-up methods are less precise but parallel and cheap

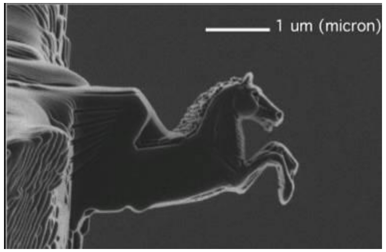
Nanotechnology: top-down vs. bottom-up

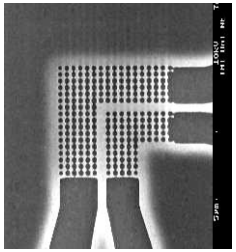


- Classical (micro-) fabrication of MEMS (Micro Electro Mechanical Systems)

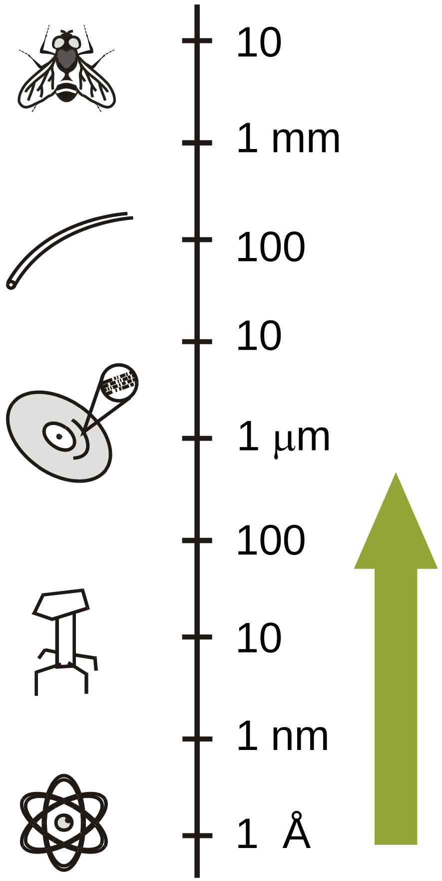


- Lithography:
  - VIS
  - UV, X-ray, e – beam
  - FIB (serial)



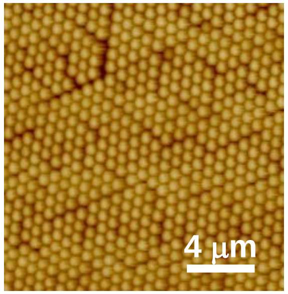


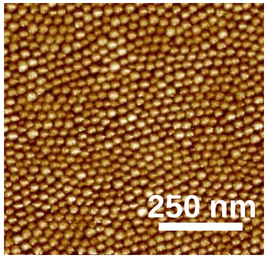
Nanotechnology: top-down vs. bottom-up



- NEMS
- “molecular nanotechnology”

  (self-assembly, supra-molecular chemistry)





Using bottom-up, self-organisation, methods

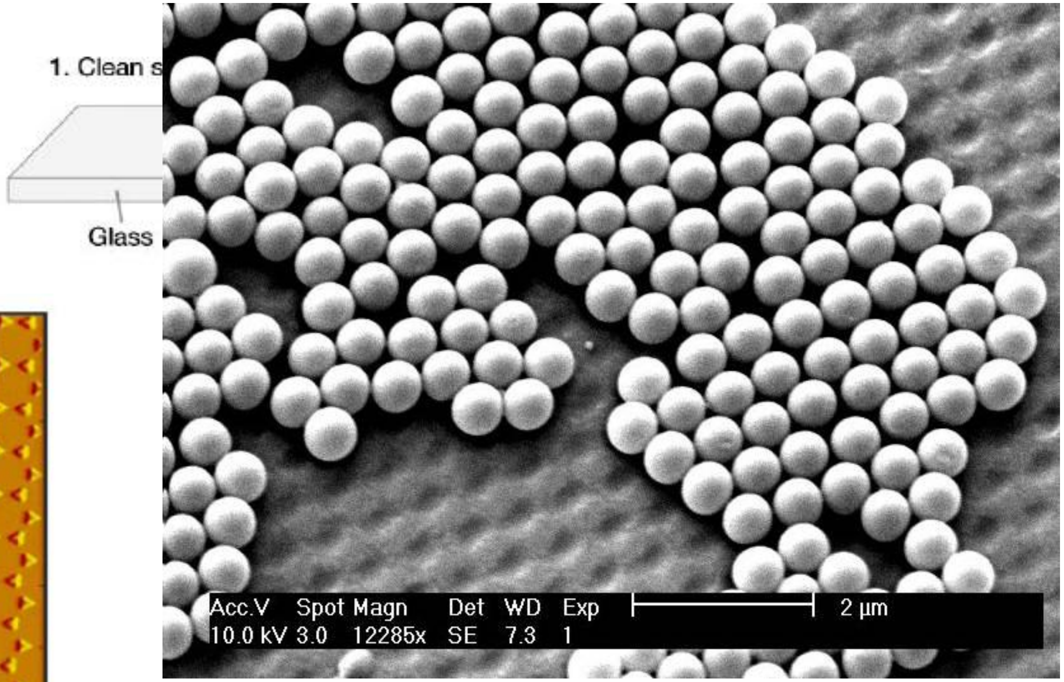
- We can cover large areas with bottom up methods
- We can get good (but not perfect) order

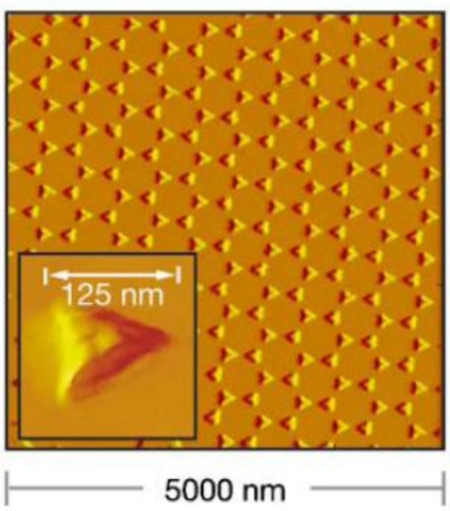
- We can not have precise positioning of features
- We can not have non-uniform surfaces

- We must target applications where these aspects are not critical

Fabrication of plasmonic nanostructures:

- Arrays of sub-micron spheres act as a mask
- Ag evaporation and lift-off

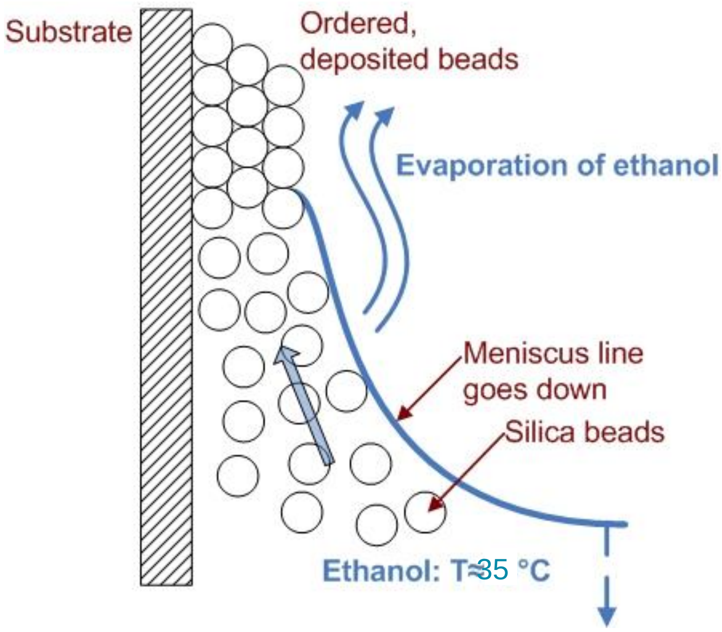


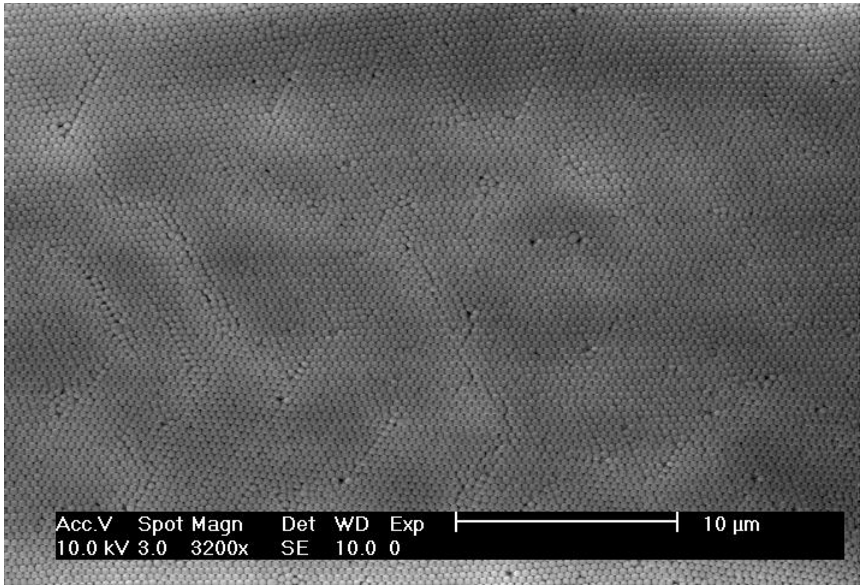


K.A. Willets & R.P. Van Duyne,
Annu. Rev. Phys. Chem. 2007. ***58****:267*

Colloidal self-assembly: vertical deposition

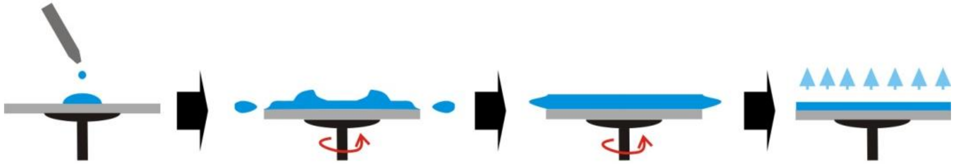
- For small particles, surface tension is the biggest force
- D ~ 1micron, surface tension ~ 10^{-7} N, weight ~ 10^{-11} N
- High order requires monodisperse spheres

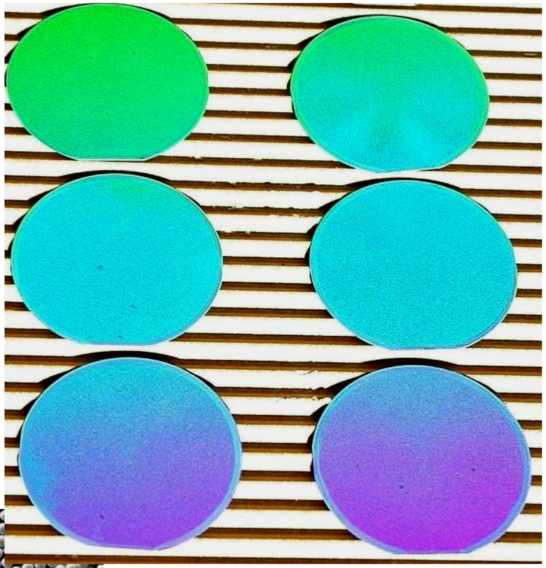


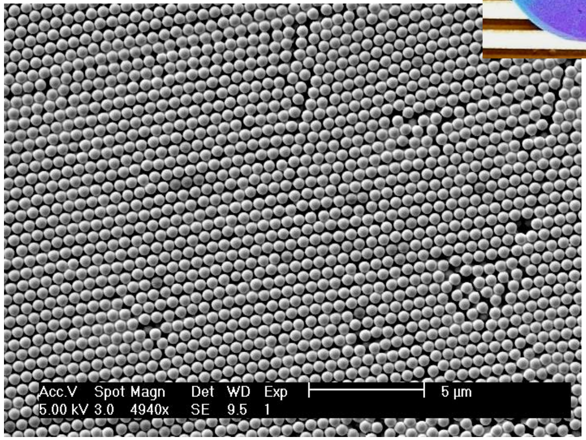


Colloidal self-assembly: spin coating

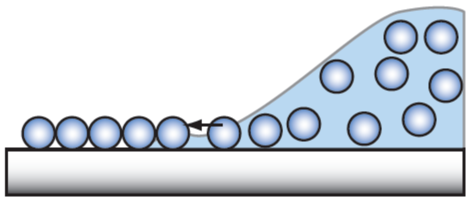
- Fast
- Compatible with clean room technologies
- Defect density OK
- Uniform coating over the whole surface



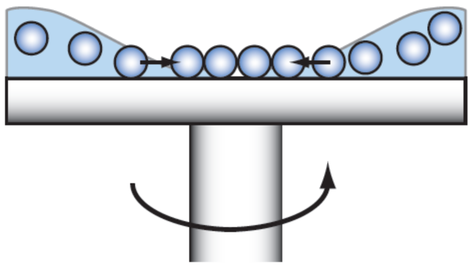




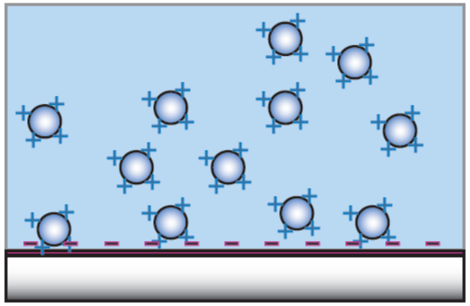
Bead deposition methods



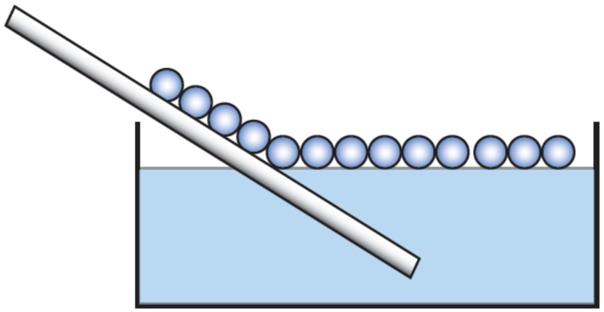
Convective self-assembly



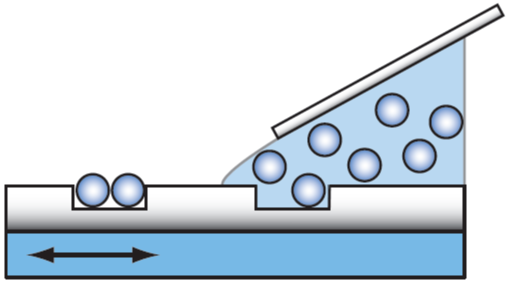
Spin-coating



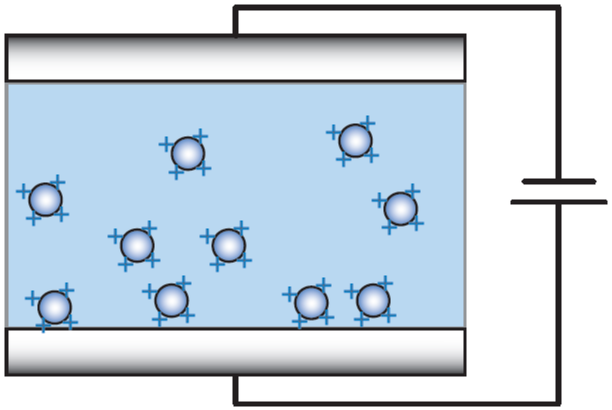
Electrostatic self-assembly



Langmuir-Blodgett

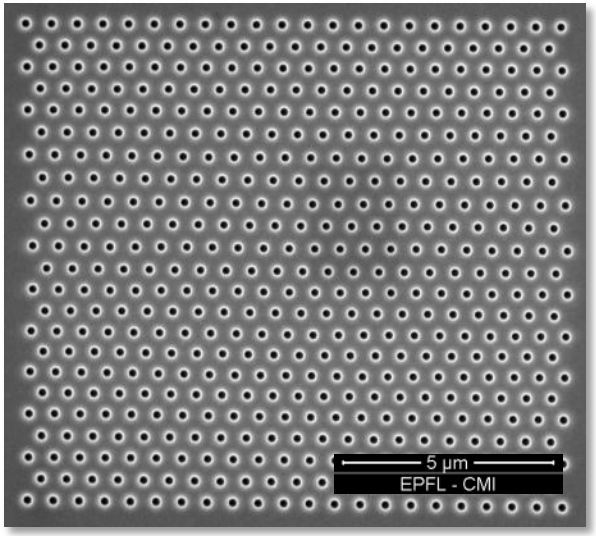


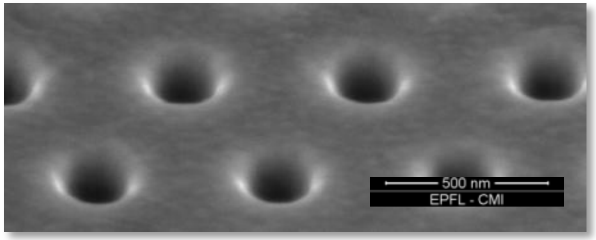
"CAPA"



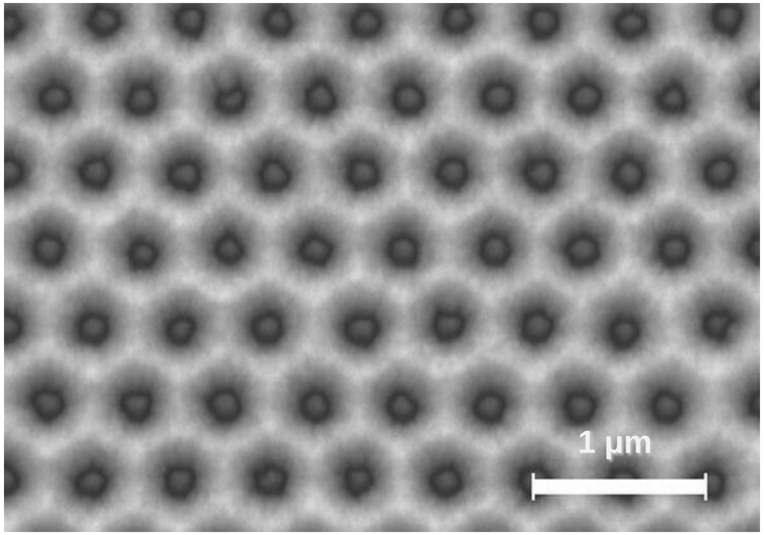
Electrophoretic deposition

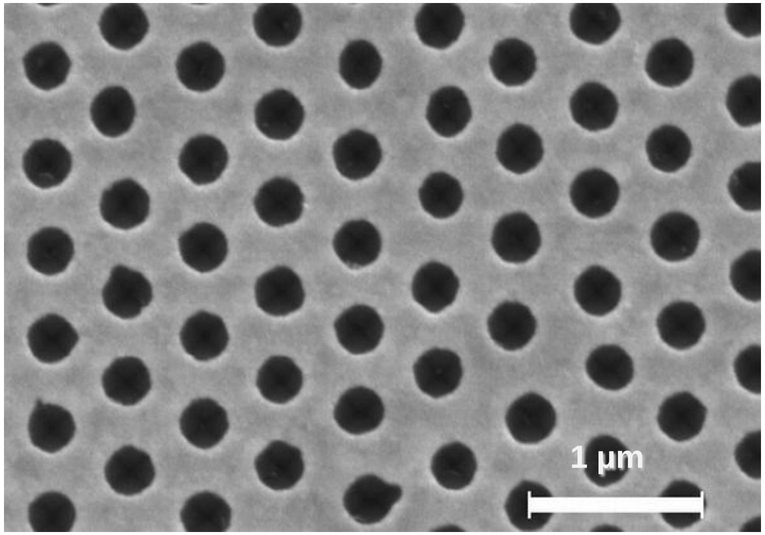
Holes arrays using FIB and using nanospheres?





Etching vs lift-off:



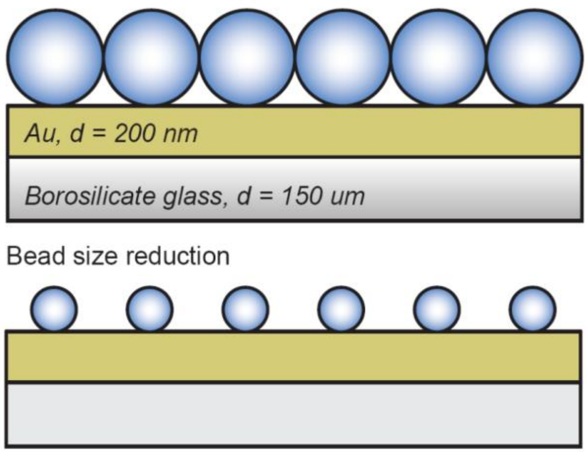


Size, spacing and organisation:

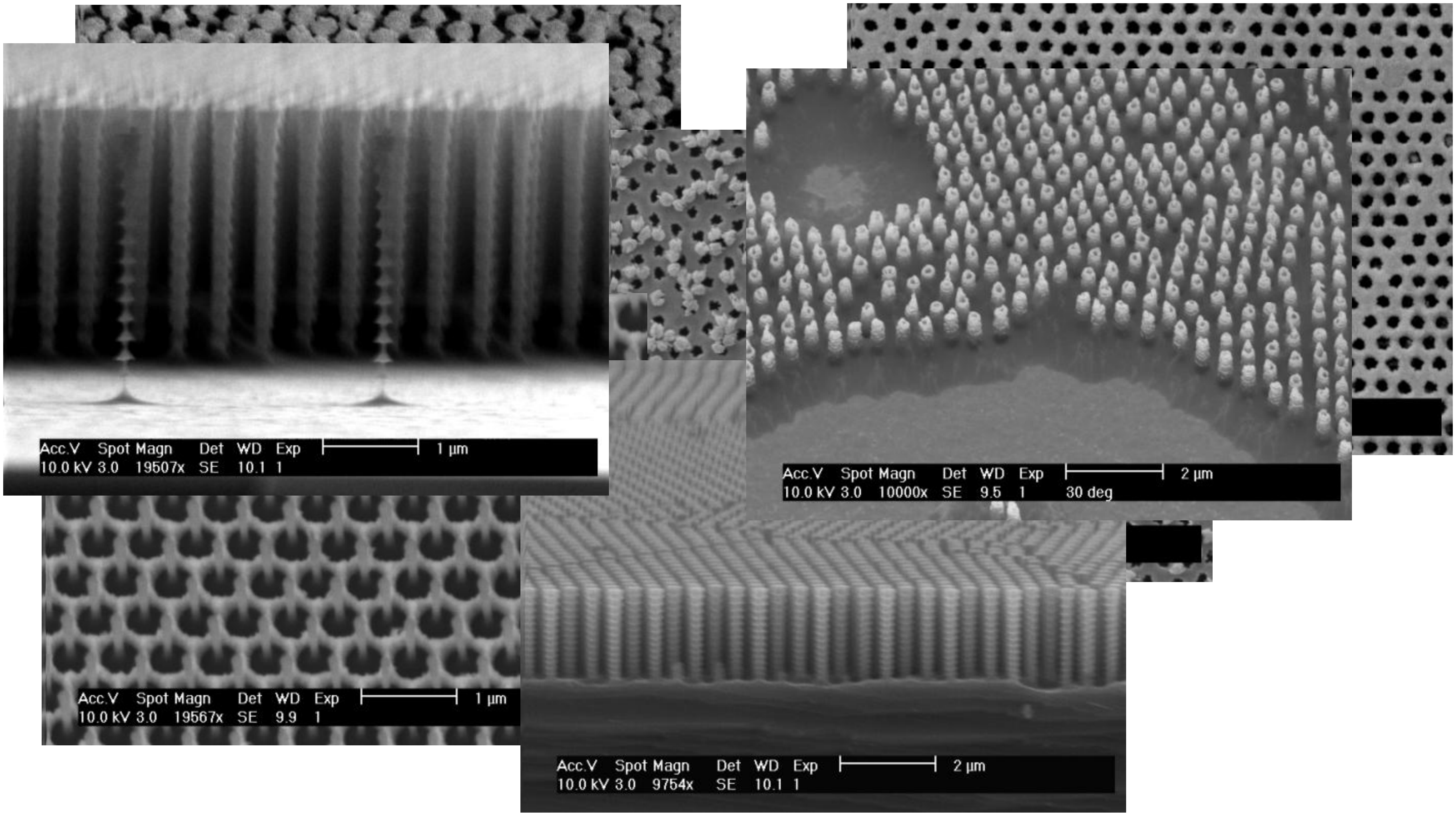
- Monodisperse spheres in a range of polymers and inorganics and of sizes (20nm – 10 microns) available commercially

- Polystyrene beads can be etched selectively (oxygen plasma only removes organics)
- Sphere spacing and diameter independent

- Spin coating can give non- hexagonal packing

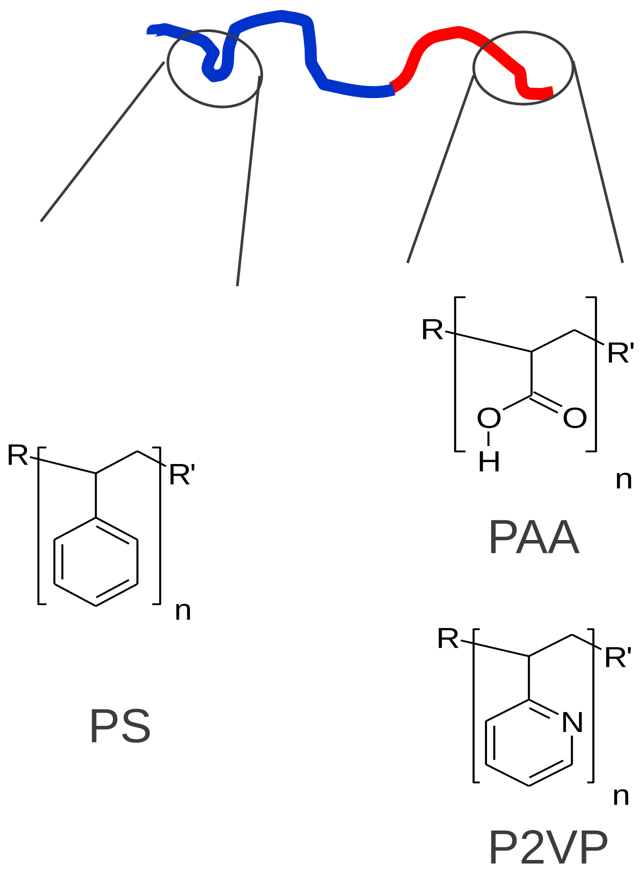


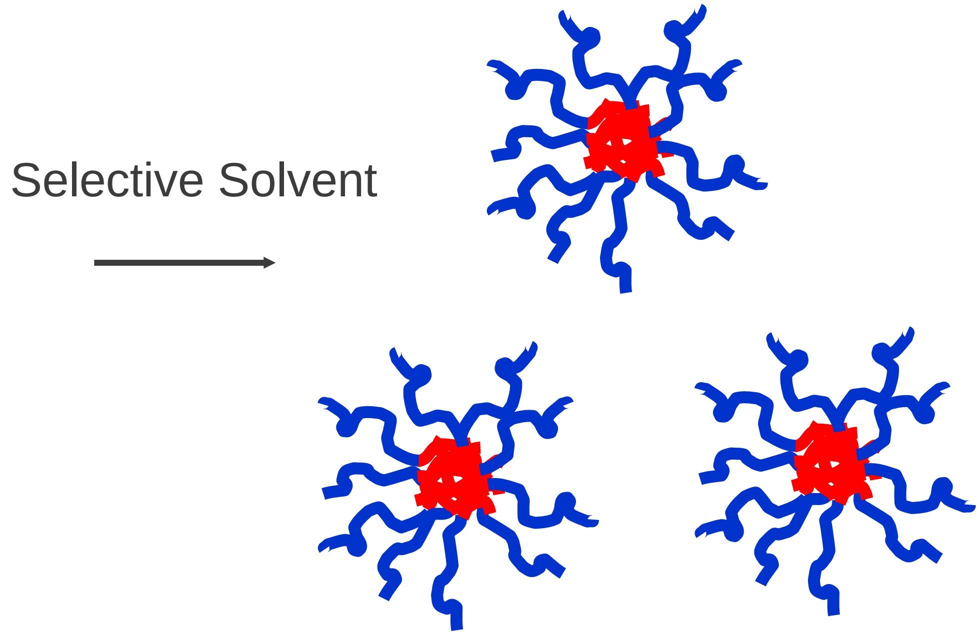
Not as easy as it looks!



Smaller structures: using block-copolymers

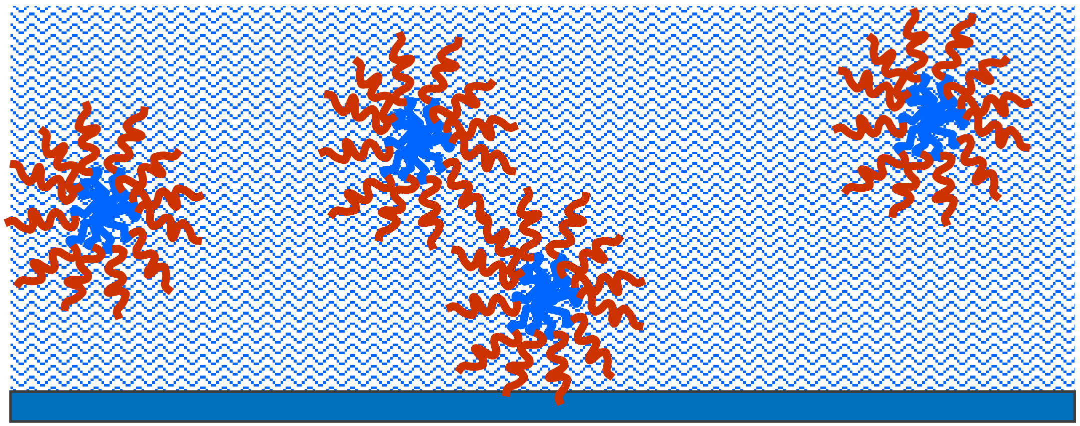
Organization in solution

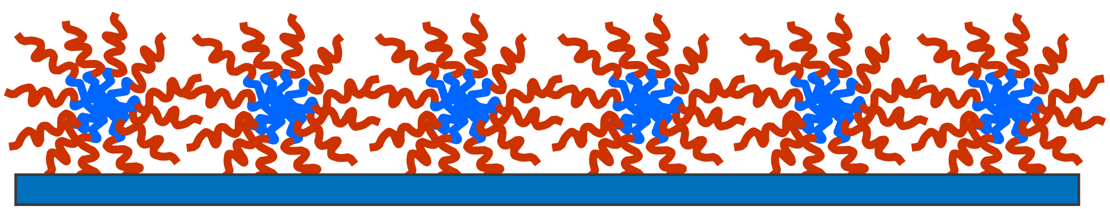




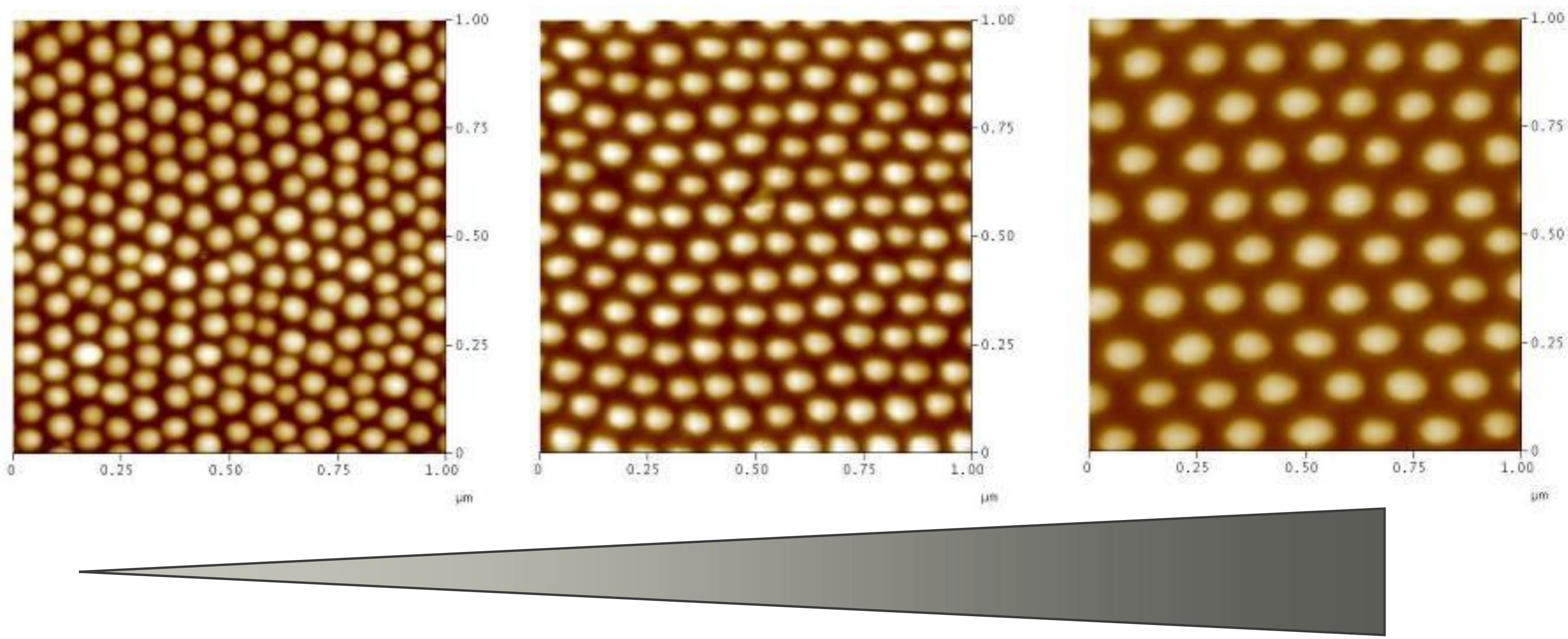
Smaller structures: using block-copolymers

- Polymer micelles form in solution and are deposited by spin-coating





Tuning size using solvent mixtures



toluene-
o-xylene
1:1

toluene-
o-xylene
1:2

toluene-
o-xylene
1:10

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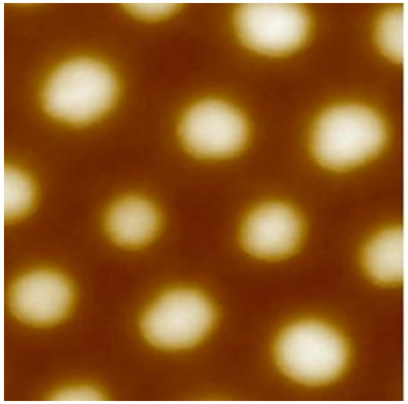
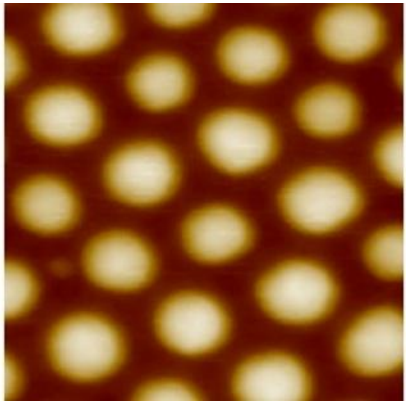
Adv. Funct. Mater.(2006) ***16,*** *1469*

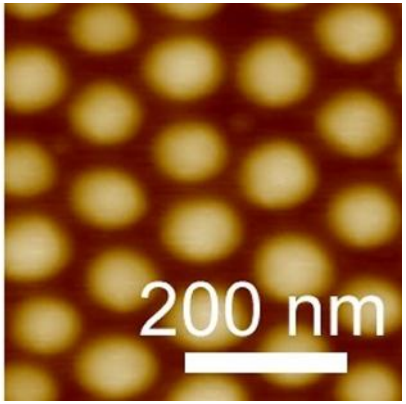
Tuning spacing using concentration



141nm

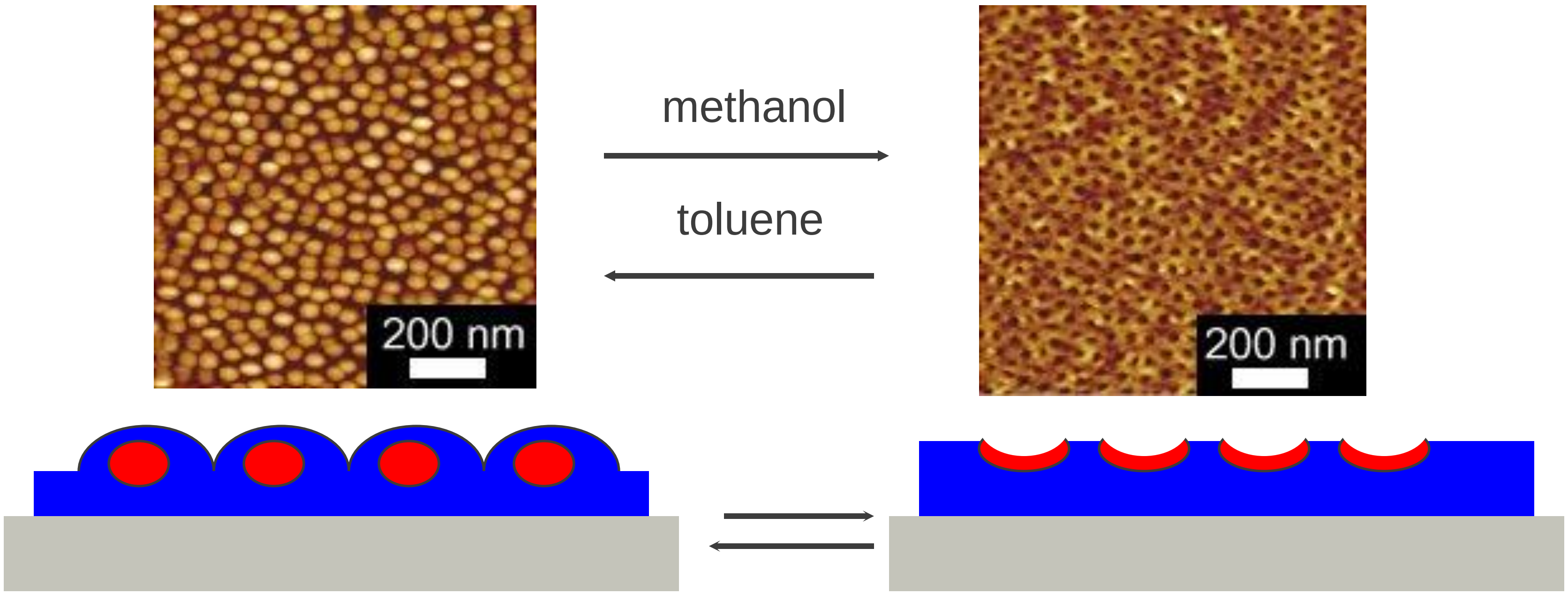


112nm



93nm

Responsive surfaces: PS-P2VP micelles



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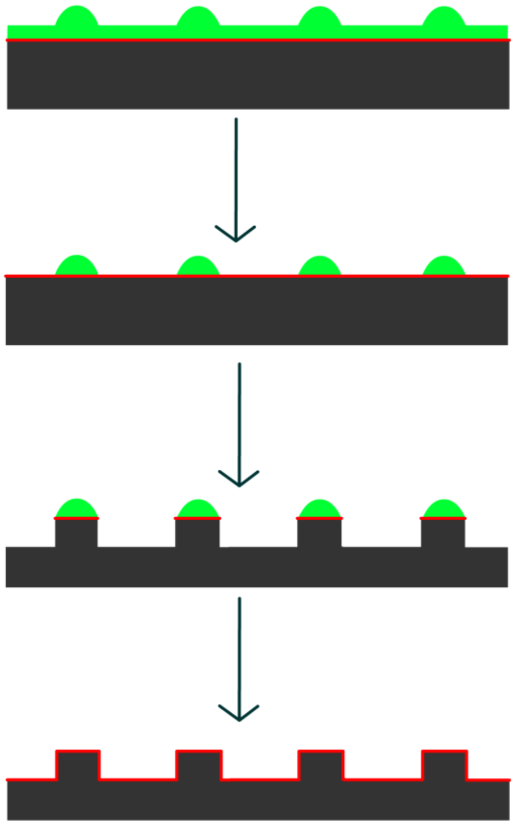
Langmuir (2006) ***22,*** *3450*

Block copolymers as etch masks: nanopillars



- PS-P2VP micelles on silicon
- Short oxygen plasma removes a thin layer of organics
- Fluorine plasma – etches silicon
- Remove remaining organics (solvent/oxygen plasma)

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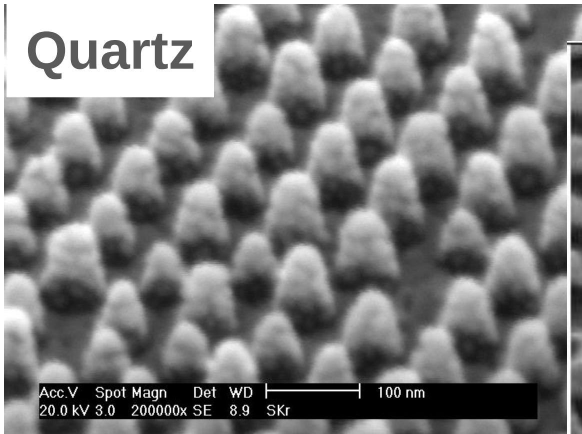
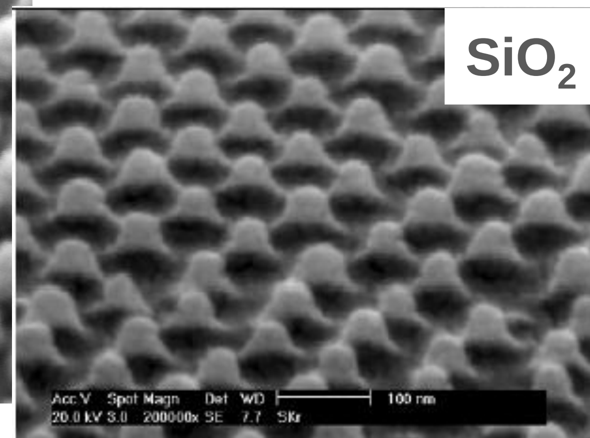
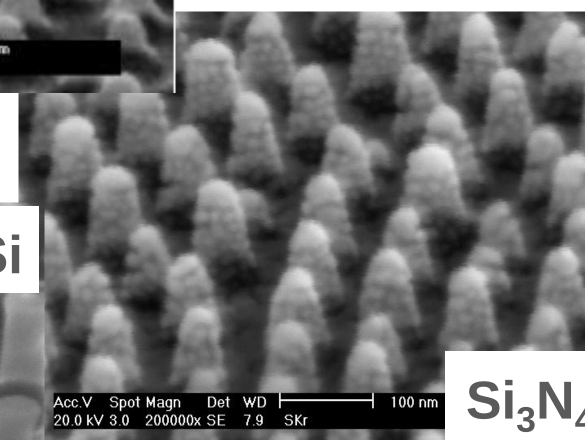
Langmuir (2006) ***22,*** *3450*

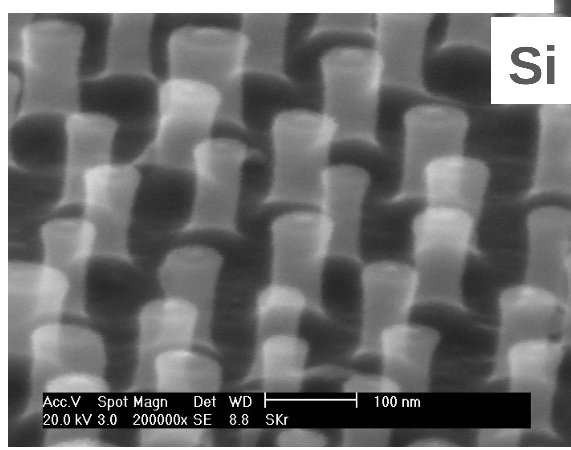
Block copolymers as etch masks: nanopillars





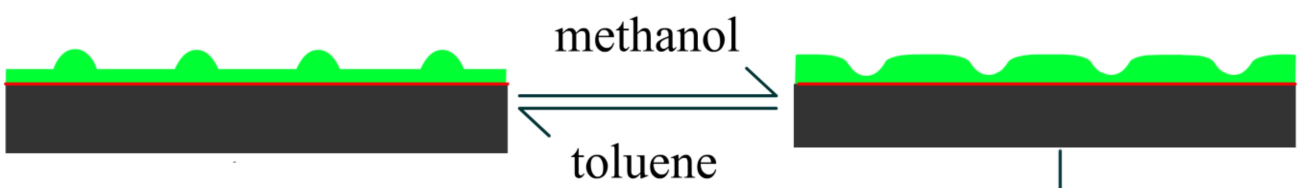
S. Krishnamoorthy et al
Langmuir (2006) ***22,*** *3450*

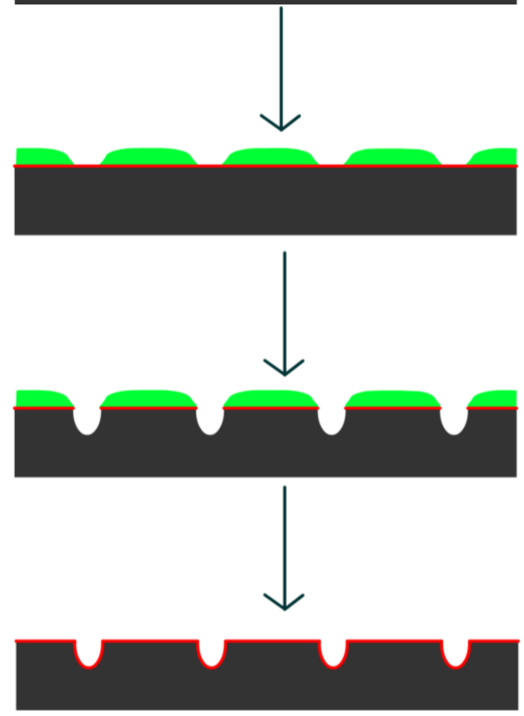




S Krishnamoorthy,
Nanotechnology ***19*** *(2008) 285301*

Block copolymers as etch masks: nanopores

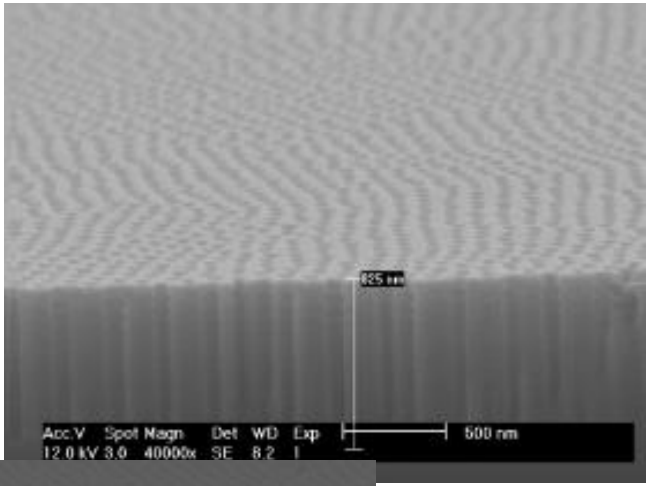


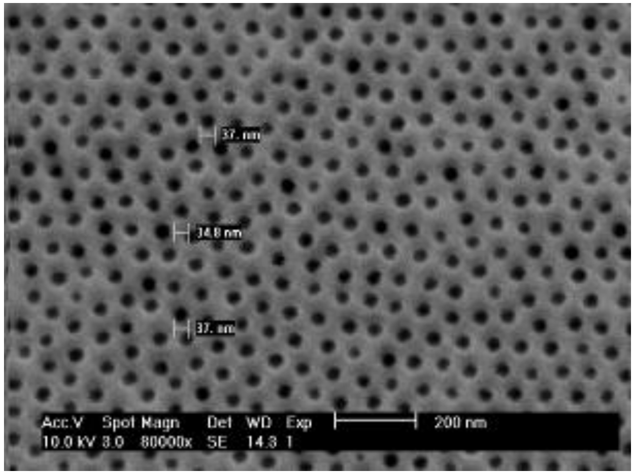


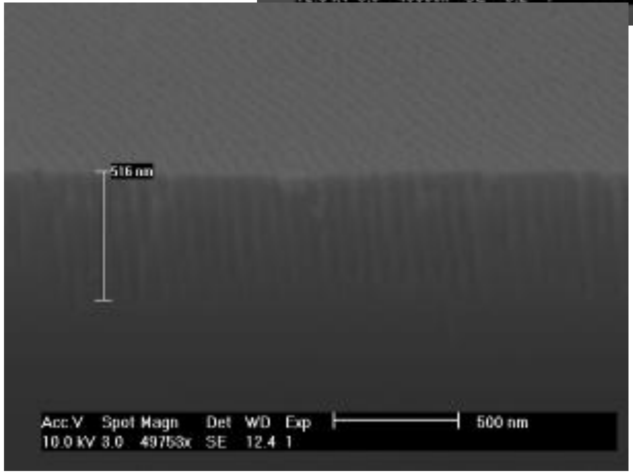
- Short oxygen plasma removes a thin layer of organics
- Fluorine plasma – etches silicon
- Remove remaining organics (solvent/oxygen plasma)

Block copolymers as etch masks: nanopores

- In silicon
- Aspect ratio ~11
- Pore diameter 80nm, depth 850nm
- Pore diameter 40nm, depth 470

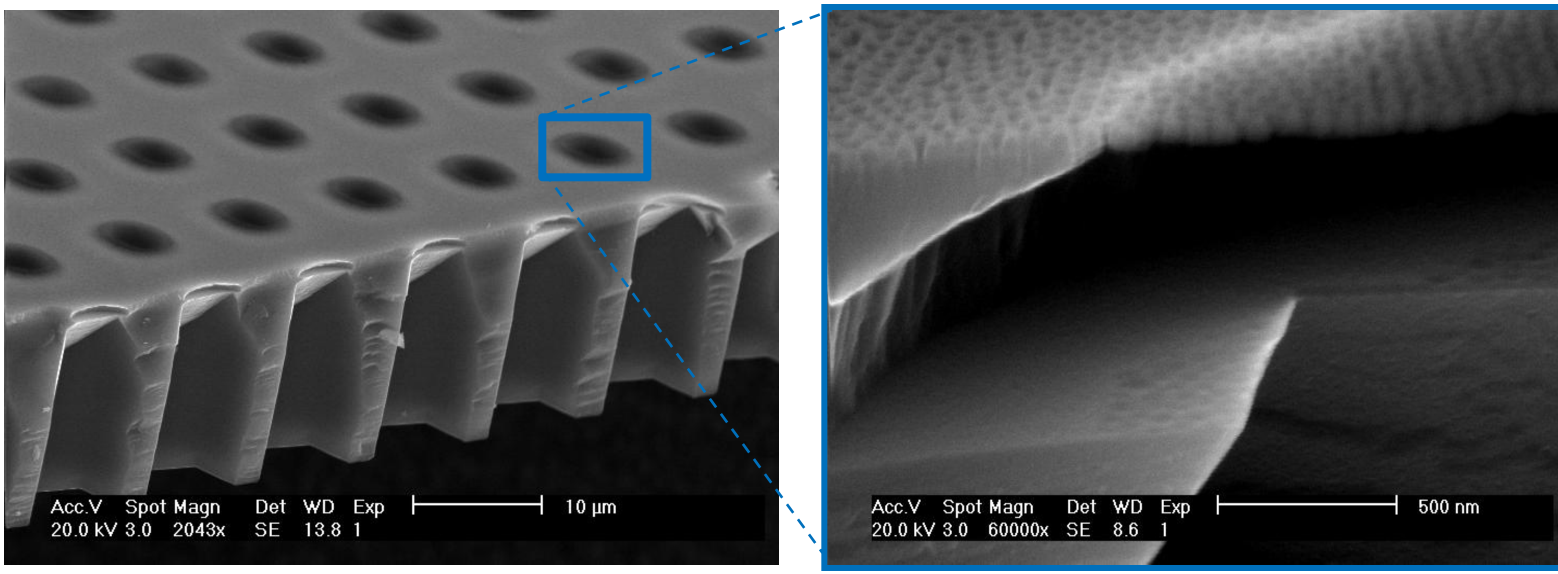






From nanostructured surface to nanoporous membrane

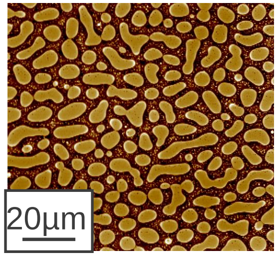
- Combination with microfabrication processes for the fabrication of suspended nanoporous membranes as part of new MEMS

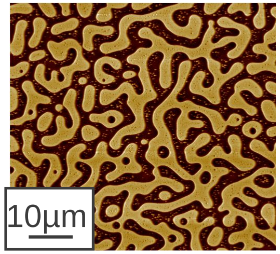


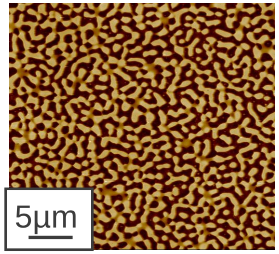
- The membrane is supported by 20 μm thick silicon beams which are spaced about 7 μm to withstand a few bars differential pressure
- Applications: ultra-filtration, molecules separation

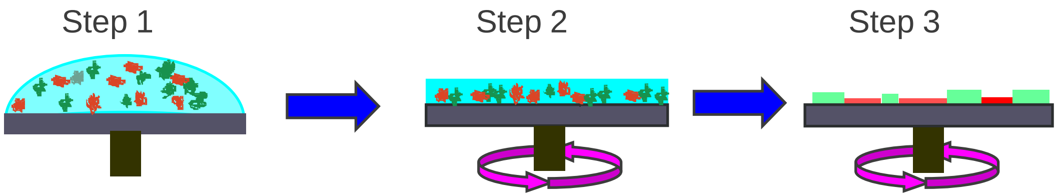
Templates: polymer demixing

- Solution of two polymers spin-coated
- As solvent evaporates, polymers separate into two phases
- Phase structure depends on many parameters
- Typical polymers: polystyrene, polyvinylpyridine, polymethylmethacrylate



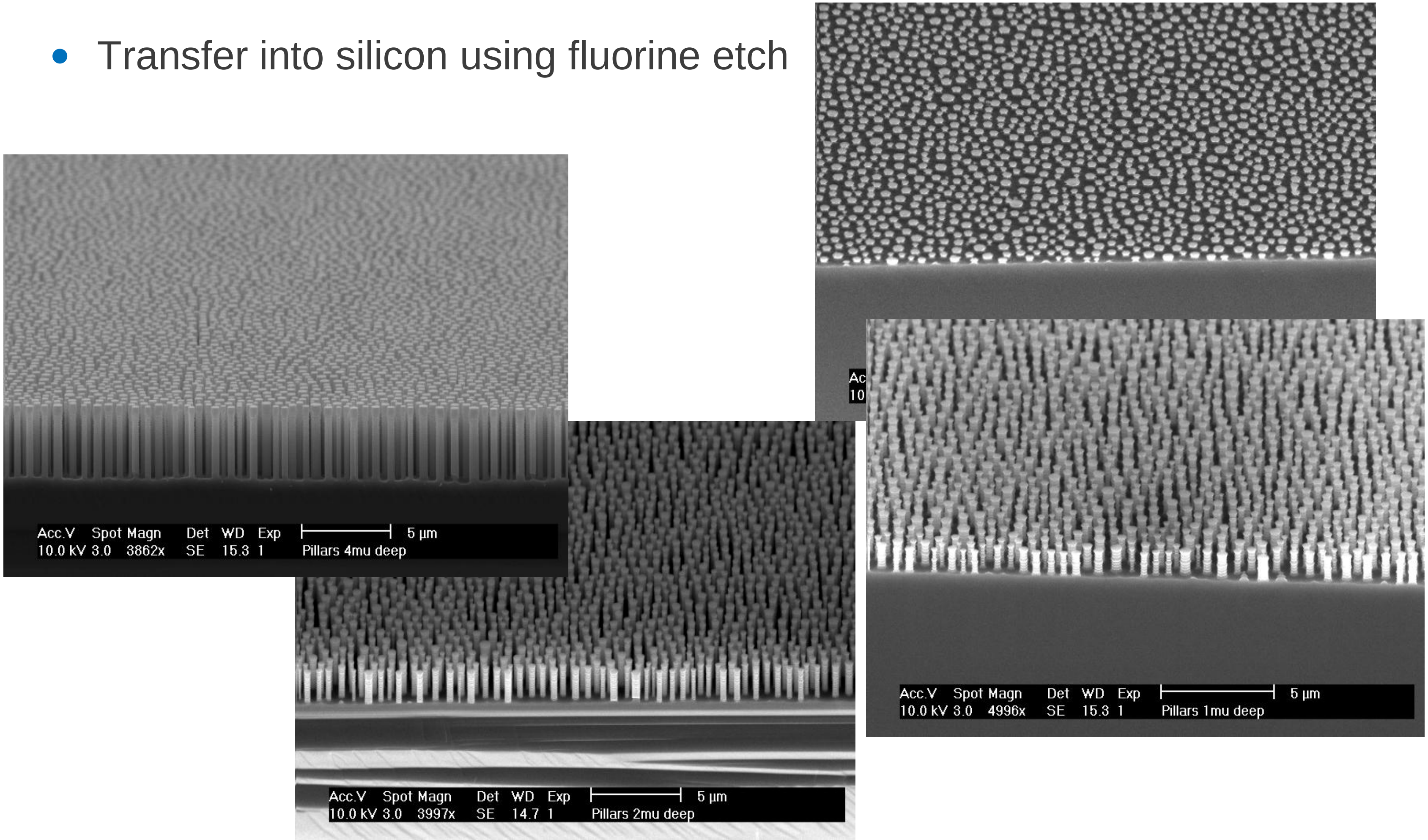






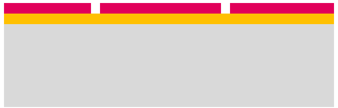
Demixed polymers as etch masks

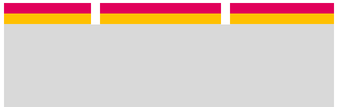
- Transfer into silicon using fluorine etch

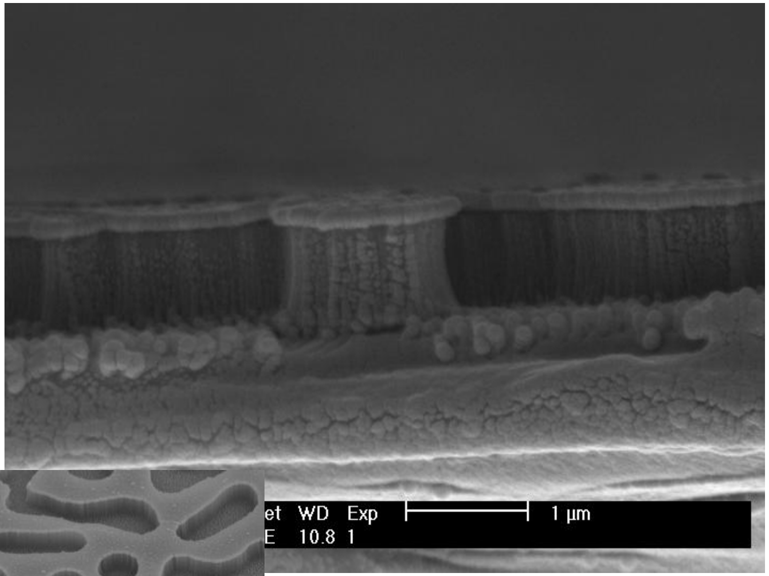


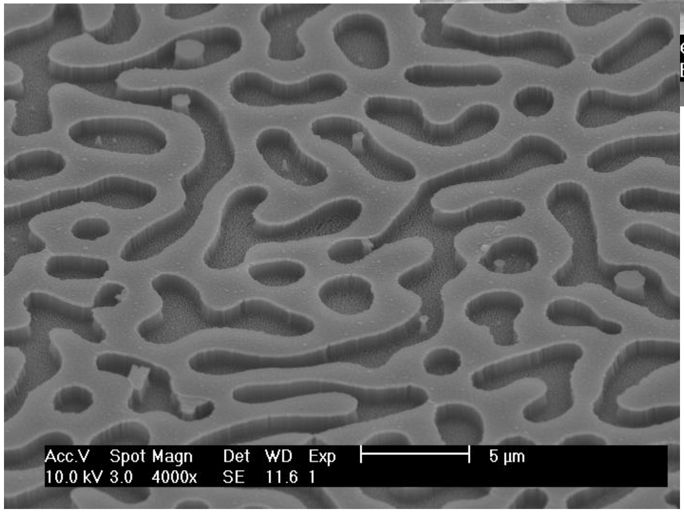
Demixed polymers as etch masks

- Etch into polymers
- Use intermediate mask layer.



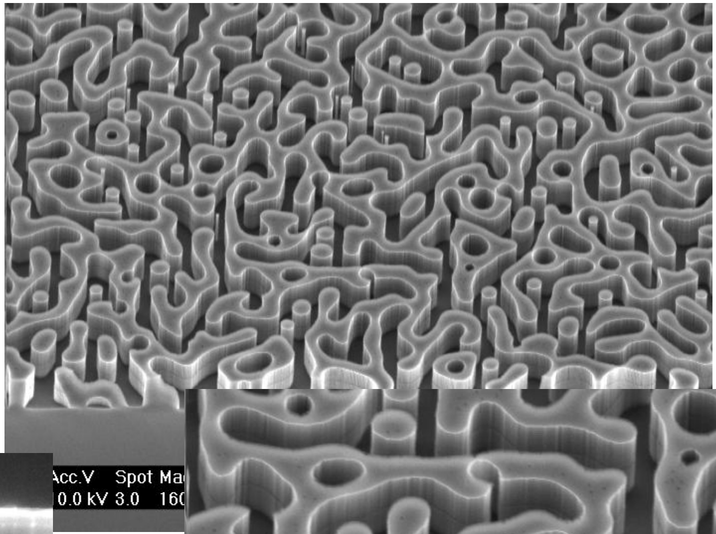


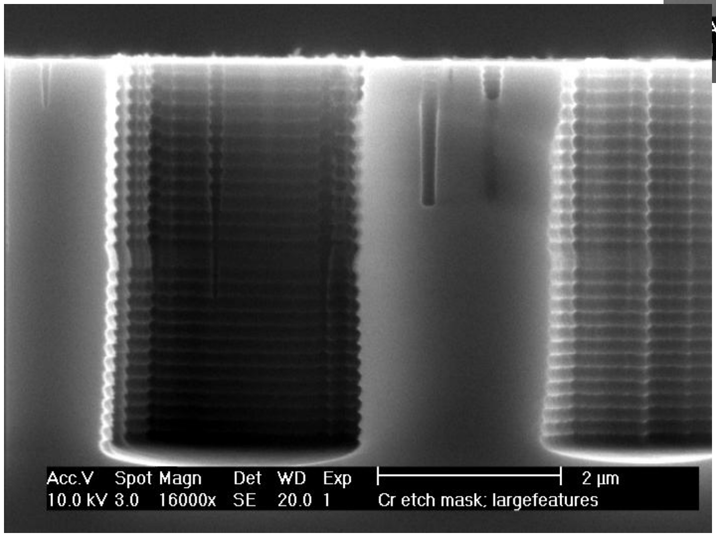


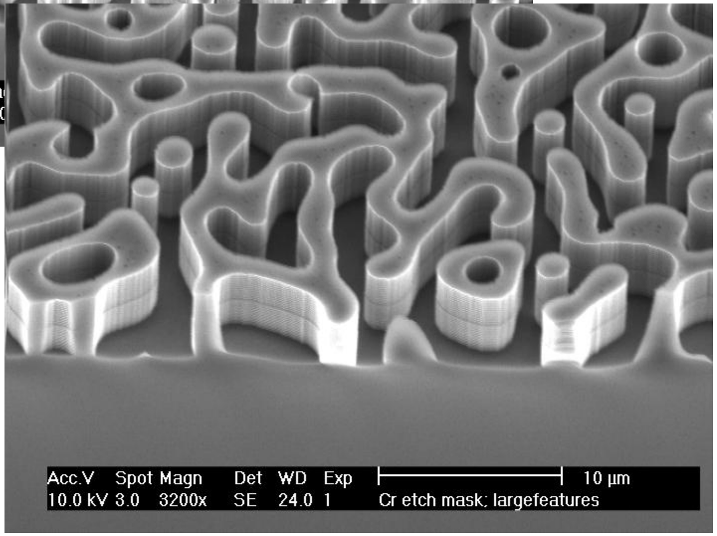


Demixed polymers: etch and then replicate

- Spinodally demixed polymer layer etched quasi-vertically into silicon.
- The silicon ‘master’





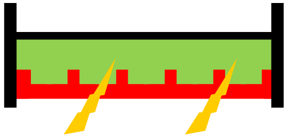


Demixed polymers: etch and then replicate

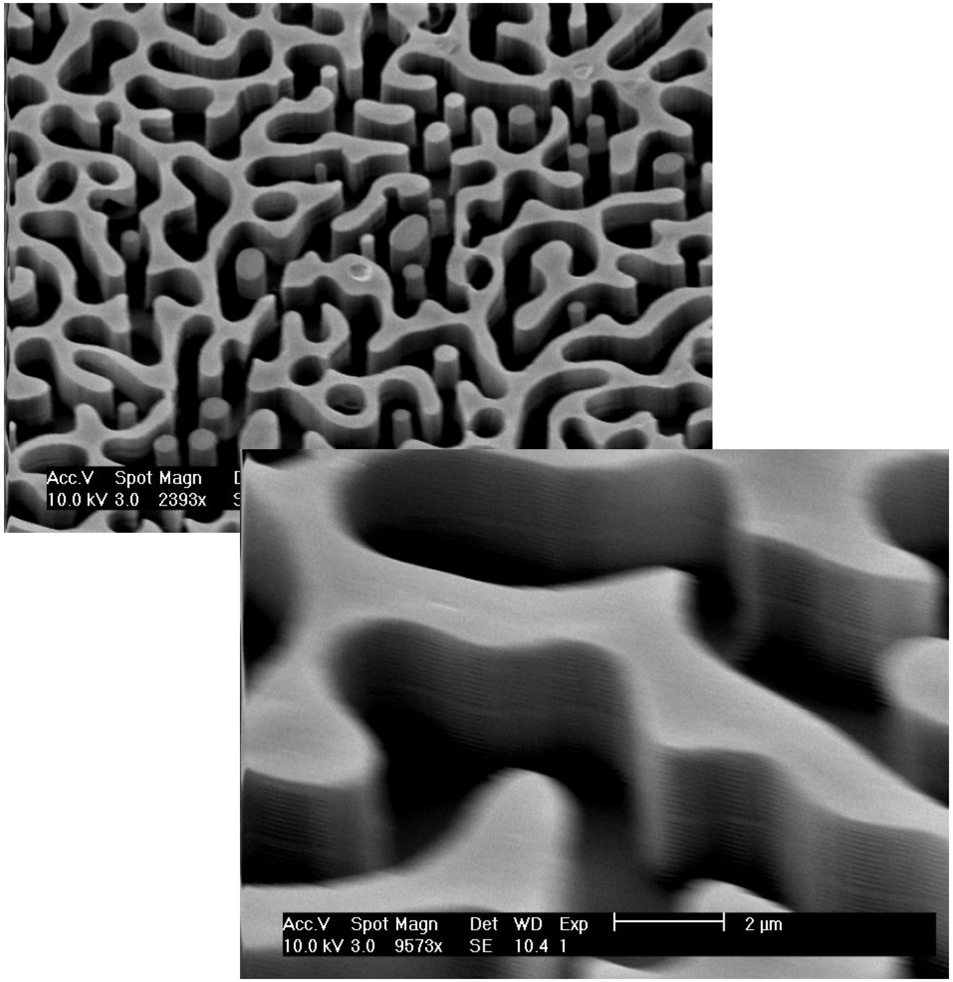
- Master in silicon

- Replication in PDMS

- UV-casting in Bis-GMA/TEGDMA

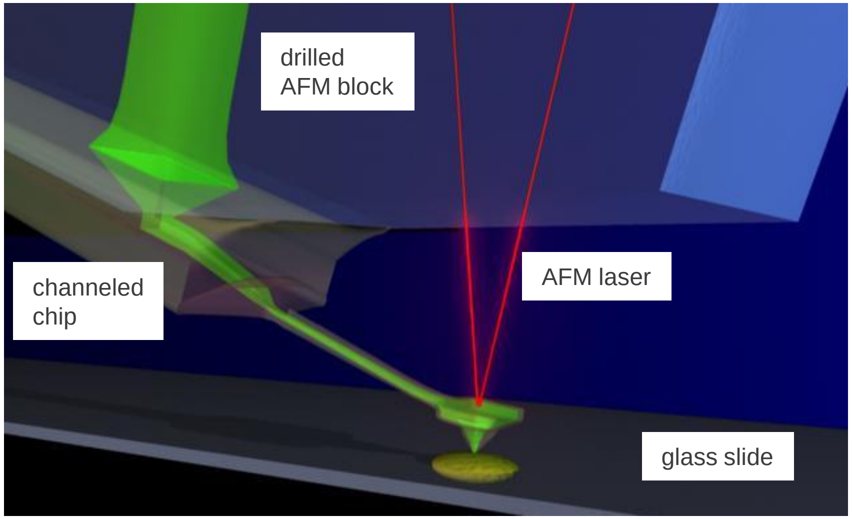


- Demoulding

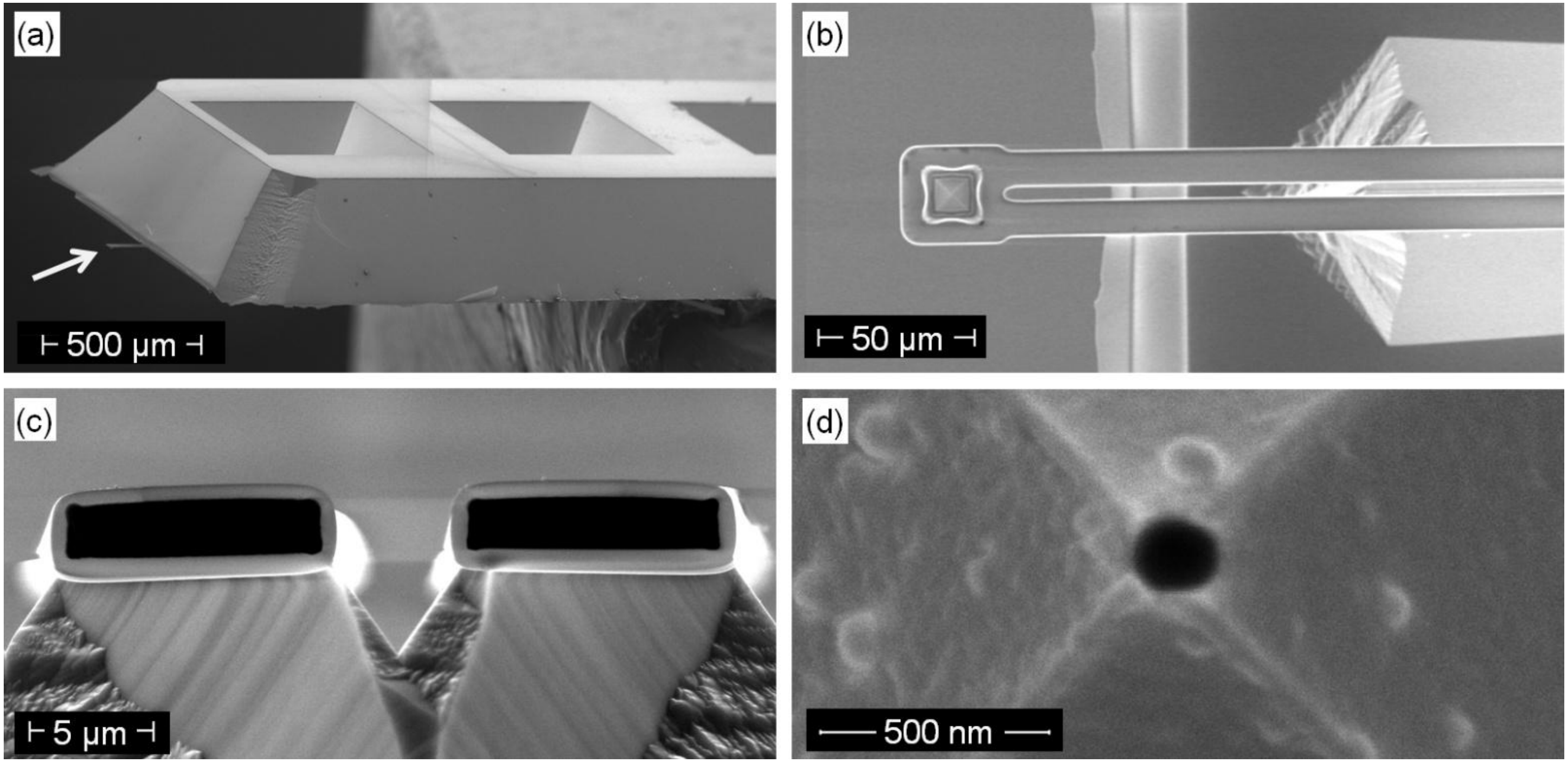


Modifying individual structures:

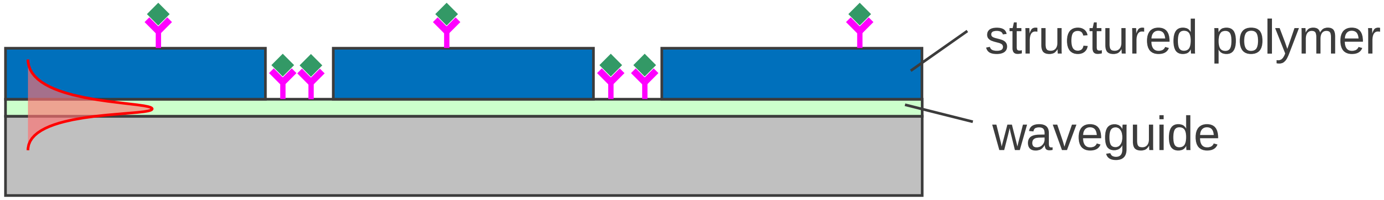
- Microfabricated hollow AFM cantilevers
  - Open at the AFM tip
  - Reservoir in the silicon chip
- AFM force feedback
  - Gentle contact to fragile samples
  - Imaging of small objects
- Liquid dispensing in air and water

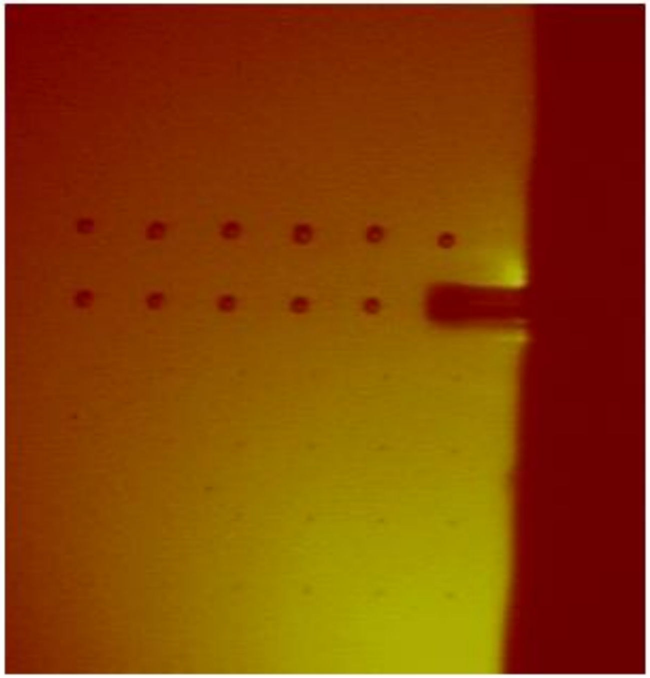


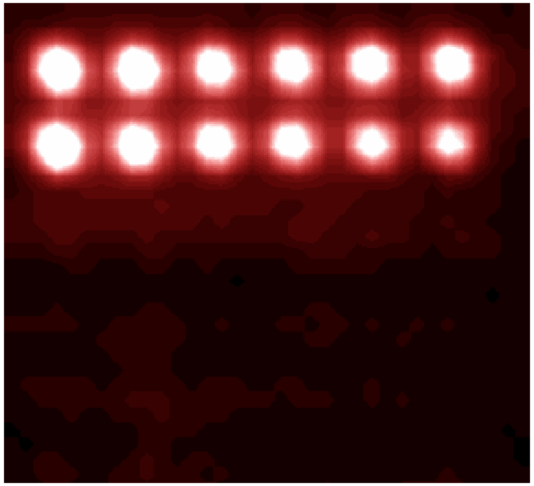
Nadis

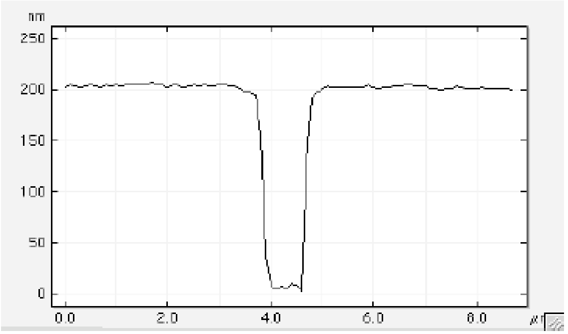


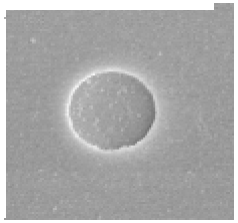
Functionalization of high-sensitive microarrays











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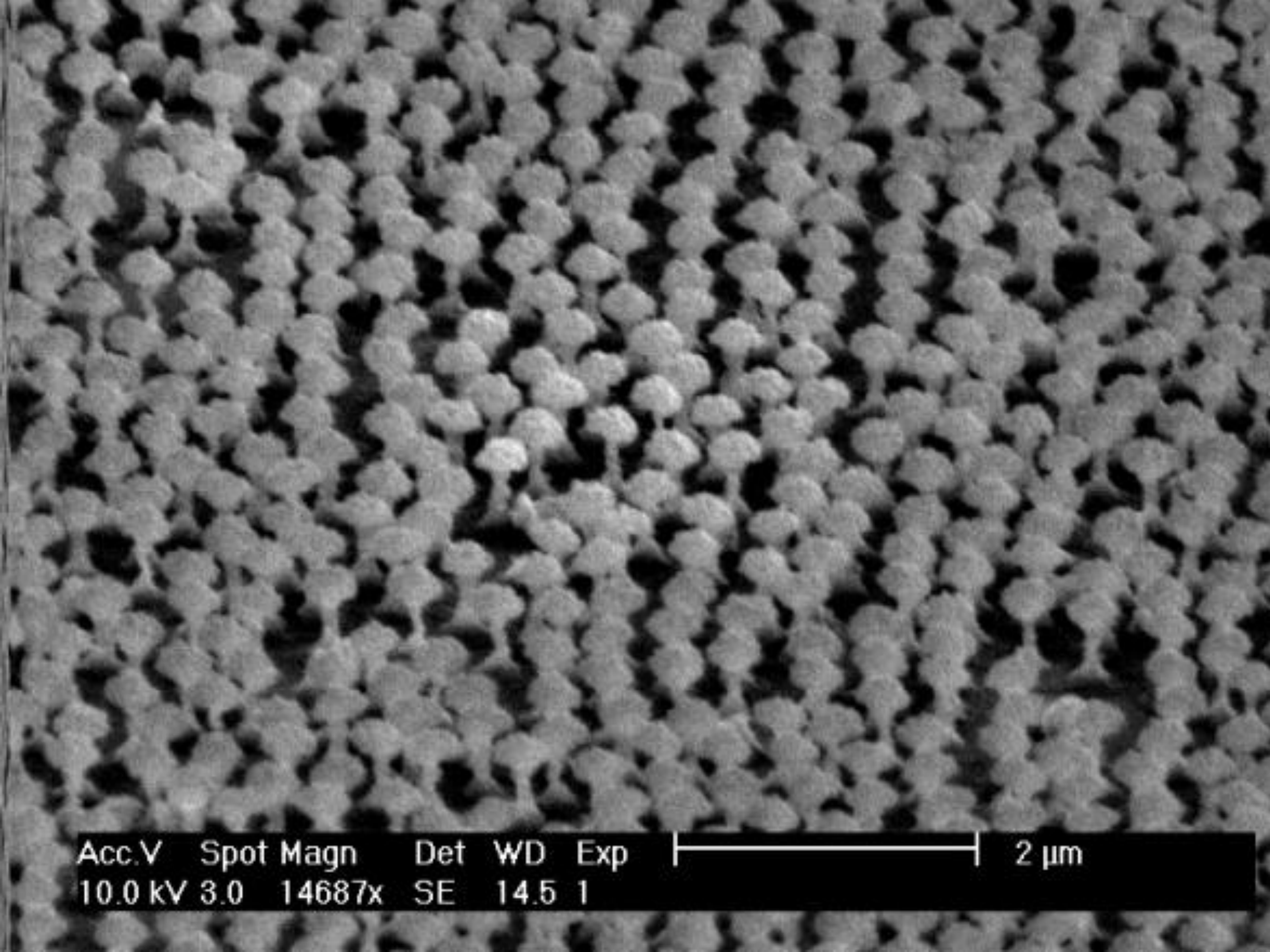
- Mickaël Guillaumée
- Nicolas Blondiaux
- André Meister
- Christian Hinderling
- Raphael Pugin
- Harry Heinzelmann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