"DNA and Proteine Microarray Technologies Developed on Porous Silicon Substrate"

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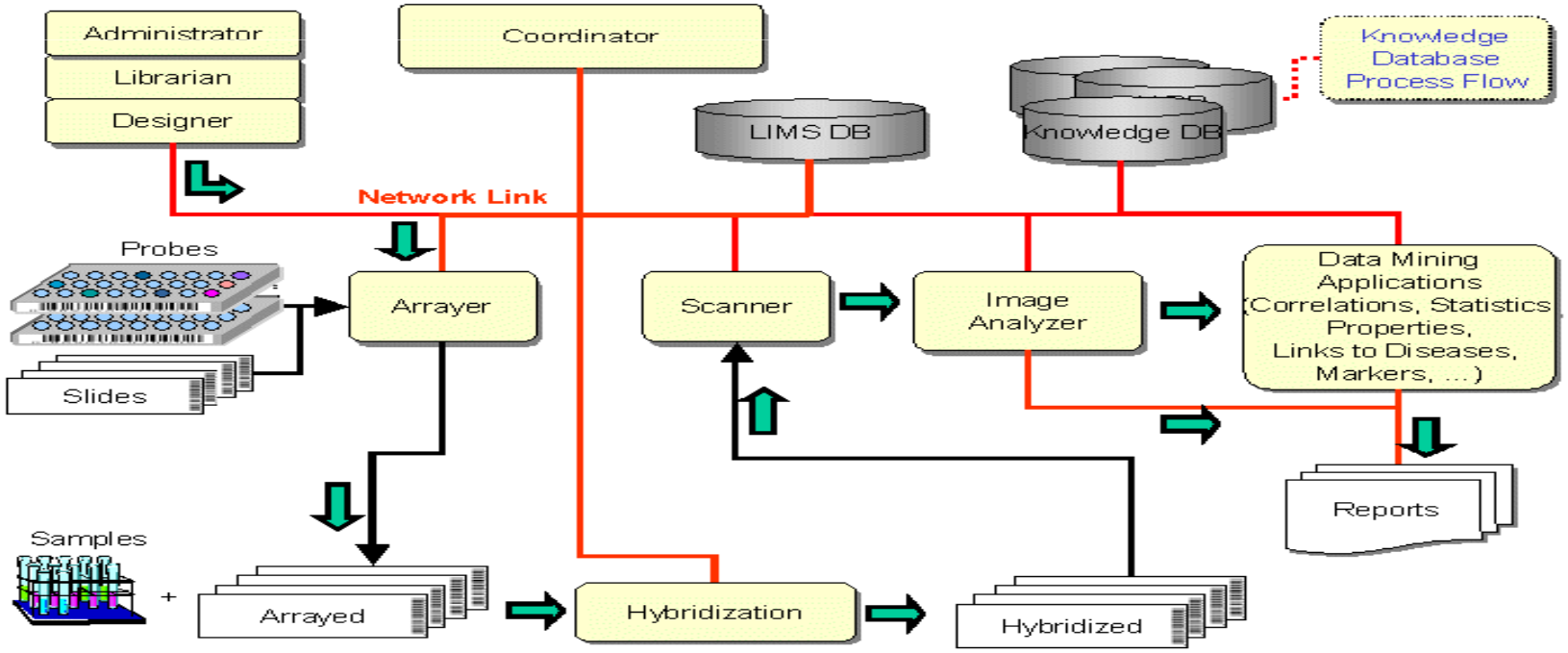
OmniGrid Micro (Digilab)

- printhead and wash/dry stations
- Positioning System with stepper motors (XYZ)
- Real-Time Computer System
- humidity control during processing

Microarray technique evolved from Southern blotting, where fragmented DNA is attached to a substrate and then probed with a known gene or fragment

Maskos, U; Southern, EM (11 Apr 1992). "Oligonucleotide hybridizations on glass supports: a novel linker for oligonucleotide synthesis and hybridization properties of oligonucleotides synthesised in situ". *Nucleic Acids Res.* (Maskos U, Southern EM.) **20** (7): 1679–84

→ **Microarray technology**

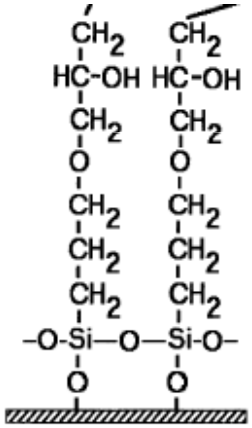


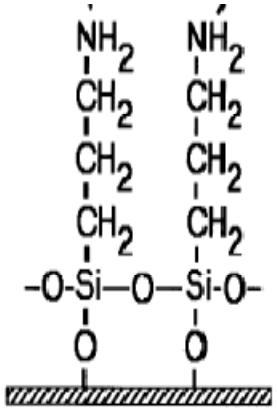
Components of Microarray Fabrication

The complete process involved in making DNA microarrays has the following main steps:

Preparation of samples -DNA extraction/DNA syntheses

Substrates preparation – specific chemistry modification





Construction of the arrays

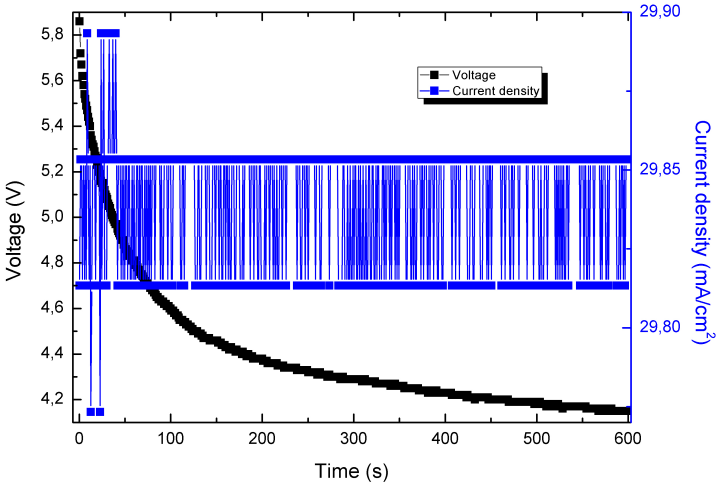
Preparation of the probes

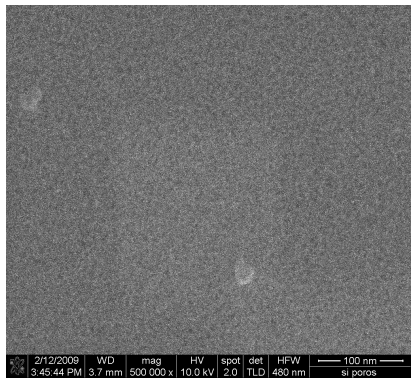
Hybridisation

Substrates preparation

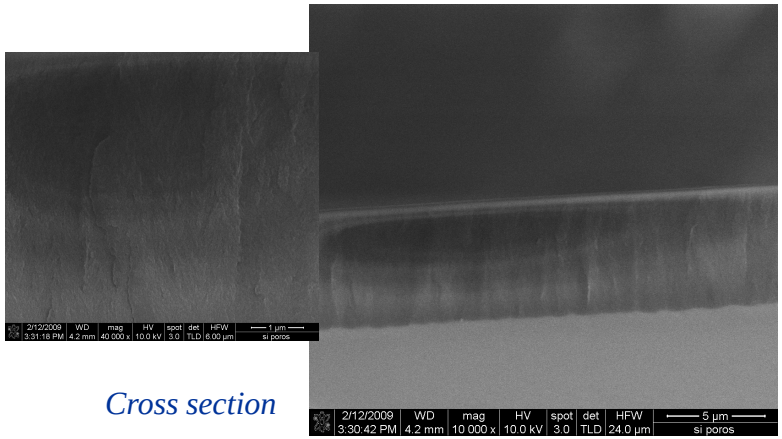
PS layers/Si fabrication

Porous silicon layer was obtained by electrochemical etching of silicon p type substrate (100) crystallographic orientation with 5-10 Ω cm resistivity using the following parameters: HF:Etanol (3:1) electrolyte solution, 300 mA/cm^2 current density, process time 600 ms.





top view



Cross section

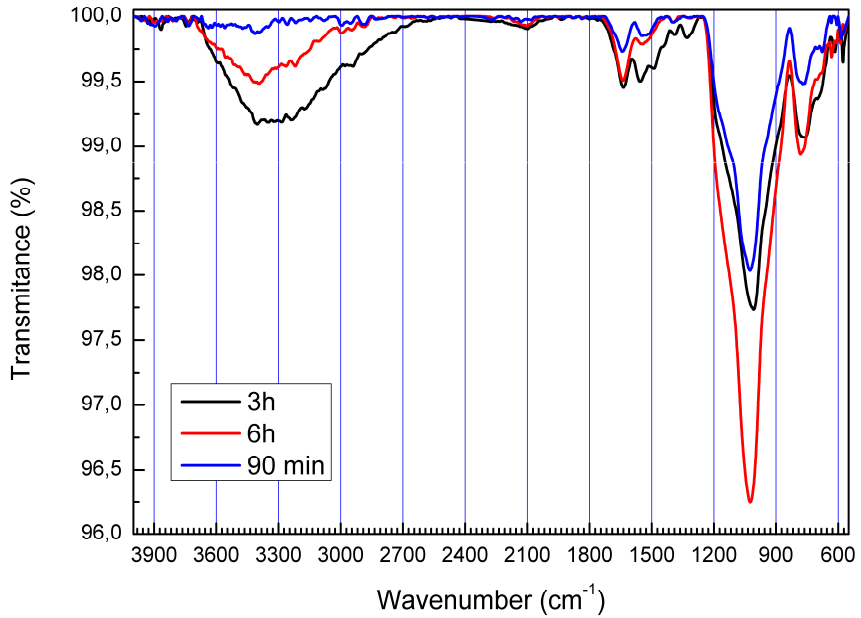
SEM images of nanoporous porous silicon (nanoPS) obtained by electrochemical porosification of Si substrate

Specific chemistry modification

PS surface chemistry

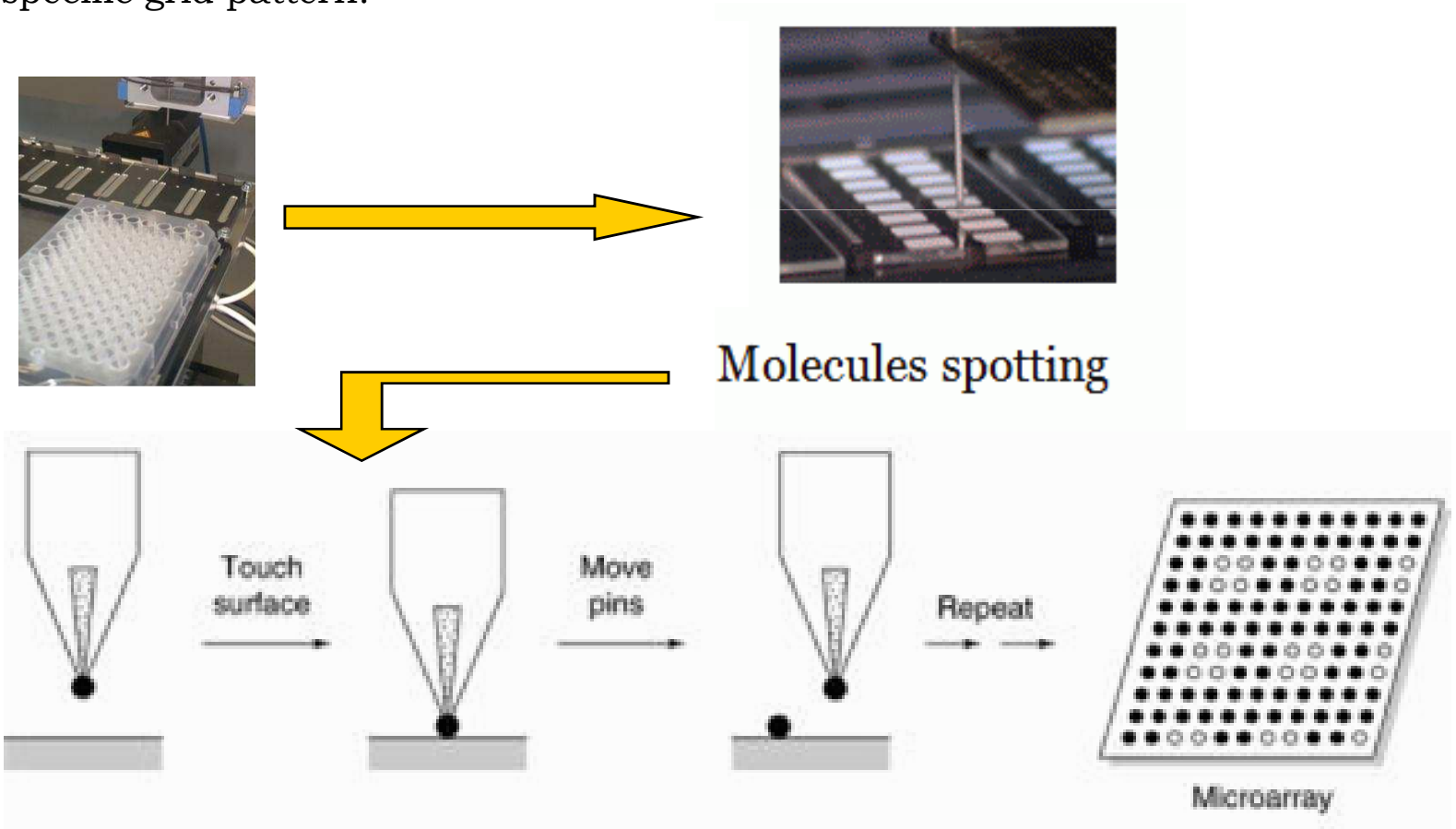
Two type of samples were prepared: The porous silicon substrate and the porous silicon substrate coated with 3-aminopropyiltriethoxysilane (APTS). APTES layers was obtain by immersion of PS in 5% APTS in ethanol solution.

FTIR spectrum of PS with APTES

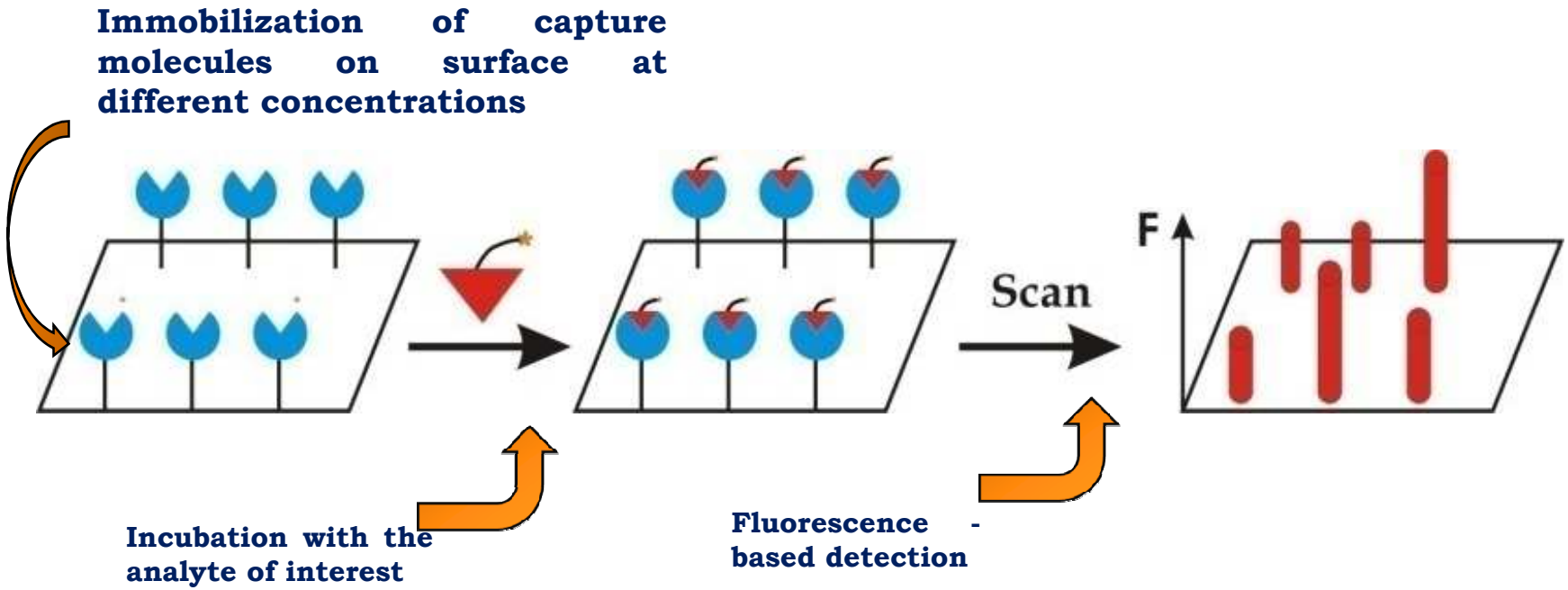


Construction of the Arrays

The DNA sequences are printed onto the microscope slides robotically, in the specific grid pattern.

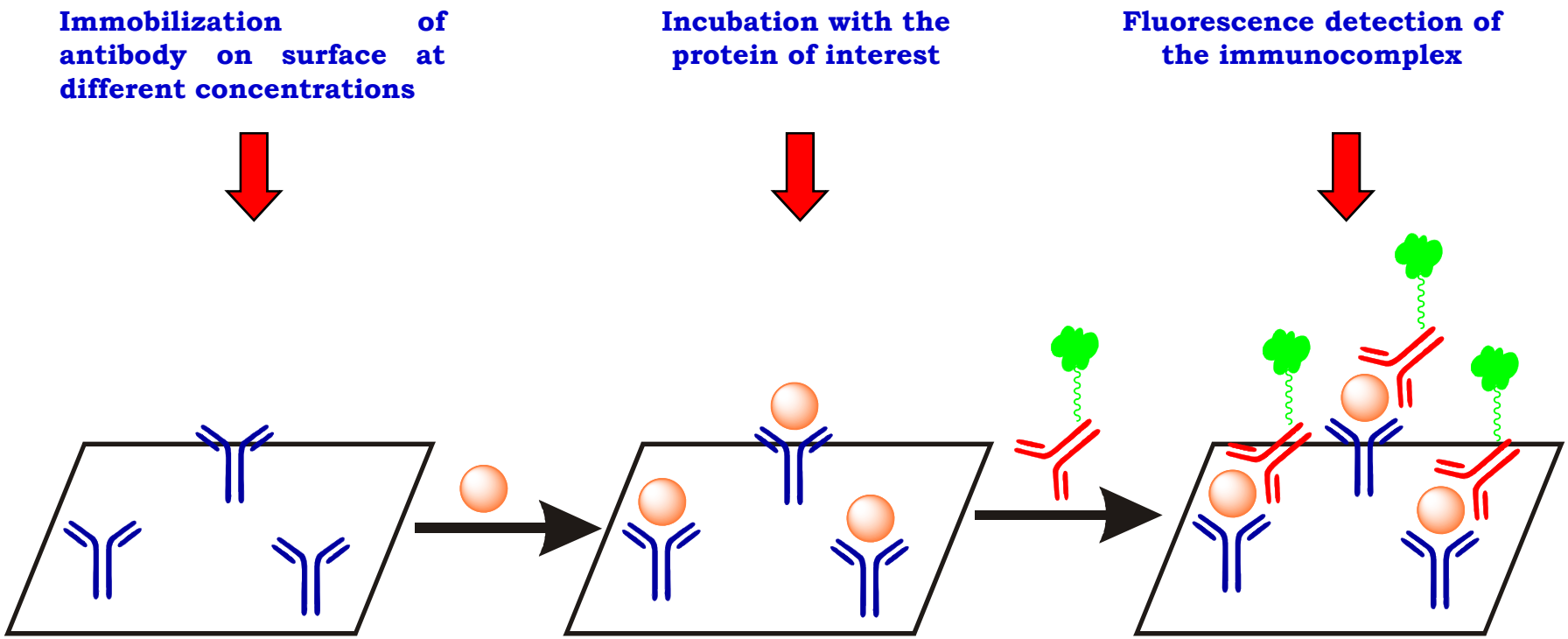


Principles of microarray



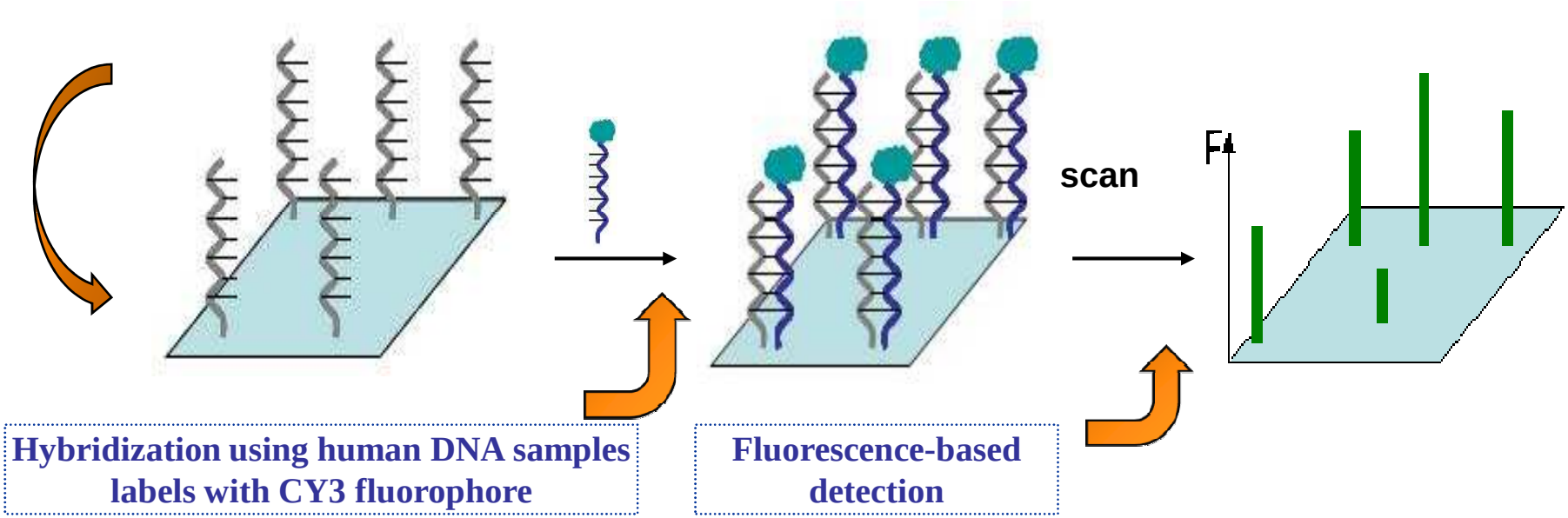
Applications

Principles of protein microarray



Applications

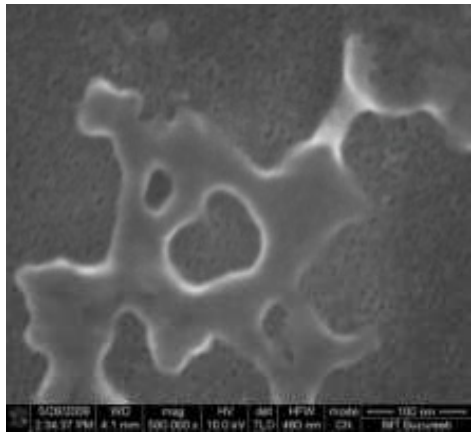
Principles of DNA microarray



Results

Protein microarray

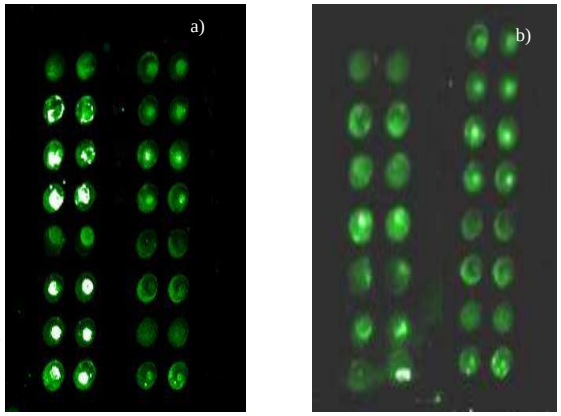
The PS surfaces were used for the immobilization of two Cy3 labelled CRP human antibodies: monoclonal rabbit (*anti*-mCRP) and polyclonal goat (*anti*-pCRP).



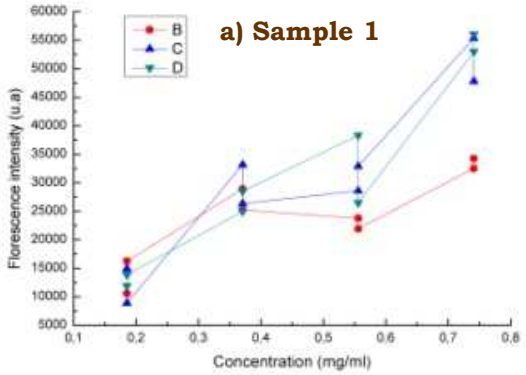
Solid pin size: 200 microns
Spot sizes: 200-200 microns, distance between spots 100 microns

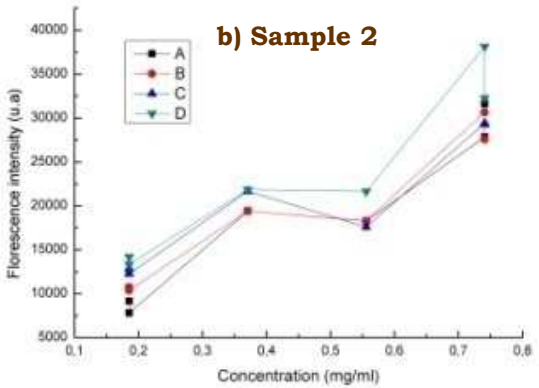
SEM image of PS onto which aCRP was immobilized

Scanned image with immobilized fluorescent antibodies on the two PS surfaces



Reproducibility tests between arrays (fluorescence intensity as a function of the capture antibody concentration): a) PS Sample 1; b) PS Sample 2



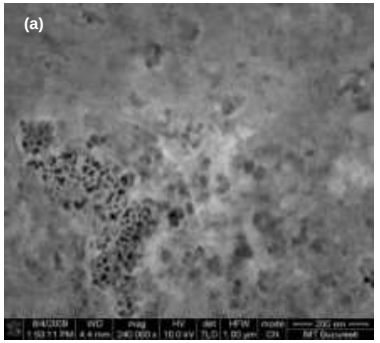


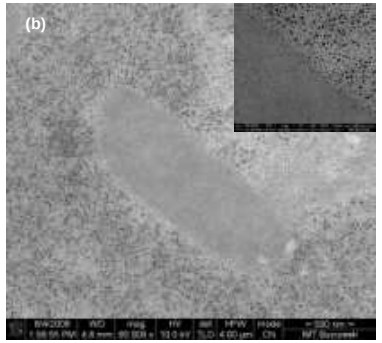
Results

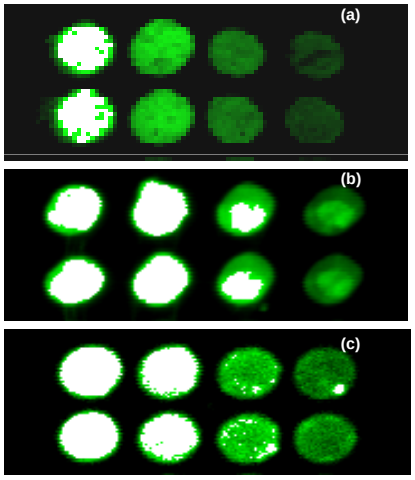
Protein microarray

The proteins activity on surfaces was tested by reaction with labelled antibody directly on surfaces.

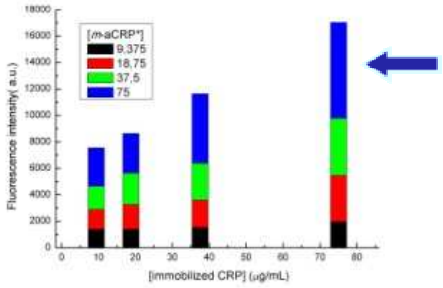
SEM image of PS surface: (a) after immobilization of CRP; (b) after reaction with labelled antibody





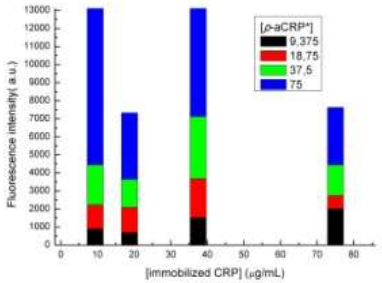


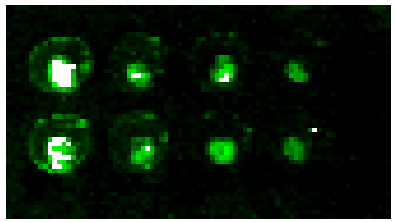
Scanned image with immobilized Cy3 labelled m-*a*CRP on (a) commercial aldehyde-functionalised glass slides; (b) commercial hydrophibic polymer functionalized glass slides; (c) house-made porous silicon



Fluorescence intensity as function of the immobilized m-*a*CRP.

Fluorescence intensity as function of the immobilized p-*a*CRP.





Scanned image with spotted antigen – labelled antibody reaction at different concentration of spotted protein and antibody.

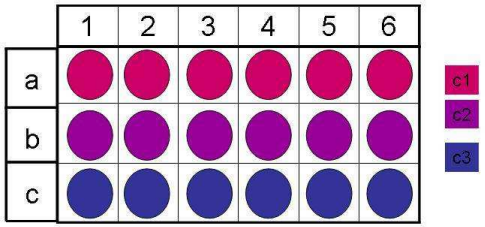
Fluorescence intensity as function of the immobilized protein concentration increases with increase of the hybridization antibody amount. The trend is observed to conserve more for m-*a*CRP than p-*a*CRP.

Results

DNA microarray

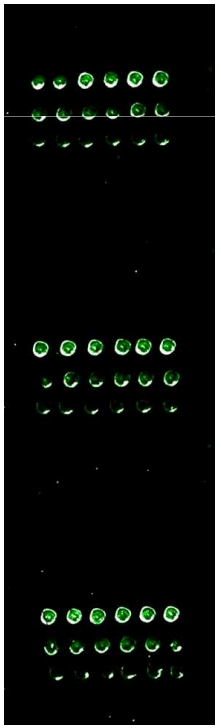
Complementary DNA sequence used for hybridization label with Cy3
5'-CGAATTGTGAGCGGATATCCTGGTT-3',

DNA printed on different surfaces on serial dilution

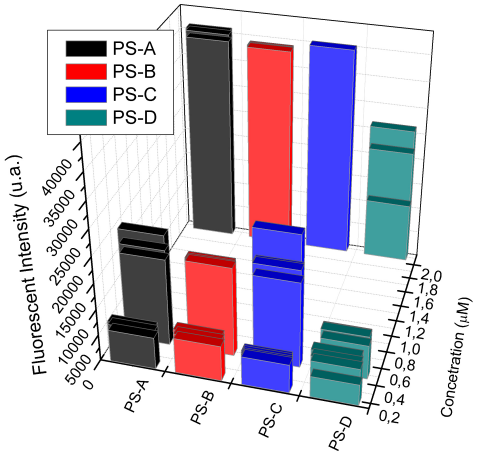


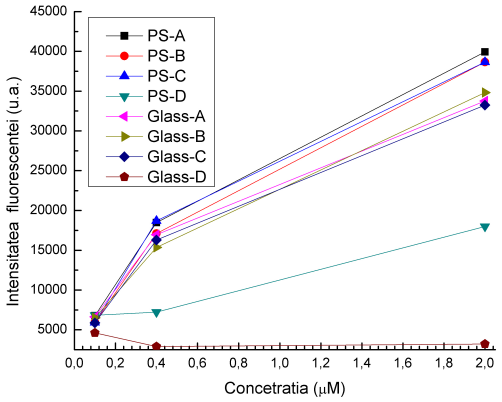
Design array

Hybridized array- fluorescent image



Scanned image of PS functionalized and glass with DNA hybridization





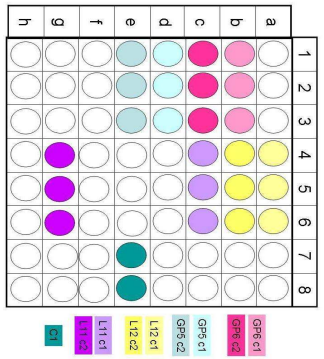
a) Reproducibility tests between arrays (fluorescence intensity as a function of the hybridized DNA); b) spot signals corresponding to tree different concentrations DNA for glass and PS surface

Results

DNA microarray

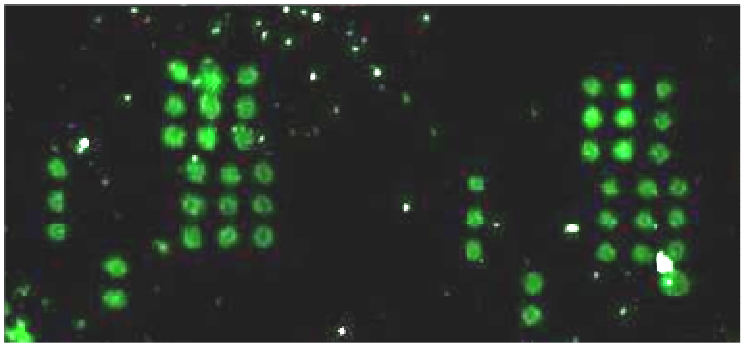
HPV –DNA sequence printed

Primer name	Sequence (5'–3')*a*
GP5+	5' TTT GTT ACT GTG GTA GAT ACT AC 3'
GP6+	5' GAA AAA TAA ACT GTA AAT CAT ATT C 3'
HPV L11:	5' TTT GTT ACT GTG GTA GAT ACT AC 3'
HPV L12	5' GAA AAA TAA ACT GTA AAT CAT ATT C 3'

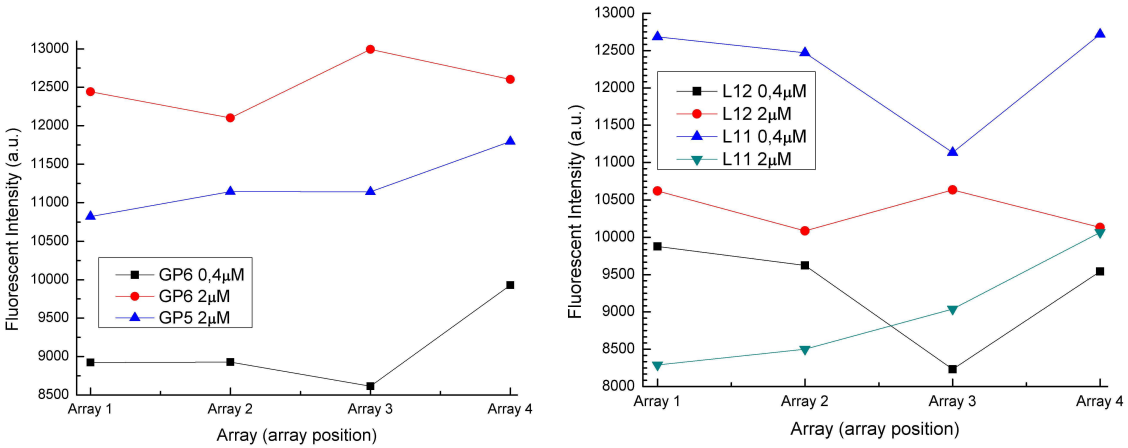


Design array

Scanned image after hybridization with DNA labeled with Cy3



We performed HPV DNA microarray hybridizations with human DNA probes labeled with fluorescent Cy3. DNA samples were selected from patients infected with HPV viruses confirm by diagnosis methods.



Fluorescent hybridization signal for: a) GP5-6 and b) L11-12

For the GP6-5 - DNA immobilized the signal is high and in accordance with the concentration. For the other two ssDNA (L11-12) relationship between concentrations immobilized on the surface is inversely proportional to the signal obtained this is due to high concentration of complementary probe leads to blocking the free bases and an incomplete hybridization