Aplicaciones de Litografía de Nanoimpresión

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Applications:

- DNA detection by stretching in nanochannels.
- DNA detection by electrochemical detection
- Biosensors for protein detection
- Tissue engineering
- Cells separation in blood
- Organic lasers
UV-Lithography
Thin Film Deposition (sputtering, e-beam evaporation)
Reactive Ion Etching and Deep Reactive Ion Etching
Electroplating
Bonding
Nanoimprint machine
Dicing
High resolution FE-SEM
SPM techniques
Mechanical and Interferential Perfilometry. High sensitive fluorescent microscopy.
Surface Plasmon Resonance equipment

90 square meters of class 100/1000 cleanroom
Approach towards NANOTECHNOLOGY

TOP DOWN

Controllably Accessible Area

10000
1000
100
10
1

Atomic World

Macroscopic World

nm

BOTTOM UP

objects

self-
assembly and organisation

supra-
molecular structures

atoms, molecules

microtechnique

thin film technology

submicron-techn.

lithography & pattern transfer
Fabrication Methods for Small Structures

Technologies for Structure Generation:
- Mechanical Tooling
- Optical Lithography
- Particle based Lithography
- Scanning Probe Lithography, Self-assembly

Resolution:
- 10^{-3}
- 10^{-6}
- 10^{-9} m

Typical Objects:
- Milli: Watch Components, LIGA-Gears
- Micro: Micro chips, Nano electronics
- Nano: Molecules
Thermal imprint lithography

1. Imprint
   - Press Mold
   - Remove Mold

2. Pattern Transfer
   - Oxygen RIE

\[ h_r = h_0 - h_{pr} \nu \]


The general idea behind the DNA stretching methods is to use nanofluidic channels to linearize double strands of DNA and read information. This information can be read either optically, by using fluorescent labels, or electrically, by including electrical probes inside the channels.

1) DNA detection by stretching in nanochannels
DNA is a long chain polymer (0.34 nm/bp) naturally coiled into a ball (few microns).

The DNA is a extremely long molecule (cm long), composed by sequences of nucleotides, that is naturally coiled into a ball of several microns to fit inside the cell nucleus. The current methods of analysing DNA requires chopping in shorter segments, replicating each segment a million times, sorting by size and piecing the original sequence back together in a time consuming and costly process. In contrast, Austin and Chou groups at Princeton developed the concept of stretching single molecules of DNA to analyse then.
Nanofluidic channels are currently being used for DNA stretching and direct visualization of the genomic length. This method provides important advantages such as rapid DNA restriction mapping in short times, reduction of the DNA sample down to single molecule genomic content, parallel analysis and more sensible detection.

\[ R_g = (PwL^3)^{1/5} \]

bulk solution

\[ R_g >> D > P \]

\[ L_z = L \left( \frac{pw}{D^2} \right)^{1/3} \]

0.34 nm/base pair

50, 100 and 200 nm line width and 80 nm in depth.


*When D>P, the molecule is free to coil in the nanochannel and the elongation is due entirely to excluded volume interactions between segments of the polymer greatly separated in position along the backbone (fig. a).

De Gennes theory developed a scaled argument for the average extension of a confined self-avoiding polymer

\[ r \approx L(wP/D^2)^{1/3} \]

*When D<P, the physics is dominated not by excluded volume but by the interplay of confinement and intrinsic DNA elasticity. In the strong confinement limit D<<p, backfolding is energetically unfavourable and contour length is stored exclusively in deflections made by the polymer with the walls. These deflections occur on average over the Odijk scale \( \lambda \approx (D^2P)^{1/3} \) and assuming that the average deflection made by the polymer with the walls is small, \( r = L[1-A(D/P)^{2/3}] \) (A≈0.36).


Log-log plot of the best power law fit
Advantages of using micro/nanofluidics:

- Short reaction time
- Reduction of the DNA sample (ng->fg)
- Parallel analysis and high sensitivity
- Uniform stretching (no external force)

Confined DNA (stretched)

Native DNA (coiled)

$D \approx 100\text{nm}$
Nanofluidic channels are currently being used for DNA stretching and direct visualization of the genomic length. This method provides important advantages such as rapid DNA restriction mapping in short times, reduction of the DNA sample down to single molecule genomic content, parallel analysis and more sensitive detection.

**Chips manufacturing process**

1. **Silicon**
   - Imprint NIL resist and residual layer etching

2. **Pyrex**
   - Deposit Pt and lift-off

3. **RIE of nanochannels**

4. **Photolithography and RIE of microchannels**

5. **Si thermal oxidation**

6. **Inlet and outlet laser ablation**

7. **Anodic bonding**
Silicon stamp with post and "channel-lines"

Top-view of the entry to the "channel-lines"

Pattern transfer on silicon

Cross-sections view of the nanochannels
Silicon-glass chip with electrodes defined in Pt on the pyrex.

Top-view of the nanochannels through the glass (63X in oil. NA=1.4)

Different packaging of the chip to introduce the DNA sample
Optical image of the filling of the device

Filling with a fluorescent solution of rhodamine B

Stretching of λ-DNA molecules (48502 bp = 16.5 μm) in nanochannels. Fluorescent label YOYO-1 (1:5) nicking recoil
Histogram of the measured extension lengths of a single \( \lambda \)-DNA molecule. The average extension length, based on an analysis of 300 consecutive frames, is 5.8 \( \mu m \)

Extension factor: 0.3

\[
L_{\text{ext}} \cong L_{\text{dye}} \left( \frac{w_{\text{eff}} P_{\text{dye}}}{D^2} \right)^{\frac{1}{3}} \quad \Rightarrow \quad D = 130 \text{ nm}
\]

The developed technology is a good alternative for the fabrication of biochips for single molecule DNA stretching applications.
Guías nanofotónicas
2) Detección de DNA mediante detección electroquímica

1. **Voltamperometría**: corriente electroquímica generada por reacciones red/ox en los dígitos.

2. **Espectroscopía de Impedancias**: medida de la resistividad y capacidad de la disolución en función de la frecuencia.
Fabricación de chips a escala micro y a escala nano
Detección de hibridación de DNA característico de escherichia coli

- Electrodomos comerciales/Electrodomos thin-films
- ¿Baja el LoD si electrodomos interdigitados nano?
- ¿Fiabilidad de la medida?. Comparativa con métodos ópticos.

Detección de proteínas y patógenos a través de la inmovilización de proteínas
3) BIOSENSORS FOR PROTEIN DETECTION

Biofunctionalised PMMA copolymer (Tg=86°C. Viscosity=6.7cp) with functional group: succinimidil imide.

- Imprinting
- Demolding
- Residual layer etching

Covalent binding between succinimidil imide and amino group of proteins

ELISA tests to check the differential affinity of protein adsorption between the copolymer and the antiadhesive layer coating

Stabilisment of optimal conditions

F<sub>13</sub>-TCS

Streptavidin protein adhesion

Antiadhesive layer coating with F<sub>13</sub>-TCS (tridecafluoro-(1,1,2,2)-tetrahydrooctyl-trichlorosilane) developed. α ~114°
A fluorescence labeled streptavidin Alexa-Fluor 488 conjugated was put on the i) micro (grating with 10 \( \mu \)m period and equal line width and space) and ii) nano (200 nm width lines spaced some micrometers) patterned areas of the surfaces.

**BIOSENSOR TO DETECT IgG**

- Rabbit anti-IgG with Alexa Fluor 488
- Biotinilated rabbit anti-immunoglobine antibody

**Threshold detection better than 200 ng/ml**

Selective binding of SAv to the activated surfaces is achieved by first binding a biotin linker with an activated carboxylic acid function to the formed amino groups. Binding tests were carried out with fluorescent labelled SAv. The fluorescence intensity was much higher in the plasma treated PS, while much less protein binding was found when the NH$_3$ plasma step was omitted.

Biotin linker
Sandwich-type immunoassay to detect the presence of the IgG protein on a sample

The developed immunoassay was used to quantify the IgG content of several solutions, yielding a detection limit at about 50 ng/ml.

Los SP son muy sensibles a cambios del índice de refracción en la vecindad de la superficie

Hay que inmovilizar proteínas en oro.

\[
 k_{\text{SPP}} = \frac{\omega}{c} \sqrt{\frac{\varepsilon_m n^2}{\varepsilon_m + n^2}},
\]

\[
 k_{\text{photon}} = \frac{\omega}{c} n_2 \sin \theta,
\]

\[
 \theta = \arcsin \left( \frac{n}{n_{\text{glass}}} \sqrt{\frac{\varepsilon_m}{\varepsilon_m + n^2}} \right).
\]
1) Secondary antibody
tNF
antiTNF
amine coupling
gold

Proteínas TNF-α e IL-6

5-500 pg/ml
Transmisión extraordinaria de luz a través de redes de nanoagujeros

Fabricación de las redes mediante nanoimpresión y deposición de oro.
4) TISSUE ENGINEERING

Morphological and biochemical changes of cells: (Tekniker-3B)

Objective: To study how the interaction between topography and growth factors (cytokines) on the extracellular matrix (PLGA, PCL, natural polymers based on polysacharides) lead to morphological and biochemical changes in cells (fibroblasts, osteoblasts, human bone marrow stem cells).

Surface area

<table>
<thead>
<tr>
<th>Pitch structures between 3 and 12 µm</th>
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</table>

<table>
<thead>
<tr>
<th>Surface area</th>
<th>Cell length</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.3 µm</td>
<td>1.3 µm</td>
</tr>
<tr>
<td>2.7 µm</td>
<td>2.7 µm</td>
</tr>
<tr>
<td>5 µm</td>
<td>5 µm</td>
</tr>
</tbody>
</table>

Z-axis (µm)
5 \mu m

2.7 \mu m

1.3 \mu m
Human bone marrow stem cells aligned on structured hyaluronic acid substrates

Strepatavidin and BSA proteins patterned on Hyaluronic acid substrates.

**ALP quantification from hBMSCs cultured on PLGA patterns under osteogenic differentiation conditions**

Alkaline Phosphatase

<table>
<thead>
<tr>
<th>ALP conc. (µM)</th>
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<tbody>
<tr>
<td>160</td>
</tr>
<tr>
<td>110</td>
</tr>
<tr>
<td>60</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>-40</td>
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</tbody>
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Blank and different patterned substrates
5) Cells separation in blood

Use of microfluidic devices to separate cells attending its size. The separation technique is based on the concept of displacement lateral deterministic (DLD), in which the stochastic process such as diffusion is minor.

1. Separation of white blood cells, red blood cells and platelets from blood.

2. Devices to enrich fetal cells from maternal blood, as a tool to perform a non-invasive prenatal diagnosis

\[ \varepsilon = \frac{d}{\lambda} = \tan \theta = \frac{1}{N} \]

Under certain simplifications and with \( N \) large and \( h \gg D_c \); \( D_c = \alpha 2G/N \) with \( \alpha = 1/(3N)^{1/2} \)
6) Organic Distributed Feedback Lasers

- **Pump** (optical): pulsed Nd:YAG laser (10 ns, 10 Hz) operating at 532 nm.

- **Active medium**: polystyrene doped with 0.5 wt% of a perylenediimide derivative (PDI).

**Amplified spontaneous emission (ASE)**

- **Pump intensity**
- **Active medium**
- **ASE**

**Presence of ASE**

**Stimulated emission**

**Output intensity/ Arb. units**

**Wavelength / nm**

$I_{out}$

$I_{Threshold}$

$I_{pump}$

Spontaneous Emission

Estimated Emission

$I_s/40$

**Graph**

- Low pump
- High pump
Basic elements

- **Pump** (optical): pulsed Nd:YAG laser (10 ns, 10 Hz) operating at 532 nm.

- **Active medium**: polystyrene doped with 0.5 wt% of a perylenediimide derivative (PDI).

- **Resonator**: distributed feedback (DFB) grating

Light propagating in a waveguide mode is scattered from the periodic structure to create a diffracted wave propagating in some new direction.

**Bragg condition**

\[ m \lambda_{Bragg} = 2n_{eff} \Lambda \]

\[ \Lambda = 368 \text{ nm} \]

**Pump intensity**

\[ \theta \sim 20^\circ \]

**DFB emission**

\[ \text{spot} \sim 1 \text{ mm}^2 \]
PhC lasers are pumped vertically from above with a Q-switched frequency doubled neodymium doped aluminium yttrium garnet laser at 532 nm.
Most remarkable properties

Lasing threshold

Photostability

Photoestability halflife $\tau_{1/2}$:
$\sim 3 \times 10^5$ pulses (10 ns, 4 $\mu$J·p$^{-1}$) at 10 Hz

*Pump: Nd:Yag (10 ns, 10 Hz) at 533 nm.

*Threshold: 3 µJ/pulse

*Photostability: more than 100,000 pulses (i.e. 3 h at 10 Hz) with pumping energy at 6 µJ/pulse
Last results: Wavelength tunability

**SiO$_2$ gratings**

\[ 2\pi \frac{h}{\lambda} \cdot \sqrt{n_f^2 - n_{\text{eff}}^2} - \phi_c - \phi_s = w \pi \]

“**Model h**”: waveguide of thickness $h$

“**Model h+(d/2)**”: waveguide of thickness $h+(d/2)$
Last results: Wavelength tunability gratings on doped PS

“Model $h$”: waveguide of thickness $h$

“Model $h+(d/2)$”: waveguide of thickness $h+(d/2)$
2D structures to reduce threshold of laser emission.
Follow-up

i) Use of polymer-semiconductor nanocomposites based on IV-VI nanoparticles emitting in the NIR region: CdHgTe, PbS, PbSe.

ii) DFB Biosensors