MINIATURIZATION OF ELECTROCHEMICAL-ASSISTED DNA DEPOSITION USING NANOBIOPLUME SYSTEM

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Electrochemical deposition using pyrrole chemistry has proven to be highly efficient for covalent grafting of oligonucleotides, proteins and oligosaccharides on gold surfaces. This technology has been mostly used for microarrays fabrication and SPR-assisted assays [1]. Spotting tools have been designed to enable the parallel electro-deposition of biomolecules at different scales: pipette tips first, then stainless steel pins, and more recently silicon cantilevers [2]. To further reduce spots size and increase density, nanotip cantilevers with piezoresistive force controller have been fabricated. Matrices of droplets of 1 µm in diameter were deposited using this so-called NanoBioplume spotter [3].

Nanotip-based deposition and piezoresistive force control were here combined with electrochemistry to obtain matrices of oligonucleotide spots using the polypyrolle technology.

The deposited solution was an aqueous electrolyte containing a mixture of pyrrole-conjugated oligonucleotides and pyrrole monomers. A potentiostat was used for applying a +2V potential between the nanotips and the conducting surface and for measuring the resulting current variations. As shown in figure 1, chronoamperograms obtained exhibited a conventional shape, suggesting that electro-polymerization correctly happened. Due to the very small electrode cross sections, currents were in the nanoamper range, which made it necessary to design a dedicated electrical connection set-up. During matrix spotting, polymerisation time was controlled by the contact time of the nanotips with the deposition surface.

Microscope images of the nanotips before and after the deposition process are displayed in figure 2. Thanks to the piezo-resistive force control, tips were not damaged during the spotting.

Bright field images of the spots were taken immediately after the end of the deposition, thanks to glycerol which prevented them from drying (figure 3, top). The correct alignment and spot uniformity were due to the automatic piezoresistive-assisted control of matrices deposition.

To finally validate the covalent grafting of the oligonucleotides on the surface, spots were incubated with their biotinylated complementary strands and then with phycoerythrin conjugated streptavidin. Fluorescent images confirm the presence of the spots (figure 4, bottom).

This experiment proves that polypyrolle electro-polymerization can be performed at the femtoliter scale. It thus opens the way to further investigation of localized electrochemical process and can be seen as a new flexible biomolecule nano-patterning technique.

References :

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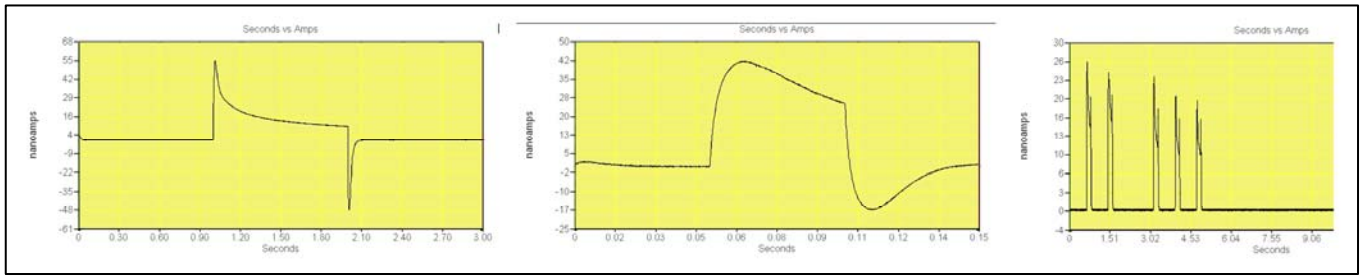
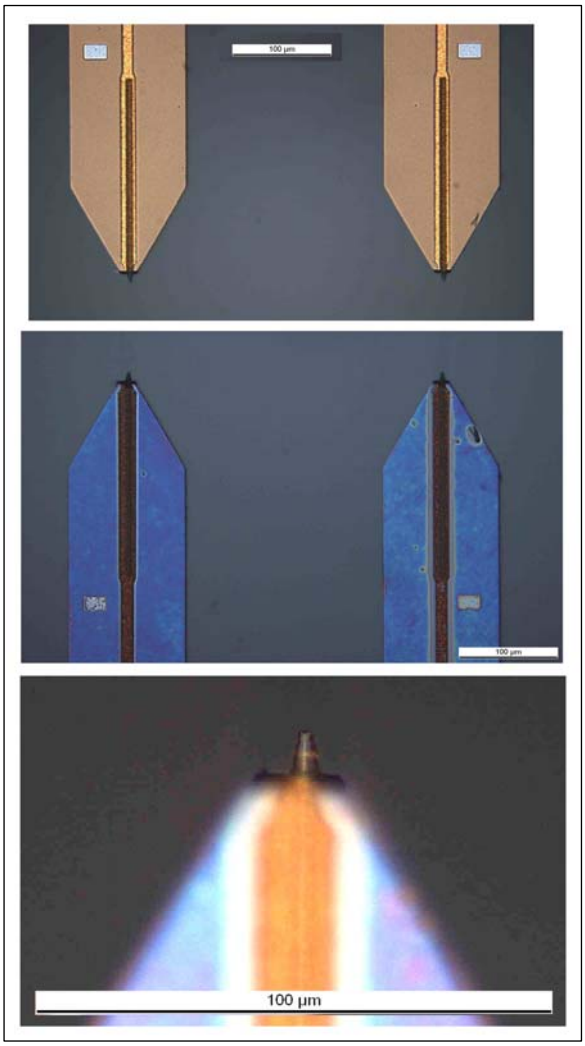
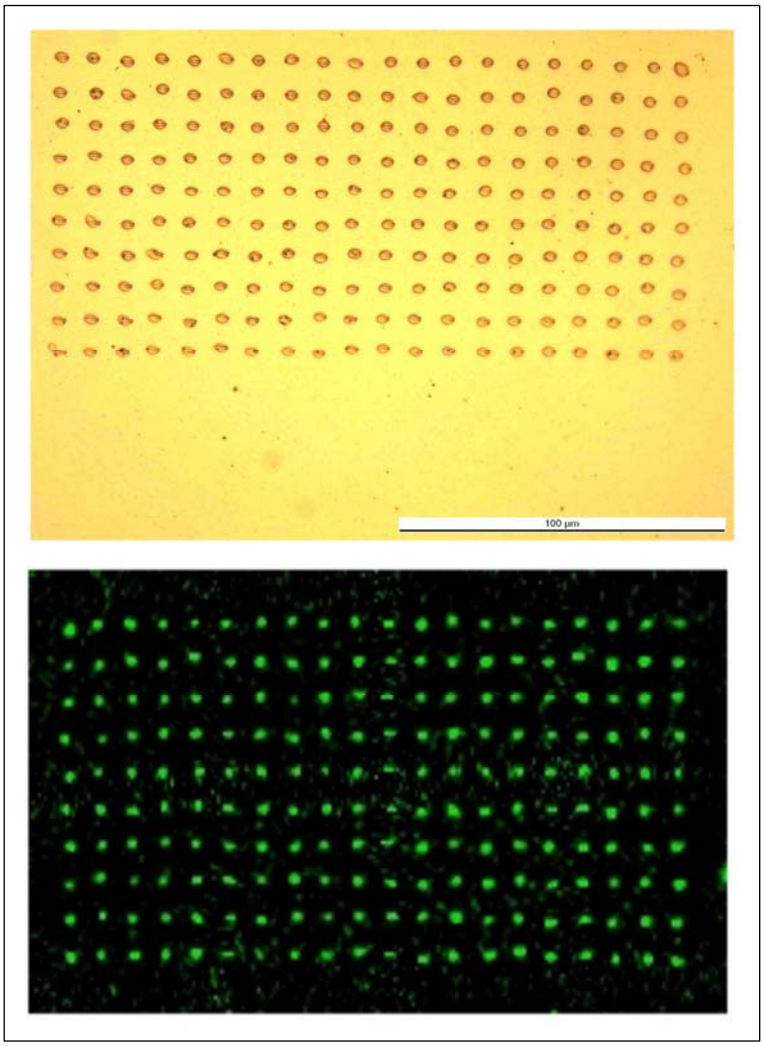


Figure 1: Chronoamperograms: current (nA) versus time (s)
From left to right: 1s polymerization, 50ms polymerization, deposition of a 5 spots matrix.
A +2V potential is applied between the surface and the nanotip.



Left / Figure 2: Optical microscope images of the nanotips.
top: before deposition
middle and bottom: after deposition.



Right / Figure 3: Matrix of 200 spots of oligonucleotides with 10µm interspot.
Top: Bright field image just after deposition.
Bottom: Fluorescence image after hybridization with complementary strand and revelation with phycoerythrin conjugated streptavidin