

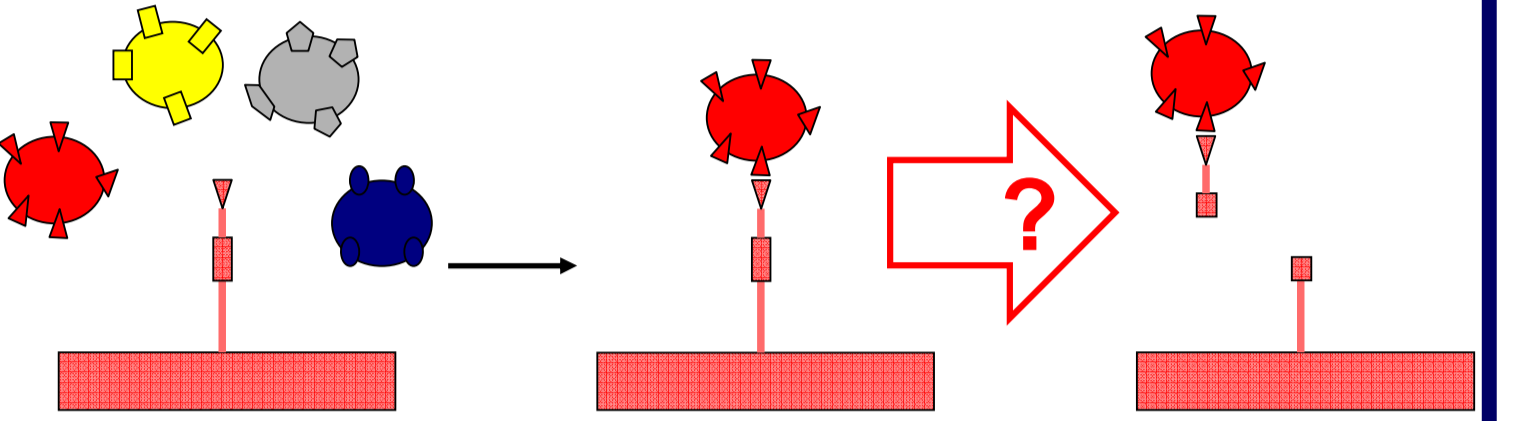
REVERSIBLE CELL CAPTURE ON A DNA BIOCHIP COUPLED TO SPR IMAGING

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Biochips for blood cell analysis

Biochips dedicated to cell analysis generates tremendous interests in the scope of developing new tools for both diagnostic and research purposes. The goals sought after are based on rapid analysis, high throughput, small sample volumes and use of bio-compatible materials to increase efficiencies of detection.

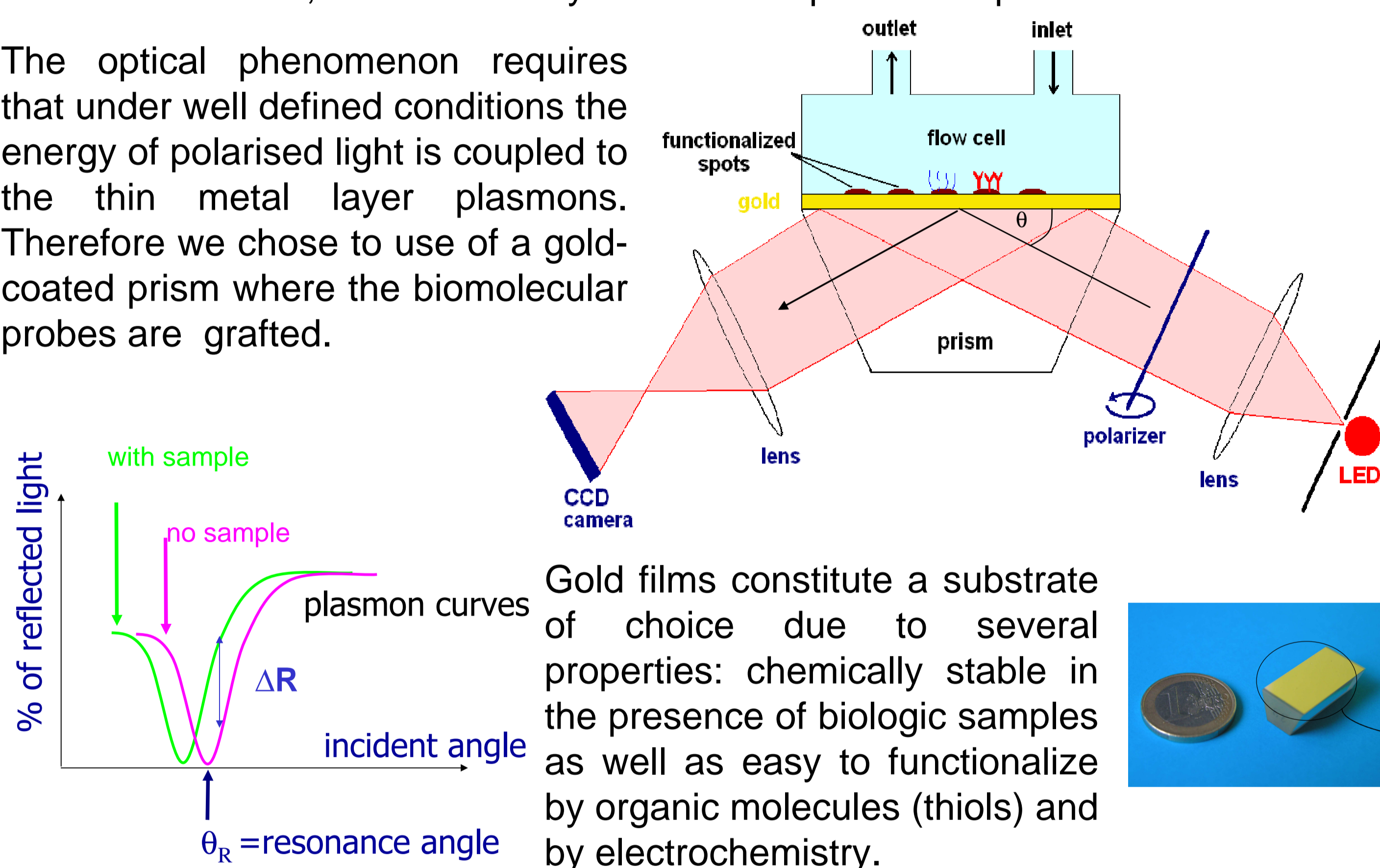
In the work we present, the aim was to develop a biochip system that ensures a selective capture and a controlled release of cells from the micro-array surface. The micro-platform was combined with the Surface Plasmon Resonance Imaging (SPRi) for the detection.



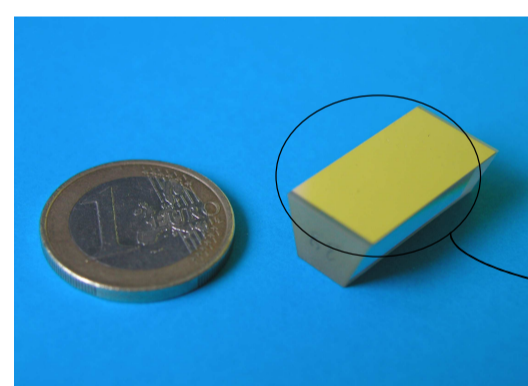
SPRi detection technique

The SPRi is a high-performance technique that allows to observe the biomolecular interaction. It combines many advantages for biosensing such as real-time detection, label-free analysis and multi-parallel response.

The optical phenomenon requires that under well defined conditions the energy of polarised light is coupled to the thin metal layer plasmons. Therefore we chose to use of a gold-coated prism where the biomolecular probes are grafted.

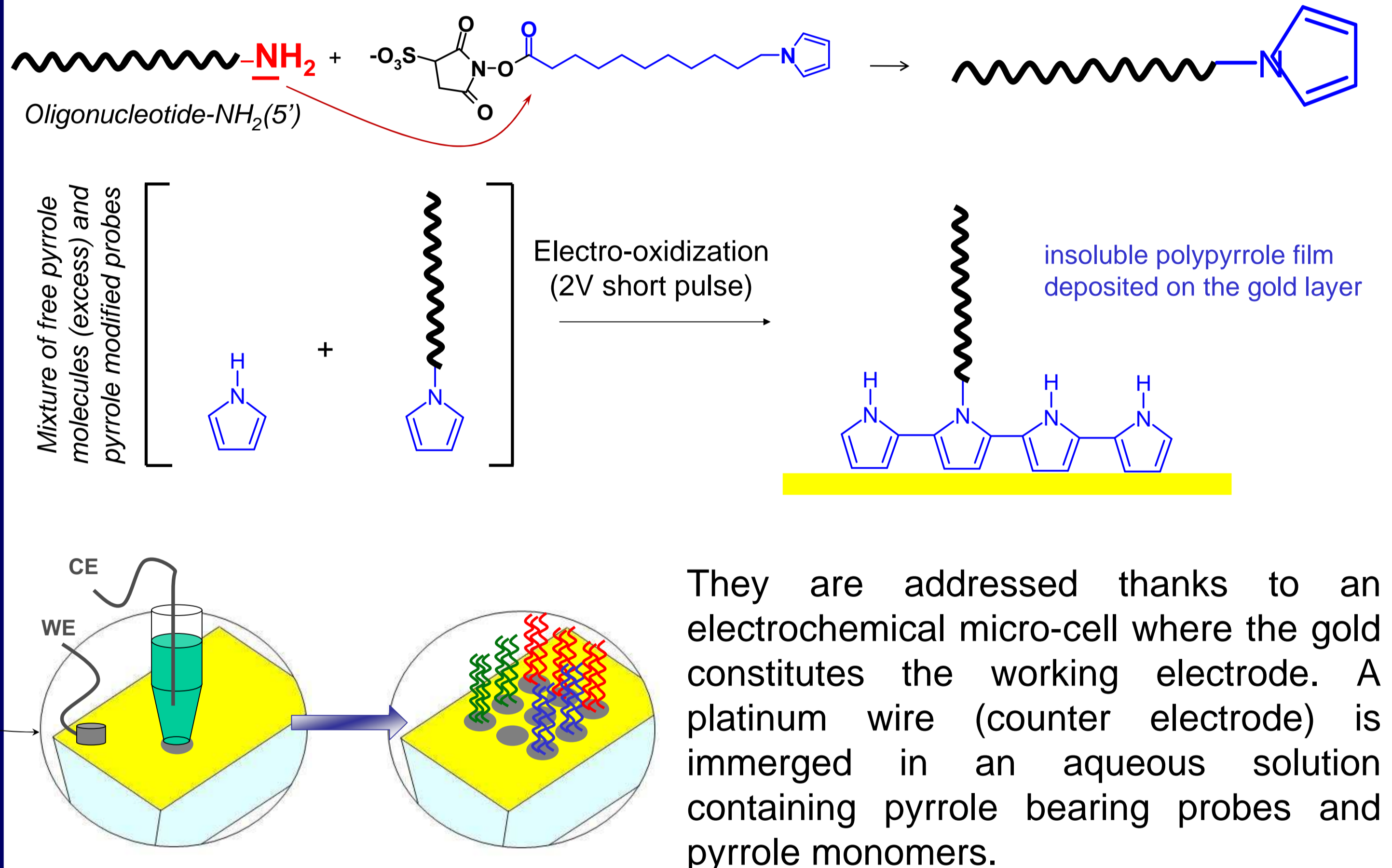


Gold films constitute a substrate of choice due to several properties: chemically stable in the presence of biologic samples as well as easy to functionalize by organic molecules (thiols) and by electrochemistry.



Biochip functionalization

Preparation of probe matrix is based on surface functionalization by polypyrrole films of nanometric thickness. The process includes an electrochemically directed copolymerization of pyrrole and pyrrole-modified probes.

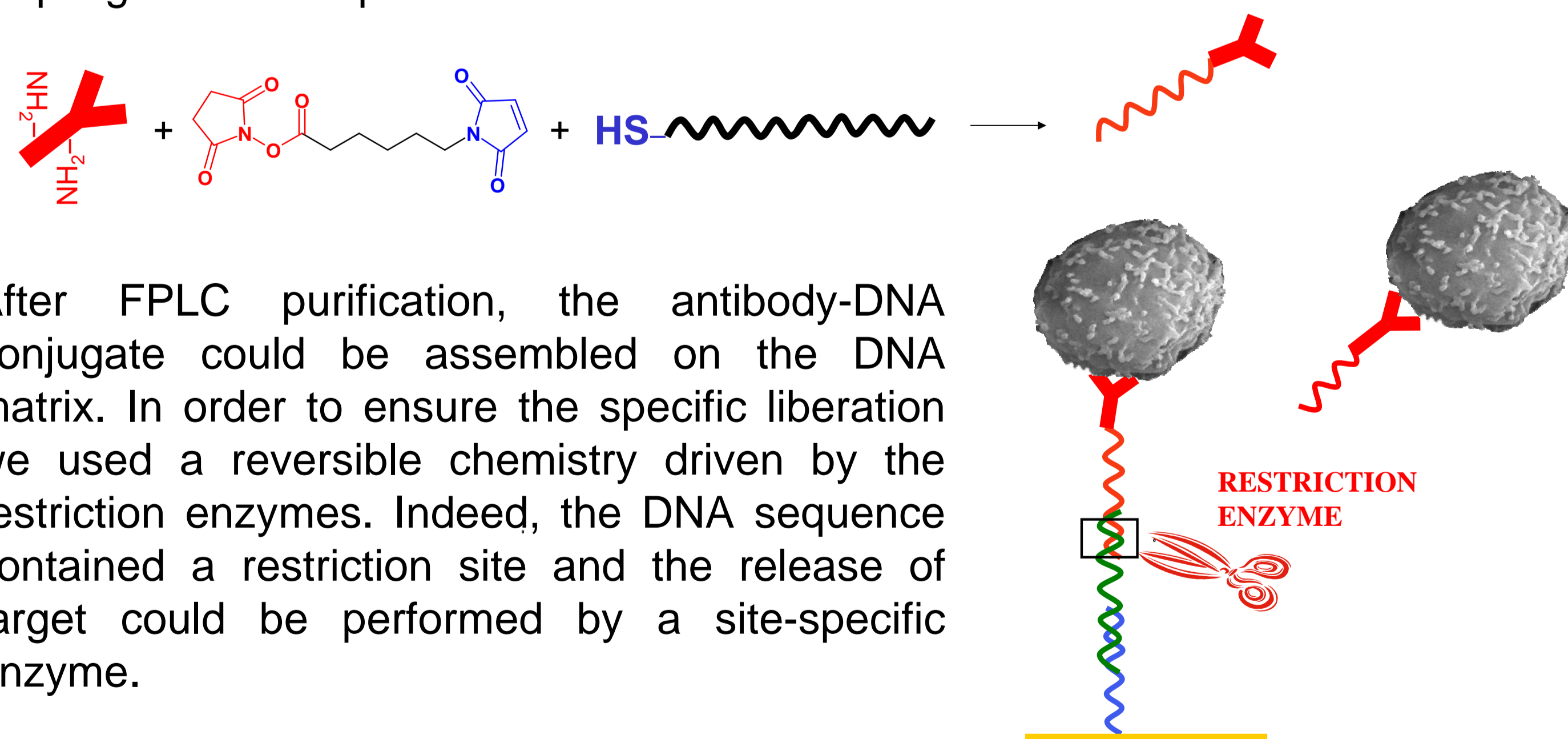


They are addressed thanks to an electrochemical micro-cell where the gold constitutes the working electrode. A platinum wire (counter electrode) is immersed in an aqueous solution containing pyrrole bearing probes and pyrrole monomers.

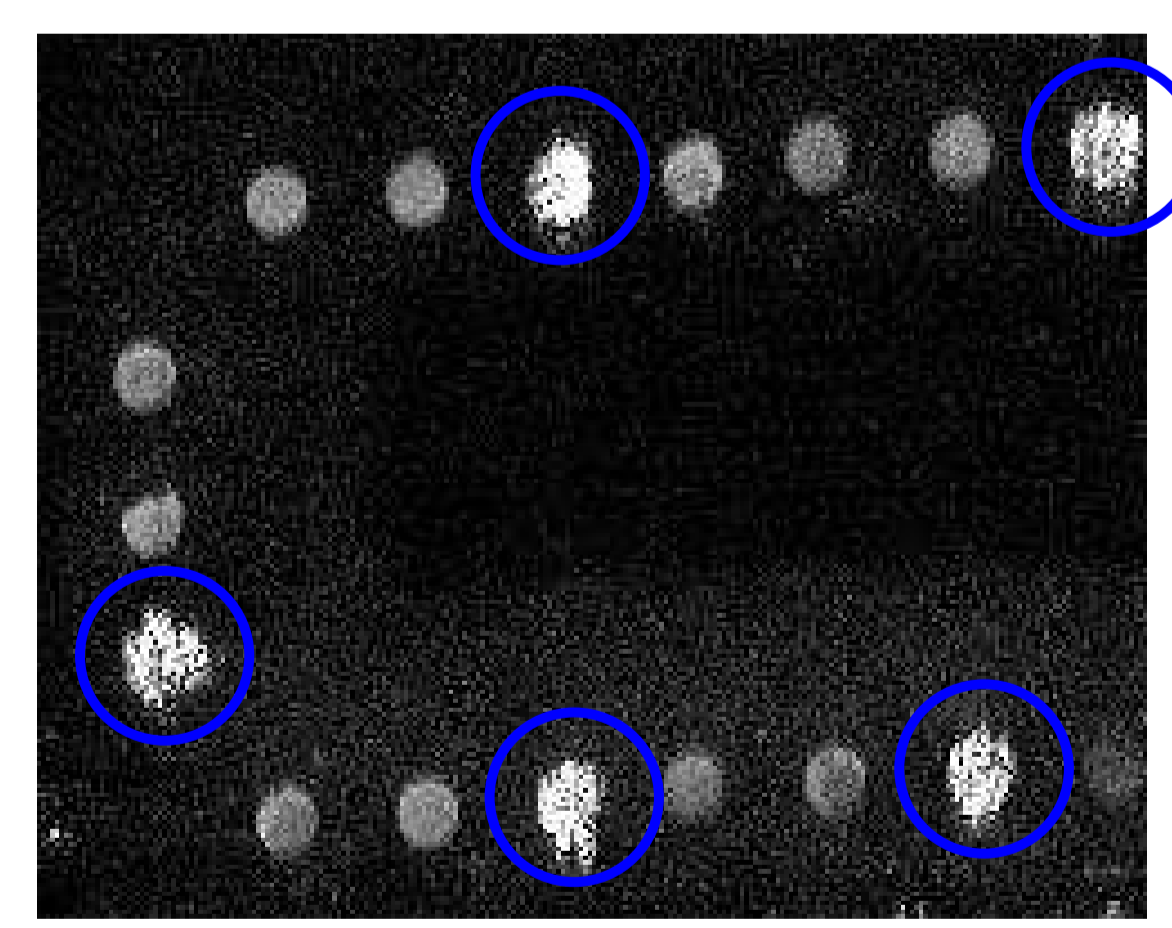
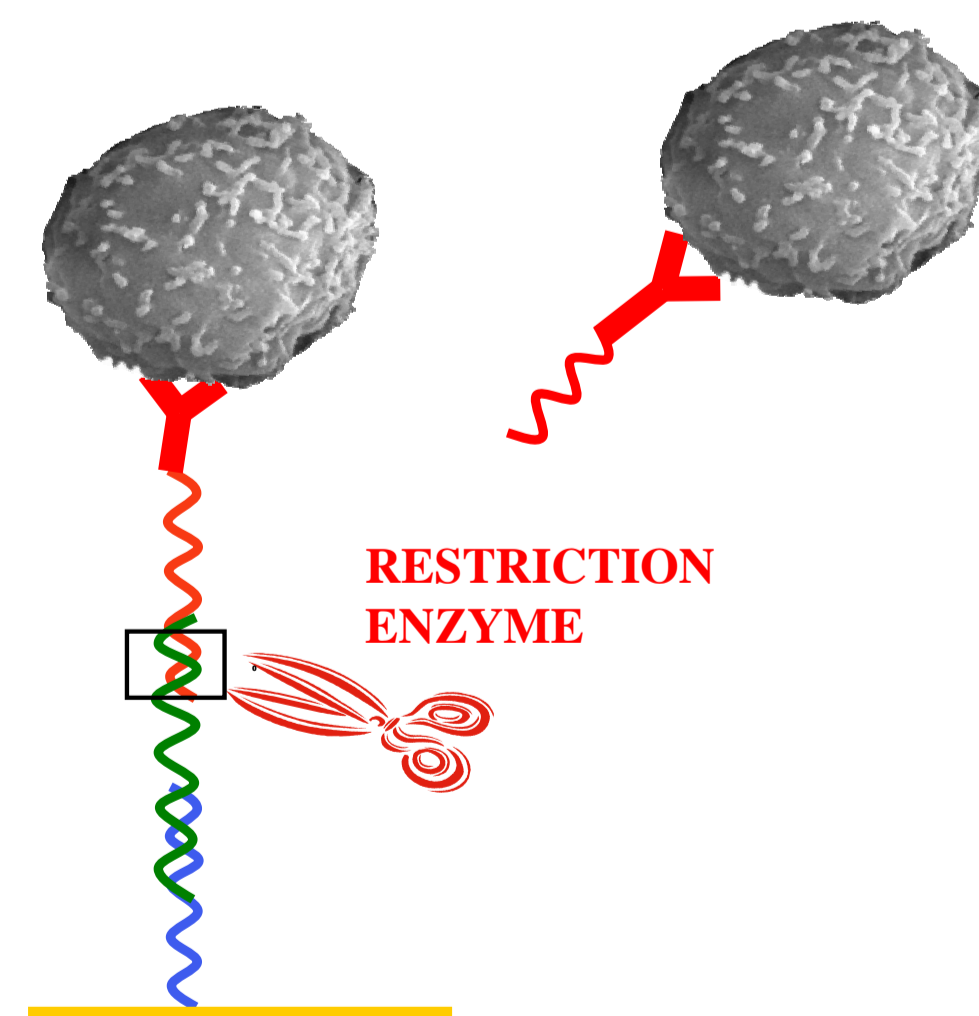
Living cell capture and liberation

Cell capture was based on the immunorecognition through specific antibodies. However, we used the DNA biochip since the DNA hybridization is a specific and precise process, so expected in the biosensors construction and programmable microassembly. Thus, the specific antibodies underwent the reaction of covalent coupling to DNA sequence.

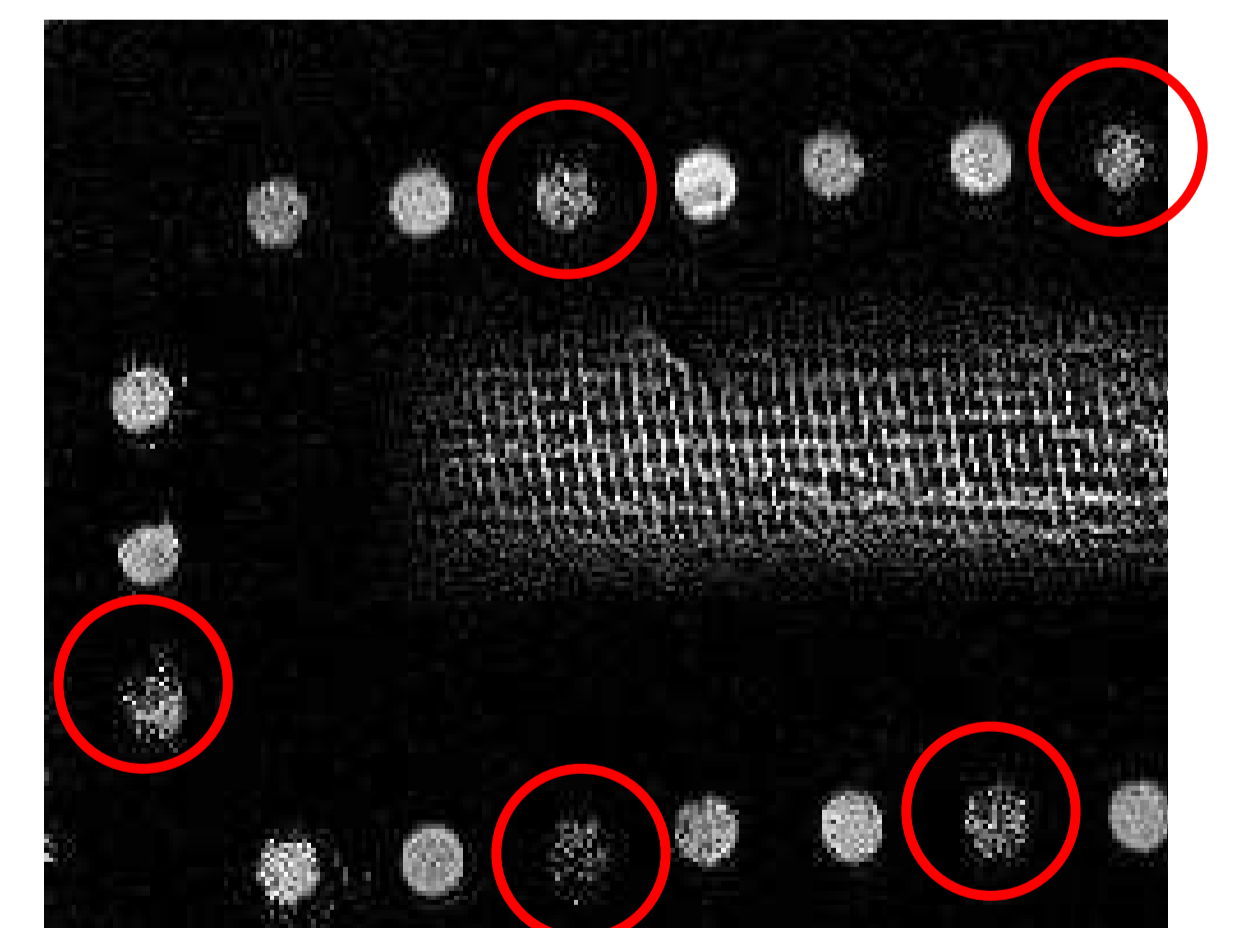
We finally demonstrated controlled capture and specific liberation of lymphocyte cells from biochip surface. For this purpose, we synthesized lymphocyte directed antibody-DNA conjugate to fulfill planned biochip assembly. We also adapted fluidics system in order to drive the cell flow over functionalized plots and to reduce non-specific interactions. Thus, the SPR imaging detection system enabled us to observe directly the immobilizing cells as well as their liberation by enzymatic cleavage.



After FPLC purification, the antibody-DNA conjugate could be assembled on the DNA matrix. In order to ensure the specific liberation we used a reversible chemistry driven by the restriction enzymes. Indeed, the DNA sequence contained a restriction site and the release of target could be performed by a site-specific enzyme.



Specifically immobilized cells



Cells released by enzymatic cleavage

Conclusions and Perspectives

This study has shown that a stable DNA biochip can be turned into the antibody microarray through the protein-oligonucleotide conjugate. Moreover, the specific assembly enables capture and controlled release of the living cells that can be monitored in the real time by SPR imaging. Apart from the enzymatic cleavage, we are going to use a physical target desorption bases on localised heating. Thus, we will apply the developed system in order to separate different cell populations and to characterise them in the term of their viability and homogeneity.