

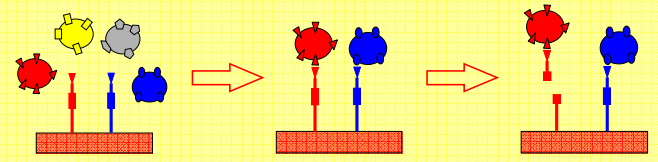
Reversible cell capture on a DNA biochip coupled to SPR imaging

Radoslaw Bombera¹*, Yoann Roupioz¹, Thierry Livache¹,
¹UMR 5819 (CNRS-CEA-UJF), INAC/CEA-Grenoble, Grenoble, France
^{*}Fondation Nanosciences, Grenoble, France (PhD financial support)
 contact: yoann.roupioz@cea.fr

Research background – The aim of project

The human blood is a source of particularly important information from the diagnostic point of view. Blood quantitative and qualitative analysis is a simple way to access different elements about the patient's pathological (or not !) condition. Blood component (cells, molecules) concentration monitoring gives as the essential data concerning numerous diseases.

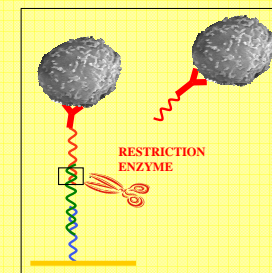
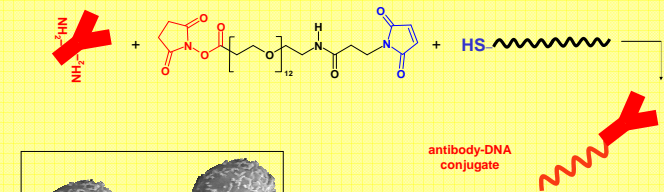
Present-day blood analysis methods involves high blood sample volumes and long analysis time. Therefore, the biochip has generated tremendous interests in the scope of developing new tools for both diagnostic and research purposes. The goals sought after are based on rapid analysis, high throughput and small sample volumes.



In this work, we developed a biochip system that ensures a selective capture and a controlled release of cells from the micro-array surface. The micro-platform is combined with the Surface Plasmon Resonance Imaging (SPRi) for the real-time detection.

Living cell capture and liberation

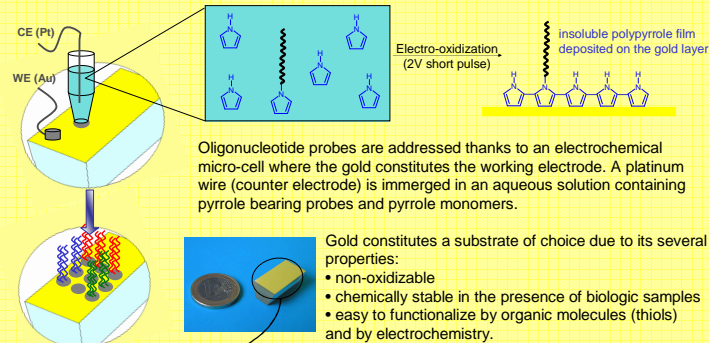
Lymphocyte B and T capture is based on the immunorecognition through specific antibodies. Since the assembly is based on the DNA biochip, the antibodies molecules are coupled to oligonucleotide sequence. The antibody-DNA conjugate is purified by anion exchange chromatography.



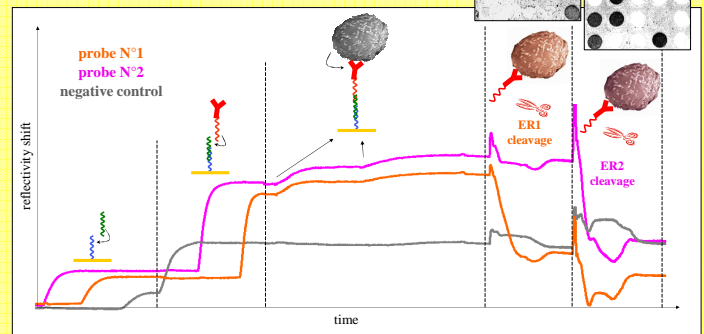
The specific hybridization of antibody-DNA conjugates enables a transformation of DNA biochip to protein matrix. Thus, the DNA biochip could be equipped with few antibodies directed to different cell type. The cell specific liberation is driven by the restriction enzymes - the DNA sequences contain the restriction site. Thus the site-specific enzyme can perform controlled release of the target.

Biochip functionalization

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



Site-specific enzymatic cleavage on the biochip assembly

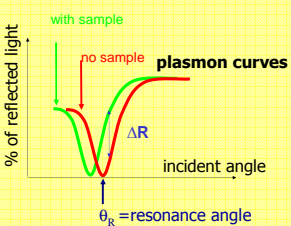


SPRi signal kinetics – B and T lymphocyte immobilization and two step enzymatic release

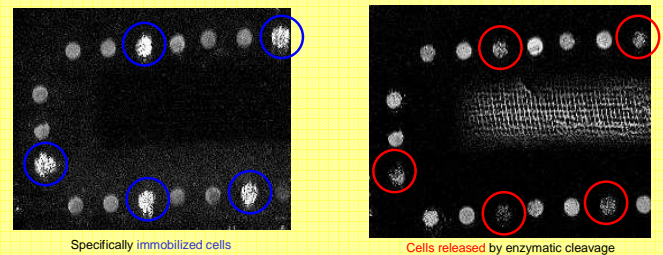
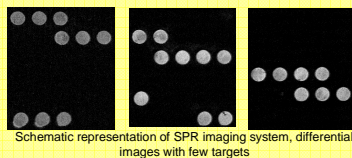
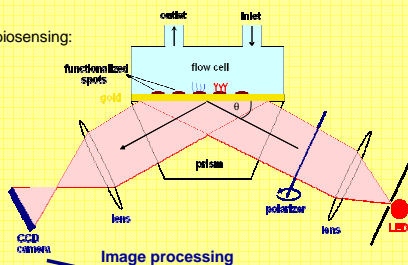
Detection by Surface Plasmon Resonance Imaging

The SPRi combines many advantages for biosensing:

- high-performance detection technique
- real-time detection
- label-free analysis
- multi-parallel response.



Since the signal is based on the optical phenomenon, the probe-target interactions are observed as reflectivity shifts.



In this case, fluidics system is adapted in order to drive the cell flow over functionalized plots and to reduce non-specific interactions. The SPR imaging detection system enable direct observations of the immobilizing cells as well as their liberation 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.