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### **Title:**

Ultraflat Gold Chips for Blood Particles Characterizations by Novel, SPRi & AFM, Hyphenated Techniques

### **Authors & affiliations:**

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**Abstract:** (Your abstract must use **Normal style** and must fit in this box. Your abstract should be no longer than 300 words. The box will 'expand' over 2 pages as you add text/diagrams into it.)

297 words

In the field of biosensors, some limitations occur at the  $\mu$ array scale depending on the substrate nanostructuration and the organization of ligands grafted on the biochip. However most of analytical methods are based on macroscopic responses without taking in consideration bio-molecular events at the nano-scale. To bridge these two dimensions, we developed a new generation of ultraflat gold chips which present high surface plasmon resonance quality. We have established the fabrication process in order to generate atomically flat terraces appropriate for biomolecular characterization by AFM while keeping plasmon phenomenon with optimal sensitivity. By this way, we recently demonstrated that the SPR monitoring of the immuno-chip building was confirmed by AFM investigations in terms of surface density and homogeneity of IgGs in the femtomole/mm<sup>2</sup> window<sup>1,2</sup>. For the first time, the counting of individual macromolecules by AFM on SPRi chips is feasible, which opens the way to a better understanding of critical phases in the biochip establishment. Moreover, it is a major interest to dispose of a successful "tool" to detect and characterize nano-bio-objects (as protein complexes, virus, particles...) enched in various biological samples, in order to qualify them.

Microparticles are produced from membrane of every cell types by a process called vesiculation. Circulating microparticles present in blood samples have emerged as a biological marker in several pathologies. Increase of circulating microparticles can be associated with autoimmune diseases or thrombosis. Thus the quantification of microparticles is crucial in various medical fields and requires new analytical solutions<sup>3</sup>.

In this presentation, first results of submicroparticles characterization in blood samples will be presented based on ultraflat arrayed chip bearing natural ligands and IgGs (Figure 1). This technique seems to be promising for the complete exploration and characterization of submicroparticles for which the usual techniques like flow cytometry fail in terms of sensitivity.

### **References:**

1) Elie-Caille C., Rauch J-Y., Rouleau A., Boireau W. Preparation of flat gold platforms for biomolecular characterizations. *Micro & Nano Letters*, 4 (2), 88-94, 2009.

2) Elie-Caille et al., in prep

3) Yuana Y, Oosterkamp TH, Bahatyrova S, Ashcroft B, Garcia Rodriguez P, Bertina RM, Osanto S. Atomic force microscopy: a novel approach to the detection of nanosized blood microparticles. *J Thromb Haemost* 2010; 8: 315\_23

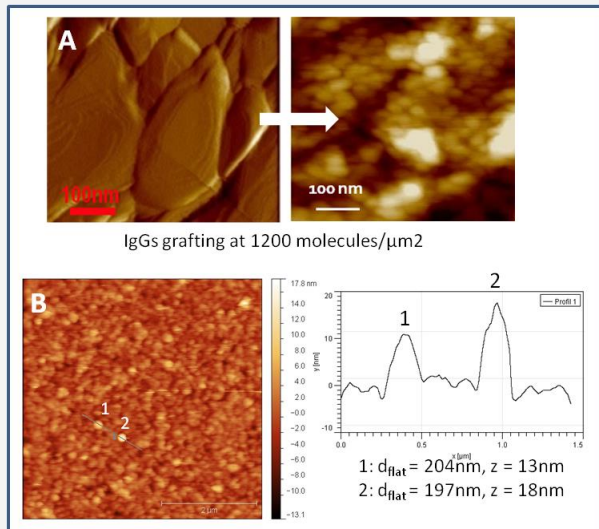
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Figure 1:



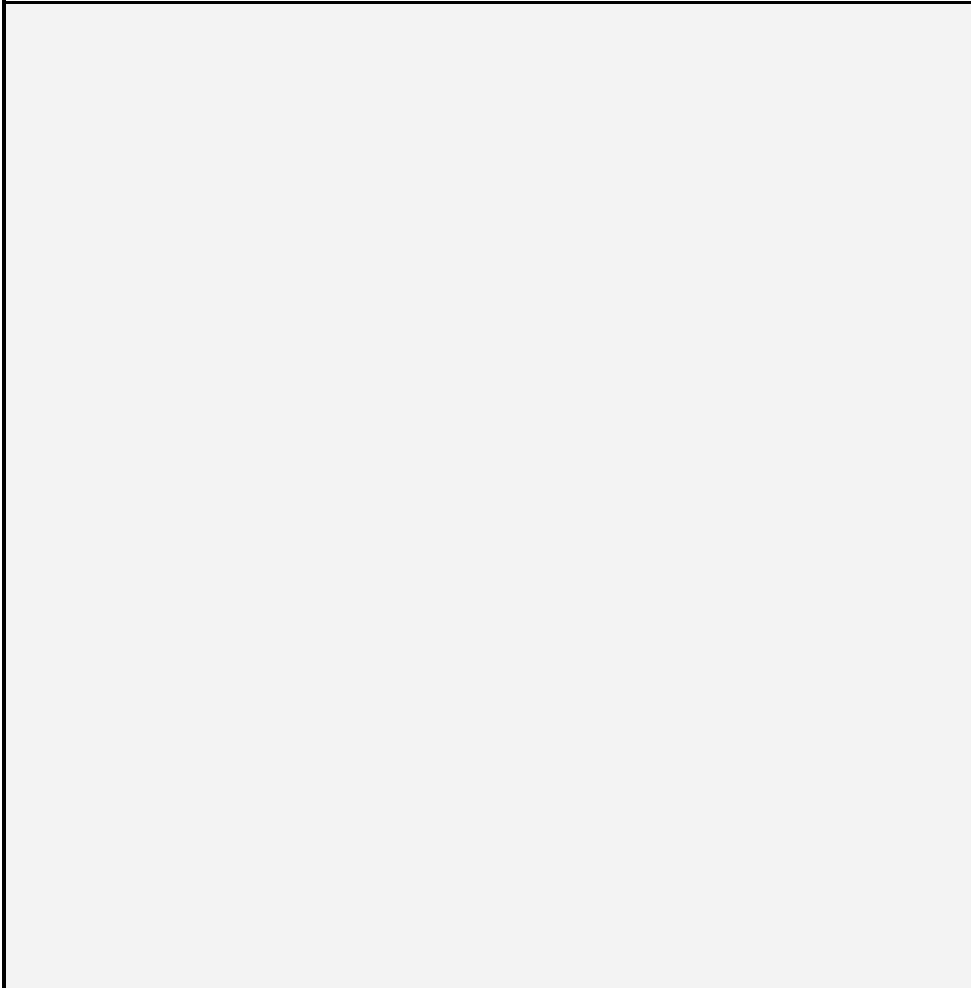
- A) Antibodies counting with AFM on ultraflat gold surface,
- B) Sub-microparticles characterizations with AFM after immunocapture from blood on SPR chip

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