# In Situ Probing of IgG Conjugated Gold Nanoparticles in Liquids by SEM and ToF-SIMS

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We report recent results of *in situ* observations of IgG conjugated gold nanoparticles in a novel portable vacuum compatible microfluidic device using scanning electron microscope (SEM) and time-of-flight secondary ion mass spectrometry (ToF-SIMS). The unique feature of the liquid flow cell is that the detection window is open to the vacuum allowing direct probing of the liquid surface. The liquid is held within the aperture by the surface tension. The flow cell is composed of a silicon nitride (SiN) membrane and polydimethylsiloxane (PDMS). We have demonstrated that it is fully compatible with vacuum operations for surface analysis. The aperture can be drilled through the 100 nm SiN using a SEM focused ion beam (FIB) or the primary ion beam in ToF-SIMS. Characteristic signals of the conjugated gold nanoparticles were successfully observed through the aperture by both energy-dispersive X-ray spectroscopy (EDX) in SEM and ToF-SIMS. Comparison was also made among wet samples, dry samples, and liquid sample in the flow cell using SEM/EDX. Stronger gold signal can be observed in our novel portable device by SEM/EDX compared with the wet or dry samples, respectively. Our results indicate that analyses of the nanoparticle components are better made in their native liquid environment, which is enabled by our unique microfluidic flow cell.

Figure 1 depicts the schematic of the three known types of cells used in imaging liquids. Immunoglobulin G (IgG) is an important antibody isotype in blood and extracellular fluid, which is also a main component in the immune system. The immunoconjugate nanoparticles can act as address tags or biosensors. It is widely used for biological and medical studies. To illustrate the potential usage of our device in biological analysis, colloidal gold reagents were chosen in this study. The details of device fabrication and interface assembly were described in our previous papers [1, 2]. The same procedure was followed to fabricate the microfluidic devices used in this study. Three protein-modified gold nanoparticle (mean diameter ~5 nm) solutions (SPI Supplies LLC.) were tested in this study. The proteins are goat anti mouse IgG (H+L), goat anti-rabbit IgG, and Strepavidin, respectively. The wet nanoparticles samples were prepared by depositing a small drop of a solution on a clean silicon wafer (100) (University wafer). The dry samples were prepared by drying a wet sample deposited on a silicon substrate under ambient conditions.

An FEI Helios Nanolab dual-beam FIB was used to drill the detection aperture through the SiN film. SEM measurements were performed using a FEI Quanta 3-D FEG dual beam microscope (Hillsboro, Oregon) at 20 kV. SEM was used in the high-pressure "environmental" mode (*e.g.*, ESEM) for the wet sample on silicon wafer. The dry sample on the silicon wafer and the liquid sample in the detection aperture are analyzed in the high vacuum mode, basically like any conventional SEM. Oxford

Instruments' Inca EDS (Energy Dispersive Spectroscopy) was used to obtain the intensity of elements at 20 keV and 0.43 nA. A ToF-SIMS V spectrometer (IONTOF GmbH, Münster, Germany) was used to probe the nanoparticles in this work. A pulsed 25 keV $Bi^+$ (beam size: ~250 nm) ion beam with an incident angle of 45 degree off the normal was used as the primary ion beam for all measurements. The SIMS measurements were performed at the beam current of ~1.0 pA with a beam width of 130 ns and a repeated frequency of 20 kHz. The main chamber vacuum pressure was $2\text{-}4\times10^{-7}$ mbar with our device inside the chamber, and the pressure only slightly increased to $3\text{-}5\times10^{-7}$ mbar during measurements. This indicates that no spraying or fast spreading of aqueous solutions occurs through the aperture.

Figure 2 depicts results from SEM EDX and ToF-SIMS. It is known that our knowledge about the detailed molecular structure of these nanoparticles interface is limited. We report that IgG conjugated nanoparticles in aqueous solution has been analyzed in SEM and ToF-SIMS via a novel microfluidic flow cell with a windowless detection area for the first time. Characteristic gold signals were observed. These signals observed through the aperture of the microfluidic flow cell device are stronger compared with individual wet or dry nanoparticle samples, demonstrating the advantage of conducting chemical imaging of nanoparticles *in situ* in their native liquid environment using surface sensitive characterization tools [3].

References:


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[4] The authors acknowledge funding from the PNNL Use of Facility Fund and the CII LDRD fund.


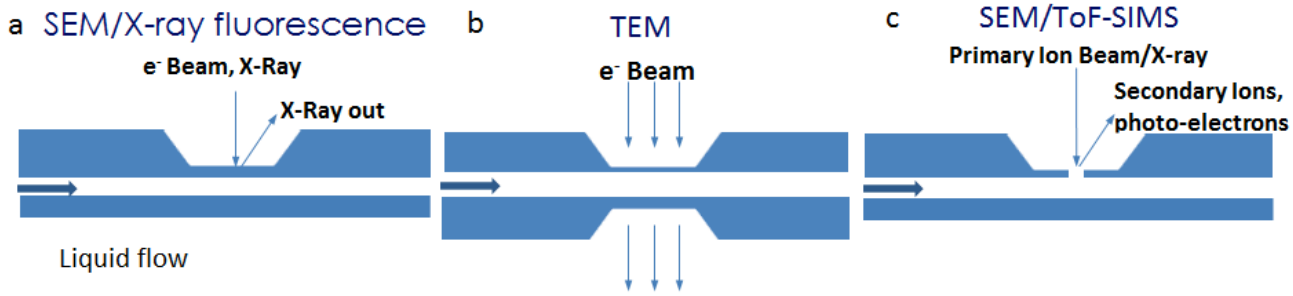


**Figure 1.** Three type of known liquid cells used in electron microscopy and spectroscopy.

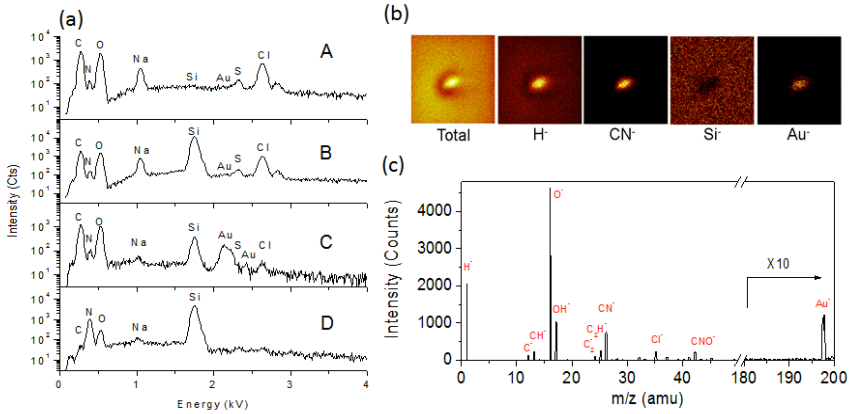


**Figure 2.** SEM/EDX (left) and ToF-SIMS (right) results of 5 nm IgG conjugated particles in liquids.