Engineering biosensors and nanoparticle based drug carriers using a novel photonic technology

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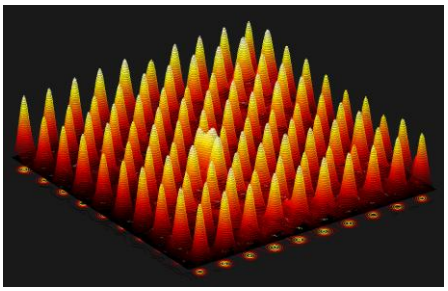
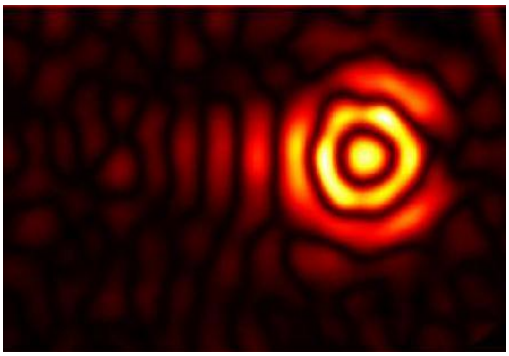
Abstract

Light assisted molecular immobilisation (LAMI) is a novel technology that results in spatially localised covalent coupling of a large variety of protein molecules and other biomolecules onto thiol reactive surfaces, e.g. thiolated glass/quartz, gold or silicon [1-4]. The reaction mechanism behind the reported new technology involves light-induced breakage of disulphide bridges in proteins upon UV illumination of nearby aromatic amino acids resulting in the formation of reactive molecules which will bind thiol reactive surfaces [5]. This new technology allows for dense packing of different bio-molecules on a surface (enzymes, antibody Fab fragments, cancer markers such as prostate specific antigen PSA) allowing the creation of multi-potent functionalised active new materials [2, 3, 6-9]. We have recently shown that the new photonic technology combined with the Fourier-transforming properties of lenses as well as with a simple millimeter scale feature size spatial mask allows for molecular immobilisation with diffraction limited resolution, achieving ~700nm resolved patterns of immobilised biomolecules [8, 9].

Protein microarray (1μm spots) carried out with LAMI

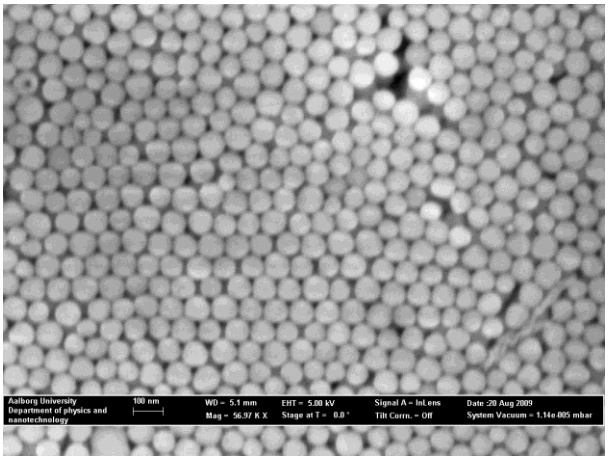




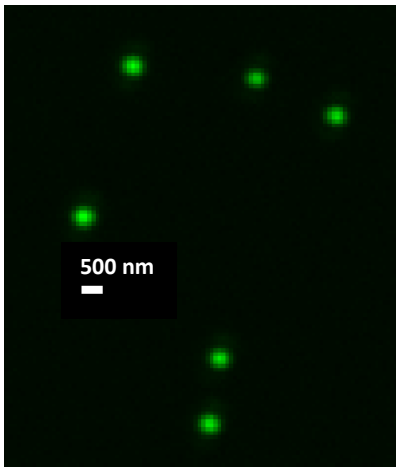
Fluorescence from proteins immobilised according to a diffraction pattern (700nm resolution)

LAMI technology is ideal to couple drugs and other bio-molecules to nanoparticles which can be used as carriers into cells for therapeutic purposes. We hereby show that this technique has the outstanding potential for nanomedicine allowing the creation of sensitive nanoprobes aimed at binding therapeutically interesting molecules. These nanoprobes have biological and medical applications such as bioseparation, biosensing and drug delivery. Thiol derivatised silica nanoparticles have been prepared. In order to explore the interesting magnetic properties of magnetite, superparamagnetic Fe_3O_4@SiO_2 Core-shell nanoparticles have also been prepared and functionalised with thiol groups. We have immobilised prostate specific antigen (cancer marker), human serum albumin, bovine serum albumin and insulin onto thiol derivatised nanoparticles using LAMI technology. All nanoparticles and the protein-nanoparticle bioconjugates were characterised with Dynamic Light Scattering, Scanning Electron Microscopy, Energy Dispersive X-ray spectroscopy, UV-Vis absorption

and Fluorescence spectroscopy, and Fluorescence microscopy. We are currently following the interaction between insulin immobilised onto superparamagnetic nanoparticle carriers with insulin receptor protein present in muscle cells and monitoring the induced cellular metabolic changes. Since magnetite based nanoparticles are magnetic in nature, we potentially can guide the molecular carrier to a particular cellular location by means of magnetic fields, being this way able of triggering an ON-OFF cellular response (work in progress).



SEM image of silica nanoparticles



Extrinsic fluorescence of PSA-AF555 immobilized onto thiol functionalized silica nanoparticles

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