PLASMONIC SUBSTRATES FOR METAL ENHANCED FLUORESCENCE

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Introduction

The quantification of serum biomarkers plays an important role in medical science. Biomarkers are used for risk stratification, detection of disease and therapy monitoring in patient cohorts in all clinical areas. Very often their concentrations in human blood are very low, which creates a permanent need for raising the sensitivity of the assay methods used.

Metal-enhanced fluorescence (MEF) is a promising technology to deliver the required highly sensitive tests [1]. It is well established, that MEF results from localized surface plasmons (LSPs) generated by the interaction of the excitation light with nanometer-sized noble metal structures, that dramatically increases the quantum yield of fluorescent molecules thus leading to highly sensitive detection systems (e.g. for fluorescent immunoassays) [2,3]. In Fig. 1 a typical MEF measurement setup is sketched.

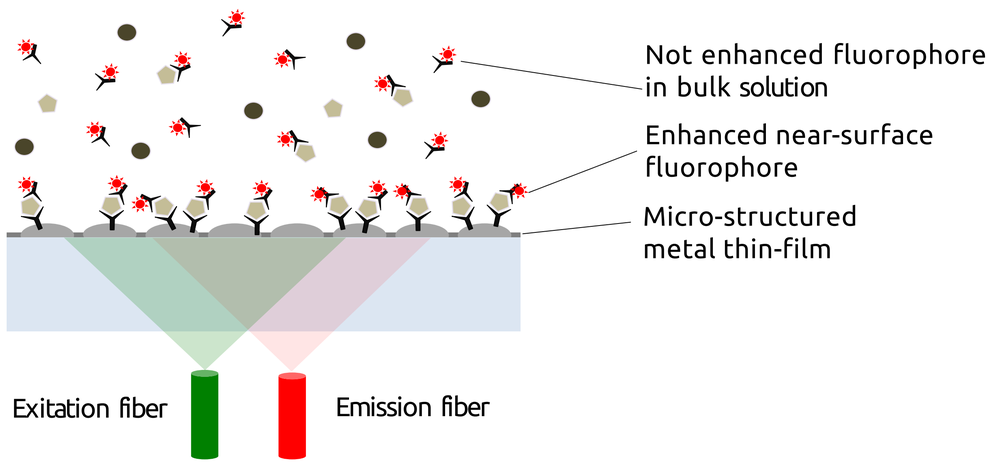

Fig. 1 Schematic representation of metal-enhanced fluorescence and measurement setup. The fluorescence of the near-surface fluorophore is drastically enhanced in contrast to the fluorophore in the bulk solution.

Design and manufacturing reproducibility of the required metal structures is the key for a) optimizing the enhancement effect and b) reliability of the assay system they are used for. This is why commercial application of this technology has failed so far.

STRATEC Consumables (former Sony DADC BioSciences) and FIANOSTICS successfully solved these problems by using highly reproducible nano-structuring technologies (patent pending), originally developed for Blu-Ray and DVD manufacturing. The manufactured MEF substrates were successfully used in the detection of fluorescence labelled antibodies and its application in fluorescence labelled immunoassays (MEF-FIAs) for biomarkers in human serum was demonstrated. This new MEF platform is compatible to any given assay format (e.g. microtiter plate, microfluidic chips, arrays or lateral flow devices).

Design of MEF substrate

The MEF substrate are polymer slides with a nanometer sized hexagonal arrays of well-like structures on the surface. The wells typically have diameters below 0.5µm, an aspect ratio of about 2 and a micron pitch. They are coated homogenously with several nanometer of Ag. By adjusting the geometry of the wells-array and the thickness of the Ag-layer the MEF effect can be optimized.

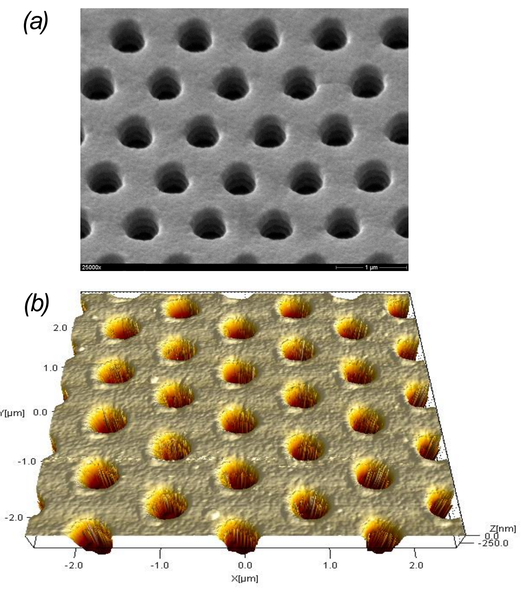

Fig. 2 (a) SEM image of an uncoated MEF substrate; (b) AFM measurement of the MEF substrate coated with several nanometer of silver

Manufacturing method

The master structures were manufactured by laser lithography, copied into a Ni stamp by electroforming and replicated in a disc format by a vario-thermal injection compression process. For the polymer a medical grade of cycle olefin was used. The disc were selectively coated with Ag-spots by a DC sputtering process and protected by a surface adhesive film to avoid degradation of the Ag layer. Subsequently slide in microscope format were milled out and laser-welded onto 96-well microtiter plates.

References

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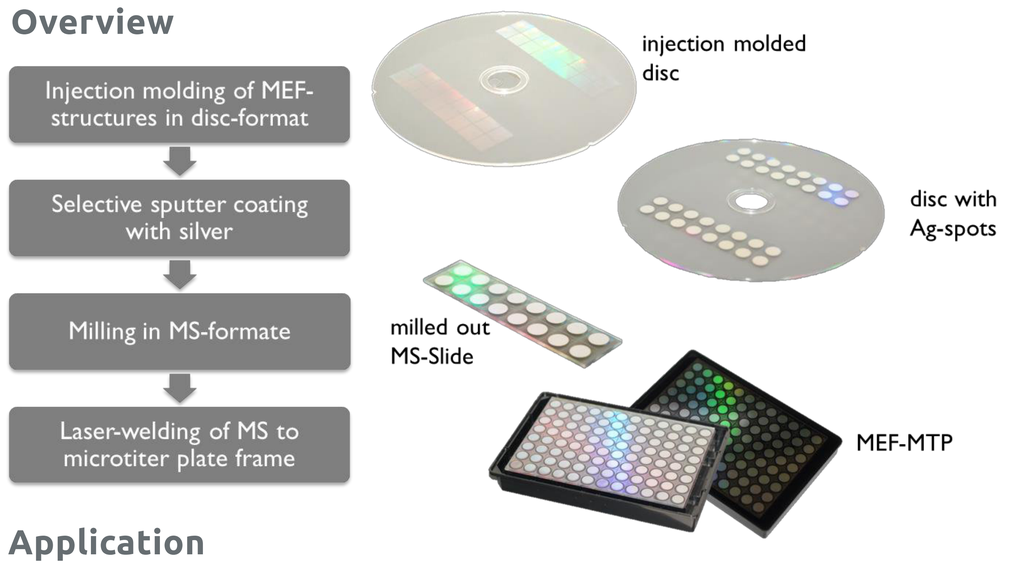
Overview



Application

To demonstrate the functionality of the MEF substrate, Cy5 labeled antibody (goat anti-rabbit) were adsorbed to the MEF substrate and measured with a commercial fluorescence microplate reader. The measurements were done in bottom configuration, meaning that excitation and emission through the bottom of the plate. Depending on the fluorophore, antibody concentration in the sub-picomolar range can be clearly detected (see **Fig. 3**).

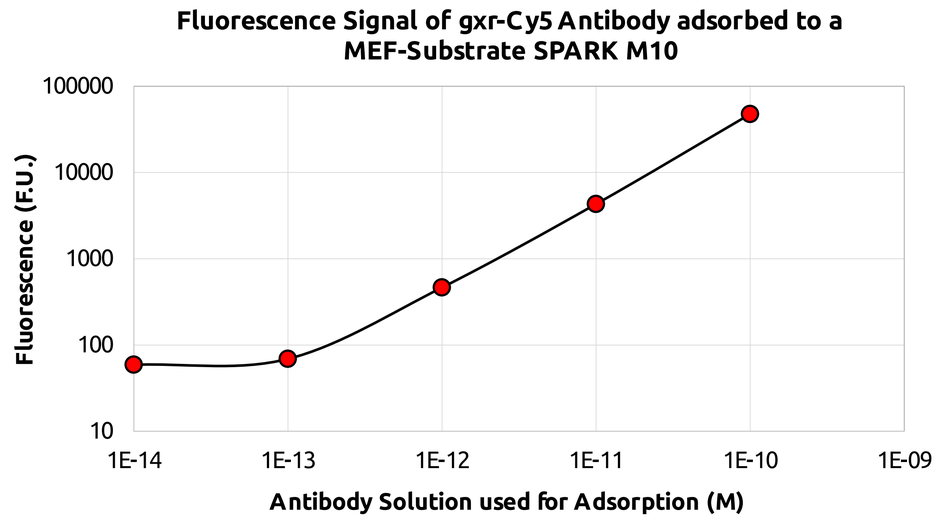


Fig. 3 Enhanced fluorescence signal of a Cy5 labeled antibody adsorbed a MEF substrate

MEF-FIA for NOGGIN and ASPORIN

The developed MEF-platform was used to develop fluorescence immunoassays for NOGGIN and ASPORIN two regulatory molecules which have a mayor impact on bone metabolism. NOGGIN acts as bone morphogenetic protein inhibitor and as such has an impact on bone/cartilage regeneration, limb development and fracture repair. From a clinical point of view the most promising area of research with NOGGIN as a biomarker may be it's involvement in the generation of bone metastasis [4]. **Fig. 4** depict a typical calibration curve for the NOGGIN assay with and without MEF enhancement.

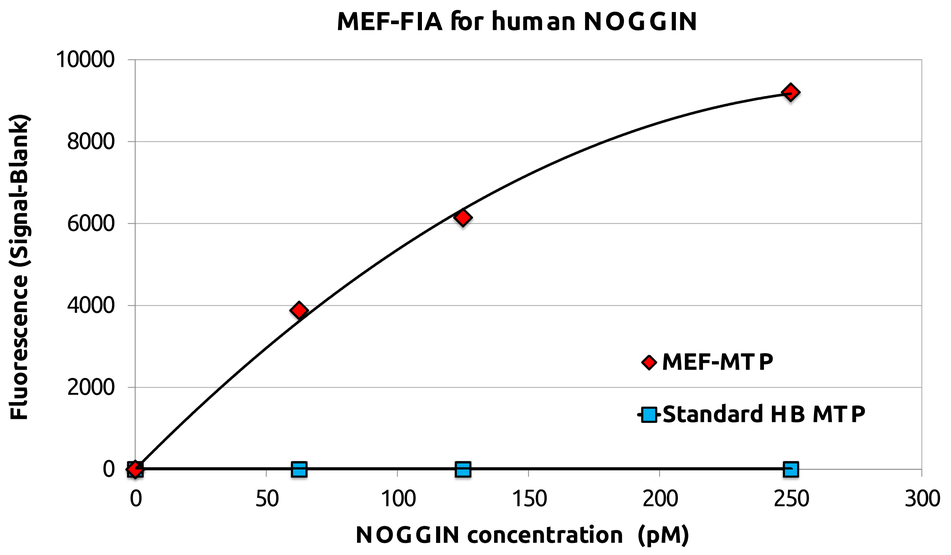


Fig. 4 Fluorescence Immunoassay for human NOGGIN protein performed on a MEF-MTP and on standard MTP showing no MEF effect

Summary and Outlook

The functionality of the MEF substrates could be successfully demonstrated in fluorescence labelled immunoassays for biomarkers in human serum. An enhancement of several orders of magnitudes could be achieved,allowing the quantification of biomolecules down to low picomolar and sub-picomolar concentrations. Preliminary tests have shown, that the MEF substrates can be also used for other fields application like SERS (Surface Enhanced Raman Spectroscopy).