

PO1070

Fluorescence Analysis for Amines on Plasma Functionalized Surfaces

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Amino groups on polymer surfaces are used as anchor groups in combinatorial chemistry for bioassays or microarrays. We present the fluorescence analysis of amino groups on polymer surfaces introduced by atmospheric pressure dielectric barrier discharge (DBD) microplasma using silicone-based plasma stamps. Our stamps produce functionalized spots with a diameter of 500 μm .

Reacting primary and secondary amino groups with fluorescent labels allows 3D intensity distribution measurements with sub-micron resolution. One of the first results is that the depth profile of the functionalization is independently of the plasma treatment time. It is about 1 μm . With the use of specific fluorophores a discrimination of primary and secondary amines was achieved.

We show that two-photon microscopy is especially suited for porous substrates with high sampling depths. With help of fluorescence lifetime measurements by ps-resolved fluorescence recording and suitable references absolute amino group concentrations, respectively densities were determined. Fluorescence anisotropy measurements enable insight into the molecular rotation of fluorescent labels. Altogether, fluorescence analysis provides valuable information about the substrate surface topography, amino group density and uniformity as well as the binding situation with the substrate in order to investigate fundamental interaction of plasma and substrate. This all together helps to optimize the plasma process to receive a homogeneously functionalized substrate.

A two-photon microscope (TPM) operating with a titanium-sapphire laser was used for time-resolved measurements of fluorescence decay. This method supplied more information about structured surfaces than can be gathered by fluorescence intensity and wavelength. With the use of an objective with 40-fold magnification and a numerical aperture of 1.3 resolution of surface features as small as 200 nm laterally and 800 nm axially were achieved.

Keywords

amino groups

fluorescence microscopy

surface topography

microplasma