Dip-in-Lens Femtosecond Laser Writing of Photoactivator-Free Proteinaceous Microstructures

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We report the dip-in-lens configuration of photoactivator-free fabrication of microstructures made from serum albumin proteins by femtosecond laser multi-photon direct write.

Amongst the 3D printing technologies, laser direct write of protein is an attractive method to create protein structures providing a complex biomimetic 3D microenvironment; such microenvironment is considered relevant for cell culture studies to understand and manipulate cell behavior by protein-cell-interaction. Similarly, protein binding selectivity in a complex 3D microenvironment might enable to enhance versatility of medical microfluidic devices. For the laser direct write of proteins, various photoactivators have been used so far to enhance the polymerization process. Some of photoactivator molecules are, however, unavoidably trapped to remain inside the protein microstructure, which is definitely undesirable for biological applications.

Here, we present our recent advances in fabrication of microstructures from pure protein without any photoactivator by direct laser write technique: Because we use high protein concentrations for photoactivator-free fabrication, light scattering and absorption within the protein solution create strong differences of exposure along the axis parallel to the laser beam (z-axis in our configuration). These differences in exposure might cause different feature sizes or cross-linking irregularities that lead to defects in the proteinaceous microstructure. Therefore, we utilize a dip-in-lens configuration to maintain a constant focal depth within the protein solution.

A 100x lens of numerical aperture 1.35 is immersed directly into the protein solution and slowly scans the desired path with a scanning speed of a few micrometers per second, as shown in Figure 1. Figure 2 indicates that photoinitiator molecules are trapped in bovine serum albumin (BSA) cross-linked with a photoinitiator, while no evidence of trapped photoinitiator for cross-linking without it.

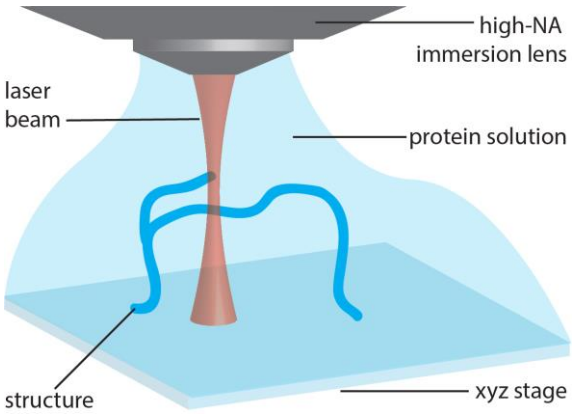

Figure 1: Schematic drawing of the dip-in-lens configuration. A 100x high-numerical aperture lens is directly immersed into the protein solution. At a fixed working distance from the lens the laser beam is focused. Within the laser focus, two-photon absorptions induces protein cross-linking. The light path is determined by the computer-controlled stage movement relative to the objective lens. As long as the immersion remains complete, the exposure condition is constant at the focus which enables homogeneous fabrication conditions.

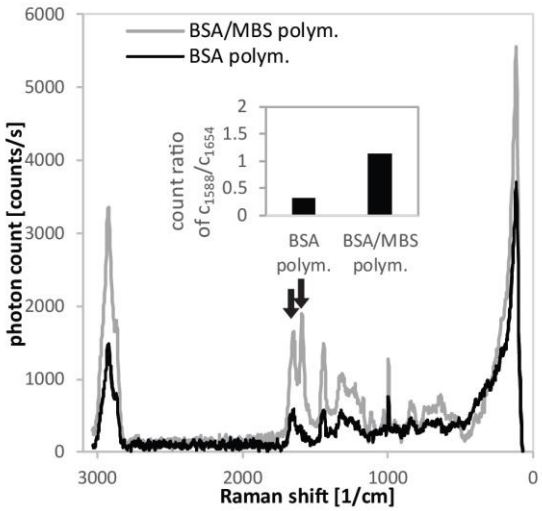

Figure 2: Preliminary raman spectra of BSA proteinaceous structures, pure (black line) and with (Sodium 4-[2-(4-Morpholino) benzoyl-2-dimethylamino] butyl benzenesulfonate) (MBS) photoiniator (grey line). Black arrows highlight the peaks that are used to evaluate MBS presence. A ratio of peak maxima is shown in the inset to account for individual sample signal strength.

Dip-in-lens laser direct write enhances the homogeneity within pure proteinaceous microstructures by maintaining the same exposure conditions throughout nearly arbitrary designs. The aspect of photo-activator-free fabrication is crucial for medical and biological applications where even the slightest traces of impurities represent a risk. We believe that the creation of pure proteinaceous microstructures from a vast diversity of available proteins offers many applications in lab-on-a-chip and total analysis systems.