Feature Size Analysis of Pure Proteinaceous Microstructures Fabricated by Femtosecond Laser Direct Write

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We report the model-based analysis of feature sizes of pure proteinaceous microstructures fabricated by femtosecond laser direct write based on multiphoton absorption.

Amongst the 3D printing technologies, laser direct write of protein is an attractive method to create protein microstructures, since they provide complex biomimetic 3D microenvironment. Specifically, such microenvironment is considered relevant for cell culture studies to understand and manipulate cell behavior by protein-cell-interaction. Furthermore, 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 [1]. Some of photoactivator molecules are, however, unavoidably trapped to remain inside the protein microstructure, which is definitely undesirable for biological applications. Recently, we developed a new technique to create pure proteinaceous microstructures using photoactivator-free, pure protein precursor [2].

Here, we present the analysis of the feature sizes in height and diameter dependent on fabrication conditions. We consider a previously applied model for photoactivator-induced fabrication of protein to remain valid because the model is based on the light-induced radical generation and includes all kinetics of solvent, protein concentration and chain reactions in the fitting parameter alpha. The role of the photoactivator is now substituted by the monomer and the simple model considering the transition to radical due to two-photon absorption remains relevant.

Fig. 1 shows preliminary height measurements by scanning electron microscopy (SEM) of structures fabricated from glycerol-water dissolved bovine (BSA), mouse (MSA) and human serum albumin (HSA). For the two-photon absorption, it is expected that the affected precursor material in volume becomes smaller than the focal volume [3]. After such absorption processes, further chemical reactions are required to cross-link protein molecules into a fabricated microstructure. The details of the cross-linking mechanism are not well-understood. To gain insight, we discuss our findings on features sizes depending on not only laser properties, but also precursor materials.

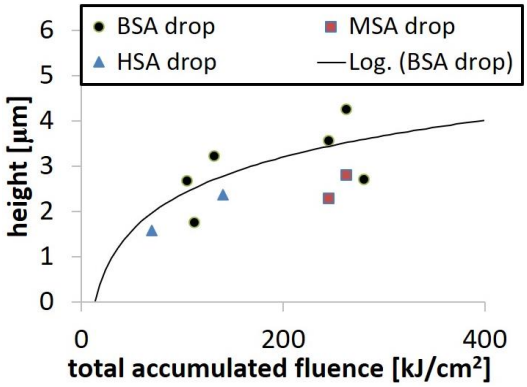

Figure 1: Feature size in height measured from SEM images for BSA, HSA and MSA fabricated from levelled glycerol-water mixtures. Numerical aperture is 0.46, estimated refractive index is 1.425. Solid line is a logarithmic fit.

Further, Fig. 1 shows that feature sizes between the different variants of serum albumin are comparable. This result might relate to the high conservation of protein structure for serum albumin variants. It is advantageous to have comparable feature sizes for different variants because fabrication can be optimized in the available variants.

With a model-based analysis, we aim to improve the feature size prediction and thereby improve the device fabrication for future biomedical applications.

References

[1] D. Serien, and K. Sugioka, *review* Opto-Electronic Advances 1(2018), 180008
[2] D. Serien, M. Abe, M. Terakawa, H. Kawano, A. Miyawaki, K. Midorikawa, and Koji Sugioka, in preparation
[3] K. Sugioka, and Y. Cheng, *review* Appl. Phys. Rev. 1(2014) 041303