Femtosecond Laser Direct Writing of Microstructures Made from Red Fluorescent Protein

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We report microfabrication of bright red fluorescent protein mScarlett by femtosecond (fs) laser direct writing (LDW). In the recent decades, light-induced multi-photon cross-linking has been applied to fabricate microstructures of proteins, but only limited types of proteins have been demonstrated for fabrication. Meanwhile, we recently reported cross-linking a natively green fluorescent protein, enhanced green fluorescent protein (EGFP), a protein which is widely used for labelling and bioimaging.

Here, we demonstrate the fabrication of layered microstructures made of bright red fluorescent protein mScarlett. mScarlett also is a natively fluorescent protein used for labelling and bioimaging. We want to broaden the applicable fluorescence wavelength range and understand the circumstances of fluorescence retention. After fabrication, we study the fluorescence with regards to native fluorescence retention and fluorescence observations based on intrinsic fluorescence. For this study, we investigate fabrication parameters laser power, scan speed and total accumulated fluence as well as imaging parameter exposure time. We believe our findings will contribute to 3D fabrication of fluorescent protein as biomarkers in vicinity of a living cell.

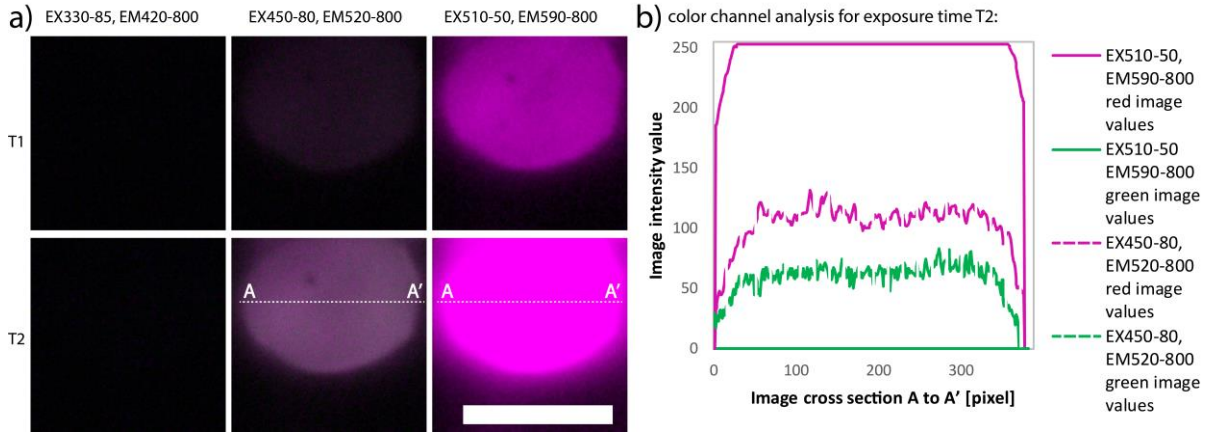

Figure 1 Fluorescence images of mScarlett microstructure (red is shown as magenta)

a) For two different exposure times of ms range, T1<T2, fluorescence images are shown with the indicated excitation (EX) and emission (EM) conditions. Note that due that emission ranges overlap partially, while excitation wavelengths are defined sharply. Red is substituted by magenta to assist the human eye. Scale bar represents 10 μm. A-A' indicates the cross section for image value profiles. b) Image intensity profile along the cross sections A-A' for exposure time T2 where the fluorescence signal of mScarlett saturated at intensity value 255 as shown (magenta, solid). For excitation with 450-80nm, red (magenta, dashed) and green (green, dashed) image values are recorded.

We successfully fabricated microstructures from mScarlett and as Figure 1 shows the native red fluorescence was retained. With increased exposure time, we observed green fluorescence signal underlying the red fluorescence, as shown in Figure 1b. The corresponding image in Figure 1a appears grey without individually identifiable green elements which indicates the co-localization of the green signal. By comparison with microstructures of EGFP and bovine serum albumin, we consider this underlying signal to be intrinsic fluorescence.

The study of femtosecond laser microfabrication from fluorescent proteins is important to reliable fabricate not only the desired design but also retain the desired fluorescence properties. Fluorescent microstructures might give a new tool to biological cell studies for intracellular tracking.