show the kinetics of antigen and antibody binding in real-time without signific-
ificant contribution of signal from background fluorescence. We also show how single molecule analysis allows determination of the labeling efficiency of the antibody bound to the surface. By analyzing the bleaching steps of individual fluorophores at a given location, we can determine the number of dye molecules attached to randomly labeled antibody conjugates. Our data using this method indicates a bias towards antibody labeled with less fluorophores. Finally, we show single molecule detection of sub-picomolar concentrations of antigen using well characterized antibody reagents.

3424-Pos  Board B579
3D-Super-Resolution Microscopy Reveals mRNA Nano-Structure in Stress Granule
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mRNAs play critical roles in gene expression with various regulations. Under stress, cytoplasmic mRNAs assemble and form stress granules (SGs), where mRNAs play critical roles in gene expression with various regulations. Under stress, cytoplasmic mRNAs assemble and form stress granules (SGs), where they are remodeled for repression of translation. However, the details of the fine structure of SG and the assembly process of mRNA have not been understood, which hinders the comprehension of physiological role of SG.
We investigated these issues by stochastic optical reconstruction microscopy (STORM), which provides super-resolution images with spatial resolution of ~20 nm in the lateral direction and of ~60 nm in the axial direction. Furthermore, we performed three-dimensional super-resolution imaging using cylindrical lens. To visualize endogenous cytoplasmic mRNAs, we microinjected Cy5-labeled linear antisense 2-0-methyl probes into the cytoplasm of COS7 cells. After the injection, cellular stress was induced by addition of 0.5 mM ar-
senite in a culture medium. To investigate the maturation of SGs, STORM im-
age were captured at various time-points during SG formation.
Three-dimensional super-resolution images showed that endogenous mRNAs were located in spherical compartments with a diameter of ~200 nm. Since these compartments were densely packed within several micrometers radius, we could not observe these structures by diffraction-limited imaging. We termed this structure “mini-granule”. With stress duration, mini-granules increased in number, while they maintained the same size. These data demonstrated that the growing process of SGs resulted from the assembly of mini-
granules. The result of this study indicated that mini-granules were responsible for the physiological functions of SGs.

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Crossing the Border towards Deep UV Time-Resolved Microscopy of Native Fluophores
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More than 20 years ago, single photon counting based techniques evolved as one recognized standard in fluorescence detection. In combination with confocal microscopy FLIM (Fluorescence Lifetime Imaging Microscopy) and FCS (Fluorescence Correlation Spectroscopy) became established techniques for in-
vestigations down to the single molecule level. Up to date, these experiments typically are carried out in the visible up to the near infrared spectral range. Based on recent advances in fiber amplified laser technology [1] and ultrasensitiv-
tive detection, we present a novel approach to extend time-correlated single photon counting (TCSPC) into the deep UV using 266 nm excitation. Hereby, direct access is granted to the native fluorescence of biomolecules originating from appropriate chromophoric groups such as the amino acids tryptophan and tyrosine within proteins. As first results, we will present label-free FLIM of cells where the aromatic amino acids within the proteins become visible. As a benchmark, also FCS with organic fluorophores in the deep UV will be shown.
Another application of time-resolved fluorescence microscopy in the deep UV includes microfluidics and thus enables label-free detection and identification of various aromatic analytes in chip electrophoresis [2, 3]. Fluorescence decay curves are gathered on-the-fly and average lifetimes can be determined for differ-
ent substances in the electropherogram with the aim to identify aromatic compounds in mixtures. Based on the time-correlated single photon counting the background fluorescence can be discriminated resulting in improved signal-to-noise-ratios. In addition, microchip electrophoretic separations with fluorescence lifetime detection can be performed with protein mixtures empha-
sizing the potential for biopolymer analysis.

References:
[1] Schoenau et al., Biomedical Optics (BIOMED), Miami, Florida, 2012