show the kinetics of antigen and antibody binding in real-time without significant 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 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 us 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′-*O*-methyl probes into the cytoplasm of COS7 cells. After the injection, cellular stress was induced by addition of 0.5 mM arsenite in a culture medium. To investigate the maturation of SGs, STORM images were captured at various time-points during SG formation.

Three-dimensional super-resolution images showed that endogenous mRNAs 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 investigations 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 ultrasensitive 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 different 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 emphasizing the potential for biopolymer analysis.

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3426-Pos Board B581

Localization Precision for Asymmetric Single-Molecule Images in Superresolution Localization Microscopy

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We present theoretical localization precision formulae for asymmetric single-molecule images in superresolution localization microscopy. Superresolution localization microscopy, such as photoactivated localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM), have demonstrated superior performances in cell imaging and enable the investigation of cellular processes at close to the molecular scale. All these techniques rely on the precise localization measurements of single-molecules at the nanoscale by using statistical estimators to fit diffraction-limited single-molecule images with the theoretical point spread function (PSF) of the imaging system, which is commonly approximated as a two-dimensional Gaussian. However, to our best knowledge, all previous theories [e.g., R. E. Thompson et al, Biophys. J. 82, 2775 (2002) and R. J. Ober et al, Biophys. J. 86, 1185 (2004)] on theoretical localization precision are developed for circularly symmetric single-molecule images. In contrast, many of the recent advances in the developments of localization microscopy have demonstrated that astigmatism can occur and result in asymmetric PSFs as a result of optical aberrations in the imaging system [S. Quirin et al. Proc. Natl. Acad. Sci. USA 109, 675 (2012)] and asymmetric molecular emission [K. I. Mortensen et al., Nat Meth 7, 377 (2010)]. Asymmetric PSF has also been implemented in localization microscopy to achieve astigmatic imaging for three-dimensional single-molecule localization techniques [B. Huang et al., Science 319, 810 (2008)]. Therefore, our new theory for asymmetric single-molecule images can be particularly useful where asymmetric PSFs have been used or observed in localization microscopy.

3427-Pos Board B582

3D STED Microscopy in Scattering Specimens

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By breaking the classical diffraction limit, Stimulated Emission Depletion (STED) Microscopy has revolutionized far-field fluorescence microscopy. 25 nm resolution and better have been achieved in two dimensions imaging cultured cells and even neurons in the brain of living mice. 3D super-resolution has also been demonstrated utilizing two opposing objectives or phase filters with a top-hat profile (see Figure), its application to tissue has however been hampered by aberrations introduced by refractive index inhomogeneities.

Here we present our latest results in 3D STED microscopy of scattering specimens enabled by the integration of adaptive optics into a custom STED microscope. We will present our current research about the physical and technical concepts of adaptive optics STED microscopy as well as the latest biological applications of adaptive optics STED microscopy.

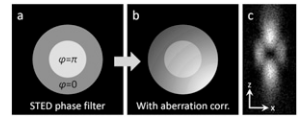

Figure. Schematic of 3D STED Microscopy with aberration correction. A top-hat phase filter (a) is modified to compensate for sample-induced aberrations (b) to achieve an optimized 3D depletion profile (c).

3428-Pos Board B583

STED Microscopy with Time-Gated Detection:Benefits and Limitations

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In a stimulated emission depletion (STED) microscope the region in which fluorescence markers can emit spontaneously shrinks with continued STED beam action after a singular excitation event. This fact has been recently used [1] to substantially improve the effective spatial resolution in STED nanoscopy using pulsed excitation, continuous wave (CW) STED beams and by sorting photons depending by them arrival-times (time-gated detection). We present theoretical/experimental data that characterize the time evolution of the effective detection volume of a STED microscope and illustrate the physical basis, the benefits, and the limitations of this new STED implementation, namely gated CW-STED (gCW-STED). Among all the STED implementations, gCW-STED provides the highest effective resolution at low light intensity and is in essence limited (only) by the reduction of the signal that is associated with gating. Time-gated detection also strongly reduces the influence of local variations of the fluorescence lifetime on STED microscopy