Here we demonstrate that SPIM-FCS/FCCS possesses the sensitivity to detect and quantify protein-protein interactions in live cells by characterizing the interaction of the subunits of heterodimeric transcription factors, c-Fos/c-Jun and IQGAP/cdc42. The protein-protein interaction clearly shows up in the cross-correlation amplitude. Analysis of the spatial distribution of diffusion coefficients of the fluorescent proteins and of their cross-correlation amplitude shows that formation of the heterodimer is correlated with regions of decreased mobility, probably related to DNA binding.

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Super-Resolution Fluorescence Imaging Reveals Nanoscale Organization of Stress Granule

Ko Sugawara[1], Kohki Okabe[1,2], Akihiko Sakamoto[1], Takashi Funatsu[1].
[1]University of Tokyo, Tokyo, Japan, [2]JST, PRESTO, Tokyo, Japan.

In response to environmental stresses, cytoplasmic mRNAs assemble and form stress granules (SGs) accompanied by several proteins affecting mRNA functions. SGs have been proposed to play an important role in mRNAs remodeling for repression of translation. However, the underlying mechanism remains unclear, since conventional fluorescence imaging cannot resolve the detailed distributions of SGs components due to the diffraction limit. Here, we investigated nanoscale organizaion of SGs by stochastic optical reconstruction microscopy (STORM), which provided us super-resolution images with spatial resolution of ~20 nanometers in the lateral direction and of ~60 nanometers in the axial direction.

Super-resolution imaging revealed that mRNAs in SGs were highly localized to tiny compartments with a diameter of less than 100 nanometers, whereas they were elusive in conventional fluorescence imaging. In mature SGs, the number of these compartments was higher than in small SGs, but the size of these compartments showed little difference. The result demonstrated that the growing process of SGs resulted from the assembly of tiny compartments. Furthermore, multicolor super-resolution imaging showed that some SG associated proteins colocalized with mRNAs and others did not. Since SGs components were densely packed within several micrometers radius, we could not observe the differences by conventional fluorescence imaging. These results suggested that SGs have highly organized composition that would be responsible for the physiological functions in cellular stress responses and that super-resolution imaging technique is a powerful tool to investigate the detailed organization of densely packed granular architectures in cells.

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Glycan Catabolism by Human Gut Symbionts involves Dynamic Protein Interactions

Krishanthi S. Karunatilaka[1], Elizabeth A. Cameron[2], Nicole M. Koropatkin[2], Eric C. Martens[2], Julie S. Biteen[1].
[1]Department of Chemistry, University of Michigan, Ann Arbor, MI, USA, [2]Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI, USA.

The human gut hosts trillions of bacteria that directly influence human health. The majority of gut microbiota play an important role in nutrition by metabolizing host-indigestible complex glycans into short-chain fatty acids. *Bacteroides thetaiotaomicron* (*Bt*), a prominent bacterial symbiont in the distal gut, metabolizes over a dozen complex glycans using membrane-associated protein complexes. The Starch Utilization System (Sus), a multi-protein complex in *Bt* that is essential for growth on starch, uses eight proteins (SusRABCDEFG) to process starch. SusCDEFG localize in the outer membrane and likely form a complex to facilitate starch binding, degradation and import. However, conventional biochemical methods have been unable to completely reveal the assembly and dynamics of these proteins in response to starch. We have applied single-molecule super-resolution imaging to characterize the Sus complex response to different sugars in live *Bt* under anaerobic conditions. Protein correlation studies performed with HaloTag-labeled SusG and other fluorescent antibody-labeled Sus proteins demonstrated that simple sugars such as glucose or maltose do not induce Sus complex assembly. Conversely, incubation of *Bt* cells with starch enhanced the co-localization of Sus proteins, suggesting starch-induced assembly of the complex. Furthermore, single-molecule tracking revealed that in the absence of starch, SusG predominately moves in a fast diffusion mode corresponding to freely diffusing SusG. In contrast, two-color single-molecule experiments performed with fluorophore-labeled starch and SusG showed that starch confines the free motion of SusG and induces the assembly of Sus proteins. Overall, our results suggest that starch catabolism involves dynamic interactions of Sus proteins, which assemble as a complex in the presence of starch in live cells.

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Orbital Tracking of Single Fluorescent Particles on a Commercial Confocal Microscope

Luca Lanzano'[1,2], Enrico Gratton[2].
[1]Nanophysics, Istituto Italiano di Tecnologia, Genoa, Italy, [2]Laboratory for Fluorescence Dynamics, Department of Biomedical Engineering, University of California, Irvine, CA, USA.

Single Particle Tracking (SPT) is a super-resolution technique used to determine the position of fluorescent particles with nanometer precision. The localization is generally obtained by analyzing the spatial distribution of fluorescence intensity emitted by the particle. In fact, the center of the distribution can be determined with an uncertainty which is much lower than the size of the distribution itself. In the orbital tracking method the position of a particle is obtained analyzing the distribution of intensity along a circular orbit scanned around the particle. In combination with an active feedback this method allows tracking of particles in 2D and 3D with millisecond temporal resolution[1]. More recently, the use of orbital tracking to perform imaging has also been proposed[2].

The orbital tracking and the other 3D SPT feedback methods are generally implemented on homebuilt microscopes which are not yet commercially available. On the other hand, commercial setups offer the advantage of a user-friendly software interface and pre-calibrated hardware components. It would be of interest to implement a SPT setup based on a feedback approach with minimal modification of a commercially available microscope. Here we explore this idea using a widely used confocal laser scanning microscope, the Zeiss LSM 510, in combination with an external piezoelectric stage scanner. We discuss advantages and limitations of this implementation of the orbital tracking method and the potential application to live cell experiments.

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Single-Molecule Fluorescence Imaging of Reco Localization and Dynamics in Bacillus Subtilis

Hannah H. Tuson[1], Yi Liao[1], Lyle A. Simmons[2], Julie S. Biteen[1].
[1]Chemistry, University of Michigan, Ann Arbor, MI, USA, [2]Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI, USA.

In all organisms, the high fidelity of DNA replication is essential for maintenance of chromosome integrity. DNA damage can be caused by polymerase errors or by external factors (e.g., X-rays or mutagenic chemicals). Thus, the cell has evolved a number of repair mechanisms to respond to different types of damage. In *B. subtilis*, repair of double-strand breaks (DSBs) in the DNA occurs through RecA-mediated homologous recombination. The mechanism by which RecA finds DSBs in vivo is not well described, but is believed to involve the proteins RecF, RecO, and RecR. This role for RecO in DSB repair in *B. subtilis* is distinct from its role in *E. coli* but analogous to that of Rad52 in eukaryotes, making *B. subtilis* an excellent model system for studying cellular response to DNA damage. Previously, bulk fluorescence studies have shown that RecO forms foci after the induction of double-strand breaks. However, RecO in undamaged cells can only be visualized when overexpressed, leaving questions about its true localization at wild type expression levels. Here, we have created cells in which PAmCherry-RecO is natively expressed from the RecO promoter as the only RecO source. We use single-molecule fluorescence microscopy in live *B. subtilis* to show that RecO rapidly forms foci following treatment with the DNA damaging agent phleomycin, but is diffuse throughout the cell under non-damaging conditions. This result suggests that, unlike several other proteins involved in DNA repair, RecO is not associated with the replisome prior to DSB recognition. Future work will examine the previously reported role of single-strand binding protein (SSB) in recruitment of RecO to DSBs.

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Versatile Pulsed 560 nm Laser Source for Time-Resolved Microscopy and Spectroscopy

Marcelle Koenig, Thomas Schoenau, Susanne Trautmann, Kristian Lauritsen, Romano Haertel, Dietmar Klemme, Rainer Erdmann.
PicoQuant GmbH, Berlin, Germany.

Fluorophores have become a standard tool in life science and biophysics as well as material sciences. Nowadays, fluorophore position and concentration are often not sufficient to answer questions in flurophore dynamics, such as diffusion behavior or molecular interactions. These aspects become accessible with the application of time-resolved fluorescence microscopy and