indistinguishable from that described previously for L-Aβ isomer. Namely, both peptides form channel-like pores with heterogeneous conductances, similar cation selectivity and both are blocked by Zn^{2+}. MD simulations show comparable β-barrel-like Aβ channels stability for D- and L-isomers, and both Aβ barrels isomers exhibit higher probability to cations in the solvated pore. Cell toxicity assays show similar toxicity levels for both Aβ isomers, consistent with earlier reports. The combined results suggest that Aβ cell toxicity is predominantly receptor-independent, non-stereo selective and imply a mechanism where increased levels of endogenous L-Aβ are toxic to cells mostly via direct pore formation. The findings presented lend support to the AD hypothesis suggesting cellular ion-dysregulation via Aβ channel formation.

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Platform: Imaging & Optical Microscopy: Cell & Tissue Imaging

3132-Plat

Towards Single Cell Optical Tomography

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Using a single-beam, oscillating Optical Tweezers we demonstrate trapping and rotation of rod-shaped bacterial cells with respect to the optical axis [1]. The angle of rotation is determined by the amplitude of the oscillation. This technique allows imaging fluorescently labeled 3D sub-cellular structures from different, optimized viewpoints. To illustrate our method we analyze the Z-ring of *E. coli* [2]. We use cells that express FtsZ-GFP and have their cytoplasmic membrane stained with FM4-64 (Figure). In a vertically oriented cell, both the Z-ring and the cytoplasmic membrane images appear as symmetric circular structures that lend themselves to quantitative analysis.

Scanning the cell alignment and using 3D image reconstruction from the corresponding images of a fluorescently labeled 3D sub-cellular structure, would make our approach analogous to that of cryo-electron tomography.

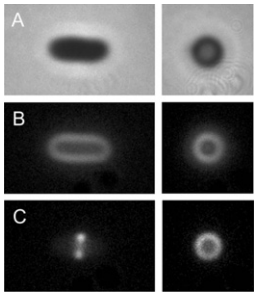

Figure - A trapped *E. coli* cell. Left: horizontal orientation; Right: vertical orientation. A) Phase contrast image, B) FM4-64 image of the cytoplasmic membrane, C) GFP image of the Z-ring.

3133-Plat

Spatiotemporal Image Correlation Spectroscopy Reveals Arp2/3-Complex-Driven Cytoplasmic Streaming in Mouse Oocytes, Maintaining Meiotic Cortical Spindle Positioning

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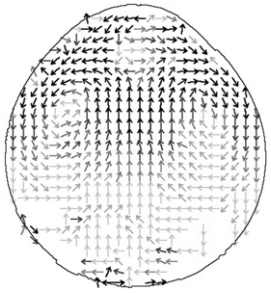
We use spatio-temporal correlation methods with transmitted light microscopy to demonstrate a novel pattern of internal-to-cortical circular cytoplasmic flow in meiosis II mouse oocytes. This flow is responsible for poising the spindle near the cortex for completion of meiosis II and successful oocyte fertilization. We show that this flow is driven by a balance of Arp2/3-complex-induced actin nucleation at the cortex and myosin-II-driven contraction of the cortex. STICS analysis of EGFP-Utrophin-labeled actin fibers shows cortical outflows with velocities consistent with those required to drive the cytoplasmic streaming. Inhibition of the Arp2/3-complex with CK-666 reverses this flow and leads to internal movement of the spindle away from the cortex. Interestingly, neither inhibition of actin polymerization, myosin-II contraction, nor microtubule depolymerization resulted in spindle internalization or large changes in cytoplasmic streaming. Kymograph analysis of the cortex stained with fluorescent Concavalin A demonstrates cortical contraction that is eliminated upon inhibition of myosin-II. Combined inhibition of myosin and Arp2/3-complex results in elimination of reversed cytoplasmic streaming.



3134-Plat

Molecular Architecture and Assembly Principles of Vibrio Cholerae Biofilms

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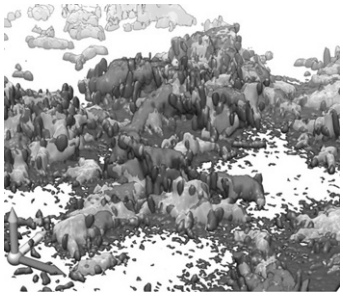
In their natural environment, microbes organize into communities held together by an extracellular matrix composed of polysaccharides and proteins. We have developed an in vivo labeling strategy that allows the extracellular matrix of developing biofilms to be visualized with conventional and super-resolution light microscopy. Here we show that Vibrio cholerae biofilms have three distinct levels of spatial organization: cells, clusters of cells, and collections of clusters. Subsequent multiresolution imaging of living V. cholerae biofilms revealed the complementary architectural roles of the four essential matrix constituents. RbmA provides cell-cell adhesion, Bap1 allows the developing biofilm to adhere to surfaces, and heterogeneous mixtures of Vibrio polysaccharide (VPS), RbmC, and Bap1 form the dynamic, flexible and ordered envelopes that encase the cell clusters. The ability to observe living biofilms develop with nanometer-scale precision and molecular specificity allows their fundamental construction and architectural principles to be established.



3135-Plat

Coherent Raman Scattering Microscopy: New Quantitative and Non-Invasive Tools for Biomedical Research

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A series of advances over the past decade have made the combination of coherent Raman scattering (CRS) with optical microscopy a highly sensitive and chemically selective tool for the label-free and noninvasive analysis of chemical species or biological components inside a sub-femtoliter probe volume [1]. By exploiting the coherent driving and detection of Raman modes in coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS), CRS microscopy allows the point-by-point chemical mapping of living cells and tissues, which is often difficult to attain by fluorescence and incoherent vibrational microscopy techniques. Here, we focus on two modalities of CRS imaging that allow label-free and quantitative biomedical research: First, single-frequency SRS imaging [2] is applied for the non-invasive mapping of lipids and proteins in skin cells and tissue tumor diagnostics. Second, in order to obtain unprecedented fast access to the full wealth of chemical and physical structure information of an a priori unknown biomolecular sample, we have also implemented hyperspectral CARS imaging [3]. Using this new technique, we demonstrate the 3D visualization of intracellular chemical composition and lipid structure properties inside a living cell, which cannot be obtained by conventional methods.

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[3] A. Volkmer, Vibrational imaging and microspectroscopies based on coherent anti-Stokes Raman scattering microscopy, J. Phys. D: Appl. Phys., 38 (2005) R59-R81. (Topical Review)